SPECIAL GUEST EDITOR SECTION

Compositional and Structural Features of the Main Bioactive Polysaccharides Present in the *Aloe vera* Plant

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***Aloe vera* (*A. barbadensis* Miller) is probably one of the most popular plants, widely studied because of numerous properties associated with the polysaccharides present in its gel. In particular, two main types of bioactive polysaccharides can be distinguished in the *A. vera* gel: an acetylated mannose-rich polymer that functions as storage polysaccharide, and a galacturonic acid–rich polymer as the main component comprising the cell walls of the parenchymatous tissue. Interestingly, most of the beneficial properties related to the aloe plant have been associated with the acetylated mannose-rich polysaccharide, also known as acemannan. However, the composition and structural features of these polysaccharides, as well as the beneficial properties associated with them, may be altered by different factors, such as the climate, soil, postharvest treatments, and processing. Further, different analytical methods have been used not only to identify but also to characterize the main polysaccharides found in parenchyma of *A. vera* leaf. Within this context, the main aim of this review is to summarize the most relevant information about the structural and compositional features of the main polysaccharides found in the *A. vera* gel as well as the most relevant analytical techniques used for their identification and their influence on the technological, functional, and beneficial properties related to the *A. vera* plant.**

Aloe vera, a member of the Liliaceae family, has enjoyed a long history of providing myriad health benefits, being one of the herbal remedies most frequently used in the treatment of different diseases (1–3). Around the world, there are more than 400 species of *Aloe*, but without a doubt the most popular and widely used is *A. barbadensis* Miller (also called *A. vera* Linne and commonly referred to as *A. vera*). Other *Aloe* species used in health and medicine include *A. arborescens* Miller (a member of the Asphodelaceae family), *A. perryi* Baker, *A. andongensis*, and *A. ferox*, among others (1, 4).

A. vera is a perennial plant with turgid green leaves joined at the stem in a rosette pattern. The *A. vera* leaves are formed by a thick epidermis (skin) covered with cuticles surrounding the mesophyll, which can be differentiated into chlorenchyma cells and thinner-walled cells forming the parenchyma (5). The parenchyma makes up most of the leaf by volume, containing the *A. vera* gel, a synonym to inner leaf, inner leaf fillet, or aloe fillet (1, 6).

The *A. vera* gel consists of about 98.5–99.5% water, and more than 200 different components have been identified in the remaining solids fraction, with polysaccharides being the most abundant type of compound (5). Other interesting chemical components, such as soluble sugars, glycoproteins, phenolic anthraquinones, flavonoids, flavonols, enzymes, minerals, essential and nonessential amino acids, sterols, saponins, and vitamins, have also been identified (4, 7). Interestingly, *A. vera* polysaccharides have been considered the main component responsible for most of the beneficial properties attributed to the *A. vera* plant (6, 8–10).

Several reports have been conducted to identify the carbohydrate composition of the aloe polysaccharides (9). In fact, various polysaccharides have been detected or isolated from the gel, including mannans (5, 9, 11–15), galactans (16), pectic substances (13, 16, 17), and different glucuronic acid–containing polysaccharides (12). However, significant variations on the aloe polysaccharide species were observed in those early studies. The reason for such discrepancies is not fully understood, but could be largely attributed to several factors, including seasonal changes, geographic location (including soil and climate), growth periods, horticultural conditions, and postharvest treatments (4, 13, 16, 18–21), and also to the particular conditions used in the different analytical determinations.

Polysaccharides Present in *A. vera*

In most of the vegetal tissues, polysaccharides are the most abundant components. They can be divided into two main classes depending upon their function in the plant. The polymers that can be found forming the cell walls are commonly known as cell

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wall polysaccharides, while the polymers able to act as the major source of energy and water in many plant organs are classified as storage polysaccharides. In the *A. vera* gel, two main types of polysaccharides can be distinguished: a mannan-rich storage polysaccharide and pectic substances that are the main component of the cell walls. In addition, cellulose and different types of hemicellulosic polysaccharides have also been identified (9).

Storage Polysaccharide: Acemannan

The majority of storage polysaccharides are located within the cells, i.e., starch, although in some instances hemicellulosic polysaccharides from the cell walls, mainly mannans, may act as food reserves (22, 23).

In most members of the Liliaceae and Iridaceae families, glucomannans have been identified as the main storage polysaccharides. Structurally, these polymers contain approximately equal amounts of glucose (Glc) and mannose (Man) residues, linked in a β–(1 → 4) backbone with side chains containing small proportions of galactose (Gal) residues (24).

In aloe plants, the inner gel has been considered a water storage tissue, being a mannan identified as the major polysaccharide present therein (5, 9, 25). This polymer has been the most widely studied polysaccharide from *A. vera*, consisting of β 1–4-linked mannose residues (5, 9, 25). Because this mannan is partially acetylated, the term "acemannan" was coined (25).

Acemannan.—Acemannan, commercially also known as Carrisyn™, is the storage polysaccharide located within the protoplast of the parenchymatous cells of plants belonging to the *Aloe* genus. This polymer is considered to be responsible of the large amount of water (approximately 99%) that can be retained within the aloe leaves (5).

