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Chapter

A Potential Alternative for Agar in In Vitro Culture Media Based on Hydrocolloids Present in Nopal: General Structure and Mechanical Properties

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Abstract

In Vitro culture is a technique commonly used for plant research. Nevertheless, it is more expensive than traditional methods of production, due to the use of the culture medium gelling agent called agar. Recent studies have been searching for alternative substances in raw materials with the same characteristics but which can be extracted easier than agar. The dietary fiber of the nopal cactus (Opuntia) is a rich source of hydrocolloids (pectin and mucilage). These hydrocolloids have the ability to gel in combination with the indicated solution. In this chapter, we will focus on the study of the hydrocolloids from nopal cactus to replace agar partially and/or totally as a gelling agent using in vitro culture media benefiting from the molecular structure and mechanical properties of the compounds.

Keywords: In vitro culture media, gelling agents, mechanical properties, molecular structure, nopal cactus hydrocolloids

1. Introduction

Due to the growing global crisis caused by climate change and greenhouse gases, which are significantly affecting food availability and price volatility in the agricultural sector, agricultural systems have been forced to implement new adaptive measures with the aim of developing alternatives that improve and accelerate traditional methods of food production through biotechnology [1, 2].

Plant biotechnology had an important development in the last decades, moving from soil cultivation to practices where ecological factors are involved with sustainable objectives such as water conservation, decrease of soil erosion, and higher yields and better-quality crops. As a result of this movement, the technique of in vitro

culture arose, in which plants are propagated inside containers under laboratory conditions [3] and has open the possibility of enhancing two conditions in agricultural crops, firstly, the asepsis, which is the absence of germs, and secondly, the control of factors that affect plants growth.

However, although it is a technique that has been widely used in agricultural development and plant research, the in vitro culture technique is more expensive than traditional methods of plant production, due to the components used for the preparation of the culture medium, commonly the gelling agent called agar [4–6].

The culture media must meet the nutritional requirements necessary for the development of the plant and these depend on the culture used, which is in some cases solidified to provide support to the explant through the gelling agent agar. Agar is a mixture of polysaccharides extracted from the walls of red algae (Gelidium, Gracilaria, Pterocladia, Gelidiella) and is the most widely used gelling agent in culture media due to its physicochemical properties such as porosity, thermo-reversibility, stability, gel strength, texture, elasticity, and transparency. Its use increases the total cost of the medium by 70%, due to agar overexploitation and high demand [7].

As a result of this, recent studies have made it possible to know with greater precision the availability of alternative substances to agar in naturally occurring raw materials that are easier to extract and have the same characteristics as agar. The study of natural polymers of vegetable origin has emerged as a sustainable alternative due to their properties. Starches and gums have been investigated due to their high availability in local markets and low cost [8–10], concluding that they can potentially replace agar partially and/or totally as gelling agents due to their physicochemical composition, and can reduce costs of this technology due to their greater efficiency, ease of extraction and acquisition [11].

The nopal cactus (Opuntia ficus-indica), a native plant distributed throughout Latin America, is a natural polymers supplier that has been studied in numerous investigations for its properties, since phenolic compounds had been found in the composition, which can be used in various industries, including pharmaceutical, construction, agrotechnology, bioenergy, and biotechnology industries [12]. One of the most important components of nopal cactus is dietary fiber, which is a rich source of hydrocolloids (pectin and mucilage), named as such because of their great capacity to capture and retain water.

Pectin is mainly composed of galacturonic acid, and mucilage is composed of arabinose, galactose, xylose, rhamnose, and galacturonic acid (classified as an acid mucilage) [13]. These are hydrocolloids that have the ability to gel and form gels in combination with the indicated solution. In addition, it is well known that one of the main functions of mucilages is to promote seed germination since when they come into contact with water, they increase their volume, forming a moist layer around the seed, which facilitates germination and protects the plant from external damage [14, 15].

Recently, nopal cactus hydrocolloids have been used as a thickening, stabilizing, encapsulating, and moisturizing agents in different research projects; however, in this chapter we will focus on the study of the nopal cactus hydrocolloids for their potential to substitute (partially or fully) the gelling agent agar for in vitro culture media applications, benefiting from the molecular structure and the mechanical properties of those compounds.

First, we will describe in vitro culture and the important features for the development of this technique, followed by the gelling agents and hydrocolloids mostly used to improve the technique and the recently studied substitutes, including their characteristics. Finally, we will discuss the potential application of nopal cactus

hydrocolloids as gelling agents in in vitro culture and describe their molecular structure and mechanical properties.

