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Effects of pasteurization on bioactive polysaccharide acemannan and cell wall polymers from *Aloe barbadensis* Miller

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ABSTRACT

Physico-chemical modifications promoted by pasteurization treatments, performed at 65, 75 and 85 °C, for 15 and 25 min, on acemannan, the main bioactive polysaccharide from Aloe vera (*Aloe barbadensis* Miller) parenchyma, and cell wall polymers (CWP) were evaluated. The fresh Aloe samples were characterized by a relatively low content of acemannan (107–139 mg/g dm) probably due to the irrigation system used for its cultivation. Pasteurization seemed to increase the yields in acemannan content. However this effect was probably due to the decrease observed in ethanol-soluble mannose for all treatments. Deacetylation and loss of galactose side-chains might have contributed to the formation of new hydrogen bonds between mannose oligosaccharides and the long chains of acemannan. On the other hand, fresh Aloes exhibited a high content of pectic polysacharides, mainly homogalacturonans, accounting for up to 59% of total CWP. Further, pasteurization also affected the CWP, mainly the pectic moieties, in two different ways. On the one hand, a slight degradation of pectins was observed for samples treated at 65 °C which may be due to enzymatic degradation. On the other hand, the marked decrease in the pectic polymers (mainly homogalacturonans), observed for samples treated at 85 °C, may be due to their thermal degradation. Compositional and structural modifications on the different polysaccharide types were reflected by the significant changes occurring in the related functional properties, such as swelling (Sw), water retention capacity (WRC), and fat adsorption capacity (FAC). Swelling values were "exceptionally" high for fresh Aloe samples (over 200 mL water/g alcohol insoluble residue (AIR)), and pasteurized samples exhibited even higher Sw values. WRC and FAC values were also very high and exhibited similar trends; only samples pasteurized at 85 °C presented a significant decrease in comparison to the values determined for fresh samples.

1. Introduction

Aloe vera is one of the oldest healing plants known to mankind. Many authors have considered Aloe to be a member of the Liliaceae family, although, according to Rodríguez, Darias, and Díaz (2010), it comes from a family of its own called Aloaceae. However, this plant is related to plants such as onion, garlic, and asparagus, which are known to have medicinal properties (Lawless & Allan, 2000). Most of these plants originated in the dry regions of Africa, Asia, and Southern Europe, especially in the Mediterranean regions (Urch, 1999). Due to the numerous beneficial effects attributed to the Aloe plant, its production is an emerging industry for making cosmetics, functional food, and drugs (Eshun & He, 2004), and due to its medicinal properties it is being cultivated in other areas with different climatic conditions. In fact, Mexico is the main producer of Aloe, followed by Latin America, China, Thailand, and the United States (Rodríguez, 2004). There are over 360 known species of Aloe, but *Aloe barbadensis* Miller, also known as Aloe vera Linné or Aloe vulgaris Lamark is the most popular and the most widely cultivated (Rodríguez et al., 2010; Urch, 1999).

Aloe vera gel is the mucilaginous gel obtained from the squeezing of the clear jelly-like substance of the parenchyma tissue. Aloe vera gel has been reported to have multiple beneficial properties for wound healing, including the abilities to penetrate and anesthetize tissue, preclude bacterial, fungal, and viral growth, act as an anti-inflammatory agent and enhance blood flow (Christiaki & Florou-Paneri, 2010). It is now known that the gel representing approximately 70–80% of the weight of the whole leaf, serves as

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the water and energy storage component of the plant (Boudreau & Beland, 2006).

Acemannan, an ordered linear polymer consisting of substantially acetylated mannose monomers, is a storage polysaccharide present in the protoplast of the Aloe vera parenchyma, and is considered by many to be one of the major active ingredients of the Aloe vera plant (Femenia, Sánchez, Simal, & Rosselló, 1999; Rodríguez et al., 2010). The activities of acemannan as an antiviral agent, an immunomodulator, an agent in reducing opportunistic infections and stimulating the healing processes have been reported (Christiaki & Florou-Paneri, 2010; Ni, Turner, Yates, & Tizard, 2004). Apart from acemannan, the presence of a considerable number of other polysaccharides, mainly cell wall polymers (CWP), such as pectins, hemicelluloses and cellulose has also been reported (Femenia et al., 1999; Ni et al., 2004). In the last decade, there has been a great interest focused on the biological activity of CWP, which is greater and more diverse than previously realized (Gu et al., 2010).

When Aloe vera gel is exposed to air, the gel rapidly oxidizes, decomposes, and loses much of its biological activity (Eshun & He, 2004). Therefore, the potential use of Aloe vera products often involves some type of processing, e.g. heating, pasteurization, dehydration.

Pasteurization is a widely used technique for the preservation of food products (Lemmens et al., 2011). Along with the intended effects on pathogenic and spoilage microorganisms and deteriorative enzymes, it is known that thermal processing may affect food quality. Optimal process and production factors need to be identified in order to diminish undesired quality losses and to maximize the desired quality improvement as much as possible, while still ensuring microbial safety (Holdsworth, 2004).

In fact, pasteurization is probably the most common processing technique applied by the Aloe vera industry. However, in the scientific literature, studies based on the effects of pasteurization on bioactive components of Aloe are very limited. In fact, most of the processing studies involving the application of heat treatment to the Aloe plant are focused on dehydration processes.

Thus, recent studies have reported that minor alterations to the physicochemical and nutritional properties of Aloe vera gel were produced when samples were dehydrated at temperatures within the range of 60–70 °C, resulting in the production of a high quality gel (Miranda, Maureira, Rodriguez, & Vega-Gálvez, 2009; Miranda et al., 2010). Similarly, Gulia, Sharma, Sarkar, Upadhyay, and Shitandi (2010) recommended a temperature of 70 °C to obtain Aloe vera powder in a hot air oven with optimal physico-chemical and functional properties.

