



Universitat
de les Illes Balears

TESIS DOCTORAL
2023

**ALOE VERA: TÉCNICAS DE CULTIVO, MÉTODOS
DE PROCESADO Y APLICACIONES EN
ALIMENTACIÓN.**

Francesca Comas Serra



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Doctorado en Ciencia y Tecnología Química

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Francesca Comas Serra

Director: Antoni Femenia Marroig

Director: José Rafael Minjares Fuentes

Tutor: Antoni Femenia Marroig

Doctora por la Universitat de les Illes Balears

La Tesis doctoral titulada: “**ALOE VERA: TÉCNICAS DE CULTIVO, MÉTODOS DE PROCESADO Y APLICACIONES EN ALIMENTACIÓN**” de la Sra. Francesca Comas Serra se presenta en formato de compendio de artículos, los cuales se citan a continuación:

- Minjares-Fuentes, R., Femenia, A., Comas-Serra, F., Rodríguez-González, V.M. (2018). Compositional and Structural Features of the Main Bioactive Polysaccharides Present in the *Aloe vera* Plant. *Journal of the AOAC International*, 101 (6), 1711-1719. IF 1.201, Q3 (Food Science and Technology) and Q4 (Chemistry Analytical). Citado por 42. DOI: <https://doi.org/10.5740/jaoacint.18-0119>
- Comas-Serra, F., Martínez-García, J.J., Pérez-Alba, A., Sáenz-Esqueda, M.A., Candelas-Cadillo, M.G., Femenia, A., Minjares-Fuentes, R. (2023). A New Functional Food Ingredient Obtained from *Aloe ferox* by Spray Drying. *Foods*, 12, 850. IF 5.2, Q1 (Food Science and Technology). Citado por 3. DOI: <https://doi.org/10.3390/foods12040850>
- González-Delgado, M., Minjares-Fuentes, R., Mota-Ituarte, M., Pedroza-Sandoval, A., Comas-Serra, F., Quezada-Rivera, J.J., Sáenz-Esqueda, A., Femenia, A. (2023). Joint water and salinity stresses increase the bioactive compounds of *Aloe vera* (*Aloe barbadensis* Miller) gel enhancing its related functional properties. *Agricultural Water Management*, 285, 108374. IF 6.7, Q1 (Agronomy and Water Resources). Citado por 2. DOI: <https://doi.org/10.1016/j.agwat.2023.108374>

- Comas-Serra, F., Estrada, P., Minjares-Fuentes, R., Femenia, A. (2023). Evaluation of Acemannan in Different Commercial Beverages Containing *Aloe vera* (*Aloe barbadensis* Miller) Gel. *Gels*, 9, 552. IF 4.6. Q1 (Polymer Science). DOI: <https://doi.org/10.3390/gels9070552>

Asimismo, se incluye un artículo que se encuentra en revisión en la siguiente revista:

- Comas-Serra, F., Miró, J.L., Umaña, M., Minjares-Fuentes, R., Femenia, A., Mota-Ituarte, M., Pedroza-Sandoval, A. (2023). Role of acemannan and pectic polysaccharides in saline-water stress tolerance of *Aloe vera* (*Aloe barbadensis* Miller). En Revisión en *Carbohydrate Polymers* (IF 11.2).

Fuente de las citas:

https://scholar.google.es/citations?view_op=list_works&hl=es&user=TKUmJaYAAAAJ



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Dr. Antoni Femenia Marroig, de la Universitat de les Illes Balears

DECLARO:

Que la tesis doctoral que lleva por título "*Aloe vera: técnicas de cultivo, métodos de procesado y aplicaciones en alimentación*", presentada por Francesca Comas Serra para la obtención del título de doctor, ha sido dirigida bajo mi supervisión.

Y para que quede constancia de ello firmo este documento.

Firma

Palma de Mallorca, 02 de agosto de 2023



Universitat
de les Illes Balears

Dr. José Rafael Minjares Fuentes, de la Universidad Juárez del Estado de Durango

DECLARO:

Que la tesis doctoral que lleva por título "*Aloe vera: técnicas de cultivo, métodos de procesado y aplicaciones en alimentación*", presentada por Francesca Comas Serra para la obtención del título de doctor, ha sido dirigida bajo mi supervisión.

Y para que quede constancia de ello firmo este documento.

Firma

México, 02 de agosto de 2023

“La vida no es fácil para ninguno de nosotros. Debemos tener perseverancia y, sobre todo, confianza en nosotros mismos. Debemos creer que estamos dotados para algo y que esto debe ser alcanzado”

Marie Curie

A la meva família, amics i companys

Agradecimientos

La realització d'aquesta tesi ha estat recolzada per diferents projectes i ens agradaria agrair, en primer lloc, al Ministeri d'Economia i Competitivitat en el camp de Ciència i Tecnologia d'Aliments per al recolzament financer al projecte: *Obtención de ingredientes funcionales a partir de Aloe vera y subproductos agroalimentarios. Aplicación de ultrasonidos de potencia* (AGL2012-34627).

En segon lloc, a l'Institut Nacional d'Investigació i Tecnologia Agroalimentària (INIA) i al Fons Europeu de Desenvolupament Regional (FEDER) per al recolzament financer al projecte: *Revalorización integral de subproductos en función de sus usos potenciales: Extracción de compuestos de interés mediante aplicación de US de potencia i estudios de bioaccesibilidad in vitro* (RTA2015-00060-C04-03).

Finalment, al Ministeri de Ciència, Innovació i Universitats i a la AEI (10.13039/201100011033) per al recolzament financer al projecte: *Integration of revalorised agro-food by-products into an enhanced circular economy model: Value-added vegan foods for the agro-industrial and HORECA sectors* (PID2019-106148RR-C43).



Aquesta tesi no hagués estat possible sense el recolzament i l'ajuda de moltes persones, que m'han donat suport en tot moment.

En primer lloc m'agradaria donar les gràcies als meus directors, Dr. Antoni Femenia i Dr. Rafael Minjares, per no deixar-me enfonsar i estar al meu costat durant tots aquests anys, no només de tesi, sinó també des dels meus inicis, com estudiant de Llicenciatura, fent col·laboracions els estius i darrer any a l'àrea de Química, en el grup d'Enginyeria Agroalimentària.

També estic molt agraïda per l'ajuda i ànims de les Dres. Carmen Rosselló, Susana Simal i Valeria Eim. Els seus consells i recolzament, han fet que seguís endavant fins arribar a aquest moment.

Un agraïment especial a la Dra. Reyes González, amb qui vaig passar molts d'anys al seu costat, una gran mentora però, sobre tot, una gran amiga.

Moltes gràcies a totes les companyes i companys del grup d'Enginyeria Agroalimentària, especialment Espe, Mónica, Cristina, Xisca, Bea, África, Óscar, Simó..., per tots els moments viscuts plegats, per totes les ajudes rebudes per ells i haver pogut coincidir amb aquestes grans persones en el laboratori, fent dinars, xerrades de passadís...

Agrair als companys dels serveis científicotècnics de la UIB per tota l'ajuda rebuda, en especial a na Trinidad García, de cromatografia de gasos i a nen Biel Martorell, de ressonància magnètica nuclear.

A les meves amigues i amics, que sense ser conscients del que feia, han estat al meu costat i m'han donat molt de suport, animant-me a continuar endavant i no deixar-ho córrer.

A la meva família, tiets, cosins, moltes gràcies per tots els moments viscuts.

A la meva germana, un pilar de la meva vida. Sempre m'ha donat suport i coratge per seguir endavant.

A les meves pradines, grans persones que m'ensenyaren a valorar el més simple que tenien i gaudir dels que tenim al nostre costat.

I agrair en especial, als meus pares. Són el meu pilar del dia a dia, els qui m'han ensenyat que la vida no és un camí recte, sinó que ens podem trobar amb molts d'obstacles però que aquests es poden superar amb esforç.

Tampoc oblidar a la meva família política, però que també és la meva família, que m'ha fet costat en tot moment.

Finalment, a vosaltres, Cristòfol i Núria, espòs i filla, però sobre tot, companys de viatge. Començarem un viatge fent el Camí de Santiago i hem arribat fins aquí formant la nostra família, el nostre camí. Sou l'alegria de la meva vida, amb qui he plorat moltes llàgrimes de defalliment però moltíssimes més d'alegria, qui m'heu escoltat en els pitjors moments però amb qui he compartit molts de grans moments i, encara ens queda molt de camí per compartir plegats. Simplement vosaltres!!!

“Caminante no hay camino, se hace camino al andar”

Antonio Machado

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RESUMEN

El *Aloe vera* destaca por sus propiedades beneficiosas, en gran medida debido al acemanano, su principal compuesto bioactivo, y a los polisacáridos que conforman su pared celular. No obstante, es relevante considerar que la composición y estructura de estos polisacáridos, así como sus inherentes virtudes saludables, son susceptibles a cambios originados por diversos factores como las condiciones de cultivo, los procedimientos utilizados durante su procesamiento y la incorporación de extractos de la planta en distintos productos alimentarios.

En este marco, la presente Tesis Doctoral se basa sobre tres pilares fundamentales. En primer lugar, se propone evaluar los efectos del estrés abiótico sobre los principales compuestos bioactivos presentes en el gel de *Aloe vera*. En segundo término, pretende estudiar los principales efectos derivados del procesamiento orientado a la obtención de ingredientes con funcionalidades específicas, tanto del *Aloe vera* como del *Aloe ferox*. Finalmente, busca evaluar el compuesto bioactivo acemanano en el contexto de una amplia variedad de bebidas comerciales, en cuya formulación el *Aloe vera* asume un papel principal.

En relación a los estudios sobre el impacto del estrés abiótico en los compuestos bioactivos del *Aloe vera*, las plantas se sometieron a diferentes niveles de humedad (alto y bajo) y salinidad (0, 20, 40, 60 y 80 mM NaCl). Se observó como el estrés inducido incrementó no sólo la concentración de aloína sino también de los polisacáridos pécticos e hidrosolubles, particularmente el acemanano. Análisis más detallados revelaron un aumento en la acetilación del acemanano junto con un aumento en la esterificación de las pectinas (>60%). También se identificó una mayor presencia de

ramnogalacturonanos tipo I en la estructura de la pared celular, lo cual aumentó las propiedades funcionales relacionadas con estos compuestos bioactivos.

En relación a los efectos derivados del procesamiento, los mucílagos de *Aloe ferox* y *Aloe vera* fueron sometidos a secado por atomización a temperaturas de 150, 160 y 170°C. Aunque la morfología de ambos mucilagos secados por atomización fue similar, el *Aloe ferox* presentó una mejor conservación del color y un aumento tanto de las propiedades funcionales como de la capacidad antioxidante. En términos de la composición de los carbohidratos, se observó la presencia de un polisacárido rico en manosa altamente acetilado (>90%), predominante en el *Aloe ferox*, cuya composición química y características estructurales, son semejantes al polímero acemanano característico del *Aloe vera*, convirtiéndolo en un nuevo y prometedor ingrediente funcional.

Por último, se evaluó el contenido de acemanano y su grado de acetilación en bebidas comerciales que contenían *Aloe vera* en concentraciones variables (30-99.8%), de acuerdo con su etiquetado. Se encontró que las bebidas aromatizadas (gel de *Aloe vera* <77%) presentaban niveles reducidos de acemanano y en su mayoría carecían de acetilación. Estas bebidas también contenían cantidades considerables de otros biopolímeros debido a la inclusión de ingredientes adicionales. Por el contrario, en las muestras sin aromatizantes (gel de *Aloe vera* $\geq 99.5\%$) el contenido de acemanano osciló entre 10 y 260 mg/100 g de muestra fresca. Sin embargo, solo una de estas muestras presentó un alto grado de acetilación en su acemanano (>99%), mientras que en las demás fue inferior al 35%, indicativo de una menor calidad.

En conjunto, este estudio resalta la oportunidad de maximizar la producción de compuestos bioactivos, en particular acemanano y pectinas, con calidad superior mediante el uso adecuado de agua salina en el cultivo. También señala el potencial de *Aloe ferox* como alternativa funcional al *Aloe vera* y la necesidad de regulaciones, centradas en el análisis del contenido de acemanano y de su grado de acetilación, para asegurar la calidad de los productos alimentarios presentes en el mercado basados en el *Aloe vera*.

RESUM

L'*Aloe vera* destaca per les seves propietats beneficioses, en gran part gràcies a l'acemanà, el seu principal compost bioactiu, i als polisacàrids que formen la seva paret cel·lular. Malgrat això, és rellevant considerar que la composició i l'estructura d'aquests polisacàrids, així com les seves virtuts saludables inherents, són susceptibles de canvis originats per diversos factors com les condicions de cultiu, els procediments utilitzats durant el seu processament i la incorporació d'extractes de la planta en diferents productes alimentaris.

En aquest context, la present Tesis Doctoral es basa en tres pilars fonamentals. En primer lloc, es proposa avaluar els efectes de l'estrès abiòtic sobre els principals compostos bioactius presents en el gel d'*Aloe vera*. En segon terme, pretén estudiar els principals efectes derivats del processament orientat a l'obtenció d'ingredients amb funcionalitats específiques, tant de *Aloe vera* com de *Aloe ferox*. Finalment, busca avaluar el compost bioactiu acemanà en el context d'una àmplia varietat de begudes comercials, en la formulació de les quals l'*Aloe vera* assumeix un paper principal.

Pel que fa als estudis sobre l'impacte de l'estrès abiòtic en els compostos bioactius de l'*Aloe vera*, les plantes es van sotmetre a diferents nivells d'humitat (alt i baix) i salinitat (0, 20, 40, 60 i 80 mM NaCl). Es va observar que l'estrès induït no només va augmentar la concentració d'aloïna sinó també dels polisacàrids pèctics i hidrosolubles, particularment l'acemanà. Anàlisis més detallats van revelar un augment en l'acetilació de l'acemanà juntament amb un augment en l'esterificació de les pectines (>60%). També es va identificar una major presència de ramnogalacturonans tipus I a l'estructura

de la paret cel·lular, la qual cosa va augmentar les propietats funcionals relacionades amb aquests compostos bioactius.

Pel que fa als efectes derivats del processament, els mucíl·lags d'*Aloe ferox* i *Aloe vera* es van sotmetre a assecat per atomització a temperatures de 150, 160 i 170 °C. Tot i que la morfologia d'ambdós mucíl·lags assecats per atomització va ser similar, l'*Aloe ferox* va presentar una millor conservació del color i un augment tant de les propietats funcionals com de la capacitat antioxidant. En termes de la composició dels carbohidrats, es va observar la presència d'un polisacàrid ric en manosa altament acetilat (>90%), predominant a l'*Aloe ferox*, la composició química i les característiques estructurals del qual són semblants al polímer acemanà característic de l'*Aloe vera*, convertint-lo en un nou i prometedor ingredient funcional.

Finalment, es va avaluar el contingut d'acemanà i el seu grau d'acetilació en begudes comercials que contenien *Aloe vera* en concentracions variables (30-99.8%), d'acord amb la seva etiqueta. Es va trobar que les begudes aromatitzades (gel d'*Aloe vera* <77%) presentaven nivells reduïts d'acemanà i en la seva majoria mancaven d'acetilació. Aquestes begudes també contenien quantitats considerables d'altres biopolímers probablement a causa de la inclusió d'ingredients addicionals. Per contra, en les mostres sense aromatitzants (gel d'*Aloe vera* ≥99.5%), el contingut d'acemanà va oscil·lar entre 10 i 260 mg/100 g de mostra fresca. Malgrat això, només una d'aquestes mostres va presentar un alt grau d'acetilació (>99%), mentre que en les altres va ser inferior al 35%, indicatiu d'una menor qualitat.

En conjunt, aquest estudi ressalta l'oportunitat de maximitzar la producció de compostos bioactius, en particular acemanà i pectines, amb qualitat superior mitjançant l'ús adequat d'aigua salina en el conreu. També assenyala el potencial de l'*Aloe ferox* com a alternativa funcional a l'*Aloe vera* i la necessitat de regulacions, centrades en l'anàlisi del contingut d'acemanà i del seu grau d'acetilació, per assegurar la qualitat dels productes alimentaris presents al mercat basats en l'*Aloe vera*.

ABSTRACT

Aloe vera stands out for its beneficial properties, largely due to acemannan, its main bioactive compound, and the polysaccharides that make up its cell wall. However, it is relevant to consider that the composition and structure of these polysaccharides, as well as their inherent health benefits, are susceptible to changes caused by various factors such as cultivation conditions, processing procedures, and the incorporation of plant extracts into different food products.

In this context, the present Doctoral Thesis is based on three fundamental pillars. Firstly, it aims to evaluate the effects of abiotic stress on the main bioactive compounds present in *Aloe vera* gel. Secondly, it intends to study the main effects resulting from processing aimed at obtaining ingredients with specific functionalities, both from *Aloe vera* and *Aloe ferox*. Lastly, it seeks to assess the bioactive compound acemannan in the context of a wide variety of commercial beverages, where *Aloe vera* plays a primary role.

Regarding the studies on the impact of abiotic stress on the bioactive compounds of *Aloe vera*, the plants were subjected to different levels of humidity (high and low) and salinity (0, 20, 40, 60, and 80 mM NaCl). It was observed that induced stress not only increased the concentration of aloin but also of pectic and water-soluble polysaccharides, particularly acemannan. More detailed analyses revealed an increase in the acetylation of acemannan along with an increase in pectin esterification (>60%). A greater presence of type I rhamnogalacturonans in the cell wall structure was also identified, which enhanced the functional properties related to these bioactive compounds.

Regarding the effects resulting from processing, the mucilages of *Aloe ferox* and *Aloe vera* were subjected to spray-drying at temperatures of 150, 160, and 170°C. Although the morphology of both spray-dried mucilages was similar, *Aloe ferox* exhibited better color preservation and an increase in both functional properties and antioxidant capacity. In terms of carbohydrate composition, a highly acetylated mannose-rich polysaccharide (>90%) was observed in *Aloe ferox*, which was predominant and exhibited chemical composition and structural characteristics similar to the characteristic acemannan polymer of *Aloe vera*, making it a new and promising functional ingredient.

Finally, the acemannan content and its degree of acetylation were evaluated in commercial beverages containing *Aloe vera* in varying concentrations (30-99.8%), according to their labeling. It was found that flavored beverages (*Aloe vera* gel <77%) had reduced levels of acemannan and mostly lacked acetylation. These beverages also contained significant amounts of other biopolymers probably due to the inclusion of additional ingredients. In contrast, in unflavored samples (*Aloe vera* gel \geq 99.5%), the acemannan content ranged from 10 to 260 mg/100 g of fresh sample. However, only one of these samples exhibited a high degree of acetylation (>99%), while in the others, it was less than 35%, indicative of lower quality.

Overall, this study highlights the opportunity to maximize the production of bioactive compounds, particularly acemannan and pectins, with superior quality through the proper use of saline water in cultivation. It also points out the potential of *Aloe ferox* as a functional alternative to *Aloe vera* and the need for regulations, focused on the

analysis of acemannan content and its degree of acetylation, to ensure the quality of *Aloe vera*-based food products present in the market.

INTRODUCCIÓN

1. ALOE

El Aloe, también conocido como áloe o sábila, es un género de plantas suculentas, perteneciente a la familia Asphodelaceae (Chase et al., 2016).

El nombre Aloe deriva de "alloeh" en árabe y "halal" en hebreo, que significa sustancia brillante amarga. La mayoría de las plantas de aloe no son tóxicas, pero algunas son extremadamente tóxicas, ya que contienen una sustancia parecida a la cicuta (Sánchez-Machado et al. 2017).

Durante mucho tiempo, el Aloe ha sido utilizado empíricamente como remedio medicinal para diversas enfermedades, lesiones y trastornos. En los últimos años, se han logrado avances significativos que han permitido comprender parte del mecanismo de acción del Aloe en la prevención y alivio de enfermedades, además de identificar algunos de los compuestos que tienen efectos beneficiosos para la salud (Park et al., 2009; Pandey & Mishra, 2010; Calderón-Oliver et al., 2011; Fayisa Diriba & Mirete Deresa, 2022). Se ha determinado que algunas de las propiedades antioxidantes, antiinflamatorias y antibacterianas del Aloe están relacionadas con sus efectos beneficiosos. No obstante, es importante destacar que solo algunas de las más de 400 especies de Aloe conocidas presentan efectos beneficiosos. Entre ellas se encuentran *Aloe arborescens* Miller, *Aloe perryi* Baker, *Aloe ferox* Miller o *Aloe capensis*, y, en particular, el *Aloe barbadensis* Miller, también conocido como *Aloe vera* Linné o Aloe vulgaris Lamark (Rodríguez et al. 2010; Guo & Mei 2016).

1.1 ALOE VERA

El *Aloe barbadensis* Miller, conocido como *Aloe vera*, es una planta perenne y suculenta, de la familia de las liliáceas (*Liliaceae*) o *Aloaceae* (Bozzi et al. 2007; Ahmed & Hussain, 2013; Sánchez-Machado et al., 2017).