2. The history of in vitro culture

The term "in vitro tissue culture" means growing explants in a glass bottle in an artificial environment in which asepsis, growth, and development must be controlled. Plant culture has undergone a significant evolution in the last 100 years. The knowledge and research related to cell theory has reached a significant development, which has generated a disruptive innovation in traditional culture.

The history of in vitro culture began in 1887 with Schleiden and Schwan, who explained that a cell could subsist by itself if the external conditions were favorable for its growth (cell theory). A decade later, in 1902 Herbertland, pioneer of in vitro culture and called the father of the technique, said that plants were capable of reproducing their growth from isolated cells. Herbertland proposed that it was possible to grow free vegetative cells and pollen tubules together by adding nutrient solutions supplemented with extracts of vegetative apices or with fluids from embryo sacs, and although he could not prove it due to the simplicity of his culture media, it was a breakthrough that gave the guideline to start investigating the technique [16].

In addition, Herberlat was the first to mention cell totipotency, which is defined as the ability of a cell to generate an individual completely identical to the mother cell from a single cell, which contains the same genetic material of the plant to which it belongs, therefore, it has the potential to generate a completely new plant [17]. By 1924, Blumenthal and Meyer demonstrated callus formation using carrot slices and lactic acid.

In the 1930's White, Gautheret and Nobécourt decisively demonstrated the possibility of cultivating plant cells in vitro, discovering two important characteristics, the identification of auxins as regulators of plant growth and the importance of B complex vitamins in plant growth [18]. By 1940 Blakeslee, Conklin, and Van Overbeek studied a semisynthetic liquid medium with coconut milk, which had a good proliferation response, which promoted biochemical research.

In 1962 Murashige and Skoog made a medium for tobacco tissues that contained all the nutrients necessary for the growth and proliferation of cays, and a high concentration of salts that benefited the growth of somatic embryos [19]. Subsequently, research was carried on somatic embryogenesis, explant types, the culture of microspores and meristems, and obtaining hybrids.

Culture media had been an important object of study, in 1984, Wetherell proposed that high levels of sucrose up to 12%, nitrate, and iron are essential for somatic embryos to develop to maturity [20]. However, nowadays most culture media consist of five groups of ingredients: organic nutrients, carbon source, vitamins, and growth regulators such as phytohormones and their inhibitors (auxins, gibberellins, cytokinins, abscisic acid, ethylene, brassinosteroids, polyamines, jasmonic acid, and salicylic acid).

Auxins promote cell elongation and cytokinins stimulate cell division [20]. These media are mostly used to promote organogenesis and their composition is determinant for growth. The Murashige & Skoog (MS) medium designed for tobacoo cells is the most used due to its success for agar culture [21] although there are other media such as Schen and Hildebrandt, Heller, MS, and Eriksson, among others, which contain certain macro and micronutrient content that are also used for plant cell culture.

Another factor that influences the growth of tissues in solid culture media is their consistency, which is the result of the selection of the gelling agent [22]. There is a great

variety of gelling agents, the most used are agar, agarose, gellan gum, or calcium alginate [23]. Agar is commonly used in proportions of 0.6% to 1.0%, due to its composition, purity, and properties, which do not interfere with the growth of the culture [24].

3. Commercial gelling agents

Gelling agents such as agar-agar, phytagel®, gelrite® or gellam gum, natugel®, agarose, alginate and isabgol, and gums that have been combined to be substitutes for agar, such as guar gum, cassia, xanthan gum, and katyra gum, which function as thickening, gelling, foaming and stabilizing agents, are available commercially (**Table 1**).

Gelling agent	Chemical Composition	Category	Application	Cost
Phytagel / Gelrite/ Gellam gum	Fermentation product of various Pseudomonas species	Heteropolysaccharides secreted by bacteria of the genus Sphinogomonaso.	Plant tissue culture medium. Agar substitute	250 g 90.79 USD
Natugel	Seaweed Kappaphycus alvarezii	Increased viscosity and consistency	Gel-forming agents, use in various research areas	250 g 225.81 USD
Agarose	Consisting of repeating units of a molecule called agarobiose.	Polysaccharide from algae of the genera Gellidium and Gracillaria.	Gels that allow the separation of DNA molecules by electrophoresis, cell culture, and microbiology.	100 g 245.78 USD
Alginate	Organic polymers derived from alginic acid.	Anionic polysaccharide from brown algae	Gelling and spherification agent	454 g 73.69 USD
Isubgol	It comes from the mucilage of Plantago ovata seeds.	Plantago Ovata seed hulls, rich in fiber with high mucilage content.	Gelling agent, diuretic, weight loss.	220gr 23.59 USD
Guar	Galactose (bound by α1–6) and mannose (bound by β1–4).	Seed flours Cyamopsis tetragonolobus	Stabilizer, thickener, and emulsifier. Gelling agent together with isubgol.	227 g 9.85 USD
Cassia	Galactose (bound by α1–6) and mannose (bound by β1–4).	Sennao btsifolia seed	Thickener and gelling agent in combination with agar.	150 g 10.07 USD
Xanthan	D-glucopyranosyl chain linked β1–4 bond, branching of D-mannopyranosyl trisaccharides and D-glucopyranosyluronic acid.	Fermentation Exudate of the bacterium Xanthomonas campestris B-1459	Stabilizer, thickener, emulsifier, emulsifying agent, stabilizing agent, thickening agent, emulsifying agent to provide shape and foaming and gelling agent.	250 g 12.68 USD