In addition, Femenia, García-Pascual, Simal, and Rosselló (2003), in a previous study, observed that structural modifications on the bioactive acemannan, and also affecting CWP, occurred when Aloe samples were dehydrated using temperatures above 60–70 °C. These modifications were reflected in the significant changes occurring in the related functional properties, such as swelling, water retention capacity, and fat adsorption capacity, which exhibited a significant decrease for samples treated at temperatures above 70 °C.

Further, Chang, Wang, Feng, and Liu (2006) reported that total polysaccharide content from Aloe vera exhibited a maximal stability when samples were treated at 70 °C decreasing either at higher (80–90 °C) or lower (50–60 °C) temperatures.

In order to attain a better understanding of the major changes that take place during Aloe vera pasteurization, the objective of this investigation was to evaluate the main effects on the physico-chemical properties of the main type of polysaccharides, i.e. acemannan and CWP, present in Aloe vera plant.

2. Materials and methods

2.1. Place and study period

This collaborative research was carried out in the following laboratories: (1) Postgraduate Department of Biochemical Engineering of the Technological Institute of Durango (Mexico); (2) Chemical Engineering Area of the Department of Chemistry of the University of the Balearic Islands (Spain); (3) Food Engineering Laboratory of the Faculty of Sciences of the University Juárez from the State of Durango, located in Gómez-Palacio (Mexico); and (4) in the facilities of the factory "Hacienda de Pedriceña", SPR de RL of CV located in Durango (Mexico). The experimental work was carried out from November 2008 to April 2010.

2.2. Material

Fresh whole Aloe vera (*A. barbadensis* Miller) leaves obtained from the "Hacienda de Pedriceña" industry located in Durango (Mexico) were used as the raw material to carry out all the experiments. The Aloe leaves, of between 35 and 50 cm of length, corresponded to 4-year old plants. Whole leaves were washed with distilled water to remove dirt from the surface. The spikes, along their edges, were removed before slicing the leaf. The epidermis (or skin) was carefully separated from the parenchyma using a scalpel-shaped knife. The filets were washed thoroughly with distilled water to remove the exudate from their surfaces. Approximately 415 kg of filets were obtained out of the ~970 kg of the fresh Aloe leaves used in this study. Regardless of the relative quality of the plant, the best results are obtained when the leaves are processed immediately after harvesting (Eshun & He, 2004). Therefore, fresh Aloe filets were stored no longer than 1 h at 1 °C prior to pasteurization treatments.

2.3. Pasteurization

Washed filets were trimmed and then cut into small pieces and blended in a mixer. These blended filets were then used to obtain the pasteurized samples. In each of the pasteurization treatments ~22 kg of filets were used. The process was performed in a double jacket tank with agitator, electronic temperature controller and a solenoid valve for steam. Based on existing bibliographical data about heat processing of Aloe vera, and also taking into account the broad experience of the *Hacienda de Pedriceña* factory, the samples were pasteurized at 65 °C, 75 °C and 85 °C. Two pasteurization periods were evaluated for each temperature, in particular 15 and 25 min. Three replicates were carried out for each experiment. A fresh sample of Aloe vera gel was used as a reference for each of the pasteurization treatments applied.

Microbiological studies ensuring the efficiency of pasteurization, based on the study of microorganisms and spoilage bacteria such as fungi and yeasts, total coliforms and aerobic mesophilics, were carried out by the collaborating factory. All pasteurized samples used in the study exhibited appropriate levels of the microbiological safety parameters analysed.

Identification of samples was carried out as follows: an initial letter, indicating either fresh (F), or pasteurized (P) samples, then a first number indicating the temperature of pasteurization (65, 75 or 85 °C), followed by a second number indicating the time of pasteurization (15 or 25 min). Thus, for example, the code P65/25 indicates a sample of Aloe vera filet pasteurized at 65 °C for 25 min. Due to expected variability among Aloe samples, a fresh sample was considered and used as a reference for each individual batch of pasteurized samples (therefore, in the case of sample P65/25, the corresponding fresh sample F65/25 was used to evaluate the effects of pasteurization).

2.4. Alcohol insoluble residues (AIRs)

AIRs from fresh and pasteurized Aloe vera filet samples were obtained by immersing the samples in boiling ethanol (final concentration 85% (v/v) aqueous) as described by Femenia, Robertson, Waldron, and Selvendran (1998). Prior to further analysis, the AIRs were milled using a laboratory type grain mill and passed through 0.5 mm aperture sieve.

2.5. Ethanol soluble material

In order to quantify the amount of mannose that was soluble after extraction with ethanol 85%, the ethanol-soluble material from each sample, either fresh or pasteurized, was concentrated and liophylized, before being used for sugar analysis.

2.6. Isolation and purification of acemannan

Isolation and purification of acemannan was carried out as described in Femenia et al. (2003). AIR preparations from fresh and pasteurized Aloe samples (300 mg) were suspended in distilled water (200 mL) and stirred for 2 h at room temperature. The supernatant (containing the acemannan) was recovered and extensively dialysed (MW cutoff 10,000–12,000). Further purification of the acemannan was carried out through gel permeation chromatography. The elution of dialysed fractions containing acemannan was performed on a column (100 cm × 1 cm) of Sephacryl S-400-HR at a flow rate of 16 mL/h. The fractions were dissolved in 2 mL, 50 mM potassium–phosphate buffer, pH 6.5, containing 0.2 M NaCl. Fractions (2 mL) were collected and aliquots (20 μL) were assayed for carbohydrate by the phenol–sulphuric acid method. The appropriate fractions containing purified acemannan were combined, dialysed, concentrated, and an aliquot was freeze dried for FTIR, sugar and methylation analysis. The remaining material was stored at −20 °C.