Generalmente, cada planta cuenta con un promedio de 12 a 16 hojas, las cuales pueden tener una longitud entre 25 y 50 cm de largo, dependiendo de la edad de la planta y las condiciones de cultivo. Sus hojas se caracterizan por ser verdes, rígidas, anchas y triangulares que forman una roseta alrededor del tallo. Éstas presentan dientes en el margen exterior de aproximadamente 2 mm, duros, gruesos y de color más claro que la propia hoja (Ahlawat & Khatkar 2011; Manvitha & Bidya 2014; Sánchez-Machado et al. 2017) (Figura 1).

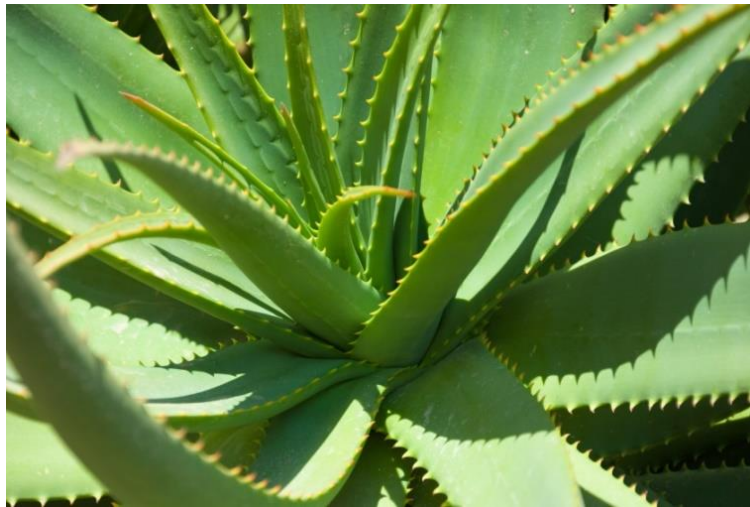


Figura 1. Planta de *Aloe vera* (*Aloe barbadensis* Miller)

Las hojas de esta planta están compuestas por una gruesa epidermis, o piel, cubierta de cutículas que rodean el mesófilo, el cual puede diferenciarse en clorenquima y células de paredes más finas, formando el parénquima (Femenia et al. 1999). La epidermis o corteza es una cutícula gruesa que representa alrededor del 20-30% en peso de toda la hoja de la planta. Está compuesta por hasta 18 capas de células intercaladas con cloroplastos, donde ocurre la síntesis de carbohidratos, lípidos y proteínas. Justo debajo de la corteza se encuentra la pulpa externa de la hoja, una delgada capa mucilaginosa que contiene haces vasculares. Estos haces vasculares funcionan como el sistema de transporte de la planta. Están formados por tres tipos de estructuras tubulares: el xilema,

responsable de llevar agua y minerales desde las raíces hasta las hojas; el floema, encargado de transportar los minerales sintetizados hacia las raíces; y el túbulo pericíclico, que almacena y transporta un látex amarillo amargo, conocido como savia o exudado, a lo largo del margen de la hoja. Éste último es conocido por sus propiedades laxantes (Saccù et al. 2001; Bozzi et al. 2007). Los componentes principales y activos del látex de aloe son derivados hidroxiantracénicos, que representan entre un 15% y un 40% de su composición, incluyendo antraquinonas como la aloína A y B. Por otra parte, la mayor parte del volumen de la hoja está constituida por el parénquima, que contiene el gel de *Aloe vera*, también conocido como jugo interno, filete interno o filete de aloe (Boudreau & Beland, 2006; Guo & Mei, 2016). El gel de *Aloe vera* consiste en aproximadamente el 98.5-99.5% de agua mientras que los sólidos restantes contienen más de 200 ingredientes diferentes, incluyendo una combinación de polisacáridos y sus derivados acetilados, glicoproteínas, antraquinonas fenólicas, flavonoides, flavonoles, enzimas, minerales, aminoácidos esenciales y no esenciales, esteroides, saponinas y vitaminas, siendo los polisacáridos los componentes mayoritarios (Eshun & He 2004; Rodríguez et al. 2010). En este contexto, cabe destacar que la mayoría de las propiedades farmacológicas del gel de *Aloe vera* han sido atribuidas al polisacárido acemanano (Choi & Chung 2003; Ni et al. 2004; Rodríguez et al. 2010; Radha & Laxmipriya 2015; Pothuraju et al. 2016). El polímero acemanano es el principal polisacárido presente en el gel de *Aloe vera*, el cual se encuentra dentro del protoplasto de las células parenquimatosas, por lo que es considerado como un polisacárido de reserva o almacenamiento de *Aloe vera* (Femenia et al., 1999). Así mismo, el gel también contiene una cantidad considerable de otros tipos de polisacáridos cuya función principal es la formación de las paredes celulares, siendo las sustancias pécticas o

pectinas, el tipo de polímero más abundante en las paredes celulares del gel de *Aloe vera* (Femenia et al. 2003; Simões et al. 2012).

El procesamiento del *Aloe vera* ha experimentado un auge en todo el mundo, impulsado por su diversidad de usos en la industria cosmética, farmacéutica y alimentaria, debido principalmente a la amplia gama de propiedades beneficiosas, entre las que destacan su capacidad para tratar el estreñimiento, la tos, las úlceras, la diabetes, los dolores de cabeza, la artritis y la mejora de las deficiencias del sistema inmunitario, entre otras (Vogler & Ernst, 1999; Eshun & He, 2004; Javed & Atta-Ur, 2014). En la industria cosmética y de cuidado personal, el gel de *Aloe vera* se ha convertido en una base fundamental para la elaboración de una gran cantidad de productos, tales como cremas, lociones, jabones, champús y limpiadores faciales. Mientras tanto, en el ámbito farmacéutico, los productos más destacados basados en el *Aloe vera* incluyen pomadas tópicas, geles, comprimidos y cápsulas (Eshun & He, 2004; Christaki & Florou-Paneri, 2010). Además, el *Aloe vera* también ha encontrado un amplio espacio en la industria alimentaria como un recurso valioso para la incorporación de ingredientes funcionales en una gran diversidad de productos convirtiéndolo en uno de los remedios herbales más utilizados (Guo & Mei, 2016; Pothuraju et al., 2016). Es especialmente utilizado en la preparación de bebidas saludables y otros tipos de bebidas, incluyendo el té (Christaki & Florou-Paneri, 2010). De esta manera, el *Aloe vera* se ha convertido en un componente importante en una amplia variedad de productos procesados, abarcando distintos sectores y beneficiando a numerosos consumidores en todo el mundo, con una gran aceptación entre los consumidores que buscan adoptar un estilo de vida más saludable (Vega-Gálvez et al., 2011; Javed & Atta-Ur, 2014; Guo & Mei, 2016).

1.2 ALOE FEROX

El *Aloe ferox* es una planta perteneciente a la familia de las *Liliaceae*, comúnmente conocido como Aloe del Cabo o aloe amargo. Al igual que el *Aloe vera*, es una planta suculenta perenne con enormes hojas sin tallo, de ápice agudo, carnosas y gruesas en forma de lanza (Steenkamp & Stewart, 2007). Se caracteriza por un tallo único con racimos erectos de flores de color naranja, rojo, amarillo y trozos de flores blancas que se extienden por hojas curvadas con espinas (O'Brien et al. 2011) (Figura 2).



Figura 2. Planta de *Aloe ferox*

Esta variedad es especialmente popular en Sudáfrica, región de origen, pero también se utiliza ampliamente en países como Japón, China y Corea. El *Aloe ferox* destaca como una de las plantas medicinales más populares en la actualidad y goza de una reputación legendaria en el mundo de las hierbas curativas. Esta especie exhibe la acción desintoxicante más potente entre todos los tipos de aloe. De manera particular, en África y Europa, gel de *Aloe ferox* tiene aplicaciones medicinales muy variadas y que abarcan desde el tratamiento de enfermedades como la gota, el cáncer de colon, el cáncer de piel, la tromboflebitis, el estímulo de la inmunidad, la diabetes, el reumatismo, el cáncer de pulmón, la leucemia, la candidiasis digestiva hasta la obesidad. De hecho, en 2002, la

Food and Drug Administration (FDA) de Estados Unidos aprobó su uso como aditivo alimentario directo para consumo humano como sustancia aromatizante natural (Kumar & Kumar 2019). Debido a sus propiedades curativas y su amplia gama de usos, el *Aloe ferox* se mantiene como una opción valiosa para la salud de diversas culturas y tradiciones médicas (Grace et al. 2008; Van Wyk et al. 2010; Chen et al. 2012). No obstante, existen pocos estudios basados en los compuestos bioactivos de esta especie y, a diferencia de *Aloe vera*, la composición química de los polisacáridos de *Aloe ferox* permanece en gran medida inexplorada. Los primeros estudios sobre polisacáridos en *Aloe ferox* mostraron como los polisacáridos pécticos, concretamente los arabinogalactanos, eran los polímeros predominantes, seguidos por los glucomananos (Mabusela et al. 1990). Posteriormente, otros estudios observaron como los polímeros predominantes estaban compuestos principalmente por glucosa y galactosa, sugiriendo la galactosa como posible huella dactilar de *Aloe ferox* (O'Brien et al. 2011). Todas estas diferencias en la composición química de los polisacáridos del *Aloe ferox* crea una ventana para llevar a cabo más investigaciones en este campo y la posibilidad de llegar a usarse como un ingrediente alimentario funcional, como es el caso de *Aloe vera*. Así también, sería muy interesante realizar estudios enfocados a los cambios en la funcionalidad de los compuestos bioactivos del *Aloe ferox* durante el procesado, principalmente en procesos de deshidratación.

2. CULTIVO

El *Aloe vera* es una planta nativa de regiones áridas del sureste de África (Reynolds 2009; Rodríguez et al. 2010; Calderón-Oliver et al. 2011). Su cultivo se ha extendido a otras regiones del Mediterráneo, de Asia y de América, entre las que destacan países como España, México, China y Estados Unidos (Rodríguez et al. 2010) (Figura 3).



Figura 3. Mapa mundial con las regiones de cultivo de plantaciones de *Aloe vera*

(Asocialoe, 2010)

La planta crece en una gran variedad de climas, incluidas zonas templadas y subtropicales aunque no puede sobrevivir a temperaturas bajo cero (Sahu et al. 2013; Manvitha & Bidya 2014; Sánchez-Machado et al. 2017). El *Aloe vera* es una planta que no requiere grandes cantidades de agua, lo que lo convierte en una opción favorable para regiones donde la disponibilidad de este recurso hídrico es limitada o su calidad baja, llegando a ser un cultivo económicamente atractivo para los agricultores de regiones que presenten condiciones agroclimáticas áridas y semi-áridas (Manvitha & Bidya, 2014). Las hojas pueden ser cosechadas con un cuchillo afilado después de 7-8 meses desde la plantación. No obstante, el International Aloe Science Council estipula que una planta de *Aloe vera* es productiva a partir de 1.5 años de edad, esperando alrededor de 11 kg de hoja por planta por año, con una vida útil de aproximadamente 12 años (Eshun & He, 2004; Ahlawat & Khatkar, 2011).

En las últimas décadas, el *Aloe vera* se ha convertido en una de las plantas medicinales comercializadas más importantes del mundo (Habeeb et al. 2007; Grace et al. 2008; Manokari et al. 2021). De hecho, el procesamiento de las hojas de *Aloe vera* generó

unos ingresos de 2400 millones de dólares, solo en 2019, una cantidad que se espera que aumente hasta 3200 millones de dólares en el período desde 2020 a 2027 (Market Growth Reports, 2022). El mercado mundial de *Aloe vera* está liderado por Estados Unidos (65%), seguido de India (10%) y China (10%) (Manokari et al. 2021), y su explotación en todo el mundo se debe principalmente a la amplia gama de propiedades beneficiosas asociadas a los diferentes compuestos bioactivos presentes en la planta (Manvitha & Bidya 2014; Baruah et al. 2016; Minjares-Fuentes & Femenia 2016; Martínez-Burgos et al. 2022).

No obstante, es importante considerar que el contenido y la composición de los principales compuestos bioactivos, así como su actividad biológica, pueden verse considerablemente afectados no solo por las condiciones agroclimáticas de las regiones de cultivo (Kumar et al. 2016; Kumar et al. 2017a; Kumar et al. 2017b; Kumar et al. 2017c) sino también por los diferentes métodos de manejo agrícola utilizados para incrementar el rendimiento de las cosechas o la productividad de la planta (Hazrati et al. 2017; Kumar et al. 2017a; Minjares-Fuentes et al. 2017a; Habibi 2018).

En la actualidad, con el cambio climático global, el uso de agua salina en el riego ha aumentado de forma considerable. Así pues, la escasa disponibilidad de agua para los cultivos industriales, combinada con el creciente uso de agua salina en el riego, ha dado lugar a la interacción de dos tipos de estrés abiótico: el déficit hídrico y la salinidad. De manera general, se ha observado que estas condiciones agroclimáticas son capaces no solo de modificar la morfología de la planta sino también pueden alterar el contenido, la composición y la estructura de los diferentes biocompuestos de la planta. En particular, se ha documentado que la falta de agua es capaz de modificar el peso molecular de los polisacáridos hidrosolubles de *Aloe vera* sin alterar la composición de los mismos (Minjares-Fuentes et al. 2017a), mientras que la salinidad promueve el incremento en la

concentración de aloína en la planta (Rahi et al. 2013). Sin embargo, el impacto de la interacción de diferentes estreses abióticos sobre la composición y la estructura de los compuestos bioactivos y las propiedades funcionales asociadas a estos, sigue siendo desconocido.

3. PROCESADO

En la producción de los extractos de *Aloe vera*, se utilizan diversos métodos que permiten obtener productos destinados a aplicaciones cosméticas, farmacológicas y alimenticias (He et al. 2005; Ramachandra & Rao, 2008; Ahlawat & Khatkar, 2011). Los métodos más comunes para aumentar el período de la vida útil de los alimentos líquidos involucran tratamientos térmicos, con el objetivo de inactivar microorganismos patógenos y enzimas capaces de degradar los diferentes productos.

En la actualidad, la industria del *Aloe vera* suele utilizar principalmente dos procedimientos: la pasteurización para obtener jugo de *Aloe vera* y la deshidratación para la producción de polvos. Aunque sean efectivos, estos tratamientos térmicos conllevan ciertas desventajas, ya que el calor puede provocar la pérdida irreversible de nutrientes esenciales, debido a la modificación de la estructura original de los diversos componentes bioactivos, lo cual puede conducir a la alteración de las propiedades fisicoquímicas y funcionales de los alimentos, resultando en la modificación o pérdida de sus propiedades fisiológicas y farmacológicas (Farnworth et al. 2001; Femenia et al. 2003; Eshun & He 2004; Ramachandra & Rao 2008; Gulia et al. 2009; Nindo et al. 2010; Ahlawat & Khatkar 2011; Dhingra et al. 2012; Chokboribal et al. 2015; Hamed et al. 2015; Minjares-Fuentes et al. 2017b).

3.1. SECADO

A pesar de que el proceso de pasteurización es ampliamente utilizado para la conservación de alimentos, los productos deshidratados presentan numerosas ventajas, entre las que destacan la reducción de volumen, una mayor estabilidad durante períodos largos de almacenamiento debido al bajo contenido de humedad, y su fácil manejo y transporte (Okos et al. 1992; Vega et al. 2007; Ramachandra & Rao, 2008; Miranda et al., 2010).

Sin embargo, es crucial asegurarse que la calidad no se vea comprometida al aplicar cualquier método de secado (He et al. 2005; Nindo et al. 2010; Lemmens et al. 2013; Javed & Atta-Ur 2014); ya que los productos alimentarios son, en general, muy susceptibles a la temperatura, la cual puede conducir a la pérdida de características no solo organolépticas, tales como textura, color y sabor, sino también nutricionales y funcionales (Attanasio et al. 2004; Sablani, 2007; Luangmalawat et al. 2008; Miranda et al., 2010; Onwude et al. 2017), dando como resultado un producto final inaceptable para el consumidor (Miranda et al., 2010). No obstante, para poder obtener la certificación internacional de productos de *Aloe vera* destinados al consumo humano, el International Aloe Science Council (IASC) (IASC, 2016), estipula que las soluciones desarrolladas a base de sólidos de *Aloe vera* (0.5 g *Aloe vera*/ 100 mL) deben presentar un contenido de aloína inferior a 10 mg/kg. En general, para lograr este objetivo es necesario procesar el gel o jugo de *Aloe vera* a altas temperaturas, en muchas ocasiones superiores a los 98°C, con la finalidad de reducir o eliminar el contenido de aloína en el producto final (He et al. 2005; IASC 2016; Minjares-Fuentes et al. 2017b).

En el ámbito industrial, el secado es uno de los procedimientos tecnológicos más utilizados. En este sentido, el secado por atomización o aspersion (spray-drying) ha sido el procedimiento usado con mayor frecuencia, seguido por la liofilización (freeze-

drying). Sin embargo, en las últimas décadas se han desarrollado e implementado nuevas tecnologías de secado entre las que destacan el secado por ventana de refracción (refractance window drying) y el secado por zonas radiantes (radiant zone drying), con el objetivo no solo de preservar la calidad del producto final sino incluso de mejorarla, a la vez que pueden reducirse los costes de producción (Nindo et al. 2010).

3.1.1. Spray-drying (SD)

El SD es un proceso de deshidratación ampliamente utilizado en la industria de la transformación alimentaria. En la mayoría de las ocasiones se suele realizar en una sola etapa con el objetivo de convertir un producto líquido, tal como suspensiones, soluciones, emulsiones entre otros, en productos sólidos con un tamaño de partícula considerablemente pequeño (<1 mm), los cuales exhiben una amplia gama de aplicaciones (Lee, 1983; Filkova & Mujumdar, 1995; Murugesan & Orsat, 2012; Phisut, 2012).

Generalmente, el SD se lleva a cabo mediante el paso de un fluido a través de una rueda giratoria o boquillas fijas, donde se atomiza o pulveriza, generando pequeñas gotas que entran rápidamente en contacto con el aire caliente. De esta manera, se aumenta significativamente la superficie de evaporación, acelerando el proceso de deshidratación debido a la elevada tasa de transferencia de agua. Posteriormente, las partículas sólidas son separadas del aire caliente de secado y recolectadas en un recipiente que evita la hidratación del polvo obtenido (Figura 4). Este método es eficaz para producir un producto final de alta calidad, con una baja actividad del agua, producido a gran escala y simplificando su almacenamiento y transporte. Además, de su habilidad de procesar el material de forma ágil mientras se conservan las propiedades y características del material original (Lee 1983; Filkova & Mujumdar 1995; Walker et al. 1999; Bruschi et

al. 2003; Broadhead et al. 2008; Obón et al. 2008; Phisut 2012; Chen et al. 2013; Stunda-Zujeva, et al. 2017; Höhne et al. 2018).

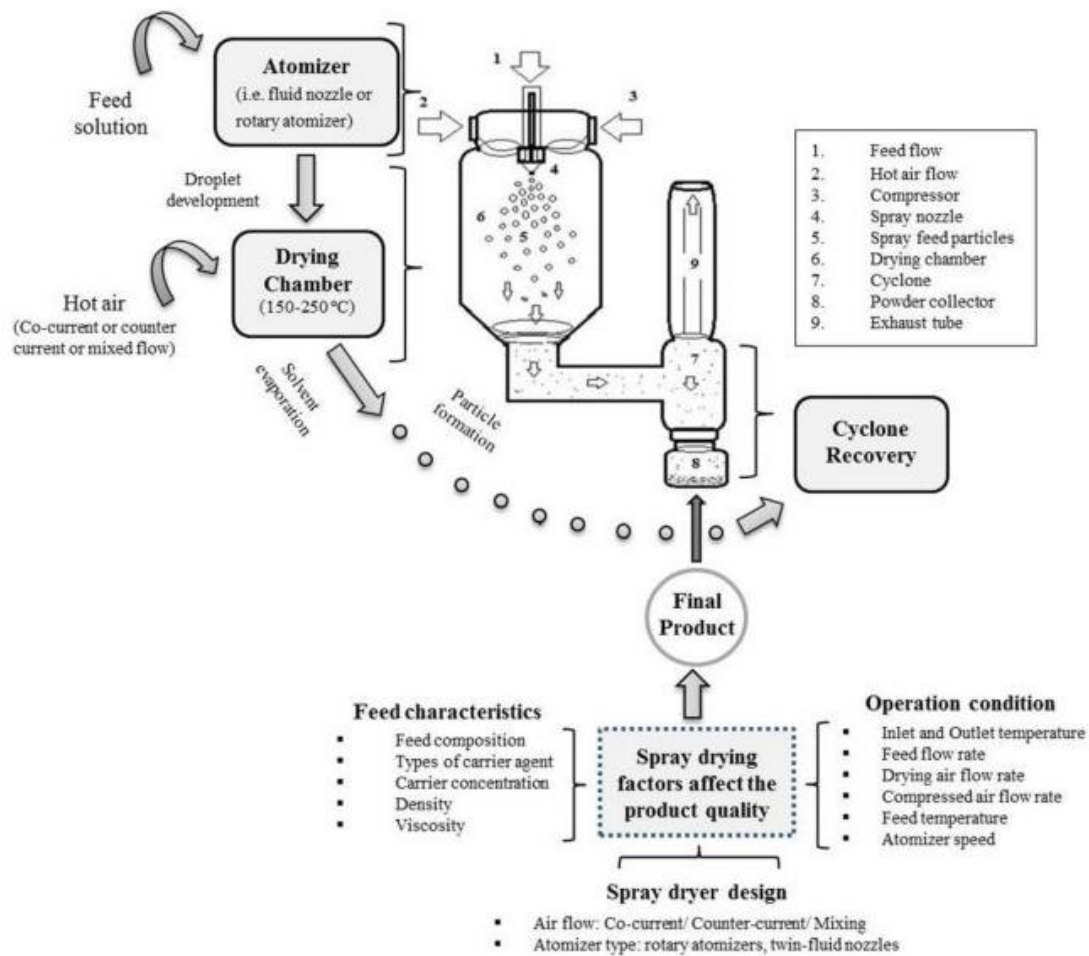


Figura 4. Diagrama esquemático del secado por spray-drying y los principales factores que afectan a la calidad del producto (Shishir & Chen, 2017).