Gelling agent	Chemical Composition	Category	Application	Cost
Agar	Agarose (galactoses) and agaropectin (anhydrogalactose partially esterified with sulfuric acid)	Gelidium cartilagineum, Gracilaria confervoides and Peteroclaia capillacea algae extract.	Stabilizer, thickener, emulsifier and gelling agent.	75 g 326.24 USD
Katira Rubber	D-galactose, D-galacturonic acid and L-rhamnose in molar ratio 2:1:3	Plant extract Exudate of Cochlospermun religiosum	Emulsifier or thickener and gelling agent.	100 g 680.29 USD

Table 1.
Commercial gelling agents. Own elaboration.

4. Agar

The development of solid culture media was fundamental not only for bacteriology but also for biotechnology. Robert Koch and his assistant Walter Hesse are known as the inventors of solid media, in 1882 they replaced animal gelatin with agar gelatin, however, this technique already had precursors. Some authors mentioned that agar was discovered in Japan in 1658 by Tarazaemon Minoya, agar was used at the time as a gelling agent for food [25]. Agar is extracted from the cell membrane wall of red algae in a fibrous crystallized form and is mainly composed of sulfated galactan [26].

Around the world, there are different genera of algae such as Gelidium, Gracilaria, Gelidiella, Pterocladia, Gracilariopsis, Ahnfeltia, and their respective species. The characteristics of each of these provide the extracted agar with different properties. However, the best quality agar is derived from Gelidium, algae that are only found in the wild. It is important to mention that the composition of the agar is not affected by seasonality, but the yield is [27].

Agar is classified as a non-branched, high molecular weight, reserve natural polysaccharide with mineral content including Copper (Cu), Zinc (Zn), Manganese (Mn), Calcium (Ca), and Magnesium (Mg). Agar is composed of agaropectin and agarose, the latter is the main constituent (70%). Both components have the same basic structure, formed by alternating units of D-galactose and 3,6-anhydro-L-galactopyranose linked by α-(1–3) and β-(1–4) bonds [28].

Agarose has the following formula [$C_{12}H_{14}O_5(OH)_4$], and is classified as a neutral polysaccharide. The α and β galactoses present in their molecular structure are responsible for the mechanical property of gelation which is linked to the formation of gels, caused by the interaction of helicoids. Agarose and agaropectin are differentiated by the presence of sulfate and pyruvate residues. Agaropectin is classified as a charged polymer, where D-galacturonic acid and pyruvic acid provide it with viscosity property [26, 29]. The aforementioned composition is similar to that of the natural polymer starch [30].

4.1 General structure and properties of agar

The FTIR spectrum of agar (Gelidium) has vibrations in the absorption bands at 3291–3390 cm^{-1} associated with O-H stretching, followed by vibration at 2932–2922 cm^{-1} for CH_2 stretching, and the absence of a band at 2845 cm^{-1} corresponding to the O-CH_3 groups indicates a degree of low methoxylation. A vibration in the

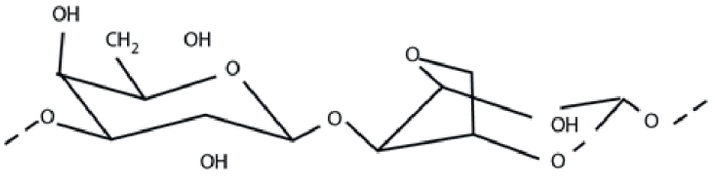

Figure 1.
Chemical structure of agar [33].

