Chapter

Extraction and Characterization of Pectins From Peels of Criolla Oranges (*Citrus sinensis*): Experimental Reviews

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Abstract

The citriculture in the international field is important not only for its nutrient and vitamin characteristics but also for its valuable source of raw materials to obtain pectin, since it was found in the internal and external parts of the citrus peel. In our country, there are several varieties of orange, from which you can obtain by-products other than juice, such as essential oils, fertilizers, concentrates, and pectin. Pectin has different uses in the pharmaceutical industry for the preparation of suspensions, emulsions, cosmetics, capsules, etc. In the food industry, it is used as a film in packaging, thickener, and gelling agent in the manufacture of jellies and preserves, in wines as dehydrating plant tissues, in milk to precipitate casein, etc. The pectin extraction was carried out by basic or acid hydrolysis and then proceeds to purify and clarify. Once purified, the pectin was characterized in aqueous solution to determine its physicochemical properties such as molecular weight, hydrodynamic radius, hydration value, shape factor, etc. Thermal and mechanical characterizations were also performed, to assess its ability to form films.

Keywords: pectin, *Citrus sinensis*, hydrolysis, films

1. Introduction

Pectin is a structural polysaccharide found in all higher plant fruits as citrus, apples, grapes, plums, etc. and is, therefore, part of the natural man diet. Many aspects of plant physiology and pathology, food texture, and even wine production involve pectin and its fate in materials and organisms. Commercial preparations of pectin are usually derived from citrus or apple peels, by-products of juice manufacture. The production involves aqueous extraction under mild acidic conditions, followed by precipitation by the addition of a di- or trivalent metal alcohol or ions.

Most of the world production of pectin is used for the preparation of jams and jellies, but a growing part is used in confectionery products, beverages, and acidified milk drinks. Pectin is suitable for applications in acidic food products due to its good stability at low pH values [1]. For this same reason, it is currently also used in the pharmaceutical industry as in the manufacture of capsules, films, and biodegradable patches.

Pectins are complex heterogeneous polysaccharides; it is used plurally because it differs from its composition from one species to another (the pectin obtained from a citrus differs, e.g., from the apple; even within citrus, there are small differences). Like most other vegetable polysaccharides, it is both polydispersed and polymolecular, and its composition varies with the source and conditions applied during storage. In any pectin sample, parameters such as weight or content of particular subunits will differ from molecule to final molecule.

All pectin molecules contain linear segments of α (1 → 4)-D-galactopyranosyl uronic acid units linked with some of the carboxyl groups esterified with methanol (**Figure 1**). In the pectin of some sources, some of the hydroxyl groups of the galacturonosyl units (0–2 and/or 0–3, oxygen bonded to carbon 3) are esterified with acetic acid [2].

Pectins are a mixture of acidic and neutral branched polymers. They constitute 15–30% of the dry weight of the primary wall of plant cells. They determine the porosity of the wall and therefore the degree of availability of the substrates of the enzymes involved in the modifications of the same. Pectins also provide charged surfaces that regulate pH and ion balance. In the presence of water, they form gels. Pectins have three main domains:

1.1 Homogalacturonans (HG)

Compounds by D-galacturonic acid (GalU) residues are bound by an α (1 → 4) bond. The carboxyl groups of C6 (carbon number 6 of the GalU) can be methyl esterified or remain free. The free carboxyl groups, if dissociated, give rise to calcium bonds between the neighboring HG chains, forming the so-called egg box structure. For a region of HG to be sensitive to calcium binding, 10 molecules of unesterified GalU are required, the formation of bonds of this type is related to the arrest of the cell wall and, therefore, with the cessation of growth and increase of stiffness of the wall. The GalU can be found acetylated in O2 (oxygen number 2 of the GalU) or in O3 [2].