En las industrias relacionadas con la alimentación y, en concreto, en la producción de ingredientes funcionales elaborados a partir de polisacáridos que presentan cierta bioactividad, el secado por atomización, spray-drying (SD) ha jugado un papel muy relevante (Fazaeli et al. 2012). Esto se debe a que esta técnica ha demostrado ser eficaz en la preservación de diversas cualidades, como el sabor y el color, así como en la preservación de diferentes nutrientes, dando un producto final de mejor calidad en comparación con el obtenido aplicando otras tecnologías de deshidratación (Nindo &

Tang 2007; Nindo et al. 2010; León-Martínez et al. 2011; Caparino et al. 2012; García-Cruz et al. 2013; Krishnaiah et al. 2013; Artunduaga et al. 2021). Específicamente, en el caso del *Aloe vera*, el SD es la metodología más utilizada para la elaboración de productos pulverizados (Devi & Rao, 2005).

Diferentes investigaciones han sugerido que el secado por atomización ofrece mejores resultados que otras técnicas de secado, un mayor rendimiento de producción, bajos costes operativos, polvos de mayor calidad, más adecuados para el envasado, el transporte y con una vida útil más prolongada (Yingngam et al. 2019; Sasikumar et al. 2020; Artunduaga et al. 2021).

Gracias a las combinaciones de tiempo de secado corto y baja temperatura del producto obtenido (90-110°C), el SD permite utilizarlo en productos sensibles al calor a presión atmosférica (Filkova & Mujumdar, 1995; Toledo, 2007). No obstante, hay que tener en cuenta que los parámetros utilizados por SD, como la temperatura de entrada, la velocidad del flujo del aire, la velocidad del flujo de alimentación, la velocidad del atomizador, los tipos de agentes portadores y su concentración, juegan un papel muy importante en las características del producto final, tales como el tamaño de partícula, la densidad, el contenido de humedad, el rendimiento o la higroscopicidad entre otras (Chegini & Ghobadian 2005, 2007; Yousefi et al. 2011; Phisut 2012). Particularmente, se ha observado que la alta fuerza de cizallamiento durante la atomización es capaz de promover la degradación de diferentes compuestos bioactivos, lo cual puede ser un factor muy importante a considerar en productos sensibles a los daños mecánicos (Nindo & Tang 2007; Nindo et al. 2010).

Previamente, Medina-Torres et al. (2016) evaluaron el efecto de la temperatura del aire de entrada (T_i : 150 - 170°C), la velocidad de giro del atomizador (A_s : 23,000 – 27,500 rpm) y el flujo de la alimentación (F_f : 1.5 – 1.7 L/h) en un proceso de SD de gel de *Aloe*

vera, a nivel de planta piloto. Estos autores observaron como el SD llevado a cabo en condiciones óptimas (Ti: 150°C; As: 27,500 rpm Ff: 1.5 L/h), permite la elaboración de un polvo, a partir de gel de *Aloe vera*, el cual presentó buenas propiedades antioxidantes además de un buen comportamiento mecánico después de ser reconstituido. Este hecho fue asociado a la conservación de algunas características estructurales de los polisacáridos del gel, tales como el peso molecular y la relación de carbohidratos presentes en el producto deshidratado. Posteriormente, se observó como el acemanano, el principal compuesto bioactivo presente en el gel de *Aloe vera*, fue modificado drásticamente por procesos de secado llevados a cabo a escala industrial, resaltando principalmente la reducción en el peso molecular y la pérdida de grupos acetilo. Estas alteraciones fueron atribuidas a las extensas condiciones de procesamiento, en particular la elevada fuerza de cizallamiento y la alta temperatura utilizada (> 150°C) dentro de la cámara de secado, a las que fue sometido el gel de *Aloe vera*. También se observó la pérdida de importantes propiedades tecnológicas y funcionales, tales como su comportamiento reológico, así como la capacidad para absorber agua y moléculas orgánicas (Minjares-Fuentes et al., 2016; Minjares-Fuentes et al., 2017b).

En definitiva, la calidad de los alimentos secados por SD depende en gran medida de los parámetros operativos aplicados. Por ello, es importante identificar los factores que influyen en las diferentes propiedades y/o características del producto final, con el objetivo de lograr la optimización del proceso para la obtención de productos con mejores características sensoriales y nutricionales, así como el logro de un mejor rendimiento del proceso (Phisut, 2012; Shishir & Chen, 2017).

4. ALIMENTACIÓN

En los últimos años, el consumo de *Aloe vera* como ingrediente en diferentes tipos de bebidas ha aumentado sustancialmente, debido al creciente interés del consumidor por sus presuntos beneficios para la salud (Kim et al., 2023). Particularmente, se ha observado que los productos procedentes de plantas son ampliamente utilizados como suplementos nutricionales para la prevención de enfermedades o para llevar un estilo de vida más saludable. Entre el 2009 y el 2016, la SEFAP (Sociedad Española de Farmacéuticos de Atención Primaria) observó una tendencia al alza en el consumo de complementos alimenticios en Italia a base de *Aloe vera* (Figura 5), aumentando en un millón el número de envases de complementos con *Aloe vera* puestos a la venta.

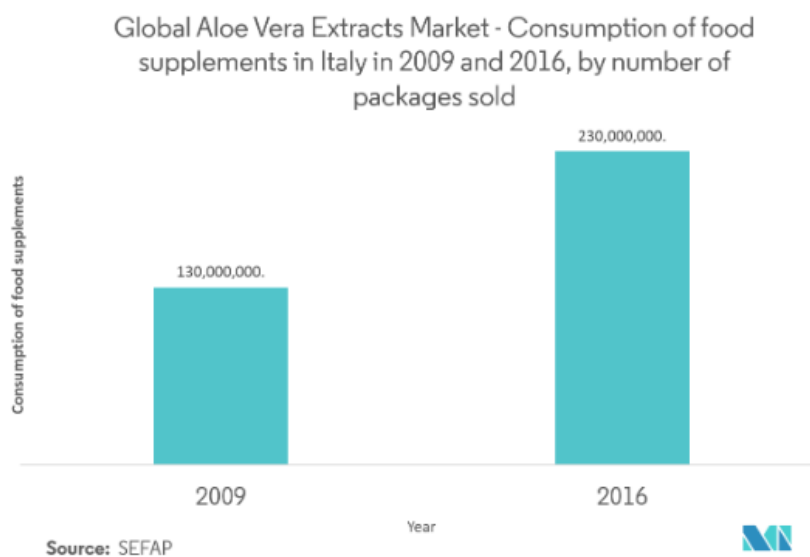


Figura 5. Gráfica del consumo de complementos alimenticios en Italia entre 2009 y 2016, por número de envases vendidos (SEFAP)

Específicamente, el gel de *Aloe vera* ha experimentado un notable crecimiento en la industria alimentaria, donde se ha convertido en un recurso fundamental para la elaboración de ingredientes funcionales, especialmente aquellos destinados a la

preparación de leche, helados, yogur y, en particular, diferentes tipos de bebidas saludables, como el popular zumo de *Aloe vera* o el té (Eshun & He, 2004; Ramachandra & Rao, 2008; Ahlawat & Khatkar, 2011; Habtemariam, 2017; Alvarado-Morales et al., 2019).

En 2011, Ahlawat & Khatkar (2011) realizaron la recopilación documental sobre las principales aplicaciones del *Aloe vera* en la industria alimentaria. Estos autores, realizaron diferenciación de las partes de aloe y su aplicación como materia prima para el desarrollo de productos alimentarios que contengan *Aloe vera*, denotando una amplia gama de aplicaciones y una gran versatilidad en la preparación de diferentes productos (ver Tabla 1).

Tabla 1. Aplicaciones alimentarias de diferentes productos elaborados con *Aloe vera* (Ahlawat & Khatkar, 2011).

Partes del <i>Aloe vera</i>	Aplicaciones alimentarias
Concentrado	Zumo, mermelada, jalea, el concentrado de <i>Aloe vera</i> también se puede mezclar con té, agua o zumo
Filete de gel	Caramelos, barritas, masticables, chicle, gránulos instantáneos de té de <i>Aloe vera</i> , goma de <i>Aloe vera</i> para encías doloridas o sangrantes, vitaminas de aloe en forma de dulces, batidos de frutas de <i>Aloe vera</i>
Jugo	Bebida lista para servir, bebida saludable, refresco, bebida laxante, sorbete, bebida deportiva (con electrolitos), bebida dietética con fibra soluble, bebida contra la resaca con vitaminas B, aminoácidos y acetaminofén, mezcla saludable de jugo de vegetales, yogures, mezcla de <i>Aloe vera</i> para whisky u otras bebidas alcohólicas, pan blanco con <i>Aloe vera</i> y jugo de pepino con <i>Aloe vera</i>
Polvo	Yogur, cuajada, ‘lassi’, helado y ‘laddu’ de <i>Aloe vera</i>

De esta forma, el *Aloe vera* es reconocido como suplemento alimenticio por diversas autoridades del sector agroalimentario, como el Codex Alimentarius, el “Federal Office of Consumer Protection and Food Safety” (BVL), el proyecto BELFRIT (que incluye a Bélgica, Francia e Italia) y el Intercambio de Información Médica de Trenton (THIE). Cabe mencionar que existen compuestos de *Aloe vera* cuya presencia está prohibida en la Unión Europea. Según el artículo 8 del Reglamento (CE) N° 1925/2006 de la AESAN (AESAN, 2006), enmienda publicada en el Diario Oficial de la Unión Europea (UE) el 18 de marzo de 2021, la aloe-emodina y cualquier preparado que contenga esta sustancia, así como los preparados de la hoja de diversas especies de Aloe que contengan derivados hidroxiantracénicos, no pueden ser utilizados en la fabricación de alimentos dentro de la UE y ha llevado a la Agencia Europea de Seguridad Alimentaria (EFSA) a realizar diversas evaluaciones sobre los efectos de estas sustancias (Figura 6).

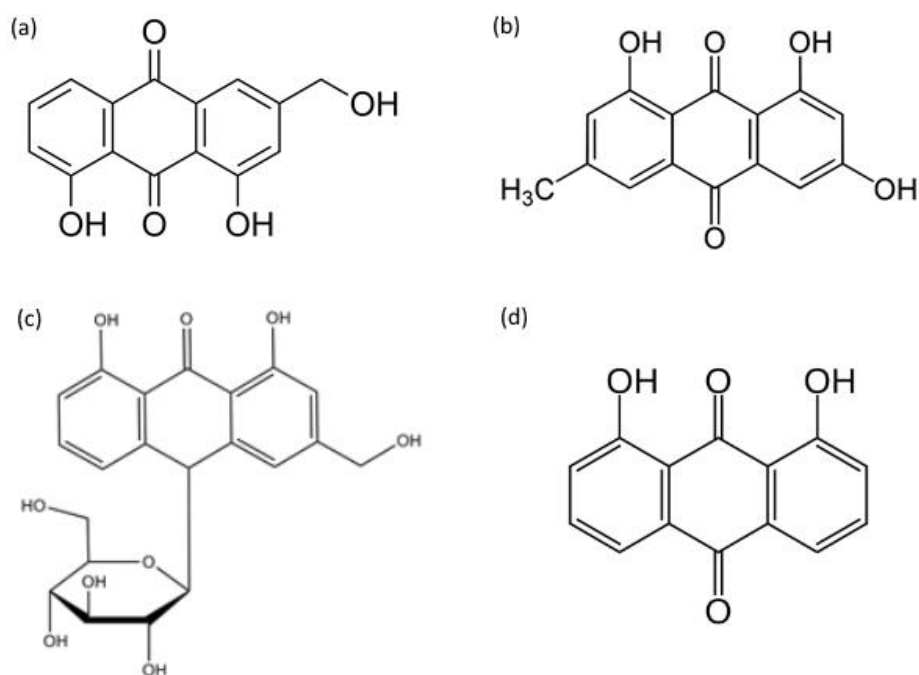


Figura 6. Estructura química de la aloe-emodina (a), emodina (b), aloína (c) y dantrona (d), derivados hidroxiantracénicos

El consumo de estas sustancias en dosis superiores a las que se esperarían en una dieta equilibrada puede suponer un riesgo para la salud del consumidor, debido a sus efectos laxantes y alergénicos (Domínguez-Fernandez et al. 2012), o incluso a sus efectos carcinógenos, observados en diferentes ensayos *in vitro* en ratas (Boudreau et al. 2013). Debido a este riesgo de genotoxicidad y carcinogenicidad, el proceso de producción de extracto de *Aloe vera* de grado alimenticio ha experimentado mejoras continuas (Pressman et al. 2019). Como se ha mencionado anteriormente, el International Aloe Science Council (IASC, 2016) ha establecido un estándar de calidad para su programa de certificación que no debe superar los 10 ppm de aloína A y B para todos los ingredientes de zumo de hoja de *Aloe vera* destinados a productos de consumo oral. Para evitar la inclusión de estos compuestos en el proceso de producción, las capas externas de la planta de *Aloe vera*, las cuales contienen la mayor concentración de estos derivados, son eliminadas antes de proceder a la obtención del gel, como se menciona en los estudios de Lachenmeier et al. (2005) y Ramachandra y Rao (2008). No obstante, en un estudio más reciente se evaluó el potencial genotóxico de la aloína y de diferentes bebidas basadas en *Aloe vera* mediante ensayos *in vitro* e *in vivo* en ratas. Los resultados obtenidos indicaron que los extractos de *Aloe vera* de grado alimenticio comercial (extracto de gel de hoja interna y extracto de hoja entera decolorizada) con valores inferiores a 10 ppm de aloína no presentaban genotoxicidad (Kim et al., 2023). La gran demanda de productos alimentarios a base de *Aloe vera* también se ha visto últimamente potenciada en el mercado mundial, siendo Asia y el Pacífico los mayores demandantes de estos productos, como puede observarse en la Figura 7 (Mordor Intelligence, 2023).

Aloe Vera Extracts Market – Market Size, by Region, Global, 2018



Figura 7. Mapa del mercado de extractos de *Aloe vera* en 2018

5. COMPUESTOS BIOACTIVOS

En muchos casos, los compuestos bioactivos no son tratados específicamente como elementos nutricionales, aunque ofrecen ventajas adicionales para la salud que van más allá del valor nutricional fundamental del producto (Hamzalioglu & Gökmen, 2016). Los compuestos bioactivos se pueden encontrar tanto en animales como en plantas, y en cantidades reducidas en los alimentos, principalmente en frutas, verduras y cereales integrales (Kris-Etherton et al., 2004; Santos et al. 2019). Particularmente, se puede encontrar una amplia variedad de compuestos bioactivos con características diversas, entre los que destacan tanto los compuestos antioxidantes como la fibra alimentaria.

Los compuestos antioxidantes son compuestos fitoquímicos que además de desempeñar roles específicos en el crecimiento y supervivencia de las plantas, presentan actividad fisiológica particularmente como compuestos capaces de inhibir las especies reactivas de oxígeno (reactive oxygen species: ROS), las cuales son consideradas como las principales promotoras del envejecimiento celular y daño al ADN, cuando son

consumidos por los seres vivos (Coronado et al. 2015). Por otra parte, la fibra alimentaria o fibra dietética es definida como “el material procedente de las células de origen vegetal, presente en nuestra dieta, resistente al proceso de hidrólisis llevado a cabo por las enzimas del aparato digestivo humano” (Trowell, 1976). Posteriormente, la definición fue ampliada a “todo el conjunto de polisacáridos y lignina presentes en la dieta que no son digeridos por las secreciones endógenas del sistema digestivo humano” (Trowell et al., 1976). Desde un punto de vista analítico, el término dietética se refiere principalmente a los polisacáridos, excluyendo al almidón, y a la lignina presente en la dieta. Desde el punto de vista fisiológico, los polisacáridos que integran la fibra dietética pueden reducir los niveles de colesterol en suero y glucosa en sangre, y pueden tener un impacto positivo en el índice glucémico, entre otros efectos beneficiosos (Courtin & Delcour, 1998; Huseini et al. 2012; Choudhary et al. 2014; Kumar & Tiku, 2015; Liu et al., 2015).

En las últimas dos décadas, se han publicado varios artículos científicos sobre los diferentes compuestos bioactivos presentes en la planta *Aloe vera*. En la mayoría de estos estudios, los diferentes compuestos con capacidad antioxidante, principalmente aloína y otros compuestos fenólicos, así como los polisacáridos bioactivos, en particular el polímero de almacenamiento conocido como acemanano, y las pectinas de la pared celular, parecen ser los componentes clave para explicar la mayoría de las propiedades funcionales atribuidas a la planta *Aloe vera*.

5.1. COMPUESTOS ANTIOXIDANTES

Los compuestos antioxidantes, en gran parte de origen sintético, se utilizan para limitar la peroxidación lipídica, la cual representa uno de los principales mecanismos de degradación de los alimentos durante el procesamiento y almacenamiento. No obstante,

los antioxidantes naturales poseen diversas propiedades biológicas que permiten proteger el cuerpo humano de los radicales libres, ya que estos pueden causar algunas enfermedades crónicas como el cáncer o enfermedades cardiovasculares (Baydar et al. 2007).

En las plantas de carácter medicinal encontramos una amplia variedad de moléculas captadoras de radicales libres, tales como compuestos fenólicos, compuestos nitrogenados, vitaminas, terpenoides, carotenoides y otros metabolitos secundarios con actividad antioxidante (Cotelle et al. 1996; Velioglu et al. 1998; Zheng & Wang 2001; Cai et al. 2003).

Ninguna metodología analítica es capaz por sí misma de cuantificar totalmente la capacidad antioxidante, recomendando siempre el uso de diferentes técnicas para determinar la capacidad antioxidante de las muestras de una forma más global (Pellegrini et al., 2003). Por eso, se combinan diferentes técnicas para estimar y determinar la presencia de todos estos constituyentes bioactivos en muestras de origen vegetal (Kumar et al. 2017c). De hecho, un estudio realizado por Nejatizadeh-Barandozi (2013) demostró como los polifenoles no flavonoides contribuyen a la mayor parte del contenido total de polifenoles en diferentes muestras de *Aloe vera*, los cuales han sido beneficiosos para aliviar o prevenir los síntomas asociados a las enfermedades cardiovasculares, el cáncer y la diabetes.

Actualmente se sabe que la gran mayoría de los compuestos con capacidad antioxidante presentes en *Aloe vera*, se localizan en el látex o exudado, el cual se encuentra distribuido en los tejidos vasculares situados entre la capa exterior de la planta (corteza) y la pulpa interior. Comúnmente, este exudado exhibe un matiz amarillo pardoado, posee un sabor amargo y se han contabilizado aproximadamente hasta 80 componentes químicos diferentes utilizando técnicas de cromatografía líquida. Entre estos, los

compuestos antioxidantes más abundantes son las antraquinonas C-glucósido, las antronas, las cromonas, las fenilpironas y los derivados de naftaleno. Los derivados antraquinónicos C-glucósido son considerados elementos característicos del exudado presente en las hojas de *Aloe vera*, aunque en realidad no se encuentran en todas las especies (Kanama et al., 2015). Cuando están presentes, tienden a ser la parte principal del exudado y están mayormente representados por la aloína. Este compuesto se ha identificado en la mayoría de las especies de Aloe en cantidades que varían del 0.1% al 6.6% del peso seco de la hoja, abarcando entre el 3% y el 35% del exudado total (Reynolds, 1985; Kanama et al., 2015). La aloína parece ser exclusiva del exudado de las hojas, siendo además el compuesto amargo principal, el cual ha sido descrito como un derivado C-glucósido de la aloe-emodina (Reynolds, 1985, 2004).

En las últimas décadas, se ha observado un destacado potencial terapéutico de la aloína en el ámbito de la prevención y curación del cáncer. Específicamente, se han identificado efectos quimioprotectores contra lesiones preneoplásicas inducidas por 1,2-dimetilhidrazina en el colon de ratas Wistar (Hamiza et al., 2014). En concreto, se ha observado como la aloína puede inhibir la secreción del factor de crecimiento endotelial vascular (VEGF) en células cancerosas, lo que conduce a la inhibición de la proliferación y migración de las células endoteliales. El VEGF es una de las citosinas proangiogénicas más cruciales y ampliamente reconocidas por su capacidad para inducir la formación de vasos sanguíneos en tumores (Pan et al, 2013). En este mismo sentido, se ha observado como la aloe-emodina posee la capacidad de inhibir la proliferación en ciertos tipos de células cancerosas, como las presentes en el cáncer de pulmón, carcinoma escamoso, glioma y neuroectodérmicas (Lin et al., 2011; Suboj et al., 2012; Masaldan & Iyer, 2014).