1642 cm^{-1} band is associated with the stretching vibration of the conjugated peptide bond formed by amine (NH) and acetone (CO) groups. Vibrations for CH_2 groups are found at 1413–1370 cm^{-1}, and a vibration that is identified for bridging a sulfated ester at 1179 cm^{-1}. In addition, the most represented vibration band in the agars is found at 930 cm^{-1} and attributed to the bridging of 3,6-anhydro-galactose [30–32]. A schematic of the agar structure is shown in **Figure 1**.

Agar is a hydrophilic hydrocolloid that has different properties, including the ability to form colorless, thermo-reversible gels that do not lose their original characteristics with changes in temperature; the gel forms at 30°C and dissolves at temperatures between 75°C and 90°C. The gels are not digested by plant enzymes. Important properties of these gels, such as yield and gel strength, may vary depending on the genus and location of the algae (**Table 2**).

For the extraction of agar there are different methodologies that can alter the above-mentioned properties (yield and gel strength). The most used methodologies are direct extraction in the water bath, the Freeze–Thaw method, syneresis method, and the alkaline treatment. Recent extraction processes are photobleaching, microwave-assisted extraction, and enzymatic method assisted by hydrogen peroxide [40, 41].

On the other hand, agar has important uses in different industries in addition to the elaboration of solid culture media for plant growth and micropropagation. It was the first hydrocolloid used as a gelling and stabilizing agent in the food industry. Agar is also used in the dental industry for the manufacture of the mold, in the medical industry for pharmaceutical formulations, in other industries for biodegradable films as packaging film; and its use in the cosmetic industry is well known [32].

The industry of agar production continues growing, Asia and the Pacific dominate the manufacture of this product, Indonesia and China play an important role, but Chile has prospered in that sense. The main suppliers of Gelidium are Spain, Portugal,

Species	Performance (dry weight) %	Gel strength (g cm^{-2})	Author
Gracilaria verrucosa	4.3	225.8	Montilla-Escudero et al. [34]
Gracilaria cornea	36.6–46.1	251	Freile-Pelegrín et al. [35]
Gelidium coulteri	24.0–39.0	263–288 g	Macler & West [36]
Gelidium robustum	45.0–37.0	268–288	Hurtado et al. [37]
Gelidium sesquipedale	40.0–45.0	1000	Mouradi-Givernaud et al [38]
Gracilariopsis tenuifrons	39.57	1231	Zecchinel et al. [39]

Table 2.
Yield and strength properties of gel in different species of algae.

Morocco, Japan, Republic of Korea, China, Chile, and South Africa [42]. Annually, 55,000 tons of seaweed are extracted, which produce 7500 tons of agar with a value of US$ 132 million [43]. The growth of this industry has caused ecological problems that have hindered agar temporalities and supply; therefore, scientists have been inclined to find total or partial substitutes for agar for different industries. The following section explores some commercial gelling agents and natural agar substitutes with successful results.

4.2 Agar substitutes for gelling agents in culture media

Currently, research is seeking to reduce the ecological effects of agar extraction, while reducing the costs of this biotechnology [6]. Therefore, in 1986, the carrageenan was used as a substitute for the gelling agent agar in plant tissue cultures; according to the results obtained in the research, the tissues grew better on this gel than when the medium was solidified with agar [44].

In Cuba, the Center for Research and Development of Medicines (CIDEM) investigated the use of Aloe vera (Aloe barbadensis miller) and sago flour (Maranta arundinacea) as solid support in culture media for medicinal plants. The research demonstrated that partial or total substitution of agar by A. vera gel or sago flour is possible. In addition, this culture medium has been used for the in vitro propagation of agraz (Vaccinium meridionale Swartz) [45, 46].

Starches from cassava (Manihot esculenta), corn (Zea mays), and rice (Oryza sativa) have been investigated for their high availability in local markets and low cost. The use of starches in the partial replacement of Phytagel® in the modified MS medium for sweet potato (Ipomoea batatas) and cassava (M. esculenta) crops was investigated in Honduras at the Plant Tissue Culture Laboratory. As a result, it can replace up to 72% of the Phytagel® dose in sweet potato (I. batatas) and cassava (M. esculenta) crops.

Isabgol, which is the seed of Plantago psyllium, a herbaceous species from Spain and Morocco, used commercially for the production of mucilage for dietary fiber, in conjunction with commercial sugar was used as an alternative agar in in vitro culture media for plantain. The results showed that not only can isabgol be a solidifying agent in culture media, but also a preservation medium for germplasm banks [5].