2.7. Analysis of carbohydrate composition

Carbohydrate analysis was performed as in González-Centeno et al. (2010) for neutral sugars. Sugars were released from residues by acid hydrolysis. Samples (either purified acemannan, ethanol-soluble material or water insoluble cell wall polysaccharides from fresh and pasteurized Aloe filets) were dispersed in 12 M H_2SO_4 for 3 h followed by dilution to 1 M and hydrolysed at 100 °C for 2.5 h (Saeman, Moore, Mitchell, & Millett, 1954). A second sample was hydrolysed only with 1 M H_2SO_4 (100 °C for 2.5 h). The cellulose content was estimated by the difference in glucose obtained by Saeman hydrolysis and this milder hydrolysis method. Neutral sugars were derivatized as their alditol acetates and isothermally separated by GC at 220 °C on a 3% OV225 Chromosorb WHP 100/120 mesh column. Uronic acids were determined by colorimetry, as total uronic acid (Blumenkrantz & Asboe-Hansen, 1973), using a sample hydrolysed for 3 h at 20 °C in 12 M H_2SO_4, followed by 1 h at 100 °C in 1 M H_2SO_4.

2.8. Methylation analysis

Methylation analysis of purified acemannan fractions was based on a modified sequential method using sodium hydroxide and methyl iodide (Ciucanu & Kerek, 1984). The modifications introduced to improve the overall methylation procedure have been described in detail by Femenia, García-Conesa, Simal, and Rosselló (1998).

2.9. Fourier transformed infrared (FTIR) spectroscopy analysis

FTIR spectra were obtained on a Bruker IFS66 instrument, at a resolution of 3 cm^{-1}, after preparing a KBr disc containing 2 mg of either AIRs or purified acemannan polymer from fresh and pasteurized Aloe filets. The single beam traversing each sample was ratioed with the single beam of the corresponding background. Equivalent samples from different experimental runs gave the same spectra in all cases. Further, the percentage of total uronic acids present in AIR samples which are esterified (DME) was determined through FTIR as described by Manrique and Lajolo (2002).

2.10. Functional properties

Functional properties included hydration properties, swelling (Sw) and water retention capacity (WRC), and fat adsorption capacity (FAC). WRC and Sw were measured using hydrated AIR samples from fresh and pasteurized Aloe filets in phosphate buffer (1 M; pH 6.3) to represent pH and buffering conditions of food products. All functional properties were measured on AIR samples from freeze dried and pasteurized Aloe filets.

Sw was measured as bed volume after equilibration in excess solvent (Kuniak & Marchessault, 1972). The sample (0.1–1.0 g) was weighed into a graduated conical tube with an excess of buffer. The suspension was stirred and after equilibration (16 h) the volume was recorded and expressed as mL/g AIR.

WRC was measured as water retained by the fibre-enriched material (Thibault, Lahaye, & Guillon, 1992). Samples (0.1–1.0 g) were suspended (24 h) in phosphate buffer (20 mL) and centrifuged (15,000 rpm; 15 min) with residual solids in the supernatant recovered by filtration (GF/C paper) and recombined with the pellet. The pellet was weighed ($P1$), and dried at 102 °C overnight. After cooling the dry weight was determined ($P2$) and hence WRC as: $P1 - P2/(P2 - k)$, where $k = a(P1 - P2)$, with $a = 28 \times 10^{-3}$ g salt (phosphate)/mL.

FAC was measured as oil retention capacity (Caprez, Arrigoni, Amadó, & Neukom, 1986). AIR samples (0.1–1.0 g) were mixed with sunflower oil (3–30 mL), left overnight at room temperature, centrifuged (15,000 rpm, 10 min), the excess supernatant was decanted, and FAC expressed as g oil/g AIR.

2.11. Scanning electron microscopy

SEM micrographs of fresh and pasteurized Aloe samples were obtained with a Hitachi S-3400N (Japan) scanning electron microscope at an accelerating voltage of 15 kV. Freeze dried samples were directly observed, without further treatment, under a pressure of 40 Pa.

2.12. Statistical analysis

Results were analysed by means of a one-way and multifactor analysis of variance, using the LSD test with a 95% confidence interval for the comparison of the test means.

3. Results and discussion

3.1. SEM observations

Ultrastructural differences, shown by SEM micrographs, were clear between fresh and pasteurized Aloe vera samples. Thus, the regular and relatively rigid structure of the parenchyma cells observed in fresh samples (Fig. 1a) was modified after the pasteurization process (Fig. 1b). Most of the cell walls were broken down and a more amorphous material could be observed in the

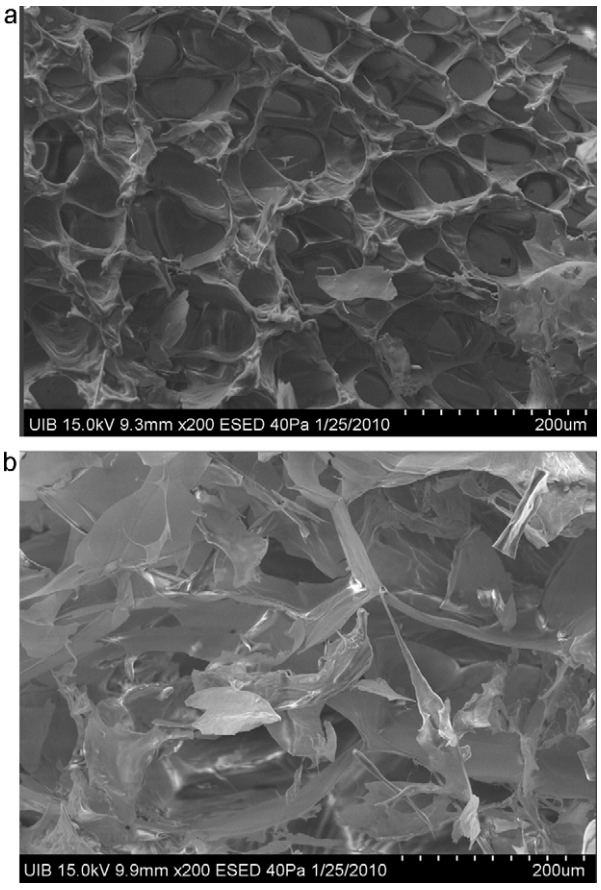

Fig. 1. SEM micrographs of a blended sample of fresh Aloe vera gel (F75/25) (a) and a sample of Aloe vera gel pasteurized at 75 °C for 25 min (P75/25) (b).

pasteurized sample. All pasteurized samples, under the different treatments, exhibited a similar appearance.