5.2. POLISACÁRIDOS

Los polisacáridos son el componente más abundante que constituye la materia seca del gel de *Aloe vera*. Debido a este hecho, estos compuestos, en ocasiones denominados nutraceuticos, han sido considerados como los principales responsables de la mayor parte de los beneficios asociados a la planta *Aloe vera* (Domínguez-Fernandez et al. 2012). Específicamente, la mayoría de las propiedades farmacológicas del gel de *Aloe vera* se han atribuido al polisacárido acetilado acemanano (Choi & Chung, 2003; Ni et al., 2004; Rodríguez et al., 2010; Radha & Laxmipriya, 2015; Pothuraju et al., 2016). El polímero acemanano es el principal polisacárido que se encuentra en el gel de *Aloe vera* (Femenia et al., 1999). Sin embargo, el gel también contiene un número considerable de polisacáridos provenientes de la pared celular, siendo los polisacáridos pécticos el tipo de polímero más abundante en las paredes celulares del gel de *Aloe vera*, seguido por la celulosa y pequeñas cantidades de hemicelulosas (Femenia et al., 1999, 2003; Simões et al., 2012). Es importante resaltar que recientes estudios han demostrado que la composición y características estructurales de los polisacáridos de *Aloe vera* juegan un papel muy importante, no solo en la interacción con otros compuestos bioactivos, sino también en las propiedades tecnológicas, funcionales, fisiológicas y en la actividad biológica. No obstante, las condiciones agroclimáticas, el manejo agronómico y el procesamiento del *Aloe vera* pueden modificar considerablemente la arquitectura y la disposición tridimensional de los polisacáridos, alterando las propiedades asociadas a estos compuestos.

5.2.1. COMPOSITIONAL AND STRUCTURAL FEATURES OF THE MAIN BIOACTIVE POLYSACCHARIDES PRESENT IN THE ALOE VERA PLANT

<https://doi.org/10.5740/jaoacint.18-0119>

SPECIAL GUEST EDITOR SECTION

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

RAFAEL MINJARES-FUENTES

University of the Balearic Islands, Department of Chemistry, Ctra Valldemossa Km 7.5, 07122, Palma de Mallorca, Spain; Universidad Juárez del Estado de Durango, Facultad de Ciencias Químicas, Av. Artículo 123 s/n, Fracc. Filadelfia, 35010, Gómez Palacio, Durango, México

ANTONI FEMENIA¹ and FRANCESCA COMAS-SERRA

University of the Balearic Islands, Department of Chemistry, Ctra Valldemossa Km 7.5, 07122, Palma de Mallorca, Spain

VÍCTOR MANUEL RODRÍGUEZ-GONZÁLEZ

Universidad Juárez del Estado de Durango, Facultad de Ciencias Químicas, Av. Artículo 123 s/n, Fracc. Filadelfia, 35010, Gómez Palacio, Durango, México

***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.**

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

A *loe 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

Guest edited as a special report on “*Aloe vera*: Chemistry, Major Chemical Components, Quantification, and Molecular Weight Determination of Polysaccharides” by Kan He.

We would like to acknowledge the financial support (RTA2015-00060-C04-03 and AAEE045/2017) of the Spanish Government (MICINN) and the European Regional Development Fund (FEDER), and the Government of the Balearic Islands for the research fellowship (FPI/1477/2012) of the “Conselleria d’Educació, Cultura i Universitats” and European Social Fund (FSE).

¹Corresponding author’s e-mail: antoni.femenia@uib.es

DOI: <https://doi.org/10.5740/jaoacint.18-0119>

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 \rightarrow 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 \rightarrow 4) acetylated Man, which also contains β -(1 \rightarrow 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 \rightarrow 4) α -D-galacturonic acid (GalA) repeating units with intermittently (1 \rightarrow 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 (Ca²⁺; 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

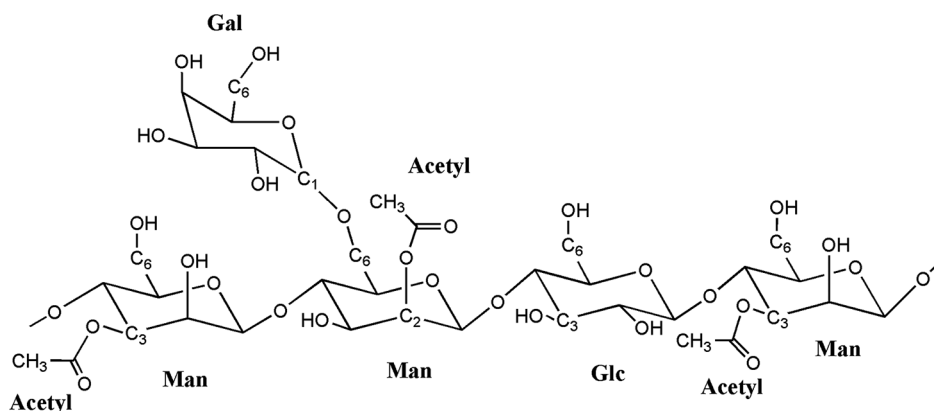


Figure 1. Chemical structure of acemannan polymer proposed by Chokboribal et al. (15).

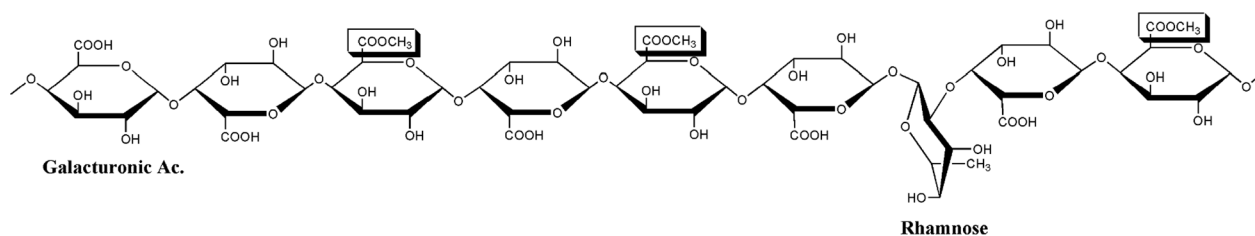


Figure 2. Schematic representation of the pectin structure. Modified from Minjares-Fuentes and Femenia (42).

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 \rightarrow 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 \rightarrow 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 \rightarrow 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, Nejat-zadeh-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, ¹H-NMR spectrometry has demonstrated capability of simultaneously detecting and quantifying an important number of constituents in

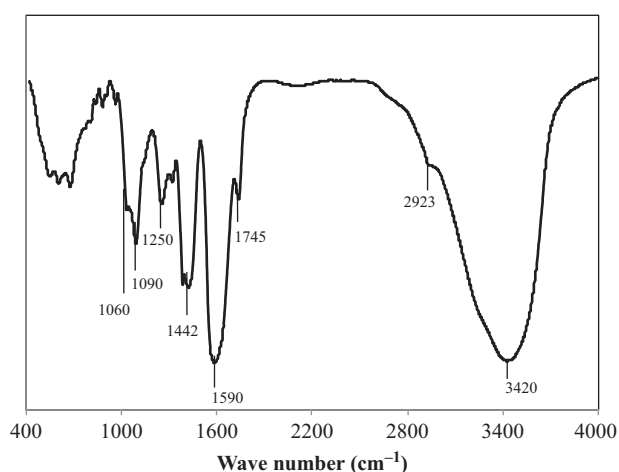


Figure 3. FTIR spectra of crude polysaccharides from *A. vera* gel.

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 ¹H-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, ¹H-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

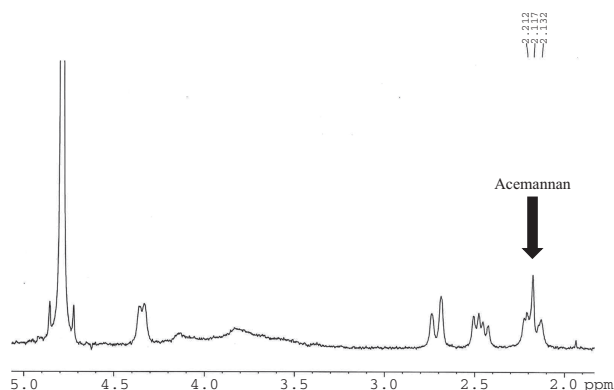


Figure 4. ¹H NMR spectra from acemannan isolated from *A. vera* gel.

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

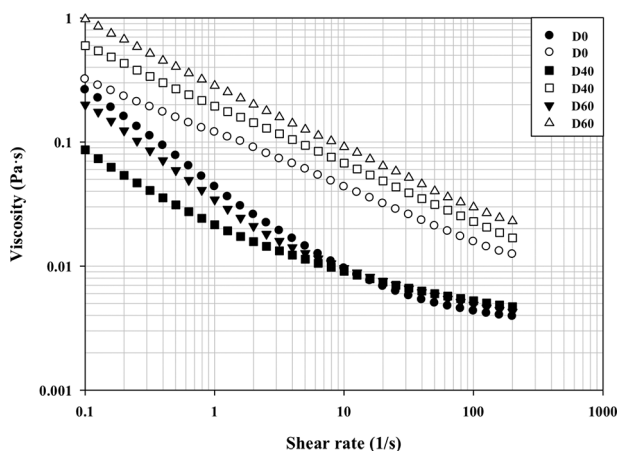


Figure 5. Flow curves describing the non-Newtonian shear-thinning flow behavior of fresh (full dots) and reconstituted (empty dots) *A. vera* mucilages treated with different water deficits: D0=aloe without water deficit; D40=aloe with 40% water deficit; and D60=aloe with 60% water deficit. Modified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

highlighted that functional properties from *A. vera* samples dehydrated at temperatures between 40 and 60°C, 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 85°C. 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 60°C for 15 min. On the other hand, *A. vera* gel obtained from 4-year-old plants, pasteurized at 75°C for 20 min, exhibited the highest WRC, whereas, for plants of the same age, pasteurization carried out at 70°C 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 pointed out that

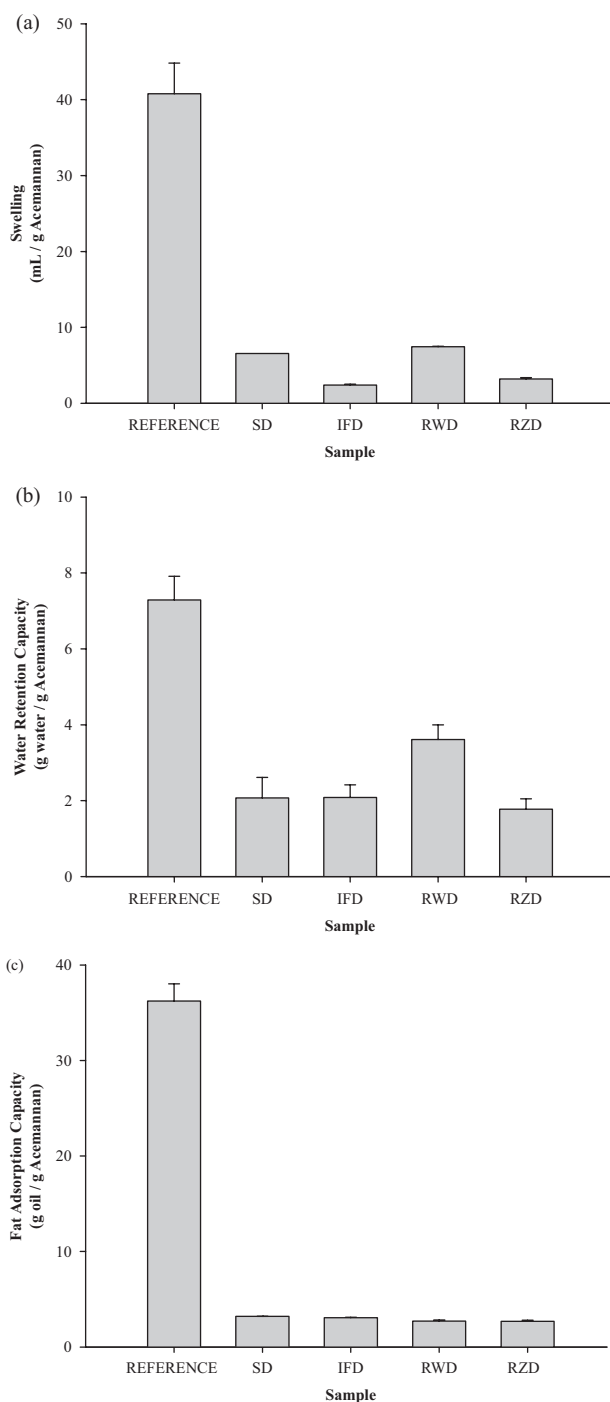


Figure 6. Functional properties determined for acemannan polysaccharide obtained from fresh *A. vera* gel (REFERENCE) and processed samples obtained by spray drying (SD), industrial freeze drying (IFD), refractance window drying (RWD), and radiant zone drying (RZD). (a) Swelling (Sw), (b) water retention 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, °C	Time, min	Sw, mL/g	WRC, g H ₂ O/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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OBJETIVOS

OBJETIVOS

El Objetivo General de la presente Tesis Doctoral se centra en profundizar en el conocimiento científico en torno a diversos aspectos vinculados al *Aloe vera*; en concreto, el estudio se ha focalizado sobre tres aspectos fundamentales: (1) las técnicas de cultivo, (2) los métodos de procesamiento y (3) la utilización del *Aloe vera* en el ámbito de la industria alimentaria.

Por tanto, el estudio se ha dividido en tres líneas principales de investigación. En la primera línea, basada en las técnicas de cultivo de *Aloe vera*, se ha evaluado la influencia del estrés hídrico y salino sobre los principales compuestos bioactivos del *Aloe vera* y, en particular, sobre el acemanano y las pectinas. En una segunda línea, basada en la investigación de los efectos del procesado, se ha focalizado en el secado por atomización (spray-drying), en particular sobre el efecto de esta técnica de secado sobre las propiedades físico-químicas, los principales polisacáridos bioactivos y la capacidad antioxidante del *Aloe ferox*, en comparación con el *Aloe vera*. Y, por último, la tercera línea de investigación se ha basado en la evaluación de las características del acemanano, como principal compuesto bioactivo en una amplia gama de bebidas comerciales que contienen el gel de *Aloe vera* como ingrediente principal.

En este contexto y con la finalidad de alcanzar el objetivo general propuesto, se definieron los siguientes objetivos específicos:

- Evaluar el efecto de la combinación de déficit hídrico y diferente grado de salinidad (estrés hídrico-salino) sobre el contenido de aloína en el gel de *Aloe vera* y su capacidad antioxidante; así como, en los polisacáridos presentes en el gel de *Aloe vera* y sus propiedades funcionales asociadas, tales como

hinchamiento, capacidad de retención de agua y capacidad de adsorción de lípidos.

- Identificar las modificaciones que ocurren en los principales polisacáridos presentes en el *Aloe vera* como consecuencia de los efectos combinados de la salinidad y el estrés hídrico, haciendo hincapié en el estudio del acemanano y las sustancias pécticas, dado que ambos polímeros son considerados como factores clave en conferir tolerancia ante el estrés abiótico.
- Evaluar los principales cambios en las propiedades fisicoquímicas, como la actividad de agua, la solubilidad, la higroscopicidad, así como en el comportamiento de flujo del gel de *Aloe vera* deshidratado obtenido mediante diferentes procedimientos de secado utilizados a nivel industrial: en particular, el secado por atomización (spray-drying), la liofilización (freeze-drying), el secado por ventana de refractancia (refractance window-drying) y el secado por zona radiante (radiant zone-drying).
- Evaluar los cambios en la composición de los polisacáridos del gel de *Aloe ferox* secado por atomización (spray-drying) y sus propiedades funcionales relacionadas, comparándolo con el *Aloe vera* secado utilizando la misma técnica. Este estudio se realizó con la finalidad no solo de desarrollar un nuevo ingrediente funcional a partir del *Aloe ferox*, sino también con la finalidad de actualizar la información científica disponible acerca de la composición de los polisacáridos presentes en esta variedad de Aloe.
- Evaluar la presencia de acemanano, en términos de cantidad y calidad, en una amplia gama de bebidas comerciales elaboradas a base de gel de *Aloe vera*, procedentes de diferentes fabricantes, procesadores y distribuidores de alimentos destacados en el mercado del *Aloe vera* a nivel local, nacional e internacional.

Para alcanzar este objetivo, se determinó, en cada una de las muestras comerciales, tanto la cantidad de acemanano presente, como la bioactividad de este polímero, la cual fue evaluada a partir de la determinación del grado de acetilación.

RESULTADOS Y DISCUSIÓN



ARTICLE INFO

Available online 28 February 2023

Keywords:
 Aloe vera
 Salinity
 Polyphenols
 Functional properties
 Water and salinity stress

ABSTRACT

The Aloe vera plant is an important source of bioactive compounds. Its growing conditions may have a remarkable effect on both its physiological and chemical characteristics. Thus, the objective of this study was to evaluate the effect of joint water and salinity stresses (water + salinity) on the main bioactive compounds of the Aloe vera gel, the relative water potential/stomatal water (stomatal water) and the related functional properties. Aloe vera plants were treated with two levels of available soil moisture (high and low) and five levels of salinity (0, 20, 40, 60 and 80 mM NaCl). Plants grown at high available soil moisture and low and medium salinity levels (0, 20, 40 and 60 mM NaCl) had the highest relative water potential and stomatal water (stomatal water) values (P < 0.05). Total phenolic compounds, flavonoid polyphenols and water-soluble polyphenols were the bioactive compounds analyzed along with the antioxidant activity, radical scavenging activity, water retention and the absorption coefficient. In general, the water + salinity stress negatively affected the development and growing process of the plants. However, it did cause a considerable increase in the total amount of dry matter (DM) in 2.4 g/100 g of fresh Aloe vera gel. This increase was mainly due to the synthesis of new polyphenols, and in particular of water-soluble polyphenols composed of monomers, polyphenols and glycosides which increased from 194 to 318.5 mg/g of water-soluble matter at water + salinity stress increased. Further, there was a significant increase in the total amount, from 10.7 to 19.8 mg/g of total DM content from Aloe vera gel. These changes in the bioactive compounds were reflected in the functional properties studied. Polyphenol properties of Aloe vera gel, such as reducing and water retention capacity, exhibited higher values for these plants treated with water deficit and salinity. The antioxidant capacity, determined by FRAP and DPPH methods, was also higher in the gel from stressed plants. Overall, these results show that water + salinity stress could be an agricultural strategy for increasing one of the sources of bioactive compounds present in the Aloe vera gel for supporting their related functional properties.

1. Introduction

In recent decades, Aloe vera (*Aloe barbadensis* Miller), a succulent plant belongs to Asphodelaceae family, has become one of the most important commercial medicinal plants in the world (Cifuentes et al., 2018; Hahn et al., 2007; Mankin et al., 2011). In fact, processing of the Aloe vera leaves generated a volume of 2.4 billion US dollars, in 2019 alone, an amount which is expected to increase by up to 5.2 billion US dollars from 2020 to 2027. The global market in Aloe vera is led by the USA (65 %), followed by India (10 %) and China (10 %) (Mankin et al., 2011), and its application worldwide is mainly due to the wide spectrum of beneficial properties associated with the different bioactive compounds present in this plant (Cifuentes et al., 2018; Martínez-Balboa et al., 2020; Minjares-Fuentes and Femenia, 2017).

* Corresponding author.
 E-mail address: rafael.minjares@uaeh.mx (R. Minjares-Fuentes).
<https://doi.org/10.1016/j.agwat.2023.108374>
 Received 12 November 2022; Received in revised form 10 May 2023; Accepted 12 May 2023
 Available online 28 May 2023
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CAPÍTULO I (A)

Joint water and salinity stresses increase the bioactive compounds of *Aloe vera* (*Aloe barbadensis* Miller) gel enhancing its related functional properties

González-Delgado, M., Minjares-Fuentes, R., Mota-Ituarte, M., Pedroza-Sandoval, A.,
 Comas-Serra, F., Quezada-Rivera, J.J., Sáenz-Esqueda, A., Femenia, A.

Agricultural Water Management

Volumen 285

Paginado 108374

Año publicación: 2023

Factor de impacto (JCR 2022): 6.7

Q1 (Agronomy 3/88 and Water Resources 7/103)

DOI: <https://doi.org/10.1016/j.agwat.2023.108374>

Role of acemannan and pectic polysaccharides in saline-water stress
tolerance of *Aloe vera* (*Aloe barbadensis* Miller)

Francesca Comas-Serra^a, José Luis Miró^b, Monica M. Umaña^a, Rafael Minjares-Fuentes^{b,c},
Antoni Femenia^a, Maria Mota-Ituarte^c, Aurelio Pedroza-Sandoval^d

^aDepartment of Chemistry, University of the Balearic Islands, Ctra. Valldemossa km 7.5, Palma
de Mallorca C.P. 07122, Spain

^bFacultad de Ciencias Químicas, Universidad Juárez del Estado de Durango, Av. Artículo 123
s/n, Fracc. Filadelfia, 35010 Gómez Palacio, Durango, México.