In 2012, a study conducted in Ethiopia investigated the efficiency of ensete (bulla) starch as a gelling agent, significantly improving the number of sprouts and saving about 72.5% in costs [47]. In another study, the partial substitution of agar by the starch of the Diacol Capiro variety in the micropropagation of lulo Solanum quitoense Lam. was carried out, with a positive result [48].

Future perspectives in the development of this biotechnology are directed towards the partial or total substitution of agar as a gelling agent by solidifying agents that can be used more efficiently, easily extracted, locally acquired, and not temporary. In addition to improving plant production in reduced spaces and the resources derived to face future challenges with micropropagation without the risk of crop contamination.

5. Nopal hydrocolloids: potential substitutes of agar

Opuntia, better known as Nopal cactus is native to the American continent and belongs to the subfamily Opuntioideae (Cactaceae) that consists of 181 known species that are present throughout the American territory in the wild and are characterized by

their easy reproduction and their ability to adapt to different climatic conditions [49]. Nopal cactus is produced worldwide and has been used by different industries in research related to natural medicine and human body benefits because it has essential nutrients for human beings such as dietary fiber, vitamin C and A, calcium, phosphorus, potassium, magnesium, chlorophyll, and antioxidants. The content of these nutrients is related to variables such as the age of the plant, the place where it grows, and the climate to which it has been exposed [50, 51].

However, it has been determined that the amino acid content of nopal cactus consists of aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, and tryptophan [52]. Within its mineral composition, there is manganese, iron, zinc, magnesium, and sodium.

Dietary fiber is the major component of the nopal cactus, which can range from 11.0 g to 23.33 g depending on the age of the stalk and has been related to health benefits in different research studies due to its high content of bioactive compounds such as phenols and carotenoids [53]. Dietary fiber is composed of insoluble fiber and soluble fiber.

Soluble dietary fiber is formed by hydrocolloids (pectin and mucilage), which are named like that for their great capacity to capture and retain water [54]. These hydrocolloids are classified as natural polymers that have recently been studied for their importance and technological advantages. They are composed of arabinose, galactose, rhamnose, xylose, and galacturonic acid residues [55]. The mucilages of the nopal cactus have a similar composition to the exudates of Sterculia trees (Sterculia and Khaya gum), which are used as stabilizers [56].

However, due to the molecular composition and characteristics of the hydrocolloids present in the nopal cactus, this work revises definitions and characteristics of mucilage and pectin from nopal cactus stalks, the extraction methods, and mechanical properties.

5.1 Structure and general properties of mucilage

Mucilage is a complex neutral polymeric substance that is part of the carbohydrates and has a yield of 1.0% to 1.2% in fresh weight and about 17.9% in dry weight [57]. It is present in the Golgi apparatus and functions as a chelator capable of binding calcium and controlling the amount of free soluble calcium [58]. Mucilage has a branched chemical structure with approximately 55 sugars where L-arabinose, D-galactose, L-rhamnose, D-xylose, and D-galacturonic acid are present in different proportions [59–61].

Sáenz et al., [62] mention that McGarvie and Parolis (1981) presented the first suggested structure for O. ficus-indica mucilage, where they describe the molecule as a linear repeating central chain of α-D-linked (1–4) and β-L-linked (1–2) rhamnose with side chains of (1–6)-β-D-galacturonic acid linked to O-4 rhamnose residues. The galactose residues substituents at the O-3 positions, or double substituents at O-3 and O-4 (**Figure 2**) [63, 64].

Sepúlveda [57] mentions that the structure of mucilage is proposed as two distinct water-soluble fractions, where one is identified as pectin with gelling properties with Ca^{2+} and the other is a mucilage without gelling properties that swells when dissolved in water and shows characteristics of high viscosity [61, 65].

The property of viscosity, a physical characteristic of fluids, has a relationship to ionic strength, pH, and slightly to temperature in the Opuntia spp. As pH increases from acidic to alkaline conditions, viscosity increases. In addition, viscosity decreases