According to the scientific literature, acemannan is mainly composed of large amounts of acetylated Man units (>60%), followed by Glc (<20%) and, to a minor extent, Gal (<10%; 5, 15, 26–29). Structurally, acemannan isolated from *A. vera* is formed by a main backbone of β−(1 → 4) acetylated Man, which also contains β−(1 → 4) linked Glc, and it may also present side chains of Gal units attached to the C−6 of the Man residues forming the backbone (5, 15, 26, 27, 30).

Recently, Chokboribal et al. (15) defined the acemannan polymer as a chain of repeating tetrasaccharide units: *O*-(acetyl-D-mannose)-*O*-(acetyl-D-mannose)-*O*-(D-glucose)-*O*-(acetyl-D-mannose) with a single-branched Gal at C6 of the second acetylated Man residue (Figure 1). However, this definition does not seem to be very accurate because it has been reported that repeating units of Glc and Man may also be present in ratios of 1:6, 1:15, and 1:22 (6, 11, 14, 27, 31).

Acetylation may occur at the C–2, C–3, or C–6 of Man residues with an acetyl:Man ratio of approximately 1:1 or even higher (10, 25, 26, 32–34). Structurally, these acetyl groups are the only non-sugar functional groups present in acemannan and seem to play a key role in the physico-chemical properties and biological activity associated to *A. vera* (14, 15, 35). In general, in most studies the MW of this polysaccharide is situated within the range from 30 to 45 kDa, although higher MWs have also been reported (up to 200 kDa; 15, 29, 36–38). It is important to highlight that the acemannan polymer is not only structurally unique but is also a characteristic compound of the *Aloe* species amongst other well-known plant mannans, which either have different side chains or are unacetylated and, therefore, highly insoluble in aqueous media (35).

Cell Wall Polysaccharides

Plant cell walls are mainly comprised by polysaccharides, including cellulose, hemicelluloses, and pectic substances or pectins. These polymers are involved in the different biomechanical properties of the cell walls (39). Several studies have shown that galacturonic acid–rich pectic substances and cellulose are the main polymers comprising the cell wall of parenchymatous tissue of *A. vera* (5, 9, 28). To a minor extent, different hemicellulosic polymers have also been reported (5, 28).

Pectic polysaccharides.—Pectic polysaccharides or pectins, which are the most abundant type of cell wall polymers present in the *A. vera* gel, are mainly found forming the cell walls of the parenchyma (5). Generally, pectins are heterogeneous polysaccharides composed primarily of (1 → 4) α–D–galacturonic acid (GalA) repeating units with intermittently (1 → 2) linked rhamnose (Rha) residues acting as branch points for neutral sugar side chains (Figure 2; 40, 41). The GalA units may be present in the acid form or may also exist as methyl esters with a certain degree of methyl ester substitution (DME), which affects their ability to form gels in the presence of multivalent ions such as calcium (Ca^{2+}; 42, 43).

A. vera pectins have been characterized as containing a very high proportion of GalA residues, usually higher than 95% with less than 5% of neutral sugars, with Rha being the most abundant sugar unit. This is indicative of a structure primarily composed of long GalA blocks with very few neutral sugar

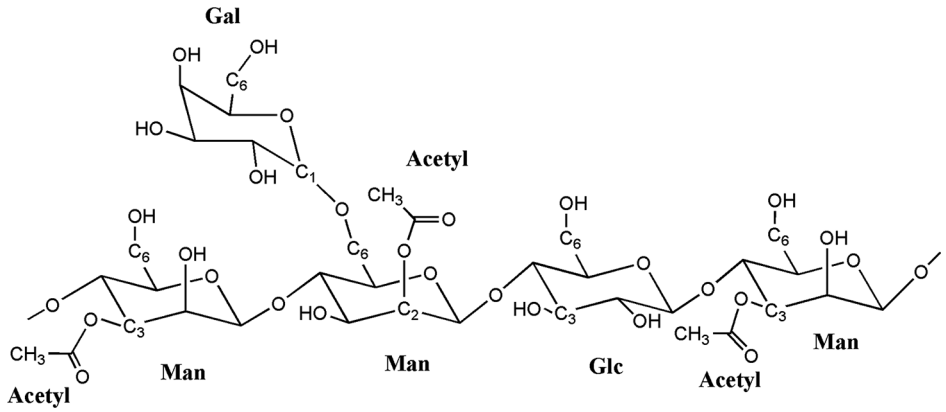

Figure 1. Chemical structure of acemannan polymer proposed by Chokboribal et al. (15).

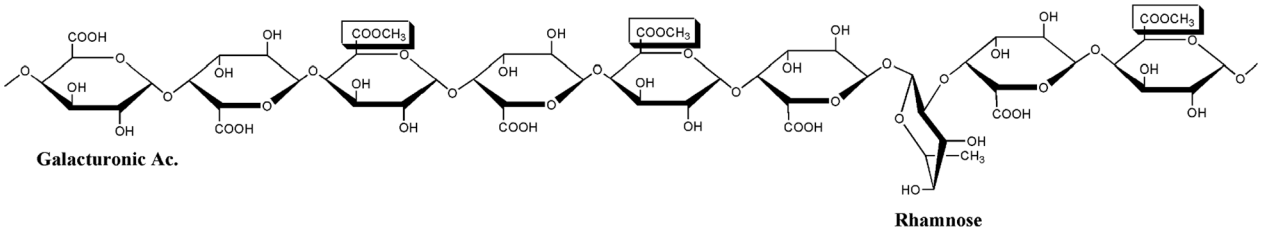

Figure 2. Schematic representation of the pectin structure. Modified from Minjares-Fuentes and Femenia (42).