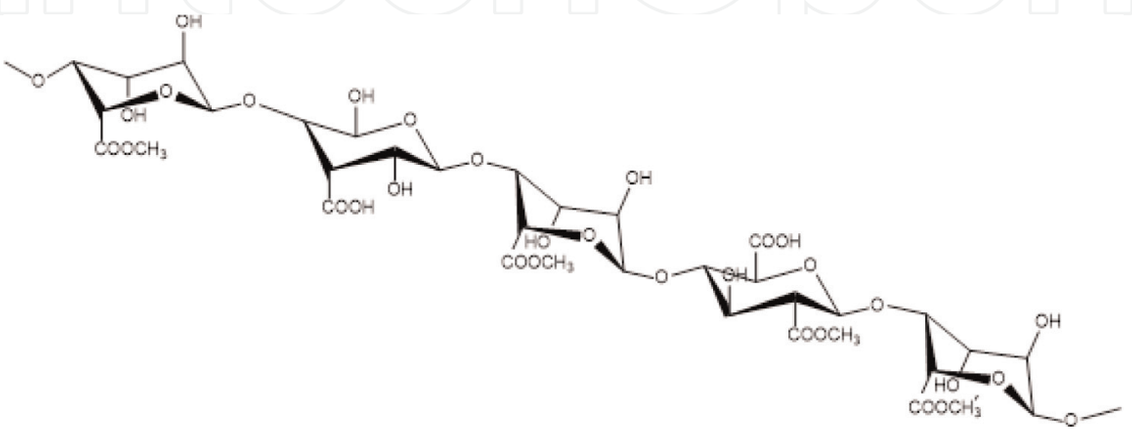

Figure 1.
Basic chemical structure of α (1 → 4)-D-polygalactopyranosyl uronic acid.

1.2 Rhamnogalacturonan-I (RGI)

GalU linked in α (1 → 4) with L-rhamnose (Rha) residues intercalated with an α (1-2) bond, that is, [(1-2)-α-L-Rha-(1-4)-α-D-GalU] n, where n can be greater than 100. These Rha residues are the anchoring of side chains, and approximately half are bound by C4 to chains of arabinans, formed by α-L-arabinose (Ara) linked in α (1 → 5) as the main axis that can be substituted with the chains Ara (1-2)-α-Ara (1-3) and/or Ara (1-3)-α-Ara (1-3) or arabinogalactan I (AGI), chains of β-(1-4)-D-galactose (Gal), with C6-Gal branches. They can also be substituted for α (1 → 5) Ara in Gal C3 [2].

1.3 Rhamnogalacturonan-II (RGII)

Small polysaccharide of very complex structure, formed by GalU, Rha, Ara, Gal, and small amounts of infrequent sugars such as apiose or acetic acid. The Rha moieties may be substituted at C3; in C3 and C4, in C2, C3, and C4 or be terminal. The arabinogalactan of the RGII presents ramifications in C3 and C6 of Gal and in C3 and C5 of Ara. The side chains contain a high number of different residues bound with various bonds. However, the RGII has a highly conserved structure and can form dimers via a borate bridge, with two ester bonds [2].

Arabinans and galactans of the RGII of the *Amaranthaceae* family can be associated to ferulic acid through an ester bond, which makes it possible to link several chains by diferulic bridges, through the action of the peroxidases. Links are also caused by the dimerization of hydroxycinnamic acids linked to arabinans and galactans of the RGI due to the action of peroxidases [2, 3].

Commercial pectins are derived almost exclusively from citrus or apple, both by-products of the manufacture of juice or cider. While the apple pomace contains 10–15% pectin on a dry matter basis, the citrus peel contains 20–30%. The pectin of citrus fruits and apples is equivalent from the point of view of the application. However, citrus pectins are light cream or light tan; apple pectins are often darker.

Other suggested alternative sources include sugar beet residues, sunflower heads (seeds used for edible oil), and mango residues. The pectin from sugar beet was produced in Germany during World War II and in Sweden and Russia in the following years. The beet pectin is inferior to the citric pectin in molecular weight.

All currently significant applications are related to (1) the degree of acetate esterification, (2) relatively low molecular mass, and (3) the presence of large amounts of neutral sugar side chains [4].

The use of pectin in traditional sugar jams is one of the best known applications, being one of the largest pectin markets. Very often the pectin is the only gelling agent allowed, with 0.2–0.4% of pectin, depending on the type and origin of the fruit. Within the European Economic Community, there are two standards in jam and extra marmalade, which contain a minimum of 30 or 45% fruit pulp, respectively. The higher quality of jam also tends to be made with better quality fruit, so it requires significantly less pectin.

The jam of citrus fruit, especially lemon or grapefruit, in which pectin has been produced in a higher than normal content, generates disadvantages. In this case, too much pectin in the fruit leads to too strong gel formation or even to pregelation and syneresis; the attempt to regulate the texture with the pH leads to a situation in which the control is extremely critical and the rejection rate to this pectin is inevitable. One solution has been to work at a pH where the pectin in the fruit no longer forms a gel. The gelation can then be achieved by adding a pectin-amide of low methoxyl content which is capable of gelling at high pH [4].

Both the selection of the correct pectin (the lower the solid solubility, the more sensitive to calcium) and the pectin content in fruit are important. Sometimes, especially at very low amounts of pectin, it may be necessary to add a calcium salt to obtain a better result. From time to time, neutral gums are added to reduce syneresis, but each attempt must be made to optimize the type and level of pectin. The exact pH before considering such addition should be considered, since the gums can mask the delicate taste of these products [4].