^cUnidad Regional Universitaria de Zonas Áridas, Universidad Autónoma Chapingo, Carretera
Gómez Palacio-Chihuahua km 38, Bermejillo, Durango, C. P. 35230, México

Abstract

This study investigates the impact of water and salinity stress on *Aloe vera*, focusing on the role of *Aloe vera* polysaccharides in mitigating these stresses. Pectins and acemannan were the most affected polymers. Low soil moisture and high salinity (NaCl 80 mM) increased pectic substances, altering rhamnogalacturonan type I in *Aloe vera* gel. *Aloe vera* pectins maintained a consistent 60% methyl-esterification regardless of conditions. Acemannan content rose with salinity, particularly under low moisture, accompanied by 90 to 150% acetylation increase. These changes improved *Aloe vera* polysaccharide functionality: pectin enhanced cell wall reinforcement and interactions, while highly acetylated acemannan retained water for sustained plant functions. This study highlights the crucial role of *Aloe vera* polysaccharides in enhancing plant resilience to water and salinity stress, leading to improved functional properties.

1

CAPÍTULO I (B)

Role of acemannan and pectic polysaccharides in saline-water stress tolerance of *Aloe vera* (*Aloe barbadensis* Miller).

Comas-Serra, F., Miró, J.L., Umaña, M., Minjares-Fuentes, R., Femenia, A. Mota-
Ituarte, M., Pedroza-Sandoval, A.

Revista: Carbohydrate Polymers

Número manuscrito: CARBPOL-D-23-04803

Bajo revisión

Año: 2023

Factor de impacto (JCR 2022): 11.2

Q1 (Chemistry Applied 3/72, Organic Chemistry 2/52 and Polymer Science 3/86)

Role of acemannan and pectic polysaccharides in saline-water stress tolerance of *Aloe vera* (*Aloe barbadensis* Miller)

Francesca Comas-Serra^a, José Luis Miró^a, Monica M. Umaña^a, Rafael Minjares-Fuentes^{b*}, Antoni Femenia^a, María Mota-Ituarte^c, Aurelio Pedroza-Sandoval^c

^a*Department of Chemistry, University of the Balearic Islands. Ctra. Valldemossa km 7.5, Palma de Mallorca C.P. 07122. Spain*

^b*Facultad de Ciencias Químicas. Universidad Juárez del Estado de Durango, Av. Artículo 123 s/n, Fracc. Filadelfia, 35010 Gómez Palacio, Durango. México.*

^c*Unidad Regional Universitaria de Zonas Áridas, Universidad Autónoma Chapingo. Carretera Gómez Palacio-Chihuahua km 38, Bermejillo, Durango, C. P. 35230, México*

Abstract

This study investigates the impact of water and salinity stress on *Aloe vera*, focusing on the role of *Aloe vera* polysaccharides in mitigating these stresses. Pectins and acemannan were the most affected polymers. Low soil moisture and high salinity (NaCl 80 mM) increased pectic substances, altering rhamnogalacturonan type I in *Aloe vera* gel. *Aloe vera* pectins maintained a consistent 60% methyl-esterification regardless of conditions. Acemannan content rose with salinity, particularly under low moisture, accompanied by 90 to 150% acetylation increase. These changes improved *Aloe vera* polysaccharide functionality: pectin enhanced cell wall reinforcement and interactions, while highly acetylated acemannan retained water for sustained plant functions. This study highlights the crucial role of *Aloe vera* polysaccharides in enhancing plant resilience to water and salinity stress, leading to improved functional properties.

Keywords: Aloe vera, acemannan, pectins, degree of acetylation, abiotic stress, functional properties

Corresponding author: Rafael Minjares-Fuentes (rafael.minjares@ujed.mx)

1. Introduction

Over the past decade, *Aloe vera* has gained significant importance as a raw material for various industries, including food, pharmaceutical, and cosmetic sectors. It has also emerged as a valuable source of diverse bioactive compounds, such as aloin and polysaccharides, owing to their wide range of beneficial properties (Minjares-Fuentes & Femenia, 2019). The increasing presence of these compounds has led to a projection that the market for *Aloe vera*-based products will experience substantial growth, with an estimated value of up to \$3.2 billion US dollars from 2020 to 2027 (González-Delgado et al., 2023).

Aloe vera gel, also known as *Aloe vera* filet, is the translucent inner tissue of the plant leaf, primarily composed of water (>98%) and various other components, including polysaccharides (>60% w/w), phenolic compounds, organic acids, enzymes, and minerals, among others (Femenia, Sánchez, Simal, & Rosselló, 1999; González-Delgado et al., 2023). The most important polysaccharides found in *Aloe vera* gel are cell wall pectins and acemannan, which significantly contribute to the technological, functional, and beneficial properties associated with the gel. The pectic polysaccharides in *Aloe vera* gel are characterized by a high percentage of galacturonic acid units (approximately 90-95%) and a low degree of methyl ester substitution (<30%) (McConaughy, Stroud, Boudreaux, Hester, & McCormick, 2008; Minjares-Fuentes, Femenia, Comas-Serra, & Rodríguez-González, 2018; Minjares-Fuentes et al., 2017a). On the other hand, acemannan, a storage polymer located within the parenchymatous cells, contains a significant amount of acetylated mannose (>60%) linked to glucose by a β -1-4 glycosidic bond, with acetyl groups being one of its main structural features (Chokboribal et al., 2015; Femenia et al., 1999; Minjares-Fuentes et al., 2018). Recent research has highlighted the importance of the acetylation of acemannan in its biological

activity. Acetylation seems to play a crucial role either in modulating the immune response against γ -radiation-induced oxidative damage (Kumar & Kumar, 2019) or inducing colorectal cancer cell apoptosis through the mitochondrial pathway (Tong et al., 2022).

Additionally, the presence of both *Aloe vera* polymers, pectin, and acemannan, significantly affects the viscous properties of *Aloe vera* gel. This phenomenon is attributed to the ability of acetyl groups of acemannan and methyl groups of pectins to interact with water molecules, which explains the high water content of *Aloe vera* gel and the remarkable tolerance of this plant to adverse agroclimatic conditions (Chokboribal et al., 2015; Minjares-Fuentes et al., 2018).

Conversely, extant literature has highlighted that several factors, including geographical location, growth periods, agricultural management practices, and climatic variations, exert significant influences on the compositional and structural characteristics of the primary polysaccharides present in *Aloe vera*. These influences, in turn, may result in important alterations to the functional properties of *Aloe vera* gel (Delatorre-Castillo et al., 2022; González-Delgado et al., 2023; Minjares-Fuentes et al., 2018; Ray, Ghosh, Ray, & Aswatha, 2015; Salinas et al., 2019).

One notable observation is that acemannan, a prominent polysaccharide in *Aloe vera* gel, undergoes metabolic changes in response to water deficit. Specifically, a loss of (1,4)-linked mannosyl residues from the acemannan polymer occurs, while the acetylation pattern remains unaltered. This metabolic modification impacts the flow behavior and viscoelastic properties of the gel, as reported by Minjares-Fuentes et al. (2017a).

Recent research has also shown that the combined exposure to salinity and water stresses leads not only to an increase in water-soluble polysaccharides containing

acemannan and pectic substances but also induces modifications in the carbohydrate composition of *Aloe vera* polysaccharides, including both cell wall and storage polysaccharides. These findings suggest that these polysaccharides play a crucial role in mitigating the effects caused by the combined stresses experienced by *Aloe vera*, as documented by González-Delgado et al. (2023).

However, despite the known drought tolerance of *Aloe vera*, the specific role of its polysaccharides in alleviating abiotic stress in these plants remains uncertain (Salinas et al., 2019). Notably, there has been a considerable increase in the interaction between two major abiotic stresses, namely hydric stress and salinity, in recent years. This has been attributed to the extensive use of saline water in irrigation systems, driven by climate change and the limited availability of fresh water resources. Within this context, the primary objective of this research was to investigate the alterations occurring in *Aloe vera* polysaccharides as a consequence of the combined effects of salinity and water stress. Particular focus was placed on studying acemannan and pectic substances, as they are considered key factors in conferring tolerance to abiotic stress in *Aloe vera*.

2. Materials and methods

2.1. Plant material

Aloe vera leaves, used as the raw material for gel extraction, were supplied by the Regional University Unit of Arid Zones from the Autonomous University of Chapingo (Durango, Mexico: latitude 23° 54' N and longitude 103° 37' W and 1,130 masl). The *Aloe vera* leaves were obtained from plants which were irrigated using distilled water with varying concentrations of NaCl (0, 20, 40, 60, and 80 mM). The moisture soil was periodic monitored in order to maintain different soil moisture: high soil moisture (HSM: 18±2.5%) and low soil moisture (LSM: 12±2.5%), with high soil moisture being

the optimal conditions for *Aloe vera* plant growth (Mota-Ituarte et al., 2023). The gel extracted from *Aloe vera* subjected to conditions of high soil moisture without the presence of saline concentration (0 mM NaCl) was used as the control sample. *Aloe vera* leaves, with a length greater than 30 cm, erect and without any mechanical damage, were used for the gel extraction. The *Aloe vera* filets were processed as previously described González-Delgado et al. (2023). The homogenized gel was freeze-dried and stored under anhydrous conditions until analysis.

2.3. *Alcohol insoluble residues*

AIRs from freeze-dried *Aloe vera* gel 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 a 0.5 mm aperture sieve.

4. *Isolation of water-soluble polysaccharides*

The water-soluble polysaccharides were isolated from AIRs following the methodology previously described Minjares-Fuentes et al. (2017a) with slight modifications. Each *Aloe vera* AIR preparation weighing 100 mg was mixed with 200 mL of distilled water and stirred for 2 h at room temperature. Afterward, the mixture was subjected to centrifugation at 13,000g for 1 h at 20 °C. The resulting supernatant containing water-soluble polysaccharides (WSP) was collected and freeze-dried. The extracts were then stored in a desiccator under dry conditions until further analysis.

2.5. *Carbohydrate analysis*

The carbohydrate analysis of AIR and WSP fractions, was performed as described by Minjares-Fuentes et al. (2017a) for neutral sugars. Approximately 5 mg of *Aloe vera* samples (AIR and WSP fractions) were dispersed in 12 M H₂SO₄ for 3 h followed by dilution to 1 M and hydrolyzed at 100 °C for 2.5 h (Saeman, Moore, Mitchell, & Millett,

1954). A second sample, from AIRs and WSP fractions, was hydrolyzed only with 1 M H₂SO₄ (100 °C for 2.5 h). Thus, the cellulose content could be 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 at 220 °C by GC (Hewlett-Packard 5890A, Waldbronn, Germany) with a FID detector and equipped with a 30 m column DB-225 (J&W Scientific, Folsom, CA, USA) with i.d. and film thickness of 0.25 mm and 0.15 µm, respectively. Uronic acids were determined by colorimetry, as total uronic acids as previously described by Blumenkrantz and Asboe-Hansen (1973), using galacturonic acid as a standard.

2.6. Degree of methyl esterification by FTIR spectroscopy

AIRs fraction, containing pectins, were desiccated prior to FTIR analysis. FTIR spectra of the samples were recorded using a FTIR spectrometer (Bruker Tensor 27, Massachusetts, USA) as previously described in Minjares-Fuentes et al. (2014). The frequency range used varied from 400 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹. FTIR spectra were processed using the OMNIC E.S.P. 5.1 software and the DME was determined using the following equations (Pappas et al., 2004):

$$\text{DME} = 124.7R + 2.2013 \quad (1)$$

$$R = A_{1740} / [A_{1740} + A_{1630}] \quad (2)$$

where A₁₇₄₀ and A₁₆₃₀ were defined as the absorbance intensities of the bands for methyl-esterified and non-methyl-esterified carboxyl groups at 1740 cm⁻¹ and 1630 cm⁻¹, respectively.

2.7. Degree of acetylation by ¹H NMR

The WSP fraction, containing the bioactive acemannan polymer, was analyzed by ¹H NMR analysis as previously described by Minjares-Fuentes et al. (2017b). The ¹H NMR spectra at 300.13 MHz were recorded on a Bruker Avance 300 spectrometer

(Massachusetts, USA), equipped with a 5 mm broadband multinuclear z-gradient (BBO) probehead. High purity (99.5%) nicotinamide standard (Sigma-Aldrich, Spain) was added as internal shift standard. The relative degree of acetylation of stressed samples in relation to the control sample was calculated using the following equation (3):

$$\text{Relative degree of acetylation} = \left(\frac{A_{\text{stressed}}}{A_{\text{control}}} \right) \times 100 \quad (3)$$

where A_{stressed} and A_{control} are the area under the curve of the signals of acetyl groups corresponding to the stressed and control *Aloe vera* samples, respectively.

2.8. Functional properties

The functional properties determined included hydration properties such as, swelling (Sw) and water retention capacity (WRC), and fat adsorption capacity (FAC). Sw and WRC of AIRs from the *Aloe vera* gel samples, were measured in a phosphate buffer (1 M; pH 6.3) while FAC was measured using corn oil, as previously described by Comas-Serra et al. (2023).

2.9. Statistical analysis

The yield of AIRs as well as the content of the different polysaccharides and the functional properties associated to these, were statistically analyzed by ANOVA with a statistical significance level of $\alpha = 0.05$. The post-hoc analysis was performed using the lower significant difference (LSD) test. All statistical analyses were performed in MINITAB® software.

3. Results and discussion

3.1. Yield of alcohol insoluble residue (AIRs)

The yield of the AIRs corresponding to the *Aloe vera* samples treated under salinity conditions at different soil moisture, are shown in **Figure 1**. As can be seen, salinity

promoted the increase of yield of AIRs, ranging from values of 4.7 mg AIR/g of *Aloe vera* gel in control sample, up to 9.5 mg AIR/g of *Aloe vera* gel in LSM-80 sample ($p < 0.05$). Interestingly, the higher yields of AIRs were observed at low soil moisture (LSM), showing a mean value 7.4 mg AIR/g of *Aloe vera* gel, whereas an average value of 5.4 mg AIR/g of fresh sample was obtained under high soil moisture (HSM) ($p < 0.05$). Previous reports have demonstrated that irrigation conditions affect the yield of the AIRs from *Aloe vera* gel (Delatorre-Castillo et al., 2022; Delatorre-Herrera, Delfino, Salinas, Silva, & Cardemil, 2010; González-Delgado et al., 2023; Minjares-Fuentes et al., 2017a). These authors have suggested that the observed increase in AIRs yield in *Aloe vera* gel may be attributed to two main factors. Firstly, the increase in solid material content in *Aloe vera* gel is a consequence of the dehydration process caused by restricted water availability in the soil. Secondly, the accumulation of salinity in the soil may further contribute to the higher yield of AIRs (González-Delgado et al., 2023; Minjares-Fuentes et al., 2017a).

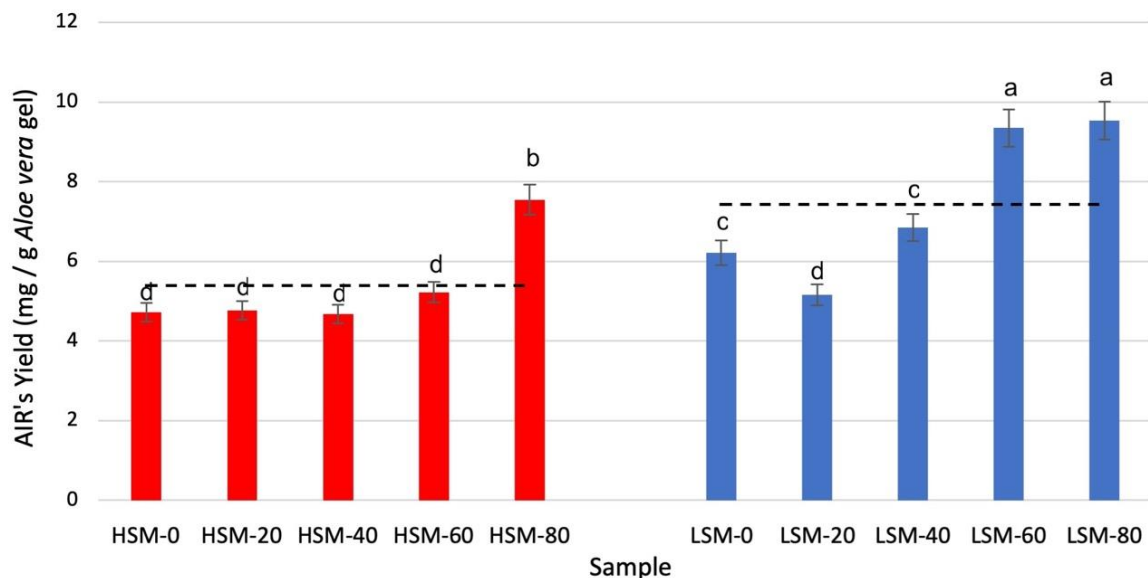


Figure 1. Yield of AIRs from *Aloe vera* gel subjected to varying levels of salinity and different soil moisture conditions. Different letters (a – d) above bars indicate statistical differences by LSD ($p < 0.05$).

3.2. Carbohydrate composition

AIRs encompass all the polysaccharides found in the *Aloe vera* samples, comprising both the cell wall polymers, commonly recognized as dietary fiber, and the reserve polysaccharides (Femenia et al., 1999). The carbohydrate composition of the predominant *Aloe vera* polysaccharides was determined using Gas Chromatography (GC) and is presented in **Table 1**.

As can be seen, uronic acids (UA) were the predominant carbohydrate, ranging from ~234 to ~426 mg/g of AIR, followed by mannose (Man) and glucose (Glc) with amounts between ~94 to ~165 mg/g of AIR and ~91 to ~123 mg / g of AIR, respectively. Galactose (Gal: 26 – 66 mg / g of AIR), xylose (Xyl: 22 – 29 mg / g of AIR), arabinose (Ara: 6.8 – 8 mg / g of AIR) and fucose (Fuc: 2.5 – 4.0 mg / g of AIR) were also found while rhamnose was detected only in trace amounts (< 2.0 mg / g of AIR).

Table 1. Carbohydrate composition (mg sugar/g AIR) of AIRs obtained from *Aloe vera* samples subjected to varying levels of salinity (NaCl: 0, 20, 40, 60 and 80 mM) and different soil moisture conditions^a.

	Rha ^b	Fuc	Ara	Xyl	Man	Gal	Glc	UA
0	1.8±0.2	3.6±0.3	6.9±0.1	23.9±0.2	129.7±1.5	26.2±1.2	97.7±2.8	261.9±69.4
20	2.0±0.7	4.0±1.1	7.6±0.2	28.6±0.9	131.5±1.5	34.6±1.0	98.6±4.5	332.4±21.7
HSM 40	1.9±0.6	3.4±0.5	7.1±0.8	23.8±0.3	138.8±2.7	34.9±0.8	104.9±0.9	367.3±18.7
60	1.8±0.2	3.6±0.4	7.4±0.7	29.3±0.7	122.6±0.8	62.4±3.9	112.7±0.4	426.2±12.0
80	2.0±0.3	3.5±0.3	6.8±0.4	24.4±0.3	153.2±3.4	62.0±3.0	99.1±2.2	336.9±29.1
0	1.3±0.1	3.6±0.4	7.2±0.7	22.9±0.1	123.4±0.4	26.0±0.3	91.8±3.3	351.8±24.0
20	1.8±0.0	3.7±0.9	7.5±0.3	26.7±1.3	127.1±0.3	45.6±1.9	109.3±12.7	244.4±27.0
LSM 40	2.0±0.3	2.9±1.1	8.0±0.9	29.4±0.2	94.0±8.9	66.6±7.8	110.0±7.2	234.1±24.7
60	1.8±0.0	3.3±0.1	6.9±0.2	25.1±2.4	157.7±0.7	55.8±4.8	116.4±13.7	248.3±68.2
80	1.6±0.2	2.5±0.2	6.8±0.4	23.0±1.3	165.4±4.6	52.9±4.2	123.6±13.8	226.6±40.0

^aSoil moisture: level of soil moisture; high (HSM) and low (LSM).

^bRha: rhamnose; Fuc: fucose; Ara: arabinose; Xyl: Xylose; Man: mannose; Gal: galactose; Glc: glucose; UA: uronic acids

In general, these results suggest a high presence of pectic polysaccharides or pectins which were inferred by the high amount of UA detected, specifically galacturonic acid (GalA), together the minor amounts of Rha, Ara and Gal (Femenia et al., 1999). On the other hand, the high content of Man in the AIRs from *Aloe vera* samples have been attributed to the presence of the main storage polysaccharide of *Aloe vera* plant, the bioactive polymer acemannan (Femenia et al., 1999; Minjares-Fuentes et al., 2017a; Rodríguez-González et al., 2011). Furthermore, around of 90% of Glc contained in the AIRs arises from cellulose, the main structural polymer formed solely by glucose. The rest of the sugars detected in the AIRs, particularly Fuc and Xyl, are considered as an indicative of the presence of small amounts of hemicelluloses (Femenia et al., 1999; Minjares-Fuentes et al., 2017a; Rodríguez-González et al., 2011).