branches (5, 9, 16, 42, 44). The MW of pectins from *A. vera* gel ranges from 200 to 523 kDa, although pectins with lower MW have also been observed (42, 43). In addition, *A. vera* pectins exhibit a relatively low DME, ranging from 2 to 20% (13, 40, 42, 43, 45). Interestingly, earlier studies carried out in *Aloe* species reported the presence of pectic substances as the main polysaccharide present in the gel (16, 17). It is also important to highlight that *A. vera* pectins have also been considered not only unique, being chemically and functionally distinct from all known pectins or other polymers (9), but also exceptional polymers because of the ability to form gels at low polymer (0.2 wt %) and calcium ion (<10 mM) concentrations in comparison with pectins from other sources (42, 44).

Cellulose.—Cellulose, the second most abundant cell wall polysaccharide found in *A. vera* gel, is a β−(1 → 4) linked glucan synthesized by enzymes located on the plasma membranes. In general, the cellulose content can be estimated by the difference in the amount of Glc obtained by Saeman hydrolysis and the content of Glc obtained with a milder hydrolysis method using H_2SO_4 1 M. In general, most studies agree that cellulose accounts for about 12–15% of cell wall material from *A. vera* gel (5, 13, 36), although minor amounts have also been reported (28).

Hemicelluloses.—Mannans and xyloglucans are the main type of hemicellulosic polymers forming the cell wall of the *A. vera* tissues, although other type of hemicelluloses, such as xylans, can also be found (5, 9). Previously, Femenia et al. (5), who carried out the complete characterization of the main polysaccharides present in the *A. vera* leaf, found that xyloglucans were the main hemicellulosic cell wall polysaccharide present in the skin, fillet, and gel. Interestingly, these authors also found minor amounts of xylans in the skin fraction, suggesting the occurrence of secondary walls, which have been associated with most of the textural differences between the skin and fillet tissues. On the other hand, the presence of mannans, mainly glucomannans, has also been identified in liquid gel, which could be released from the cell walls during *A. vera* gel processing (9). However, it is important to note that, in contrast to the storage acemannan polymer, cell wall glucomannans from *A. vera* are usually unacetylated.

Analytical Techniques Used to Characterize the Main Type of Polysaccharides from *A. vera*

Among all the constituents identified in *A. vera*, acetylated polysaccharides are considered the most important active component (5, 9, 10, 38, 46, 47). Interestingly, this type of polymer can be found in the mucilaginous parenchyma of the *A. vera* leaf.

Thus, acetylated polysaccharides have been identified as one of the key markers of the authenticity of *A. vera* inner leaf, and, in fact, good quality aloe-containing products must present the highest possible level of these acetylated polysaccharides (46, 48).

However, the composition of *A. vera* polysaccharides can exhibit a high variability depending on different factors, such as the geographic location, collection season, type of processing, and storage conditions (13, 21, 46). For instance, polysaccharides and acetyl groups can be lost or degraded during processing because of overheating, over-digestion with cellulase, or microbial contamination (47–49).

Further, marketers of *A. vera* products tend to adulterate with non-aloe polysaccharides in the final product to hide poor quality of aloe gel and sell products with a very poor content of aloe inner leaf gel that is unable to show any pharmacological effect (48). Thus, different analytical methods have been reported and/or developed for analysis of polysaccharide constituents in the mucilaginous parenchyma of *A. vera* leaf (48). Within this context, different analytical techniques (in particular colorimetric, spectrophotometric, and chromatographic methods) that have been used to characterize the main *A. vera* polysaccharides are reviewed and discussed below.

Colorimetric-Based Methods

Different colorimetric methods have been used to quantify the main aloe polysaccharides, such as phenol-sulfuric and Congo red assays (9, 50). Overall, colorimetric assays are based on the colored complex formed by the binding between the β(1 → 4)-linked polysaccharides and the dye (50). These methods show some advantages over other analytical techniques used to characterize the aloe polysaccharides because they require low investment and offer rapid responses. These advantages have probably been the main reasons that explain the wide use of these techniques in the quantification of aloe polysaccharides.

Recently, Salah et al. (49), who investigated the effect of deacetylation on the antibacterial activity of acemannan polymer, used the phenyl-sulfur colorimetric assay to characterize the neutral sugar composition of acemannan polymer after the deacetylation process took place. However, this method was not able to differentiate acetylated from unacetylated polysaccharides.

On the other hand, Congo red has also been used by several authors to quantify the acemannan polysaccharide from different *A. vera* extracts (20, 51, 52). In fact, Ray and Aswatha (20) used this colorimetric assay to determine the content of the acemannan polymer in 2-, 3-, and 4-year-old aloe plants harvested at the rainy winter season. Later, Kiran and Rao (51) also used this assay to estimate the content of acemannan in cell wall material and non-fibrous, alcohol-insoluble residue from *A. vera* gel. However, they were not able to discriminate the acemannan polymer from cell wall glucomannans.