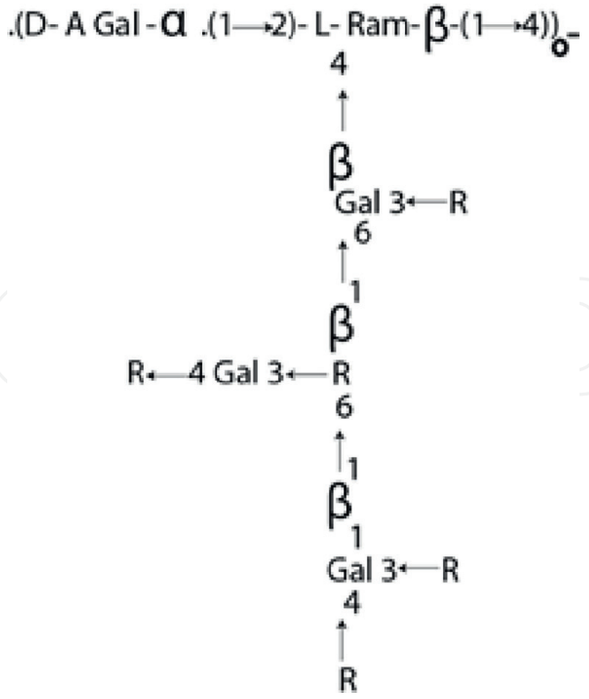

Figure 2.
Partial structure proposed for the mucilage of Opuntia ficus-indica R = arabinose or xylose [63].

as temperature increases just as it does in Xanthan gum. It is also mentioned that Opuntia mucilage has high elastic properties, the higher the concentration of mucilage, the lower the normal stresses [60]. The concentration of mucilage is important in the characterization of certain properties.

The extraction of mucilage can be carried out by different methods, and its yield depends on this. First, there is the extraction by water bath and the use of ethanol (95%) or isopropyl alcohol (95%), with a yield of 1.58% fresh weight [57]. The microwave-assisted extraction, the mechanical pressing system, and its lyophilization are other extraction methods.

The FTIR analysis of the mucilage of O. ficus indica and O. robusta performed in this study, compared with that performed in other species such as O. jonostle, O. streptacantha, O. tomentosa, O. atropes, and O. hyptiacantha shows that the functional groups present in all cactus mucilages are: absorption bands at 3500–3200 cm^{-1} that represent the carboxylic acid -OH groups involved in the intermolecular bond as mentioned by [66] Contreras-Padilla (2016). At 2975–2919 cm^{-1}, a band may appear which is related to the stretching of the -CH groups belonging to the pyranose groups, then a softer absorption band at 2850 cm^{-1}which is related to the stretching of -CH_2 groups of the carboxylic group [67]. It is shown the lack of waveband at 1749 cm^{-1} is linked to the low degree of esterification, as mentioned by [68] Rodriguez-Gonzalez et al., (2014) which indicates that the carboxyl groups are free and available to interact with water molecules and this results in their high capacity to absorb water; as well as if the free carboxyls are mixed with Ca^{2+} in the presence of water, they can form viscous structural networks. However, for O. robusta and O. atropes a slight vibration can be identified at 1730 cm^{-1}related to C=O stretching. In addition, it was found two bands at 1593 and 1388 cm^{-1} related to symmetric and asymmetric COO- stretching, which confirms the low degree of mucilage esterification. Finally, it was found a band at 1030 cm^{-1}, due to the vibration of C-O molecules attributed to the

stretching of secondary cyclic alcohols [67]. The bands below 1000 cm $^{-1}$ correspond to β-D-glucose and below 800 cm $^{-1}$ are attributed to vibrations of N-H and O-H groups (**Figure 3**) [69].

5.2 Structure and general properties of pectin

Pectin is part of the structural tissues of plants and vegetables, it is present in the skin of certain fruits such as apples or in the pulp of other vegetables such as citrus fruits, strawberries, quince, and carrots. Pectin contains mainly galacturonic acid (GalA) with residues partially esterified with methanol and is the main component of the middle lamella in plants and primary cell wall, it has the function of providing cohesion and stability to tissues [70]. Pectin is formed by long chains of α-D- (1 → 4) linked to galacturonic acid interspersed by the insertion of residues (1 → 2) linked to L rhamnose residues with side chains of neutral sugars, the linear segments are predominantly composed of homogalacturonan [71]. Within its structure homogalacturonan, rhamnogalacturonan I and II, and xylogalacturonan are identified. **Figure 4** shows the basic molecular structure of pectin, where it can be seen that each ring has a carboxyl group that can be esterified with methanol-producing methyl esters [72].

The importance of pectin lies in its ability to form gels in the presence of Ca^{+2} ions or in solute at low pH, hence its importance and multiple applications as a thickening agent, gelling agent, binder, and stabilizer in industries such as pharmaceuticals for gastrointestinal treatments, in the food industry for the production of jams and frozen foods, and recently, innovations in its use for edible coatings and foams. However, for the formation of gels, the most important characteristic is the quality of the extracted pectin, which is classified into two types: pectin with a high degree of methylation and pectin with a low degree of methylation.