Jam makers also manufacture fillings and toppings for the bakery and related industries. Many of these use pectin as a gelling agent or thickener, but, because it depends so much on their processing conditions, it is very difficult to generalize a process on an industrial scale.

In recent years, a growing area of the fruit product industry is the production of fruit for addition to yogurts and similar products. Many of these have been made with modified starch as a thickener to ensure a homogeneous distribution of the fruits together with the texture that must be extruded and the difficulties that this entails. Unfortunately, although starches are relatively inexpensive, they can mask delicate fruit flavors and lead to a mealy texture. These foods have sugar contents between 30 and 60%, so high methoxyl pectin can be used. Pectins have other uses in the dairy industry, for example, the high methoxyl pectin prevents the aggregation of casein in the heating to pH values lower than 4 or 3. It can therefore be used as a stabilizer for the drinkable yogurts treated with UHT and for mixtures of milk and fruit juices. It will also stabilize acidified soy milk drinks and whey products. The yogurt can be thickened by adding very low levels of low pectin in amidated methoxyl before cultivation. Although this is not allowed in many countries, a suitable pectin incorporated in a fruit base can, with careful formulation, have an effect comparable to a fruit yogurt. On the other hand, low-calorie soft drinks are often thin and lack the characteristic mouthfeel provided by sugar in conventional soft drinks. A low level of pectin (usually of controlled viscosity) can be used to improve the texture of these products and also to replace part of the texture due to fruit pulp in juice formulations [5].

1.4 Pectin extraction and purification

For pectin extraction two general processes are used: (1) those that separate pectins from most of the other materials by precipitation with an alcohol and (2) those that precipitate pectins as an insoluble salt with suitable multivalent metal ions (**Figure 2**). Both can be used to obtain any pectin within the two main groups, namely, high methoxyl pectin (HM-pectin) and low methoxyl pectin (LM-pectin).

The conditions chosen for the extraction depend on the raw material and the desired product. Temperatures between 50 and 90 °C, pH 1-3, and with extraction times from 30 minutes to 24 hours. Acidification can be done with sulfuric acid, sulfurous acid, hydrochloric acid or nitric acid, in accord with reference [4, 5].

Processes with a long extraction time, where the pH is low and the temperature is high, are conducive to high product yield, but the quality can be adversely affected. The combination of low temperature with long duration and low pH to obtain some degree of de-esterification (DE) in the extraction in order to produce LM-pectin or on the contrary a slow hydrolysis in time generates HM-pectin [6].

The extraction is followed by filtrations. The raw materials used, which are very soft and swellable, are separated in an initial coarse mesh filtration and sold as livestock feed. The fine suspended solids are subsequently removed from the extract by filtration through diatomaceous earth [7].

If precipitation with alcohol is used to separate the pectin from the extract, the concentration of the extract usually precedes the precipitation, the reason for which