It is important to highlight that the Congo red assay has been approved for the International Aloe Science Council (53) as a rapid method to quantify the content of glucomannans

from *A. vera* extracts. However, the results from these types of assays could generate confusion because the majority of polysaccharides, including most cell wall polymers, are β–(1 → 4)-linked (46).

Spectroscopic-Based Methods

Because of the lack of sensitivity of colorimetric methods to discriminate acetylated from unacetylated polymers, some spectroscopic methods have been applied, in particular FTIR spectroscopy and NMR. In the last decade, the use of FTIR spectroscopy has been widely used for the identification and characterization of *A. vera* polysaccharides (51, 54–56). Specifically, the identification of bands within the range of 1078–1036 cm^{-1} have been associated to the presence of polysaccharide sugars, such as Man and Glc (55). Moreover, the transmittance spectrum at around 1740, 1598, and 1248 cm^{-1} are attributed to the presence of C–O, COO–, and C–O–C stretches of acetyl groups of acetylated polysaccharides present in the *A. vera* gel, in particular to the acemannan polysaccharide (3; Figure 3).

In fact, Nejatzadeh-Barandozi and Enferadi (55) used FTIR analysis to describe the polysaccharides from *A. vera*. These authors studied the polysaccharides from skin juice, gel juice, and flowers from *A. vera* treated with different fertilizers. They observed clear differences in the polysaccharides obtained from the different portions analyzed. It is important to note that the observations of these authors have been widely used by other authors to identify changes of the chemical composition of aloe polysaccharides, in particular those affecting to acemannan and pectic polysaccharides (3, 13, 40, 43, 51, 54, 56, 57).

Interestingly, several authors have used FTIR spectroscopy to assess the effect of different drying procedures on the acemannan polysaccharide (3, 40, 57). These authors observed that the signal corresponding to acetyl groups of acemannan (1740 and 1248 cm^{-1}) decreased in most of the dehydrated samples. Thus, these authors claimed that the reduction of these bands was probably related to the deacetylation process of acemannan.

On the other hand, NMR spectroscopy has proved to be an essential tool for assessing the identity and the quality of *A. vera* gel preparations (15, 58, 59). Specifically, ^{1}H-NMR spectrometry has demonstrated capability of simultaneously detecting and quantifying an important number of constituents in a single spectrum. Interestingly, the acetyl groups of acemannan generate a characteristic signal (2.00–2.26 ppm) that can be considered the fingerprint of *A. vera* (47; Figure 4). Further, the direct ^{1}H-NMR spectrometry quantitative method presents advantages over some routine methods: simplicity, rapidity, selective recognition, and the quantitative determination of metabolites in such a complex biological matrix (48).

In 2013, Campestrini et al. (14) described for the first time by NMR analysis the polysaccharides from a fibrous fraction obtained from *A. vera*, identifying a partially acetylated 4-linked β-D-glucomannan as the main polymer. Further, these authors established the acetylation pattern of this type of polysaccharide using bidimensional NMR analysis, showing that the acetyl groups are located at C–2, C–2 and C–3, C–3 and/or C–6 positions of the mannose residues (14).

Furthermore, ^{1}H-NMR spectrometry has also been successfully used to quantify the deacetylation of acemannan polysaccharide promoted by different and novel drying procedures (47). Interestingly, the authors observed that the intensity corresponding to the signal of acetyl groups exhibited a good correlation with the losses of (1,3,4)-linked mannosyl residues observed by using GC/MS analysis.

Chromatographic Analysis

Chromatography is probably the most complete technique used to determine the content and type of polysaccharides in *A. vera* extracts or products because the monomers constituting the main *A. vera* polymers can be easily separated and analyzed either by GC or LC, including MS. The usefulness of chromatography for analysis of *A. vera* polysaccharides is based mainly in the high sensibility and high accuracy of the analysis because it is able to clearly identify the main sugar monomers released after acid hydrolysis of polysaccharides. In the last decades, the combination of chromatography and MS has been a powerful tool for the characterization of the acetylation pattern of polysaccharides, allowing inference of the distribution and even the position of the acetyl groups (25, 60), making it a valuable alternative to NMR, especially when very low amounts of material are available (10).

In fact, Gowda et al. (31) and Mandal and Das (11) used gas-liquid chromatography to elucidate the structure and composition of polysaccharides from *A. vera* gel for the first time, identifying the presence of partially acetylated Man-rich polysaccharides with different Glc–Man ratios and acetyl

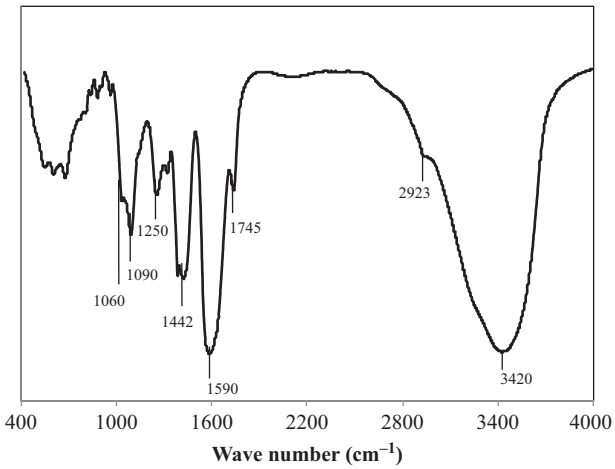

Figure 3. FTIR spectra of crude polysaccharides from *A. vera* gel.