From an industrial perspective, both *Aloe vera* gel and *Aloe vera* products are highly valued for their total content of polysaccharides, which can be assessed by measuring the concentration of sugars present in the AIRs. Thus, **Figure 2** shows the influence of salinity and soil moisture conditions on the overall polysaccharide content of *Aloe vera* gel.

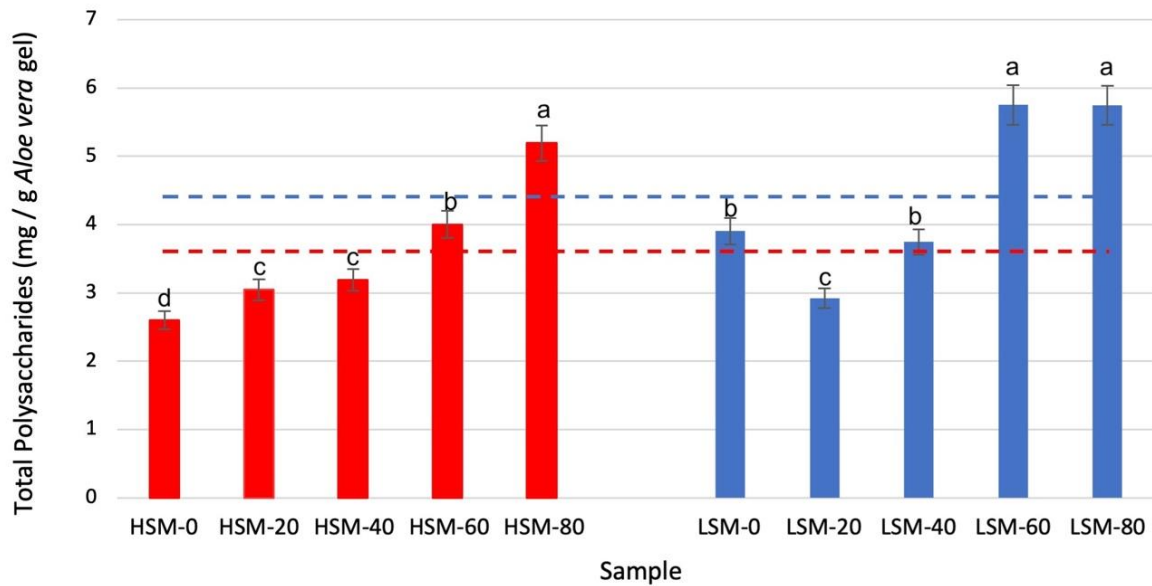


Figure 2. Total polysaccharides content of *Aloe vera* gel samples subjected to varying levels of salinity and different soil moisture conditions. Different letters (a – d) above bars indicate statistical differences by LSD ($p < 0.05$).

As observed, an increase in salinity levels resulted in a noteworthy enhancement of the total polysaccharide content ($p < 0.05$), surpassing 5 mg/g in *Aloe vera* gel. This effect was particularly pronounced under low soil moisture (LSM) conditions. Specifically, the total polysaccharide content escalated from approximately 2.6 to around 5.1 mg/g of *Aloe vera* gel under high soil moisture (HSM) conditions, and further increased to approximately 5.7 mg/g of *Aloe vera* gel under LSM conditions.

It is noteworthy to emphasize that the total polysaccharide content constituted approximately 65-70% of the AIRs (**Figure 2**), aligning with the observations made by Femenia et al. (1999) and Femenia et al. (2003). These researchers reported that *Aloe vera* polysaccharides accounted for more than 60% of the AIR fractions. The substantial quantity of polysaccharides presents in *Aloe vera* gel positions it as a promising candidate for the development of functional foods, particularly for the development of

dietary fiber-enriched products or substitutes for fat in dietary supplements (Quirós-Pozo, Ventura-Castellano, Ramírez-Bolaños, Roo-Filgueira, & Robaina, 2021).

3.4. Cellulose

Cellulose stands as the predominant polymer in nature, serving as the primary structural polysaccharide within plant tissues. This polymer is constituted by glucose units interconnected through β -1,4 glycosidic bonds (Praveen Kumar et al., 2019). Therefore, modifications in the composition of this polymer could potentially serve as indicators of distinct physiological responses initiated by the plant in response to varying stressors. Consequently, an investigation was undertaken to assess the influence of salinity at varying levels of soil moisture on the cellulose content within *Aloe vera* gel.

It is important to highlight that the majority of the detected glucose (Glc) within the AIRs extracted from *Aloe vera* gel originated from cellulose, although minor quantities might stem from the acemannan polymer. Accordingly, the distinction between glucose derived from the structural cellulose and the glucose inherent to acemannan was accomplished by quantifying the glucose residues identified in the water-soluble polysaccharide (WSP) fraction and subsequently subtracting this quantity from the overall Glc content present in the AIRs samples.

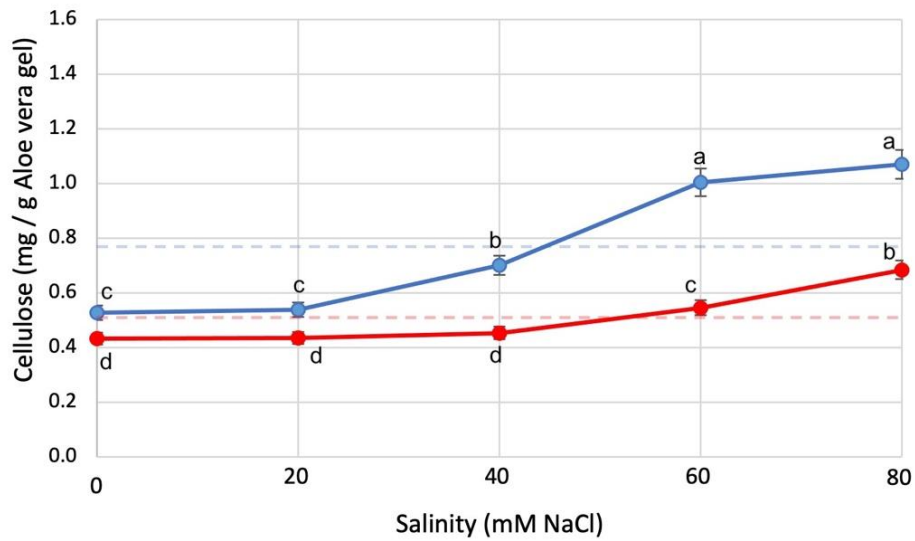


Figure 3. Cellulose content of *Aloe vera* gel subjected to varying levels of salinity and different soil moisture conditions: high (red line) and low (blue line). Different letters (a – d) indicate statistical differences by LSD ($p < 0.05$).

As can be seen in figure 3, both salinity and soil moisture exerted significant effects on the cellulose content ($p < 0.05$). Notably, *Aloe vera* gel derived from plants subjected to low soil moisture (LSM) conditions showed elevated cellulose content in comparison to *Aloe vera* gel from high soil moisture (HSM) conditions, exhibiting mean values of approximately 0.8 mg/g of *Aloe vera* gel and about 0.5 mg of cellulose/g of *Aloe vera* gel, respectively. Moreover, an increase in salinity was accompanied by an increase in cellulose content, particularly pronounced when salinity levels exceeded 60 mM ($p < 0.05$). This increment reached nearly 58% under HSM conditions and escalated up to 103% under LSM conditions.

Prior investigations have elucidated that under circumstances of salinity and osmotic stress, cellulose is utilized as a carbon source to regulate intracellular osmotic equilibrium (Iraki, Bressan, Hasegawa, & Carpita, 1989; Moore, Vitré-Gibouin,

Farrant, & Driouich, 2008b). Nonetheless, recent research has unveiled an alternative perspective: water scarcity could induce cellulose biosynthesis, serving as a defensive mechanism to uphold the structural integrity of the cell wall and turgor, thereby enabling sustained cellular growth during periods of stress (Le Gall et al., 2015).

3.6. *Hemicelluloses*

Hemicelluloses are a type of highly branched polysaccharides, comprised mainly of xylose, mannose, fucose and glucose. According to Femenia et al. (1999), three main types of hemicellulosic polysaccharides can be found in *Aloe vera* gel: xyloglucans, xylans and mannans.

An elevation in salinity levels induced a marked increase in the total hemicellulose content within *Aloe vera* gel, with this effect being particularly pronounced under low soil moisture (LSM) conditions (**Figure 4**) ($p < 0.05$). Specifically, the hemicellulose content increased from 0.2 mg of hemicelluloses/g of fresh *Aloe vera* gel to approximately 0.8 mg of hemicelluloses/g of fresh *Aloe vera* gel, as salinity levels augmented from 100 to 200 mM under conditions of water deficit. In contrast, under high soil moisture (HSM) conditions, the overall hemicellulose content remained stable (0.4 mg) within the range of 0 to 150 mM of salinity ($p > 0.05$), but exhibited an increase to 0.68 mg at 200 mM of NaCl ($p < 0.05$).

Prior investigations have revealed that the hemicellulose content within *Aloe vera* gel comprises around 10 to 15% of the total cell wall polysaccharides (Ahl et al., 2019; Femenia, García-Pascual, Simal, & Rosselló, 2003; Rodríguez-González et al., 2011).

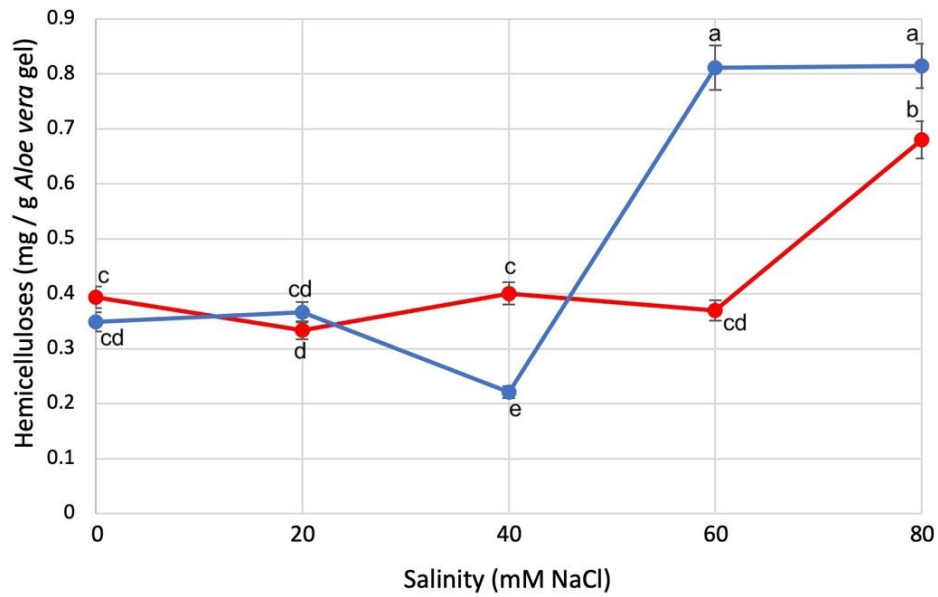


Figure 4. Hemicelluloses content of *Aloe vera* gel subjected to varying levels of salinity and different soil moisture conditions: high (red line) and low (blue line).

Different letters (a – e) indicate statistical differences by LSD ($p < 0.05$).

3.7. Pectins

Pectins represent the predominant cell wall polysaccharides found in *Aloe vera* gel, primarily composed of galacturonic acid and featuring side chains composed of rhamnose, arabinose, and galactose (Minjares-Fuentes et al., 2018).

Over the past decade, pectins extracted from *Aloe vera* gel have garnered attention as bioactive compounds due to their potential to confer health benefits, including regenerative and antitumoral properties. This has positioned them as valuable resources within the realm of tissue engineering (Gentilini et al., 2014). Nonetheless, it has been documented that the compositional and structural characteristics of pectic polysaccharides undergo significant alterations due to variations in agricultural practices and environmental conditions. These modifications consequently impact the

technological and functional properties linked to *Aloe vera* gel (González-Delgado et al., 2023; Minjares-Fuentes et al., 2017a).

Consequently, the influence of varying salinity levels under different soil moisture conditions on the pectin content within *Aloe vera* gel was investigated. On the whole, pectins were identified as the predominant polymers within *Aloe vera* gel, constituting approximately 36% of the total polysaccharide content, regardless of salinity and soil moisture conditions. However, both soil moisture and salinity exhibited a noteworthy impact on the pectin content in *Aloe vera* gel, yielding significant increases ($p < 0.05$) (see **Figure 5**).

Notably, the sole imposition of water restriction (LSM) without salinity (0 mM) led to a substantial elevation in pectin content from 1.4 to 2.4 mg of pectin/g *Aloe vera* gel ($p < 0.05$). Conversely, with the escalation of salinity from 0 to 80 mM, the pectin content expanded from 1.4 to approximately 3.0 mg of pectin/g of *Aloe vera* gel. The augmentation of pectin content in *Aloe vera* gel has primarily been attributed to the dehydration process induced by diminished water availability in the environment. This process has been observed as a crucial initial stage of the defensive mechanism of this plant against abiotic stress, leading to the metabolic utilization of storage polysaccharides (González-Delgado et al., 2023; Minjares-Fuentes et al., 2017a).

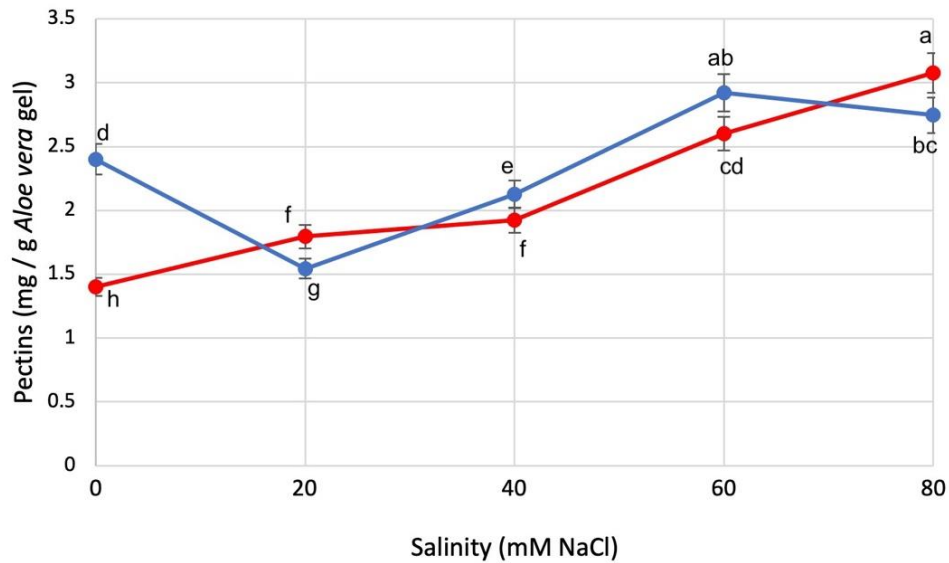


Figure 5. Pectin content of *Aloe vera* gel subjected to varying levels of salinity and different soil moisture conditions: high (red line) and low (blue line). Different letters (a – h) indicate statistical differences by LSD ($p < 0.05$).

In order to acquire deeper insights into the impact of varying salinity levels under different soil moisture conditions on the pectic polysaccharides found within *Aloe vera* gel, an in-depth analysis was conducted. This analysis encompassed the evaluation of individual carbohydrate components constituting pectins (**Figure 6**), as well as the investigation of structural attributes including linearity, the approximate extent of the rhamnogalacturonan type-I region, and the comprehensive characterization of RG-I within the pectin structure (**Table 2**).

As evidenced from the results, the primary monomeric unit within the pectin structure was UA (galacturonic acid), accounting for approximately 80%. Relatively smaller quantities of Gal (0.2 to 0.6 mg/g *Aloe vera* gel), Ara (0.03 to 0.06 mg/g *Aloe vera* gel), and Rha (0.007 to 0.017 mg/g *Aloe vera* gel) were also detected, corresponding to around 18%, 1.5%, and 0.05% of the total pectin content, respectively.

Notably, the levels of Rha (**Figure 6a**), Gal (**Figure 6b**), and Ara (**Figure 6c**) exhibited an increase with the rise in salinity, irrespective of the soil moisture conditions. Based on these findings, it can be deduced that two distinct categories of pectic polysaccharides constitute the cell wall of *Aloe vera* gel: homogalacturonans (HG) and rhamnogalacturonans I (RG-I). The former consists solely of GalA (galacturonic acid), while the RG-I type is comprised by a backbone of alternating glycosidically linked rhamnose and GalA residues with side chains consisting of either arabinogalactan polymers or linear chains of arabinans and/or galactans (Moore, Farrant, & Driouich, 2008a).

Fig. 6a

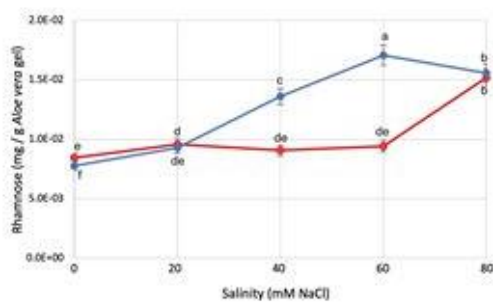


Fig. 6b

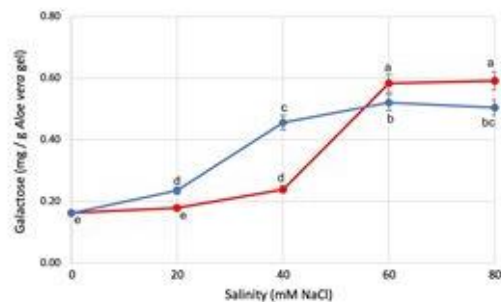


Fig. 6c

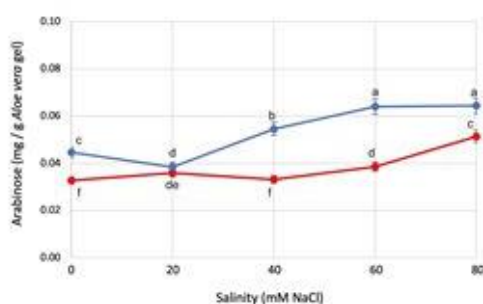


Fig. 6d

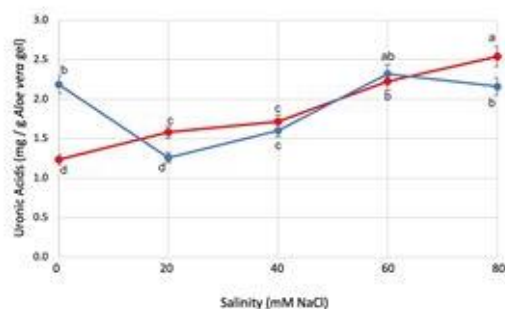


Figure 6. Individual carbohydrates comprising pectic substances from *Aloe vera* gel subjected to varying levels of salinity and different soil moisture conditions (High (red line) and low (blue line)): (a) Rhamnose, (b) Galactose, (c) Arabinose and (d) Uronic acids. Different letters (a – d) indicate statistical differences by LSD ($p < 0.05$).

The crucial role of HG and RG-I sub-units within the pectin matrix in interacting with other cell wall polysaccharides like cellulose and hemicelluloses is widely recognized (Fan et al., 2018; Kaczmarska, Pieczywek, Cybulska, & Zdunek, 2022; Liu et al., 2020; Moore et al., 2008a; Sun et al., 2019). The presence of pectins containing arabinans and arabinogalactans has been observed to be vital for the desiccation tolerance mechanism in various plants. This is because the side chains of these pectins are believed to be responsible for replacing lost water, preventing tight bonding with other cell wall polysaccharides like cellulose microfibrils and xyloglucans, as shown in studies by Moore et al. (2008a) and Zhao et al. (2019).

On the other hand, the evaluation of distinct pectin sub-components was carried out by analyzing the structural characteristics as previously described by Umaña, Dalmau, Eim, Femenia, and Rosselló (2019). Consequently, the salinity levels under varying soil moisture conditions (**Table 2**) exhibited a noteworthy impact on the linearity of pectins (measured by the ratio of GalA / Rha + Gal + Ara), as well as on the structural arrangement of the RG-I within the overall pectin structure (measured by the GalA / Rha ratio) and, also, on the length of the RG-I sub-domain (measured by the Gal + Ara / Rha ratio) ($p < 0.05$).

As can be seen, the linearity of pectins decreased as salinity increased regardless available soil moisture ($p < 0.05$), with around of 50% at HASM and near to 70% at LASM. The linearity of pectic polysaccharides has been associated to the occurrence of HG sub-domains in the overall pectin matrix. In fact, it has been exposed that cell integrity in nature is highly influenced by the presence of HGs in the cell walls since the water binding properties of GalA residues from HGs allows the hydration and swelling of pectins, maintaining the separation of those polymers forming the cell wall (Moore et al., 2008a).

Furthermore, the ratio of GalA/Rha exhibited distinct trends under varying salinity levels and soil moisture conditions. Specifically, as salinity increased, the GalA/Rha ratio rose from 146.4 to 236.5 at HASM as salinity increased, whereas at LASM, this ratio decreased from 281.4 to 118 ($p < 0.05$). The GalA/Rha ratio has been linked to the presence of the RG-I sub-domain within the pectin structure. This suggests that heightened salinity might stimulate the production of RG-I regions, potentially enhancing the interaction between pectins and other cell wall polysaccharides, thus preventing the collapse of cell walls (González-Delgado et al., 2023; Moore et al., 2008a).