3.2. Effects of the pasteurization process on the composition of the main type of polysaccharides from Aloe vera parenchyma

The most significant changes affecting the main polysaccharides from Aloe vera parenchyma tissue during pasteurization were evaluated. Thus, the bioactive polymer acemannan was purified and subjected to carbohydrate, glycosidic linkage and FTIR analysis. In addition, the water insoluble cell wall polysaccharides (CWP) from Aloe vera parenchyma were also subjected to carbohydrate and FTIR analysis. Purified acemannan and CWP from fresh Aloe vera filets were used as a reference for each of the pasteurization treatments.

One of the main features of the Aloe vera filets was their high water content (Table 1). Thus, fresh Aloe samples contained about 98.7–98.9% of water. Similar percentages, 98.6–98.8% water, were determined for pasteurized samples. No significant differences ($p > 0.05$) were found between the fresh and the corresponding processed samples.

The amounts of ethanol-soluble mannose present in the different samples were also quantified (Table 1). Thus, fresh samples contained about 92–106 mg mannose per g of dm. This mannose was probably present in the form of monosaccharides and oligosaccharides of a relatively low degree of polymerization. As can be observed in Table 1, a significant reduction ($p < 0.05$) in the amount of this ethanol-soluble mannose was detected for all the pasteurization treatments applied. Moreover, for the three temperatures tested, a longer time of pasteurization was reflected in a higher decrease of the ethanol soluble mannose, in comparison with the corresponding fresh sample ($p < 0.05$). In the case of sample P85/25, this reduction reached up to 38% of the initially soluble mannose.

Acemannan, as the main bioactive component of the *A. barbadensis* Miller (Rodríguez et al., 2010), was also quantified for all fresh and pasteurized samples (Table 1). Acemannan content for the fresh samples ranged from 113 to 139 mg/g dm. These amounts could be considered relatively low, since they represent ~42–52% of the acemannan amount reported by Femenia et al. (2003) (268 mg acemannan/(g dm of fresh Aloe filets)). Although this difference could initially be attributed to several factors such as the geographic location, annual season, climate or exposure to light (Rodríguez et al., 2010), in our opinion, the irrigation treatment applied could be a key factor to explain such differences. Thus, whereas Aloe vera plants from this study were well-irrigated, a non-irrigation treatment was applied to Aloe plants corresponding to the work of Femenia et al. (2003). This would also be consistent with the work of Yaron (1993), who pointed out that the irrigation of the Aloe plant affects the amount of mucopolysaccharides, the content being smaller in well-irrigated plants.

Contrary to what was observed for ethanol soluble mannose, the acemannan contents determined for all pasteurized samples were higher ($p < 0.05$) than those corresponding to the fresh samples. Acemannan in pasteurized samples ranged from 122 to 150 mg/g dm. The partial deacetylation of acemannan polymer (FTIR spectra (not shown) corresponding to acemannan from pasteurized samples revealed important decreases in the bands of 1740 and 1250 cm^{-1} which correspond to the C=O and C–O–C stretches of the acetyl groups) during processing might have contributed to the formation of new hydrogen bonds between the high MW chains of acemannan and the initially ethanol-insoluble mannose. However,

Table 1
Characterization of lyophilised Aloe vera fractions corresponding to the fresh and pasteurized Aloe vera filet samples (all results except dry matter are expressed as g/g dry matter of Aloe filet).

	Dry matter[a]	EtOH-soluble mannose	AIR	Acemannan	Cell wall polymers
F65/15	1.29 ± 0.03	0.093 ± 0.011	0.546 ± 0.013	0.119 ± 0.012	0.304 ± 0.023
P65/15	1.36 ± 0.16	0.074 ± 0.007	0.578 ± 0.012	0.127 ± 0.011	0,279 ± 0.013
F65/25	1.20 ± 0.05	0.103 ± 0.008	0.644 ± 0.012	0.131 ± 0.011	0.373 ± 0.015
P65/25	1.20 ± 0.08	0.078 ± 0.011	0.664 ± 0.021	0.148 ± 0.007	0.353 ± 0.012
F75/15	1.29 ± 0.20	0.096 ± 0.007	0.561 ± 0.007	0.119 ± 0.008	0.325 ± 0.009
P75/15	1.33 ± 0.08	0.077 ± 0.004	0.609 ± 0.014	0.134 ± 0.010	0.315 ± 0.016
F75/25	1.24 ± 0.03	0.106 ± 0.008	0.590 ± 0.018	0.123 ± 0.010	0.338 ± 0.021
P75/25	1.27 ± 0.02	0.072 ± 0.005	0.631 ± 0.021	0.151 ± 0.011	0.335 ± 0.012
F85/15	1.34 ± 0.24	0.092 ± 0.007	0.536 ± 0.013	0.108 ± 0.006	0.315 ± 0.014
P85/15	1.42 ± 0.21	0.065 ± 0.006	0.572 ± 0.012	0.122 ± 0.013	0.268 ± 0.021
F85/25	1.27 ± 0.03	0.098 ± 0.010	0.582 ± 0.020	0.113 ± 0.011	0.345 ± 0.011
P85/25	1.36 ± 0.19	0.061 ± 0.004	0.581 ± 0.015	0.129 ± 0.005	0.266 ± 0.015

[a] Dry matter is given as g/100 g fresh Aloe.

Table 2
Characterization of the isolated and purified acemannan from fresh and pasteurized Aloe vera filet samples (results are expressed as mg sugar/g dry matter of Aloe filet).