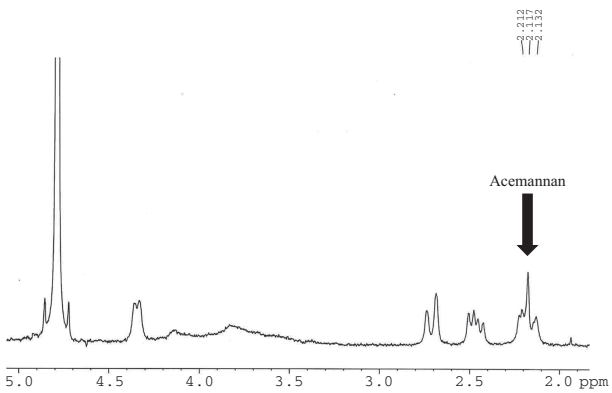

Figure 4. ^{1}H NMR spectra from acemannan isolated from *A. vera* gel.

patterns. Later, Manna and McAnalley (25) used gas-liquid chromatography/MS to determine the position of the acetyl groups of the acetylated Man-rich polysaccharides from *A. vera* gel. They observed that acetylation could occur in three different locations, at C2/C3 and C6 in a ratio of approximately 50:50.

In 1999, Femenia et al. (5) carried out a complete characterization of *A. vera* plant, dissecting the plant whole leaves in fillets and skin, using GC and GC/MS analysis. They observed that Man and cellulosic Glc were the predominant sugar residues in the alcohol-insoluble residue from all tissues, although significant amounts of sugars, such as GalA, arabinose (Ara), and Gal, were also detected. Interestingly, two main types of Man-containing polymers present in the *A. vera* plant were described. On the one hand, the polysaccharide detected in the fillet and gel fractions corresponded to a storage polysaccharide located within the protoplast of the parenchymatous cells. Its structural and compositional features corresponded to the active polysaccharide known as acemannan. On the other hand, in the skin tissue, all the mannosyl residues arose from a structural mannan polysaccharide located within the cell wall matrix. Structural and compositional differences between both polymers were confirmed by GC/MS analysis.

Later, Simões et al. (10) carried out the determination of the main structural features, including the acetylation profile, of a commercially bioactive acemannan through MS. These authors also observed the presence of Ara residues forming part of this structure. In this study, the Man units released from acemannan were highly acetylated, containing on average about two acetyl groups per sugar unit, which is double of what has usually been reported in the bibliography for this bioactive polysaccharide. Interestingly, acetyl groups from acemannan polysaccharide were non-homogeneously distributed, and even mannosyl residues with up to three acetyl groups were observed.

Several authors have successfully used GC/MS analysis to evaluate the main changes on the acemannan structure promoted by different types of processing, in particular pasteurization (28) and drying (36, 47). These authors detected, by methylation analysis, important losses of galactosyl and acetyl residues in the processed samples. Interestingly, small but significant losses of Man of acemannan from *A. vera* dehydrated using different drying methods, used at industrial scale, were observed by Minjares-Fuentes et al. (47). Further, GC/MS analysis has proved to be a useful tool to estimate the changes in the MW of acemannan. This basic estimation is based on the ratio of (1,4)-, (1,3,4)-, (1,4,6)-, and (1,3,4,6)-linked residues to terminally linked mannosyl units (5).

It should also be pointed out that size exclusion HPLC, as well as photometric or colorimetric methods, have also been reported as potential techniques to determine the *A. vera* inner leaf quality in commercial products. However, these analytical methods are not able to differentiate artificially adulterated or even non-aloe polysaccharides, such as maltodextrin, from native *A. vera* polysaccharides (48).

Influence of Compositional and Structural Characteristics of *A. vera* Polysaccharides on Technological, Functional, and Beneficial Properties

Around the world, *A. vera* processing has become a big industry because of a large collection of well-documented health benefits, such as wound healing, antimicrobial properties, anti-inflammatory properties, skin protection, hair growth, and immunomodulating properties, which have mainly been attributed to the *A. vera* polysaccharides (46). In fact, the acemannan content has been considered one of the key indicators of the quality and authenticity of aloe products. However, different aspects of this polymer, such as the structural arrangement, the MW and the degree of acetylation, should also be considered in order to assess the overall quality of *A. vera* processed products. Thus, within this context, the most relevant information published about the influence of *A. vera* polysaccharides on the technological, functional, and beneficial properties is discussed below.

Technological Properties

From a technological point of view, different rheological studies have shown that acemannan plays a key role in the pseudoplastic flow behavior of the liquid gel obtained from fresh *A. vera* gel, which may become less viscous, exhibiting typical Newtonian flow properties, when it is degraded (9, 14, 21, 61). In 1993, Yaron (21) carried out the first study about the relationship between *A. vera* polysaccharides and the rheological behavior of gel. This author observed that the viscosity of the gel decreased as the shear rate increased, denoting a non-Newtonian shear-thinning flow behavior of gel. This rheological behavior was mainly attributed to the mannose-rich polysaccharides because these polymers were the predominant polysaccharides found in the *A. vera* gel. Interestingly, the degradation of these polymers, either by physical or chemical means, promoted the modification of the flow behavior, changing from a non-Newtonian to a Newtonian behavior.