Interestingly, the highest GalA/Rha ratio was observed in *Aloe vera* gel treated with HSM-150 (approximately 236) and LSM-0 (approximately 281), while the lowest ratio was detected in gel treated with HSM-0 (around 146) and LSM-100 (about 117).

Conversely, the length of RG-I chains, determined by the (Gal + Ara)/Rha ratio, exhibited an increase with rising salinity levels regardless of soil moisture conditions. This ratio showed an increase of up to 35 at LSM and a rise up to 66 at HSM ($p < 0.05$). These findings lend support to the hypothesis that polymers containing arabinose-rich side chains could contribute to the desiccation tolerance of plants. These polymers play a role in replacing lost water and preventing the junction of cell wall polymers to avert cell collapse (Moore et al., 2008a; Zhao et al., 2019).

Table 2. Structural characteristics of *Aloe vera* pectins from samples subjected to varying levels of salinity (NaCl: 0, 20, 40, 60 and 80 mM) and different soil moisture conditions^a.

Soil Moisture ^a	Salinity mM	Gal A / (Rha + Gal + Ara)	GalA / Rha	(Gal + Ara) / Rha
HSM	0	6.0 ± 0.3 ^c	146.4 ± 7.3 ^e	23.2 ± 1.2 ^g
	20	7.1 ± 0.4 ^b	165.3 ± 8.3 ^d	22.4 ± 1.1 ^g
	40	6.1 ± 0.3 ^c	189.4 ± 9.5 ^c	30.0 ± 1.5 ^e
	60	3.5 ± 0.2 ^{fg}	236.5 ± 11.8 ^b	66.0 ± 3.3 ^a
	80	3.9 ± 0.2 ^e	167.4 ± 8.4 ^d	42.3 ± 2.1 ^b
LSM	0	10.2 ± 0.5 ^a	281.4 ± 14.1 ^a	26.5 ± 1.3 ^f
	20	4.5 ± 0.2 ^d	135.8 ± 6.8 ^e	29.5 ± 1.5 ^e
	40	3.1 ± 0.2 ^g	117.9 ± 5.9 ^f	37.6 ± 1.9 ^c
	60	3.9 ± 0.2 ^e	135.9 ± 6.8 ^e	34.3 ± 1.7 ^d
	80	3.7 ± 0.2 ^{ef}	138.8 ± 6.9 ^e	36.5 ± 1.8 ^{cd}

^aSoil moisture: level soil moisture; high (HSM) and low (LSM).

^bRha: rhamnose; Ara: arabinose; Gal: galactose; GalA: galacturonic acid.

Different letters (a – g) in columns indicate statistical differences by LSD (p < 0.05).

The degree of methyl-esterification (DME) in pectins holds a critical role in shaping their structural arrangement and functional attributes. Hence, potential variations in this parameter were investigated through FTIR analysis. The FTIR spectra exhibit pronounced intensity in the C=O stretching of carboxylic ester groups (–COOCH₃) at 1633 cm⁻¹, alongside elevated intensities at 1741 and 1433 cm⁻¹, corresponding to asymmetrical and symmetrical –COO– of carboxylic acid (–COOH) groups (**Figure 7**). These indications suggest that pectins extracted from *Aloe vera* gel, treated under varying soil moisture and salinity conditions, could be categorized as highly methoxylated pectins. In fact, quantitative analysis revealed that regardless of the salinity and available soil moisture, in all cases pectins displayed a DME exceeding 60% (Table 3).

Table 3. Structural characteristics of *Aloe vera* pectins from samples subjected to varying levels of salinity (NaCl: 0, 20, 40, 60 and 80 mM) and different soil moisture conditions.

Soil Moisture ^a	Salinity mM	DE %
HSM	0	65.4 ± 2.0 ^a
	20	65.3 ± 2.0 ^a
	40	65.0 ± 1.6 ^a
	60	65.0 ± 1.8 ^a
	80	65.0 ± 1.9 ^a
LSM	0	65.1 ± 2.0 ^a
	20	64.9 ± 1.9 ^a
	40	65.1 ± 1.8 ^a
	60	64.8 ± 1.8 ^a
	80	64.8 ± 1.9 ^a

^aSoil moisture: level soil moisture; high (HSM) and low (LSM).

Different letters in column indicate statistical differences by LSD ($p < 0.05$).

These observations contrast with the outcomes reported by McConaughy et al. (2008) and Minjares-Fuentes et al. (2017a), who found that pectins from *Aloe vera* gel exhibited low DME. In nature, it has been observed that pectins are synthesized with full methyl-esterification within the Golgi apparatus. Subsequently, upon being released into cell walls, they undergo demethylation due to plant growth, leading to a decrease in the degree of methyl-esterification. This process promotes the pliancy and softness of tissues (Yang et al., 2020). Interestingly, the elevated DME observed in pectins extracted from *Aloe vera* gel, treated across different soil moisture and salinity levels, might serve as a defensive mechanism aimed at minimizing water loss. This is because pectins with high DME values (>50) are recognized for their strong hydration attributes, facilitating swelling and viscosity, thus enhancing water retention.

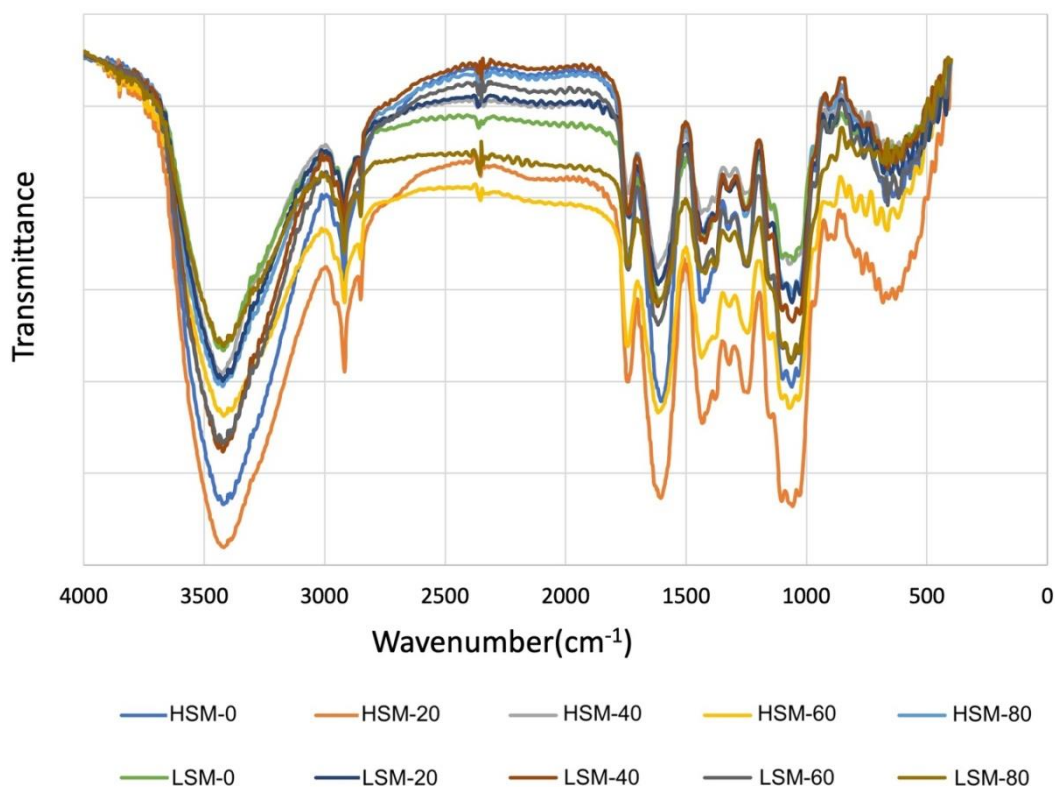


Figure 7. FTIR spectra of *Aloe vera* gel

3.8. *Acemannan*

The Acemannan polymer obtained from *Aloe vera* is composed of a primary backbone consisting of β -(1 \rightarrow 4) acetylated Mannose (Man), which is also interspersed with β -(1 \rightarrow 4) linked Glucose (Glc). Additionally, it might feature side chains of Galactose (Gal) units attached to the C-6 position of the Man residues forming the backbone (Femenia et al., 1999). This polymer is situated within the protoplast of parenchymatous cells and serves as the storage polysaccharide in plants belonging to the *Aloe* genus. One of the main functions of this polymer is based on the ability to retain water (approximately 99%) within the leaves of *Aloe vera* (Femenia et al., 1999; Minjares-Fuentes et al., 2017a). Consequently, the influence of salinity across different soil moisture levels on the main bioactive polymer found in *Aloe vera* was evaluated.

The carbohydrate composition of the bioactive acemannan polymer is presented in **Table 4**. Generally, the acemannan polysaccharide was primarily made up of mannose, accounting for approximately 90%, while glucose constituted around 7%, and minor traces of galactose were also present (<3%). More specifically, mannose content ranged from about 73 to over 100 mg/g AIR, glucose levels varied from approximately 5.2 to 11.5 mg/g AIR, and galactose content ranged from 2.1 to 3.4 mg/g AIR.

Table 4. Carbohydrate composition of acemannan polymer from *Aloe vera* gel subjected to varying levels of salinity and different soil moisture conditions.

Soil Moisture	Salinity mM	Man	Gal mg / g AIR	Glc
HSM	0	73.8 ± 10.0	2.1 ± 0.6	6.1 ± 1.2
	20	94.1 ± 20.8	3.0 ± 0.5	7.6 ± 0.5
	40	80.3 ± 8.8	2.6 ± 0.1	8.2 ± 0.4
	60	84.9 ± 4.6	3.3 ± 0.7	8.4 ± 0.9
	80	91.0 ± 5.9	2.0 ± 0.3	8.6 ± 0.9
LSM	0	93.9 ± 24.8	2.2 ± 0.3	7.1 ± 1.2
	20	86.5 ± 43.3	2.1 ± 1.3	5.2 ± 3.2
	40	96.9 ± 30.1	3.4 ± 1.1	7.8 ± 1.7
	60	99.4 ± 19.1	2.1 ± 0.6	8.9 ± 1.6
	80	105.5 ± 12.7	3.1 ± 0.0	11.5 ± 0.4

^aSoil moisture: level of soil moisture; high (HSM) and low (LSM).

^bMan: mannose; Gal: galactose; Glc: glucose

While these findings are consistent with those of other researchers who have characterized this polymer as a mannose-rich polysaccharide with over 60% mannose content (Campestrini, Silveira, Duarte, Koop, & Nosedá, 2013; Chokboribal et al., 2015; Femenia et al., 1999; Minjares-Fuentes et al., 2017a; Rodríguez-González et al., 2011; Salinas et al., 2019), it has been observed that the presence of galactose side chains within the acemannan structure could be influenced by factors such as the age of

the plants, the environmental conditions in which the plants are cultivated, and the specific plant tissue from which the gel is extracted (Campestrini et al., 2013; Salinas et al., 2019).

Conversely, both water scarcity and salinity significantly contributed ($p < 0.05$) to an increase in the acemannan polysaccharide content (**Figure 8**). Notably, the acemannan polymer content exhibited a slight rise, ranging from an average of 0.5 to 0.8 mg/g within *Aloe vera* gel due to water limitations. Similarly, elevating salinity levels from 0 mM to 80 mM led to enhanced acemannan content, with a rise from 0.4 to 0.8 mg/g in fresh *Aloe vera* gel under HSM conditions. In the case of lower available soil moisture, the acemannan content increased from 0.64 to 1.14 mg/g of *Aloe vera* gel.

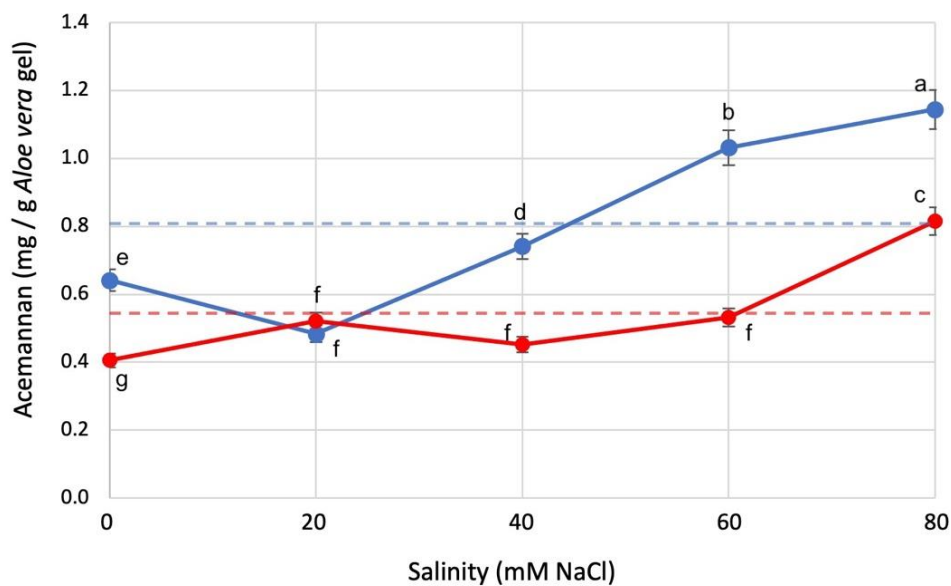


Figure 8. Acemannan content of *Aloe vera* gel subjected to varying levels of salinity and different soil moisture conditions: high (red line) and low (blue line). Different letters (a – g) indicate statistical differences by LSD ($p < 0.05$).

Previously, it was proposed that an increase in acemannan content might help to improve water retention within *Aloe vera* leaves during water scarcity (Femenia et al.,

1999; Rodríguez-González et al., 2011). However, it has also been reported that water deficit leads to the loss of (1,4)-linked mannosyl residues, implying that the acemannan polymer might be metabolized by the Aloe plant as a potential defense mechanism, primarily affecting the low molecular weight acemannan fractions (Minjares-Fuentes et al., 2017a). And more recently, it has been observed that in soils with low available moisture, salinity fosters an increase in the acemannan polymer content, facilitating better water management within the parenchymatous cells of *Aloe vera* to counteract water scarcity (González-Delgado et al., 2023).

Hence, the heightened acemannan polymer content could serve not only as a defense strategy against water scarcity but also as a fundamental biochemical and physiological response of the *Aloe vera* plant to combat the adverse effects of abiotic stress caused by insufficient water availability and the unfavorable conditions induced by salinity.

The occurrence of acetyl groups on mannose residues within the acemannan polymer is probably the main structural characteristic of this polysaccharide, resulting in a distinctive and unique polymer structure (Campestrini et al., 2013; Chokboribal et al., 2015; Minjares-Fuentes et al., 2017a; Minjares-Fuentes et al., 2017b; Salinas et al., 2019). In fact, acetylation of acemannan shows a fingerprint in ^1H NMR spectroscopy, exhibiting a characteristic signal in a range between 2.00 to 2.26 ppm (Bozzi, Perrin, Austin, & Arce Vera, 2007; Diehl & Teichmuller, 1998).

It is important to highlight that acetyl groups of acemannan plays a critical role in the interaction with other biomacromolecules, affecting the biological activity of this polymer (Chokboribal et al., 2015; Kumar & Kumar, 2019; Kumar & Tikku, 2016). Hence, acemannan polymer was subjected to ^1H NMR analysis in order evaluate the alteration in DA as a consequence of salinity at different soil moisture.

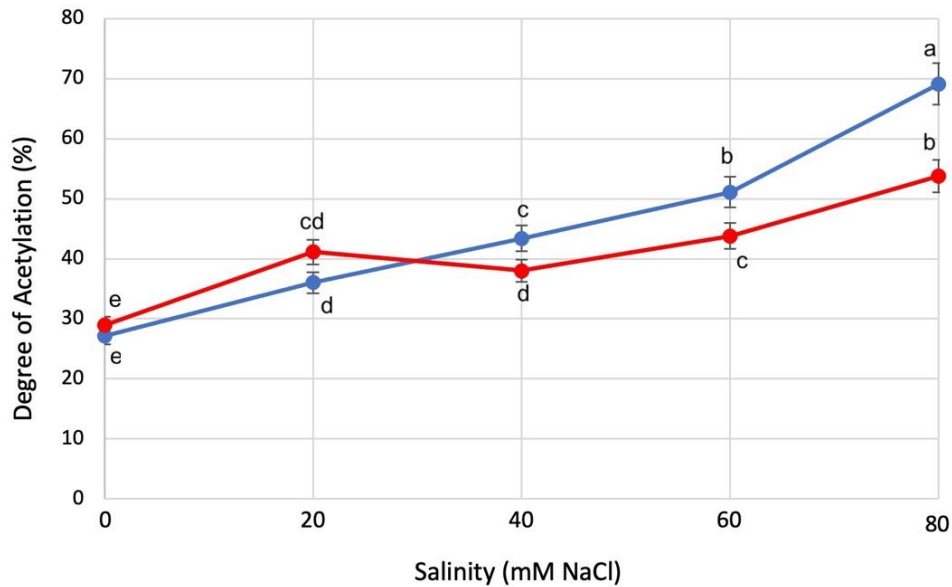


Figure 9. Degree of acetylation of acemannan of *Aloe vera* gel subjected to varying levels of salinity and different soil moisture conditions: high (red line) and low (blue line). Different letters (a – e) indicate statistical differences by LSD ($p < 0.05$).

As depicted in **Figure 9**, the DA within the acemannan structure was notably influenced by both salinity and soil moisture conditions ($p < 0.05$). Specifically, under HSM conditions, there was an approximately 96% increase in DA as salinity elevated to 80 mM. Similarly, under LSM conditions, the DA exhibited an increase of about 150% due to salinity. It is worth noting that under LSM conditions, the increase in the DA showed a linear trend as salinity levels rose.

In the context of plants, the acetylation of polysaccharides holds a pivotal role in their growth and developmental processes. Specifically, the interaction of water with acetyl groups within acemannan involves the formation of hydrogen bonds, making acetylation the primary contributor to higher concentrations within the parenchymatous tissue of *Aloe vera* gel (Femenia et al., 1999). Consequently, the increase of the DA might be a biochemical response of the *Aloe vera* plant. This response aims not only to

augment the water content but also to effectively retain a substantial amount of available water within cells, thus preventing cellular collapse and plant death resulting from prolonged water scarcity. This is particularly crucial, as reduced polysaccharide acetylation during wilting has been shown to negatively impact growth (Shahin, Zhang, Mohnen, & Urbanowicz, 2023).

3.9. *Functional properties*

The functional characteristics ascribed to any polysaccharide-rich plant material rely on two factors: the specific polysaccharides comprising it and the manner in which they are organized and connected (Jarvis, 2011). Therefore, any modifications in the composition and structure of these polymers can affect the functional attributes associated with hydration, such as swelling (Sw) and water retention capacity (WRC), as well as the capacity for adsorbing organic molecules, such as lipid adsorption capacity (FAC) (Thibault, Lahaye, & Guillon, 1992).

Therefore, the influence of combined water deficit and salinity stress on the functional properties associated with *Aloe vera* polysaccharides was evaluated. As illustrated in **Figure 10**, the combined water and salinity stress significantly impacted the functional characteristics of *Aloe vera* gel polysaccharides ($p < 0.05$). In general, the swelling (Sw) capacity linked to *Aloe vera* gel polysaccharides exhibited a notable increase due to salinity, rising from 0.30 to 0.80 mL/g of fresh *Aloe vera* gel. This effect was particularly pronounced under LSM conditions (**Figure 10a**). Specifically, under HSM conditions, Sw increased from 0.30 to 0.50 mL/g of *Aloe vera* gel as salinity escalated from 0 to 80 mM. Notably, a near 150% increment in Sw was observed when salinity was elevated under LSM conditions, with the *Aloe vera* gel treated at LSM-80 displaying the highest Sw value.

Concerning the capacity of *Aloe vera* polysaccharides to retain water (WRC), it was observed that water stress had a significant impact on WRC, while salinity did not show such an effect (**Figure 10b**). Consequently, the polysaccharides extracted from *Aloe vera* gel subjected to LSM conditions exhibited higher WRC compared to those from *Aloe vera* treated under HSM conditions. This variation in WRC among polysaccharides from *Aloe vera* gel treated at HSM ranged from 0.15 to 0.37 g H₂O/g fresh *Aloe vera* gel, as observed in cases of HSM-40 and HSM-80 treatments, respectively. Conversely, for *Aloe vera* treated at LSM with 60 and 80 mM of NaCl, the WRC ranged from 0.26 to 0.53 g H₂O/g fresh *Aloe vera* gel.