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronics
F65/15	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	1.3 ± 0.1	96.1 ± 1.8	5.7 ± 0.2	13.2 ± 0.3	1.9 ± 0.1
P65/15	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	1.0 ± 0.1	108.8 ± 2.1	3.1 ± 0.1	12.1 ± 0.4	1.2 ± 0.0
F65/25	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	1.2 ± 0.2	106.9 ± 0.8	6.5 ± 0.3	14.2 ± 0.5	1.5 ± 0.1
P65/25	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	1.0 ± 0.0	130.3 ± 0.9	3.2 ± 0.2	12.2 ± 0.1	1.2 ± 0.2
F75/15	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	1.0 ± 0.1	97.7 ± 1.3	7.0 ± 0.3	12.1 ± 0.2	1.2 ± 0.0
P75/15	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	1.0 ± 0.0	115.9 ± 1.7	3.8 ± 0.4	11.8 ± 0.3	1.0 ± 0.0
F75/25	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	1.3 ± 0.1	98.0 ± 1.0	8.0 ± 0.4	13.9 ± 0.2	1.7 ± 0.1
P75/25	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	1.2 ± 0.0	128.4 ± 1.5	5.6 ± 0.3	14.5 ± 0.5	1.4 ± 0.2
F85/15	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	1.3 ± 0.1	84.1 ± 2.2	7.3 ± 0.1	12.6 ± 0.1	1.4 ± 0.1
P85/15	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.9 ± 0.0	107.8 ± 1.5	2.9 ± 0.0	8.8 ± 0.2	1.0 ± 0.1
F85/25	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	1.1 ± 0.0	90.6 ± 1.2	7.4 ± 0.3	12.5 ± 0.3	1.4 ± 0.2
P85/25	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.9 ± 0.1	116.0 ± 1.3	2.2 ± 0.1	8.9 ± 0.4	1.1 ± 0.1

in most samples, the surplus of mannose recovered in the acemannan of pasteurized samples did not account for all the decrease observed in soluble mannose, suggesting a possible degradation of carbohydrates due to the heat treatment (Chang et al., 2006).

On the other hand, cell wall polysaccharides (CWP) accounted for 304–373 mg/g dm in the fresh Aloe filets (Table 1). Differences in CWP content were only significant ($p < 0.05$) for samples pasteurized at 85 °C (P85/15 and P85/25), which exhibited a significant decrease.

Acemannan and CWP present in the alcohol insoluble residues (AIRs), together with the ethanol-soluble mannose accounted for about 50–60% of the dry material (dm) of the fresh Aloe vera filets (Table 1). Soluble sugars (mainly glucose), proteins, lipids and mineral elements (mainly Ca, K, Na and Mg) would probably account for the remaining portion of the filets dry matter (Femenia et al., 1999).

3.2.1. Acemannan composition

The presence of mannose in purified acemannan fractions obtained from fresh Aloe filets ranged from 78.3 to 81.9% of total monomers (Table 2). Glucose and galactose were the remaining monomers accounting for 10.1–11.7% and 4.8–6.8% of acemannan component sugars, respectively. The amount of mannose is similar to the value reported by Chang, Chen, and Feng (2011), but relatively lower than the percentages reported by McAnalley (1993) and Talmadge et al. (2004). Although the presence of minor amounts of other sugars such as xylose, uronic acids, rhamnose, fucose and arabinose has been previously reported (Chow, Williamson, Yates, & Goux, 2005; Mandal & Das, 1980; t'Hart, van den Berg, Kuis, van Dijk, & Labadie, 1989), it is not clear whether the presence of these sugars is an integral part of the structure of acemannan, or whether these are a consequence of the occurrence of polluting carbohydrates.

All pasteurized samples exhibited significant changes in the acemannan related sugars in comparison with those of the corresponding fresh Aloe filet used as a reference (Table 2). In particular, the mol% of mannose units was about 6–7% higher for samples processed at 65 and 75 °C, whereas this increase was up to 12–13% for samples treated at 85 °C. Further, the presence of glucose and galactose was reduced to around 7–9%, and 2–4%, respectively. The degradation of galactose during heat treatment of Aloe has been previously observed (Femenia et al., 2003).

In order to gain more insight into the latter observations, glycosidic linkage analysis was performed on the purified acemannan

Table 3
Glycosidic linkage analysis from purified acemannan-containing fractions from fresh and pasteurized Aloe vera samples.

	Fresh[a]	P65/15	P65/25	P75/15	P75/25	P85/15	P85/25
Rhamnose							
1.2	0.1	0.1	0.1	0.1	0.1	0.1	0.0
Fucose							
Terminal	0.1	0.2	0.2	0.1	0.2	0.1	0.1
Arabinose							
Terminal-f	0.1	0.1	0.1	0.1	0.1	0.1	0.1
1.5	0.1	0.0	0.1	0.1	0.1	0.0	0.0
Xylose							
Terminal	0.4	0.2	0.3	0.2	0.3	0.2	0.2
1.4	0.8	0.8	0.7	0.7	0.8	0.6	0.5
Mannose							
Terminal	0.48	0.9	0.9	0.9	1.0	0.94	1.1
1.4	75.1	81.4	82.3	82.1	83.7	84.9	85.4
1.6	0.8	0.6	0.8	0.5	0.6	0.7	0.8
1.3.4	1.9	2.0	2.3	1.8	1.5	2.1	2.3
1.4.6	2.1	1.1	0.9	1.1	0.7	0.7	0.4
1.3.4.6	0.3	0.2	0.1	0.2	0.3	0.2	0.1
Galactose							
Terminal	2.9	1.5	1.4	1.5	1.3	1.2	1.0
1.6	0.2	0.0	0.0	0.0	0.0	0.1	0.0
1.3.4	1.0	0.2	0.3	0.3	0.2	0.2	0.3
1.3.6	1.1	0.3	0.2	0.3	0.1	0.3	0.2
1.4.6	0.4	0.1	0.1	0.1	0.0	0.1	0.0
Glucose							
1.4	9.3	8.1	7.2	8.0	7.5	5.8	5.5
1.3.4	0.5	0.2	0.2	0.1	0.2	0.2	0.1
1.4.6	2.1	1.3	1.1	1.0	0.8	0.7	0.8
Glucitol	0.2	0.2	0.3	0.1	0.2	0.2	0.3

[a] Fresh results correspond to the average values of acemannan from fresh Aloe samples.