Later, Lad and Murthy (62) studied the rheological characteristics of native gel and juice obtained from *A. vera* under dynamic and steady shear. They observed that both the elastic and viscous modules of the *A. vera* gel were influenced by the presence of weak, fibrous, and random structures of polysaccharides. Interestingly, the moduli of the gel augmented when the temperature increased whereas, in the case of the juice, this parameter decreased when temperature increased. Further, both *A. vera* gel and juice samples exhibited a shear-thinning behavior as previously described by Yaron (21); however, a plateau region was observed at high shear rates (>100 1/s). Interestingly, Campestrini et al. (14) demonstrated that acetylated glucomannan from *A. vera* exhibited higher viscoelastic properties at lower concentrations (0.03 g/L) than partially acetylated Konjac glucomannan (at 1% wt; 14). Several studies have shown that the acetyl groups of acemannan are involved in the interaction of this polymer with other biomolecules (14, 15).

Recently, Minjares-Fuentes et al. (13) studied the effect of water deficit on the main polysaccharides and the rheological behavior of *A. vera* gel. These authors observed the characteristic shear-thinning flow behavior in all the *A. vera* mucilages tested; however, the flow properties, such as the pseudoplasticity index (n) and the viscosity (η), were affected by water deficit (Figure 5). Interestingly, the flow properties increased as water deficit increased. These authors concluded that rheological properties could be governed not only by the degree of acetylation of the acemannan polymer but also by the MW of this polymer, because water deficit promoted a significant increase in

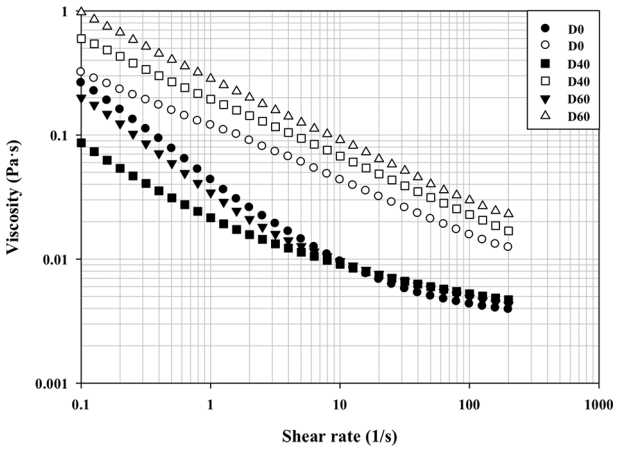

Figure 5. Flow curves describing the non-Newtonian shear-thinning flow behavior of fresh (full dots) and reconstituted (empty dots) *A. vera* mucilages treated with different water deficits: D0 = aloe without water deficit; D40 = aloe with 40% water deficit; and D60 = aloe with 60% water deficit. Modified from Minjares-Fuentes et al. (13).

the average MW of acemannan. Therefore, the rheology of the *A. vera* gel (or mucilage) could be a key aspect that should be taken into consideration when assessing the overall quality of *A. vera* processed products (15, 36, 59).

Functional Properties

The considerable industrial value of *A. vera* gel is mainly related to the high capacity of the *A. vera* polymers to retain water and/or oil (28). Functional properties closely linked to aloe plant polysaccharides, such as the ability to swell (Sw), water retention capacity (WRC), or ability to adsorb organic molecules such as fatty acids [fat adsorption capacity (FAC)] could be good indicators not only of the quality but also of the physiological and nutritional benefits of the processed *A. vera* samples (36). It should also be pointed out that the functional properties exhibited by polysaccharides present in the *A. vera* parenchyma are significantly higher than the maximum values reported for polymers obtained from different fruit and vegetables (63). In fact, the high capacity of *A. 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 *A. vera* to lower the levels of cholesterol and to retain carcinogens and other toxic compounds (28). Nevertheless, the structure and composition of the different *A. vera* polysaccharides may be altered by chemical, mechanical, and thermal processing, leading to the modification of the functional properties attributed to the *A. vera* gel (47). Dehydration, mainly applied to produce powdered samples, and pasteurization carried out to obtain *A. vera* juice, are probably the most common procedures used by the *A. vera* industry (46). Thus, several studies have been conducted to evaluate the impact of processing, either drying or pasteurization, on the main *A. vera* polysaccharides and the main effects on the related functional properties (28, 36, 47). In 2003, Femenia et al. (36) investigated the effect of convective drying on functional properties. These authors observed a significant decrease of Sw, WRC, and FAC values as temperature increased from 40 to 80 ºC. Interestingly, they highlighted that functional properties from *A. vera* samples dehydrated at temperatures between 40 and 60ºC, were significantly higher than the maximum values reported for most fruits and vegetables (36). Later, Minjares-Fuentes et al. (47) evaluated the functional properties of the acemannan polymer isolated from *A. vera* samples dehydrated using different drying procedures used at industrial scale, in particular, spray drying, industrial freeze drying, refractance window drying, and radiant zone drying (Figure 6). These authors observed that the different drying procedures tested drastically affected the functional properties of acemannan polymer. They attributed this important reduction to the deacetylation, the loss of branching (mainly galactosyl residues), and the reduction of the MW observed in the acemannan polysaccharide.