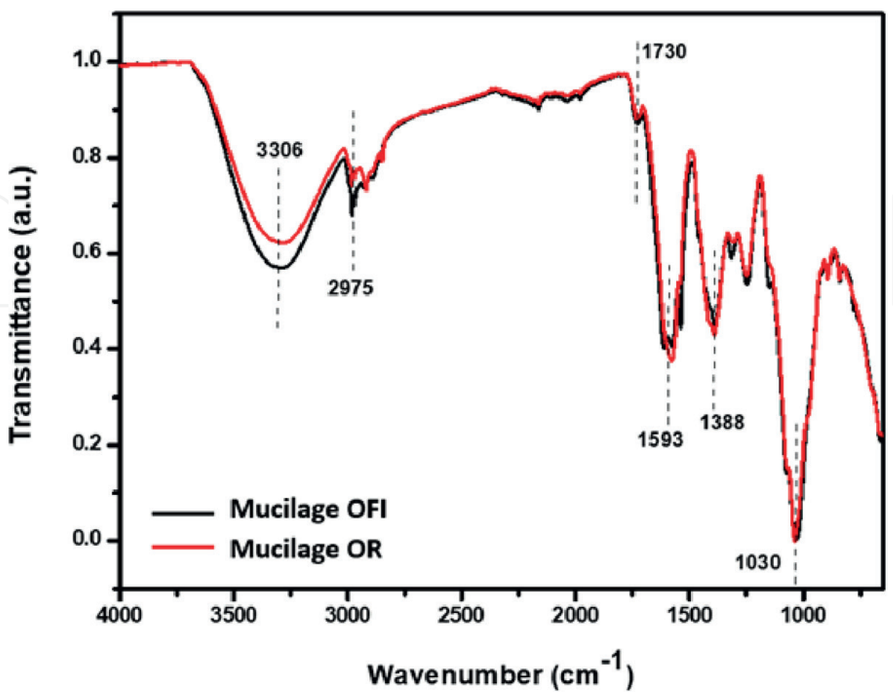

Figure 3.
ATM-FTIR mucilage extracted from Opuntia ficus-indica (OFI) and Opuntia robusta (OR) by alkaline hydrolysis.

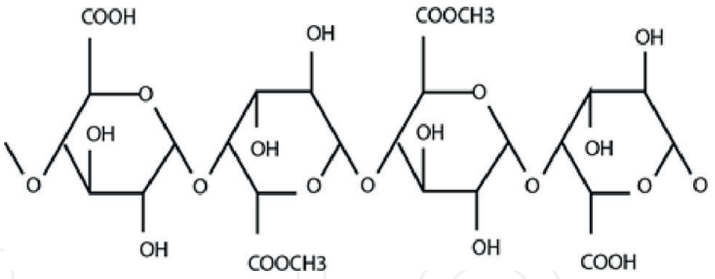

Figure 4.
Basic molecular structure of pectin [72].

The degree of methylation is the indicator of galacturonic acid residues esterified or methoxylated by the methyl group and are classified into low (<50%) and high methoxyl (>50%) pectins [73]. For low methoxyl pectin "gelation results from ionic bonding through calcium bridges between two carboxyl groups that belong to two different chains in close contact with each other. In high methoxyl pectin, cross-linking of pectin molecules involves a combination of hydrogen bonding and hydrophobic interactions between the molecules" mentions [74] Thakur et al., (2009). The yield of pectin can be from 4.42 to 10.39%, depending on conditions such as time, temperature, pH, and dry weight extraction method [75]. With the microwave-assisted extraction method, yields of 12.56% dry weight were obtained [70]. Recently, the enzymatic method with xylanase and cellulase was used for the extraction of pectin from O. ficus indica where yields of 17.91% in dry weight were obtained [76].

The conditions and method of pectin extraction directly affect the GalA content and therefore its gelation capacity; in the case of requiring pectin as a functional additive, hot acid extraction is recommended; for the use of de-esterified pectin, with