Table 4
Characterization of cell wall polysaccharides from fresh and pasteurized Aloe vera filet samples (results are expressed as mg sugar/g dry matter of Aloe filet).

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Glc (1 M)[a]	Uronics
F65/15	2.3 ± 0.0	1.5 ± 0.2	3.9 ± 0.3	12.3 ± 0.4	13.6 ± 0.3	21.0 ± 0.4	105.3 ± 1.3	(9.0 ± 0.2)	144.0 ± 3.3
P65/15	1.9 ± 0.1	1.5 ± 0.1	3.2 ± 0.1	10.9 ± 0.2	14.5 ± 0.4	20.1 ± 0.2	97.7 ± 2.1	(8.6 ± 0.3)	129.3 ± 2.7
F65/25	2.1 ± 0.2	1.3 ± 0.0	3.8 ± 0.3	11.3 ± 0.5	16.0 ± 0.5	19.7 ± 0.5	124.6 ± 1.5	(9.3 ± 0.1)	193.8 ± 3.0
P65/25	1.9 ± 0.1	1.7 ± 0.0	3.5 ± 0.2	12.0 ± 0.5	20.6 ± 0.7	18.5 ± 0.5	122.3 ± 1.7	(8.9 ± 0.1)	172.4 ± 3.3
F75/15	2.2 ± 0.2	1.6 ± 0.1	3.9 ± 0.3	11.8 ± 0.4	18.9 ± 0.6	23.9 ± 0.3	109.1 ± 1.5	(10.4 ± 0.2)	153.0 ± 2.4
P75/15	2.4 ± 0.0	1.6 ± 0.2	4.0 ± 0.1	12.5 ± 0.7	16.2 ± 0.3	22.5 ± 0.4	107.2 ± 1.2	(9.5 ± 0.2)	148.3 ± 1.9
F75/25	2.2 ± 0.1	1.3 ± 0.1	3.5 ± 0.2	10.8 ± 0.2	11.8 ± 0.8	21.2 ± 0.7	113.9 ± 2.4	(10.2 ± 0.1)	173.6 ± 3.1
P75/25	2.3 ± 0.1	1.4 ± 0.2	3.9 ± 0.1	11.6 ± 0.4	15.9 ± 0.6	22.8 ± 0.1	115.9 ± 1.5	(9.2 ± 0.1)	161.5 ± 2.3
F85/15	2.2 ± 0.0	1.7 ± 0.2	3.8 ± 0.2	11.1 ± 0.1	16.2 ± 0.3	20.9 ± 0.5	105.6 ± 1.5	(8.4 ± 0.2)	153.7 ± 4.2
P85/15	2.1 ± 0.2	1.4 ± 0.1	3.2 ± 0.1	9.7 ± 0.5	12.2 ± 0.4	17.7 ± 0.3	98.4 ± 1.6	(7.3 ± 0.3)	123.0 ± 2.7
F85/25	2.3 ± 0.2	1.4 ± 0.1	4.0 ± 0.0	12.2 ± 0.2	15.9 ± 0.7	20.7 ± 0.3	110.4 ± 1.2	(9.6 ± 0.1)	178.4 ± 1.8
P85/25	1.8 ± 0.0	1.2 ± 0.0	3.0 ± 0.1	8.9 ± 0.6	12.4 ± 0.5	14.9 ± 0.4	99.7 ± 2.3	(8.0 ± 0.3)	123.8 ± 2.5

[a] Numbers in brackets represent the composition of neutral sugars as determined by 1 M sulphuric acid hydrolysis.

fractions from fresh and pasteurized Aloe samples. The results of methylated polymers are shown in Table 3. Relative sugar mole ratios obtained from alditol acetates and partially methylated alditol acetates were in broad agreement. In addition, a complete methylation was indicated by the presence of only a few types of methylated ethers from each sugar, and, also, by the virtual absence of unmethylated monomers in the hydrolysates of the methylated acemannan fractions.

Since methylation of acemannan from fresh Aloe samples did not exhibit significant differences, the results shown in Table 3 for fresh Aloe acemannan corresponds to the average values obtained from the different fresh samples considered.

Methylation analysis revealed important structural differences among the acemmanan polymers from the pasteurized filets. Thus, although (1,4)-linked mannosyl residues were predominant in all samples, an important increase in these residues was observed in all pasteurized samples. Samples pasteurized at 85 °C (P85/15 and P85/25) exhibited the highest increase. This was accompanied by considerable decrease in (1,4,6)-linked mannosyl units. In addition, lower recoveries of galactosyl residues were detected in all pasteurized samples. These observations suggest a lower degree of branching, in particular of galactose side-chains since galactose units attached to C6 of mannose residues have been detected in Aloe acemannan (Manna & McAnalley, 1993).

The losses of galactosyl residues, together with the observed deacetylation process, would support the interaction of acemannan chains with mannose oligosaccharides by hydrogen bonding. This effect would result in mannose-rich chains of higher MW. In fact, the distribution of acetyl groups and galactosyl units along the main chain can have a significant effect on the interactive properties of mannans (Dea & Clark, 1986).

Overall, these chemical modifications may have an important influence on the physiological properties attributed to the bioactive polymer acemannan. Thus, further investigation addressed to the biological importance of such chemical variations would be of great interest.

3.2.2. Cell wall polysaccharides

One of the main features of the cell walls of the Aloe filets analysed was the clear predominance of pectic polysaccharides. Pectins, in fresh Aloe filets, ranged from 56 to 59% of total CWP. These pectic polymers were characterized by the large presence of galacturonic acid units and the lower amounts of galactose and arabinose, suggesting the occurrence of homogalacturonans, and minor amounts of rhamnogaluctoronans with a low degree of branching (Table 4). Recent studies have described the physico-chemical properties of highly purified galacturonate polysaccharides from Aloe vera (McConaughy, Stroud, Boudreaux, Hester, & McCormick, 2008; McConaughy, Kirkland, Treat, Stroud, & McCormick, 2008). The main feature of these polymers was also the high presence of galacturonic acid residues (95–98% of total sugars) (Table 4).