On the other hand, the effect of the pasteurization process on the functional properties related to the acemannan and cell wall polymers from *A. vera* was evaluated by Rodríguez-González et al. (28). They detected important changes in pasteurized *A. vera* samples depending on the conditions used during the pasteurization procedure (Table 1). Interestingly, all functional properties decreased when *A. vera* was pasteurized at 85ºC. These authors explained that the modifications observed in the functional properties could be promoted by the formation of new hydrogen bonds between Man-rich oligosaccharides, which resulted in the formation of high MW chains of acemannan.

Later, the same authors (19) carried out an optimization study aimed at obtaining the optimal conditions of the pasteurization process, maximizing the functional properties related to the *A. vera* polysaccharides. Thus, they found that the highest Sw value was obtained when *A. vera* gel, obtained from 3.6-year-old plants, was pasteurized at 60ºC for 15 min. On the other hand, *A. vera* gel obtained from 4-year-old plants, pasteurized at 75ºC for 20 min, exhibited the highest WRC, whereas, for plants of the same age, pasteurization carried out at 70ºC for 15 min promoted the highest FAC. They also highlighted the possibility of applying this pasteurization conditions for the manufacture of *A. vera* gel products with maximized functional properties.

Beneficial Properties

In the last decades, several authors have associated most of the beneficial properties of *A. vera* gel to the acetylated polysaccharide acemannan present in the gel (2, 8, 9, 33, 34, 64–67). These premises have led to the publication of numerous in vitro and in vivo studies, as well as clinical trials, with the aim of gaining more insight into the potential effects of *A. vera* polysaccharides.

In recent years, several authors have observed that polysaccharide-rich *A. vera* extracts may exhibit a radical scavenging potential comparable to that of the synthetic antioxidant butylated hydroxytoluene (8, 68). In fact, Kaithwas et al. (69) observed that *A. vera* polysaccharides have the ability to reduce the 2,2-diphenyl-1-picrylhydrazyl radical to the corresponding hydrazine by converting unpaired electrons to paired ones (70). Moreover, in vivo assays demonstrated that doxorubicin (anthracycline anticancer drug) and its metabolites produce free radical species that attacked lipid components, leading to lipid peroxidation, and co-administration of *A. vera* polysaccharides significantly prevented the increase in thiobarbituric acid reactive substances levels in doxorubicin-treated animals, which was comparable to standard vitamin E (70). It should be point out that

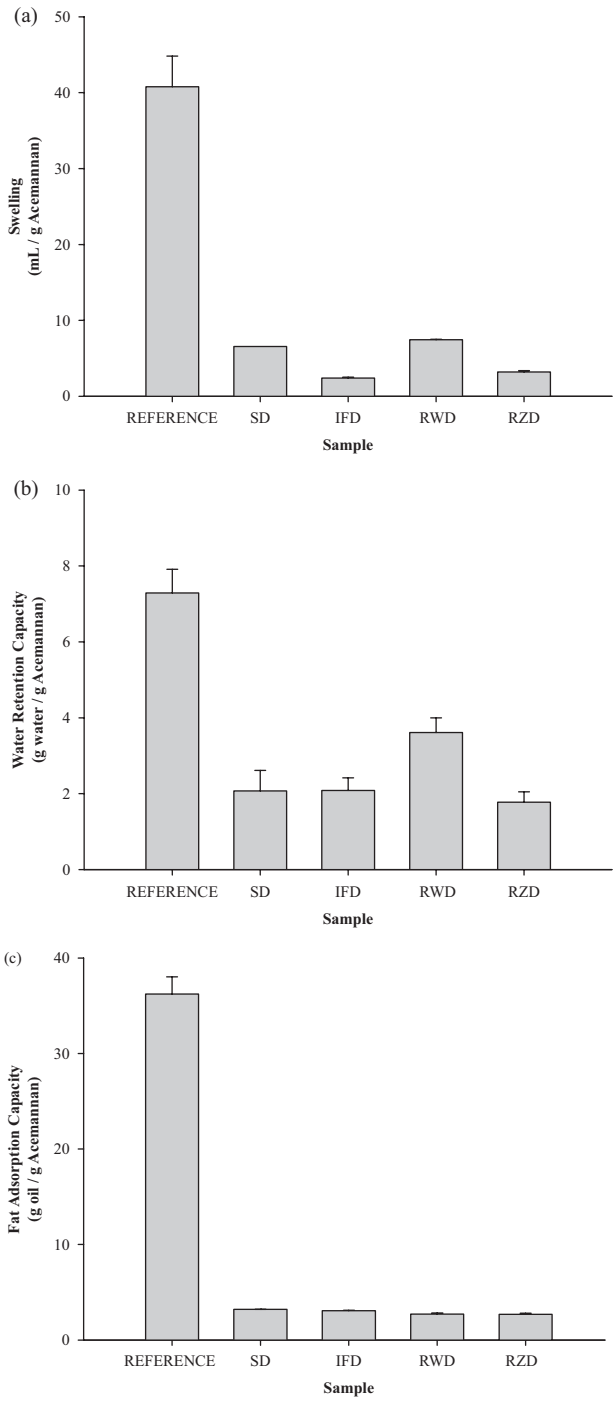

Figure 6. Functional properties determined for acemannan polysaccharide obtained from fresh *A. vera* gel (REFERENCE) and processed samples obtained by spray drying (SD), industrial freeze drying (IFD), refractance window drying (RWD), and radiant zone drying (RZD). (a) Swelling (Sw), (b) water retention capacity (WRC), and (c) fat adsorption capacity (FAC). Modified from Minjares-Fuentes et al. (47).