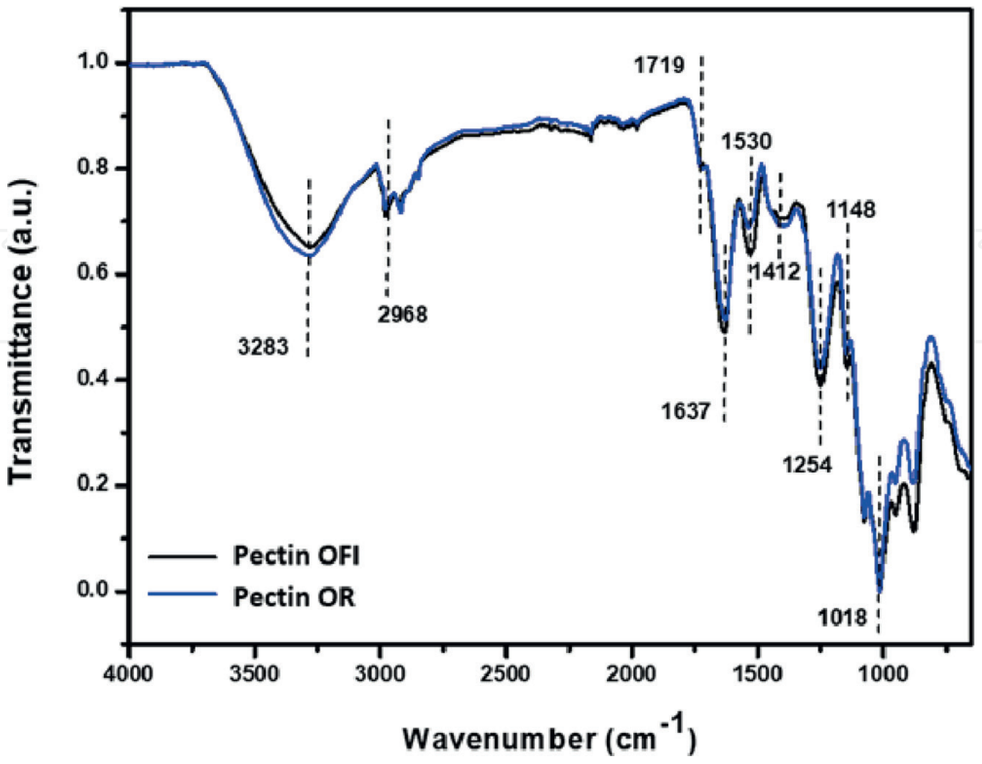

Figure 5.
ATM-FTIR of pectin extracted from Opuntia ficus-indica (OFI) and Opuntia robusta (OR) by alkaline hydrolysis.

high GalA content and gelation capacity with calcium ions, an alkaline soluble extraction is recommended [77].

According to the alkaline, extraction carried out in this study and compared to that performed by [61] Goycoolea & Cárdenas (2003), [77] Cárdenas et al., (2008) and [76] Bayar et al., (2017), the FITR analysis shows the following vibrations. A strong vibration at 3283 cm $^{-1}$ related to the stretching vibrations of the -OH groups of alcohol and carboxylic acid involved in inter-and intramolecular hydrogen bonds of the galacturonic acid polymer [78]. Subsequently at 2968 cm^{-1} a pronounced band corresponding to the absorption of the O-CH_3 extension bonds the methyl ester of galacturonic acid [79]. A 1720 cm^{-1} vibration caused by stretching vibration C=O methyl esterified carboxyl groups, at 1624 cm^{-1} vibration is related to the stretching of carboxylate ions and the relative ester band, which is more intense in pectins of a high degree of esterification. The bands found between 1600 to 1400 cm^{-1} correspond to the antisymmetric and COO - symmetric stretching characteristic of carboxylic acid salts. Some of the carboxyl group signals may also originate from phenolic compounds as indicated by the presence of peaks at 1530 cm^{-1} for aromatic ring vibrations [80]. It has been shown that the relative intensity of the last two peaks is related to the degree of methoxylation. The bands found between 1250 to 1140 cm^{-1} correspond to the C-O-C ether stretching [81]. The last strong bands found between 1140 to 1100 cm^{-1} are due to C-O-stretching of secondary alcohols and C-O- stretching of H in cyclic alcohols respectively [76] (**Figure 5**).

6. Conclusion

According to the analysis of the hydrocolloids extracted by acid hydrolysis contained in O. ficus-indica and O. robusta, analyzed by FTIR and compared with other research, it is concluded that the functional groups found in the mucilage are characteristic of proteins and polysaccharides, that have mechanical properties of viscosity, which can be used in industries such as food, pharmaceutical, construction, cosmetics, and biotechnology. However, when separating the solid residue from the mucilage extraction and performing acid hydrolysis to it, we obtain (according to the FTIR analysis) pectin with a low degree of methoxylation, because a small absorption peak is observed at 1732 cm^{-1}, which is attributed to the C = O stretching vibration of the carboxyl groups esterified with methyl. Due to this, the use of this pectin in combination with the indicated substances and the selected culture medium is suggested as a potential partial or total substitute for agar as a gelling agent in In vitro culture media for the development of plant cultures under laboratory conditions.

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Conflict of interest

The authors declare no conflict of interest.

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