Cellulose, released using Saeman hydrolysis conditions, was the second cell wall polymer in abundance, accounting for 28–31% of CWP in fresh Aloe filets.

Xyloglucans, as suggested by the presence of xylose, fucose and non-cellulosic glucose, and mannans were probably the main hemicellulosic polymers in the samples, accounting for 11–14% of total CWP.

Pectic substances were the most affected type of polysaccharides by the pasteurization treatments. Samples P65/15 and P65/25 showed a decrease of 10–11% in total uronic acids content, in comparison with the corresponding fresh Aloe samples. This loss

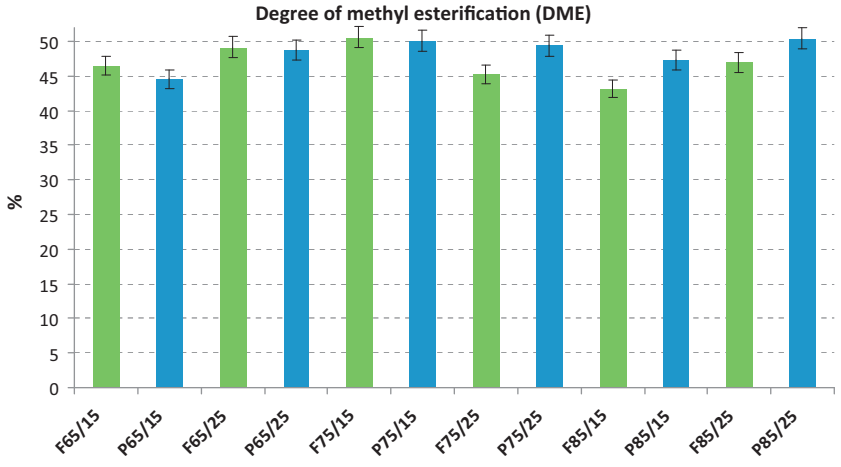

Fig. 2. Degree of methyl-esterification (DME) of pectic polysaccharides from fresh (green columns) and pasteurized (blue columns) Aloe vera filets. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

reached up to 20 and 31% for the P85/15 and P85/25 samples, respectively. The lowest losses (less than 7%) were observed for samples pasteurized at 75 °C. This would be in agreement with the works of Miranda et al. (2009, 2010) suggesting temperatures of around 70 °C in order to preserve the physicochemical properties of polysaccharides from Aloe vera.

The fact that, for samples pasteurized at 65 °C, galactose and arabinose were less affected than uronic acids, suggests that the pectic backbone might have suffered considerable modification during pasteurization, whereas pectin side-chains were less affected. On the other hand, for samples treated at 85 °C, the losses of arabinose and, in particular, of galactose residues, added to the important decrease in uronic acids, suggest that, in this case, both unbranched and branched pectin moeities were affected. Degradation of pectic polysaccharides at 85 °C may have occurred due to β-elimination reaction promoted by heating, whereas, at temperatures around 65 °C, the activity of pectic polysaccharide degrading enzymes might have contributed to the observed losses of uronic acid residues (Chang et al., 2006).

The degree of methyl esterification (DME) of pectic polysaccharides in fresh and pasteurized Aloe filets is shown in Fig. 2. DME for fresh and pasteurized samples ranged from 43 to 51%. Pasteurization at 65 °C (P65/15 and P65/25) and, also, at 75 °C for 15 min (P75/15) did not cause significant ($p > 0.05$) modification of the DME of pectins. However, pectic polysaccharides corresponding to samples treated at 75 °C for 25 min (P75/25), and, also, at 85 °C (P85/15 and P85/25) exhibited a higher DME ($p < 0.05$) than pectins from the corresponding fresh samples. Therefore, it seems that the regions of pectins with low DME could have been more easily degraded during the heat treatment. In fact, a higher thermal stability of esterified pectins than pectins with free acidic groups has previously been reported (Thakur, Singh, Handa, & Rao, 1997).

Modification of pectic polysaccharides from Aloe vera might be of special significance since one of the most characterized bioactive roles of pectins is as an anti-cancer agent (Morris, Gromer, Kirby, Bongaerts, & Gunning, 2011).

Cellulose, the main structural component of the cell wall, remained almost unchanged during the different pasteurization treatments applied. This is consistent with cellulose being the most resistant polymer forming the cell wall matrix. Hemicelluloses, probably xyloglucans, underwent only slight losses during pasteurization carried out at 85 °C, as indicated by small losses in xylose and, also, in non cellulosic glucose.

3.3. *Effects of the pasteurization process on functional properties of Aloe parenchyma AIRs*

The properties of cell wall rich materials depend on the polysaccharides comprising them, but also on the manner in which they are interlinked to form the three-dimensional, functional structure of the intact cell wall (Jarvis, 2011). Chemical, mechanical, thermal and enzymatic processing can modify the functional properties of polysaccharide-rich materials (Benitez et al., 2011). During processing, polysaccharides might undergo modifications in terms of their physical state, macrostructure, microstructure, and composition, as well as structure-dependent changes in their functional properties (Galanakis, Tornberg, & Gekas, 2010; Nindo, Powers, & Tang, 2010). Therefore, in order to evaluate possible changes in the structural arrangement of either storage or cell wall polysaccharides from Aloe vera parenchyma, hydration-related properties such as swelling (Sw) and water retention capacity (WRC), and fat adsorption capacity (FAC) were measured (Fig. 3a, b and c, respectively).