the antioxidant activity of *A. vera* polysaccharides was reported to be dose dependent (69, 70). Nevertheless, the potential free radical scavenging mechanism of *A. vera* polysaccharides is poorly understood, which could be attributed to the huge structural diversity of these polysaccharides, resulting in a major hindrance in the establishment of the structure-activity relationship (70).

Table 1. Functional properties of the polysaccharide-rich extracts from pasteurized *A. vera* gel[a]

Temperature, ºC	Time, min	Sw, mL/g	WRC, g H_2O/g	FAC, g oil/g
65	15	305 ± 3.7	23.0 ± 0.2	33.20 ± 0.5
	25	290 ± 3.5	20.8 ± 0.2	29.50 ± 0.5
75	15	272 ± 3.3	30.1 ± 0.2	32.80 ± 0.5
	25	267 ± 3.2	29.2 ± 0.2	32.00 ± 0.5
85	15	250 ± 3.0	18.0 ± 0.1	27.20 ± 0.4
	25	245 ± 2.9	15.9 ± 0.1	26.50 ± 0.4

[a] Modified from Rodríguez-González et al. (28).

Furthermore, recent studies have shown that the high MW fractions of acemannan are degraded by the intestinal microbiota to form oligosaccharides that inhibit intestinal glucose absorption (71–74), which has been associated to a significant reduction in blood glucose, blood pressure, and the improvement of the lipid profile in diabetic patients (2, 75, 76). Also, in a recent in vitro study, it was shown that acemannan could decrease the transepithelial electrical resistance of intestinal epithelial cell monolayers (Caco-2), allowing different bioactive components to be transported across the intestinal epithelium (41). Nutrigenomic studies have also been conducted to elucidate the mechanism of the hypoglycemic and insulin-sensitizing effects of *A. vera*, and the results have shown that acemannan may reduce hepatic fat accumulation, enhancing insulin signaling in adipose tissue. These studies have provided an explanation of the mechanism that increases insulin sensitivity and decreases blood glucose in diabetics and prediabetic models (77, 78).

On the other hand, several in vitro studies have shown that modified *A. vera* polysaccharides, with MWs ranging from 5 to 400 kDa, were able to increase phagocytic and proliferative activity by inhibiting the cyclooxygenase pathways and reducing prostaglandin E2 production, which plays a key role in inflammation (38, 79). Furthermore, clinical studies have demonstrated that acemannan possesses immunomodulatory properties for macrophages and monocytes with a minimal systemic toxicity following intraperitoneal or intravenous administration (1, 26, 80–83).

Recently, Chokboribal et al. (15) performed a study highlighting the importance of acetylation on the biological activity of acemannan polysaccharide. They observed that deacetylation higher than 35% significantly reduces the human gingival fibroblast cell proliferation. Further, acemannan deacetylation resulted in the considerable reduction in vascular endothelial growth factor (VEGF) expression compared with acemannan (15). Several authors have also reported that acemannan promotes wound healing by stimulating VEGF and type I collagen synthesis (81, 82).

Moreover, it has been observed that pectins with MW lower than 400 kDa may exhibit a potent macrophage-activating activity, as determined by increased cytokine production, nitric oxide release, surface molecule expression, and phagocytic activity. Interestingly, a potent antitumor activity in vivo has also been reported for this type of polysaccharide (38, 70). Recently, it has been well documented that pectins containing over 80% of GalA units, as in the case of *A. vera* pectins, possess immunostimulatory activity promoting phagocytic activity of monocyte-macrophage system in mice (84, 85).

Also, the specific intrinsic features, together with their high cytocompatibility, make *A. vera* pectins a novel and exceptional material in the development of biocomposites for biomedical applications (43, 86, 87).

Conclusions

In the last decades, *A. vera* has been the subject of numerous studies because of the wide gamut of beneficial effects that have been associated with this plant. Most authors consider *A. vera* polysaccharides as the main bioactive components present in the *A. vera* gel. In particular, two main types of bioactive polysaccharides have been distinguished in the parenchymatous tissue: the acemannan polymer and pectic substances. Interestingly, the structural and compositional features of these polysaccharides make them unique and distinct from polysaccharides obtained from other sources. The acemannan polymer is likely not only the main component of *A. vera* gel but also the most biologically active compound. The acetyl groups from acemannan seem to be the key to most of the properties associated with this polymer, enhancing its potential interaction ability with other biomacromolecules. Thus, the accurate identification of these polysaccharides, particularly acemannan, can be a useful tool, not only for assessing the authenticity of *A. vera* products, but also for assessing the biological activity of these polysaccharides. Nevertheless, the identification and quantification of these polymers is rather difficult using a single analytical technique because most of the methods used are unable to distinguish the acetylated acemannan from unacetylated mannan polymers.

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