Fig. 10a

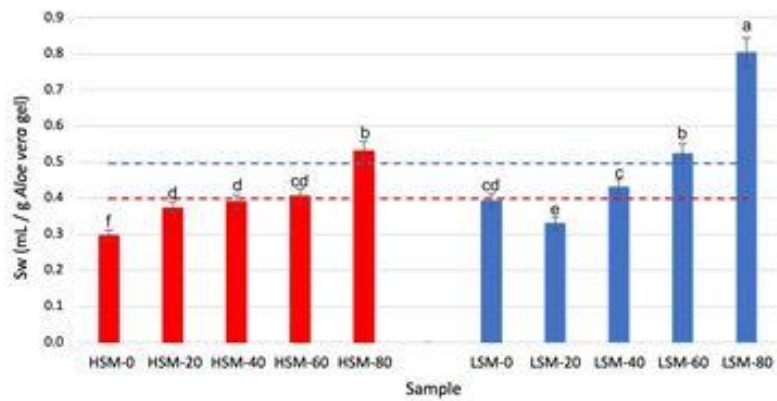


Fig. 10b

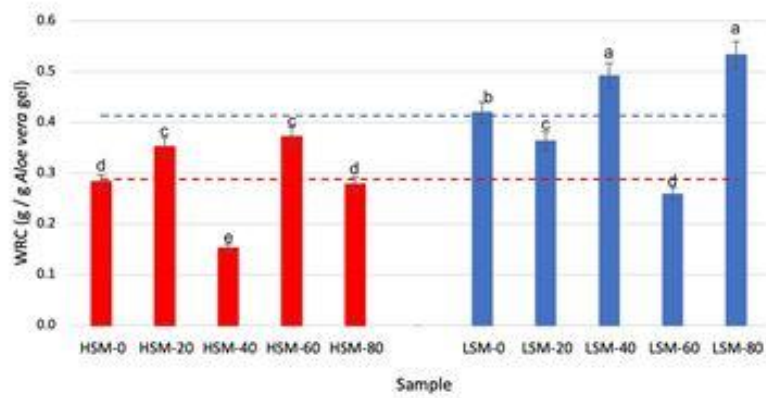


Fig. 10c

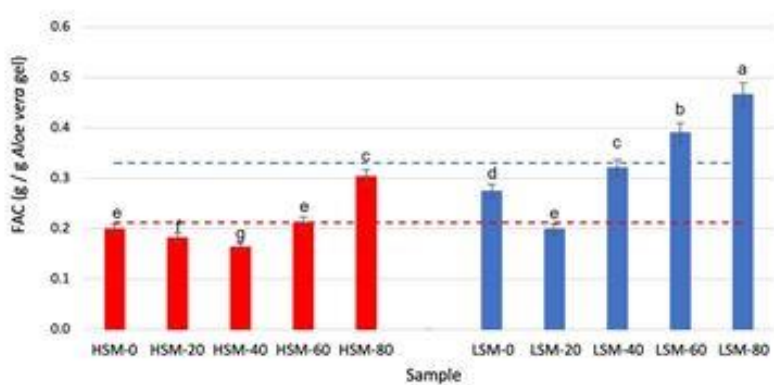


Figure 10. Functional properties of *Aloe vera* gel subjected to varying levels of salinity and different soil moisture conditions. Different letters (a – g) above bars indicate statistical differences by LSD ($p < 0.05$).

It has been reported that the hydration attributes linked to *Aloe vera* polysaccharides can significantly be influenced by their structural configurations and, also, by the presence of distinct functional groups, such as methyl or acetyl esterification (Chokboribal et al., 2015; Minjares-Fuentes et al., 2018). Hence, the augmentation in hydration properties within *Aloe vera* polysaccharides can be attributed to the increased degree of acetylation in acemannan and, also, to the heightened methyl esterification level in pectins, driven by the combination of salinity and water stresses.

Finally, the combined influence of water deficit and increasing salinity significantly affected ($p < 0.05$) the capacity of *Aloe vera* polysaccharides to entrap organic molecules, specifically lipids (FAC) (**Figure 10c**). In a broader context, the FAC values of *Aloe vera* polysaccharides displayed a range from 0.16 g/g fresh *Aloe vera* in instances where *Aloe vera* was treated under HASM conditions with 40 mM NaCl (HASM-40), to 0.47 g/g when *Aloe vera* was treated at LSM conditions with 80 mM NaCl (LSM-80). Interestingly, the FAC attribute exhibited an increase as both water restriction and salinity heightened, with a more pronounced effect observed at NaCl concentrations surpassing 40 mM.

7. Conclusions

A comprehensive investigation was undertaken to analyze the main polysaccharides present in *Aloe vera* gel originating from Aloe plants subjected to varying salinity levels under distinct soil moisture conditions. The interaction between salinity and soil moisture engendered notable alterations in the composition and structural aspects of *Aloe vera* polysaccharides, with particular emphasis on cell wall pectins and the storage polymer acemannan. These modifications affirm the crucial roles of both polymers, not

only in conferring drought resistance to *Aloe vera* but also in mitigating the deleterious repercussions induced by abiotic stresses.

Specifically, the combined impact of salinity and water stresses promoted the synthesis of pectins characterized by enhanced branching and a heightened degree of methyl esterification. These structural transformations in pectins likely serve to fortify the cellular walls, thereby impeding the amalgamation of cell wall polymers and averting cellular collapse.

Simultaneously, both stressors led to an elevation not only in the acemannan content but also in the degree of acetylation of acemannan. This augmentation in highly acetylated acemannan reinforces the efficacious retention of water within parenchymatous cells, thus counteracting cellular dehydration. This outcome substantiates the biological function of acemannan as a storage polymer within the *Aloe vera* plant.

However, further exhaustive investigations are imperative to elucidate the intricate molecular mechanisms governing the biosynthesis pathways of these polymers in *Aloe vera*. Such insights are crucial for devising novel agricultural and/or biotechnological strategies aimed at enhancing the biosynthesis of these polymer types and augmenting their functional attributes.

Acknowledgements

The authors acknowledge the financial support from the Grant PID2019-106148RR-C43 funded by MCIN/AEI/10.13039/501100011033 (Spain).

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve some paragraphs of the manuscript. After using this tool/service, the authors reviewed and

edited the content as needed and take full responsibility for the content of the publication.

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CAPÍTULO II

A New Functional Food Ingredient Obtained from *Aloe ferox* by Spray Drying

Comas-Serra, F., Martínez-García, J.J., Pérez-Alba, A., Sáenz-Esqueda, M.A., Candelas-Cadillo, M.G., Femenia, A., Minjares-Fuentes, R.

Foods

Volumen 12

Paginado 850-865

Año publicación: 2023

Factor de impacto (JCR 2022): 5.2

Q1 (Food Science and Technology 34/142)

DOI: <https://doi.org/10.3390/foods12040850>



CAPÍTULO III

Evaluation of Acemannan in Different Commercial Beverages Containing *Aloe vera* (*Aloe barbadensis* Miller) Gel

Comas-Serra, F., Estrada, P., Minjares-Fuentes, R., Femenia, A.

Gels

Volumen 9

Paginado 552

Año publicación: 2023

Factor de impacto (JCR 2022): 4.6

Q1 (Polymer Science 18/86)

DOI: <https://doi.org/10.3390/gels9070552>

CONCLUSIONES

CONCLUSIONES

El objetivo general planteado al inicio de esta investigación fue el de profundizar en el conocimiento científico de diferentes aspectos fundamentales relacionados con el *Aloe vera*. En base a los resultados y a las conclusiones obtenidas en cada una de las líneas de investigación en las cuales se ha dividido el trabajo, se puede afirmar que el objetivo se ha cumplido de forma más que satisfactoria.

A continuación se detallan las principales conclusiones obtenidas en cada una de las diferentes temáticas tratadas en esta Tesis Doctoral.

(1) Técnicas de cultivo: Influencia del estrés hídrico-salino sobre los principales compuestos bioactivos presentes en el *Aloe vera*.

- En general, el estrés abiótico, causado por la combinación de déficit hídrico con diferentes niveles de salinidad, afectó negativamente tanto al aspecto como a la morfología de las plantas de *Aloe vera*.
- La síntesis de aloína, así como de polímeros ricos en manosa fue inducida por el estrés abiótico aplicado, teniendo la salinidad un efecto más notable que el estrés hídrico.
- El contenido total de pectinas experimentó una notable disminución como resultado del estrés abiótico: en particular, los resultados más evidentes fueron observados para plantas sometidas a déficit hídrico.
- La interacción entre la salinidad y la humedad del suelo provocó notables alteraciones tanto en la composición como en la estructura de los principales polisacáridos de *Aloe vera*, en concreto en las pectinas de la pared celular y en el polímero de almacenamiento acemanano. Estas modificaciones refuerzan el papel crucial que desempeñan ambos polímeros, no sólo en conferir resistencia a

la planta de *Aloe vera* ante la sequía, sino también en mitigar las posibles repercusiones debidas al estrés abiótico inducido.

- La aplicación combinada de diferentes niveles de salinidad y humedad promovió la síntesis de pectinas caracterizadas por una mayor ramificación y un mayor grado de metil-esterificación. Estas modificaciones estructurales podrían tener como función el fortalecimiento de las paredes celulares, impidiendo así la amalgama de los polímeros de la pared celular y evitando el colapso de las células.
- Por otra parte, la aplicación del estrés hídrico-salino provocó un notable aumento no sólo del contenido de acemanano, sino también de su grado de acetilación. Este hecho refuerza la retención eficaz de agua dentro de las células del tejido parenquimático, contrarrestando así la deshidratación celular y corroborando la función biológica del acemanano en la planta de *Aloe vera*, como polímero de reserva.
- Los resultados obtenidos en este estudio podrían ser de gran utilidad para el diseño de nuevas estrategias agrícolas y/o biotecnológicas con la finalidad de aumentar no solo el contenido total de acemanano en *Aloe vera*, sino también de incrementar la bioactividad de dicho polímero.

(2) Metodologías de procesado: Evaluación de diferentes técnicas de secado y obtención de un nuevo ingrediente funcional a partir de *Aloe ferox* mediante el secado por atomización (spray-drying).

- En comparación con la muestra de *Aloe vera* fresca, las muestras de *Aloe vera* deshidratadas mediante diferentes métodos de secado, en particular: secado por atomización (spray-drying), liofilización industrial (industrial freeze-drying),

secado por ventana de refractancia (refractance window-drying) y secado por zona radiante (radiant zone-drying) mostraron una menor actividad de agua junto con valores de solubilidad e higroscopicidad más elevados.

- Además, los diferentes métodos de secado también provocaron cambios críticos en el comportamiento de flujo, pasando de un comportamiento de adelgazamiento bajo cizalla en la muestra de *Aloe vera* fresco, a un comportamiento newtoniano en todas las muestras de *Aloe vera* deshidratadas. Estas modificaciones en el comportamiento de flujo podrían ser una respuesta física a la degradación de los grupos acetilo del acemanano, promovida por los diferentes métodos de secado aplicados.
- Por tanto, el comportamiento de flujo puede ser un excelente indicador de la degradación de componentes bioactivos del *Aloe vera*, como el acemanano, lo cual podría afectar directamente tanto a las propiedades funcionales como a la bioactividad de los productos procesados de *Aloe vera*.
- Estas modificaciones deben tomarse en consideración para evaluar la calidad general de los ingredientes funcionales obtenidos del gel de *Aloe vera* antes de que sean incorporados en una amplia gama de productos en campos tan diversos como la cosmética, la industria farmacéutica o el ámbito alimentario.

A partir de los conocimientos adquiridos sobre las diferentes técnicas de secado, se procedió a la deshidratación del gel de *Aloe ferox*, mediante el proceso de secado por atomización, con la finalidad de evaluar la posible obtención de un nuevo ingrediente funcional.

- Una vez deshidratados mediante la técnica de secado por atomización, el gel de *Aloe ferox* presentó una morfología similar al gel de *Aloe vera*. Aunque, el gel de *Aloe ferox* deshidratado presentó una mejor retención del color, exhibió

valores más altos relativos a propiedades funcionales como la capacidad de hinchamiento (Sw), la capacidad de retención de agua (WRC) y la capacidad de absorción de lípidos (FAC), e incluso una mayor capacidad antioxidante.

- A partir de la composición de carbohidratos presentes en el *Aloe ferox*, se observó la presencia de un polisacárido rico en manosa altamente acetilado (grado de acetilación > 90%). En general, estas características son similares a las del polisacárido bioactivo acemanano identificado en *Aloe vera*.
- Cabe resaltar que este es el primer estudio que informa sobre un polisacárido rico en manosa, con un alto grado de acetilación, como el polímero principal presente en el gel de *Aloe ferox*. No obstante, se requiere de un análisis estructural más exhaustivo para identificar tanto el grado de acetilación como el peso molecular de dicho polímero.
- Por otra parte, los resultados y la información obtenida en este estudio podrían ofrecer nuevas alternativas para el uso del gel de *Aloe ferox* en el desarrollo de nuevos ingredientes funcionales. En este aspecto también se requieren estudios adicionales con el fin de evaluar las propiedades beneficiosas del gel de *Aloe ferox*, deshidratado mediante secado por atomización, para su posible aplicación como un nuevo ingrediente funcional en diversos productos alimentarios.

(3) Aplicaciones del gel de *Aloe vera* en alimentación: Evaluación de las principales características del acemanano en diferentes bebidas comerciales de *Aloe vera*.

- Las muestras aromatizadas evaluadas (siete muestras) con un contenido, declarado en el etiquetado, del 30 al 78% de gel de *Aloe vera* mostraron niveles muy bajos de acemanano (<20 mg/100 g de muestra fresca). Además, en la

- mayoría de estas siete muestras, el acemanano detectado estaba prácticamente desacetilado, como se observó en los análisis realizados mediante $^1\text{H-NMR}$.
- Todas las bebidas aromatizadas exhibieron la presencia de cantidades significativas de otros biopolímeros como pectinas, hemicelulosas y celulosa, aunque esto probablemente se deba a la adición de otros ingredientes en su composición, como zumo de frutas.
 - Por otra parte, el contenido de acemanano presente en las muestras no aromatizadas (8 muestras) las cuales, según su etiquetado, contenían porcentajes de gel de *Aloe vera* iguales o superiores al 99.5%, fue muy variable, oscilando entre 10 y 260 mg de acemanano/100 g de muestra fresca. En particular, solo tres muestras presentaron contenidos superiores a 160 mg de acemanano/100 g de muestra fresca; de hecho, en cuatro de ellas, el contenido de acemanano fue inferior a los 35 mg/100 g de muestra fresca.
 - Además, en estas ocho muestras no aromatizadas, excepto en una de ellas que presentó un grado de acetilación (GA) del acemanano superior al 99%, todas las demás mostraron valores de GA inferiores al 35%, lo cual es indicativo de su baja calidad.
 - Además, considerando todas las muestras examinadas, no se observó ningún tipo de correlación entre el contenido de acemanano presente en la bebida y su precio de venta al público.
 - En conclusión, la falta de legislación específica actual que regule no sólo la presencia sino también la calidad de los compuestos bioactivos, en particular el acemanano, en las bebidas comerciales a base de *Aloe vera*, provoca un estado de total incertidumbre en los consumidores en relación a los posibles beneficios reales que puedan obtenerse mediante el consumo de este tipo de productos.

ANEXOS

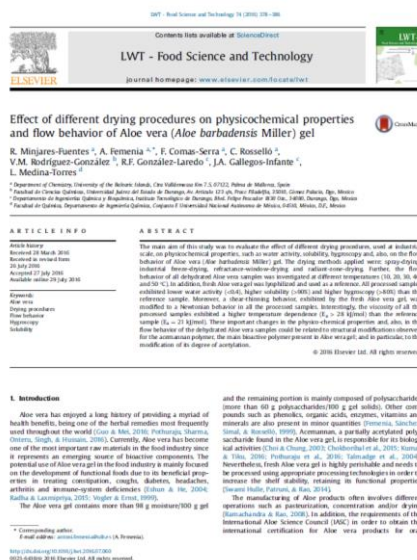
En el siguiente apartado de anexos se detallan las diferentes contribuciones o aportaciones realizadas durante el Programa de Doctorado, que han ayudado a su realización pero que no se pueden incluir de manera directa en la tesis final.

Anexo I: artículo académico sobre diferentes métodos de secado, utilizado en otra tesis doctoral, pero que ha dado paso a otros estudios que se han detallado en la tesis, como el artículo académico del *Aloe ferox*.

Anexo II: artículo académico sobre los efectos de los ultrasonidos en la pasta prensada de uva, con aportación en la experimentación, pero difiere un poco del tema de la tesis.

Anexo III: contribuciones a congresos, tanto nacionales como internacionales, con presentación de diferentes posters y colaboración en diferentes ponencias con los diferentes estudios realizados en la tesis.

Anexo IV: premios obtenidos durante el Programa de Doctorado. Premios de entidades locales, como la Asociación de Químicos de las Islas Baleares como la Comisión académica del Máster y Doctorado en Ciencia y Tecnología Química de la UIB.



ANEXO I

Effect of different drying procedures on physicochemical properties and flow behaviour of *Aloe vera* (*Aloe barbadensis* Miller) gel

Minjares-Fuentes, R., Femenia, A., Comas Serra, F., Rosselló, C., Rodríguez-González, V.M., González-Laredo, R.F., Gallegos-Infante, J.A., & Medina-Torres, L.

LWT – Food Science and Technology

Volumen 74

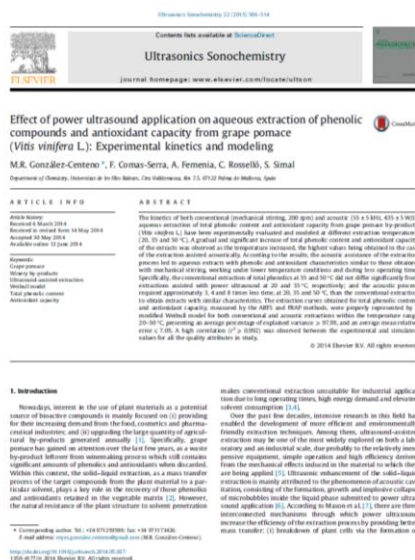
Paginado 378-386

Año publicación: 2016

Factor de impacto (JCR 2016): 2.329

Q1 (Food Science and Technology 32/130)

DOI: <https://doi.org/10.1016/j.lwt.2016.07.060>



ANEXO II

Effect of power ultrasound application on aqueous extraction of phenolic compounds and antioxidant capacity from grape pomace (*Vitis vinifera* L.): Experimental kinetics and modeling

González-Centeno, M.R., Comas-Serra, F., Femenia, A., Rosselló, C., Simal, S.

Ultrasonics Sonochemistry

Volumen 22

Paginado 506-514

Año publicación: 2015

Factor de impacto (JCR 2015): 4.556

Q1 (Acoustics 1/32 and Chemistry Multidisciplinary 30/163)

DOI: <https://doi.org/10.1016/j.ultsonch.2014.05.027>

ANEXO III

Contribuciones a congresos

(2013-2023)

CONTRIBUCIÓN A CONGRESOS NACIONALES E INTERNACIONALES

2013

1. *Ultrasounds and Temperature Effects on Extraction of Bioactive Components from Grape Pomace Winemaking Byproducts*. 2013. F. Comas-Serra, M.R. González-Centeno, S. Adrover-Obrador, A. Femenia, M.C. Garau, R. Minjares-Fuentes. 9th European Congress of Chemical Engineering/2th European Congress of Applied Biotechnology. La Haya, Holanda (poster).

2014

1. *Influence of the Drying method on the Physico-chemical properties of the main Bioactive polymers from Aloe vera gel*. 2014. R. Minjares-Fuentes, A. Femenia, F. Comas-Serra, V. Eim, C. Rosselló, M.G. Candelas-Cadillo, R.F. González-Laredo. 7th International Conference and Exhibition on Nutraceuticals and Functional Foods. Estambul, Turquía (poster).
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2016

1. *Freezing pre-treatment and ultrasonic enhancement of convective drying of beetroot: influence on drying kinetics*. 2016. F. Vallespir, F. Comas-Serra, M.A. Frau, J.A. Cárcel, C. Rosselló. 20 th International Drying Symposium (IDS). Gifu, Japón (poster).

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ANEXO IV

Premios obtenidos

PREMIOS OBTENIDOS

2016

XXI Premi Sant Albert a la Investigació Química 2016 con el trabajo que lleva por título: “¿El procesado de alimentos puede facilitar la bioaccesibilidad de los nutrientes?”

Autores: M. Dalmau, F. Comas-Serra. Asociación de Químicos de las Islas Baleares.



*Associació de Químics
de les Illes Balears*

Iris Morey Serra, amb DNI núm. 43.220.609-K, com a secretari de l'Associació de Químics de les Illes Balears, domiciliat a Palma de Mallorca, carrer Josep Rover Motta, núm. 8, baixos B,

CERTIFIC:

Que la Sra. Maria Esperança Dalmau Estelrich, amb DNI núm. 41.539.600-J i la Sra. Francesca Comas Serra, amb DNI núm. 43.206.715-L, han obtingut el XXI Premi "Sant Albert" a la Investigació Química 2016, convocat per aquesta Associació, pel seu treball titulat "¿El proceso de alimentos puede facilitar la bioaccesibilidad de los nutrientes?"

I perquè consti i tingui els efectes que corresponguin, sign el present certificat, amb el vist i plau del president.

Palma, 15 de novembre de 2016

Vist i plau



*Associació de Químics
de les Illes Balears*

Juan Frau Munar

*President de l'Associació de
Químics de les Illes Balears*

2023

II concurso de vídeos “QUIMIPROJECT”. Primer premio en la categoría de Tesis Doctoral. Comisión Académica del Máster y Doctorado en Ciencia y Tecnología Química (UIB).