Sw values for AIRs obtained from the different fresh Aloe samples did not exhibit significant differences ($p > 0.05$), ranging from 208 to 236 mL H_2O/g AIR (Fig. 2a). However, for all treatments

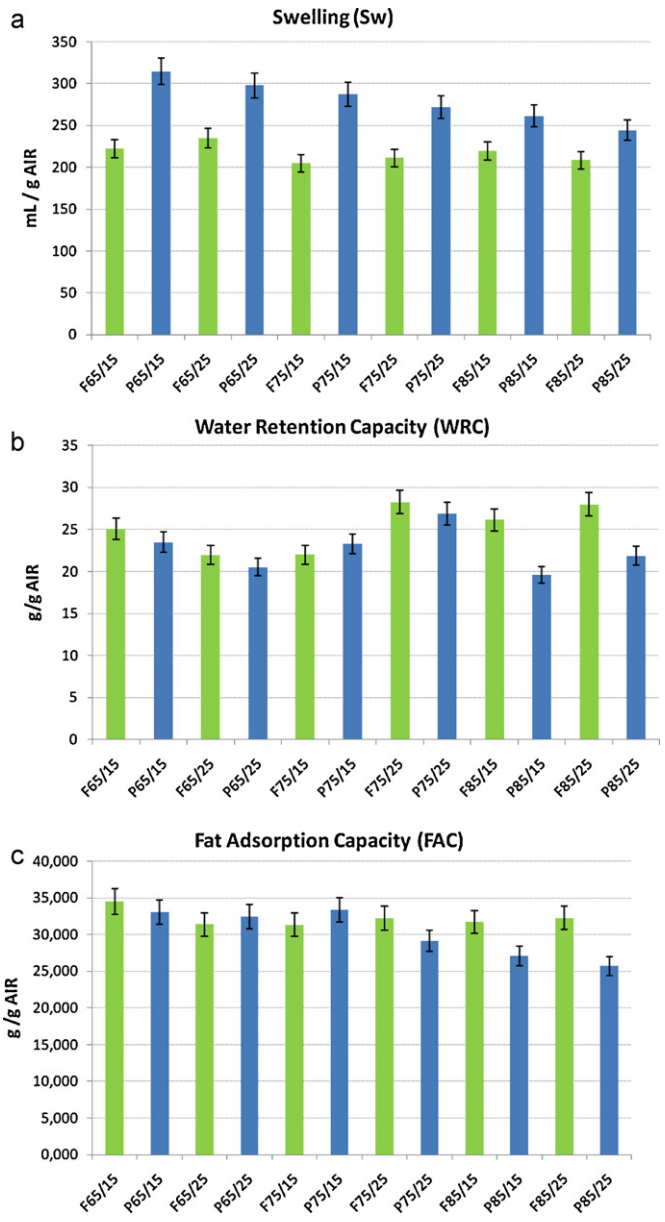

Fig. 3. Functional properties determined for AIR samples from fresh (green columns) and pasteurized (blue columns) Aloe vera filets. (a) Swelling (expressed as mL water/g AIR), (b) water retention capacity (expressed as g water/g AIR), and (c) fat adsorption capacity (expressed as g oil/g AIR). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

assayed, Sw values corresponding to AIRs from processed samples were higher ($p < 0.05$) than those corresponding to AIRs from fresh samples. Maximum Sw values were observed for samples treated at 65 °C, i.e. P65/15 and P65/25, reaching up to 320 and 296 mL H_2O/g AIR, respectively. Then, an increase in temperature promoted a clear decrease in Sw value. In addition, for a set temperature, a longer period of pasteurization seemed to cause a decrease in the Sw capacity of samples. Despite the differences between fresh and processed samples, it should be pointed out that the Sw values determined for the different Aloe samples were "exceptionally" high. Interestingly, the swelling capacity of polysaccharide-rich materials has been linked with their ability to reduce blood cholesterol (Elleuch et al., 2011).

As expected, WRC values were significantly lower than values determined for Sw capacity. There was a considerable variation in

WRC values determined for the fresh AIRs samples, ranging from 22.1 g H_2O/g AIR for F65/25 sample to 27.9 g H_2O/g AIR for F75/25 sample. Pasteurization at 65 °C and 75 °C did not cause significant alterations ($p > 0.05$) in the WRC property when values from processed and fresh samples were compared. On the contrary, WRC values of samples processed at 85 °C exhibited a marked decrease ($p < 0.05$) compared with the fresh Aloe references. It should be pointed out that the WRC of polymer-rich products has been linked to laxative effects, and also to the reduction of blood glucose (Elleuch et al., 2011).

Alongside their hydration properties, polysaccharide rich-materials from Aloe vera exhibited a high capacity to adsorb lipid molecules. Thus, FAC values determined for AIRs from fresh Aloe samples ranged from 32.3 to 34.6 g oil/g AIR, the differences among AIRs from fresh samples being non significant ($p > 0.05$). The FAC values detected for pasteurized samples followed a similar trend to the WRC values, decreasing only for samples pasteurized at 85 °C ($p < 0.05$).

It should be pointed out that the functional properties exhibited, not only by AIRs from fresh samples but also by AIRs from pasteurized Aloe parenchyma were significantly higher than the maximum values reported for most fruit and vegetables (Elleuch et al., 2011). The high capacity of Aloe vera parenchyma to retain water and oil may explain its widespread use in cosmetics. Moreover, its efficiency in binding organic molecules might play an important role in the reported capacity of Aloe vera to lower the levels of cholesterol, carcinogens and other toxic compounds.

4. Conclusions

The importance of the physico-chemical modifications detected in pasteurized Aloe vera parenchyma depended on the conditions (temperature and time) used during the pasteurization process. Regarding the chemical composition, the yields of bioactive polysaccharide acemannan apparently increased for pasteurized samples, however this effect might have been promoted by the formation of new hydrogen bonds between mannose-rich oligosaccharides, initially soluble in 85% ethanol, and the high MW chains of acemannan. Further, the structural modifications detected on the acemannan, and cell wall pectins could be responsible for the changes observed in the functional properties of pasteurized filets. The physico-chemical alterations of the main type of polysaccharides from Aloe vera parenchyma observed during pasteurization may have important implications for the physiological activities attributed to the Aloe vera plant. Therefore, further studies on the biological significance of these modifications are needed.

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