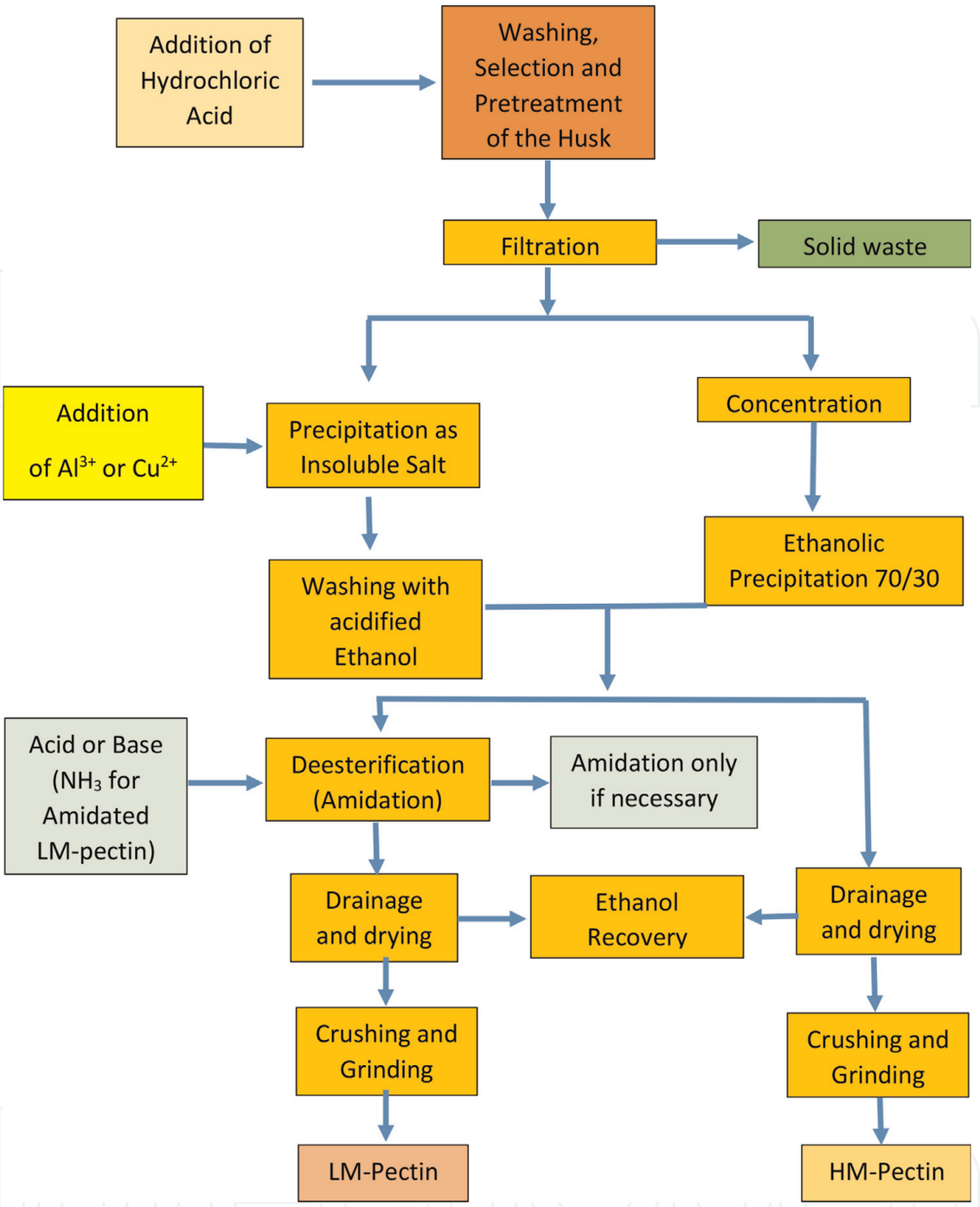

Figure 2.
Manufacturing process of pectins [4].

is saving alcohol. The concentration is usually done by evaporation, but ultrafiltration has been attempted. Al^{3+} is usually chosen if the pectin is separated from the extract as an insoluble one. Precipitation by Cu^{2+} can be an alternative. The metal ions are subsequently removed by several washes with HCl diluted in alcohol [8].

De-esterification to achieve the end of LM-pectin is usually done in an alcohol to which an acid or a base has been added. However, most of all LM-pectin are de-esterified with ammonia, thus producing amidated pectin. The traditional alcohol for the manufacture of pectin is 2-propanol (isopropanol), but also methanol or ethanol can be used. The recovery of alcohol by distillation adds considerable energy costs to production [4, 9].

A simple process of obtaining pectins is the one observed in **Figure 2**, in accordance to Ref. [4].

1.5 Films

A film is a uniform layer that can be formed either from a single component or from a mixture of polymers. The film development with biodegradable polymers is a promising technology in the food packaging industry. Food packaging is used for the preservation and protection of all types of food and its raw materials, particularly oxidative and microbial deterioration, as well as to extend the shelf life. The increased use of synthetic packaging films has led to serious ecological problems because they are not completely biodegradable and recyclable. The reduction of environmental pollution has led to a paradigm shift in the use of biodegradable materials, especially renewable raw materials in agriculture and waste processing industry food. This approach is equivalent to the conservation and recycling of natural resources, as well as the generation of new packaging of innovative design. The total packaging biodegradation generates benign products with the environment, and the permeability of CO_2 and water is a parameter to be considered at the time of the development of the films. Natural polymers cross-linked and copolymerized with synthetic monomers are other alternatives to biodegradable packaging films. For the time being, the complete replacement of synthetic plastics is simply impossible to achieve and may even be unnecessary, at least for some specific applications that require our attention in the future. Definitely, biodegradable bio-packaging will be very promising in the near future [10–14]. For this reason, the development of edible films and coatings has been extensively studied in recent decades. These structures act as a barrier between food and the environment, helping the outer packaging in its protective function. In addition, some of them are supposed to have their own sensory or nutritional characteristics. The use of fruit purees in edible films has been previously explored by conferring distinctive flavors and colors that could be exploited for applications such as sushi wraps, fruit strips, or colorful coatings for specific foods [15].

The applications of biopolymers are from foods (nutritive and dietary fibers), packaging (biodegradable bags and/or protective films), thickening agents, gelling agents, foaming agents, and emulsifiers [16]. As films or membranes, are used in separative processes, for gas separation, diffusion, filtration and reverse osmosis [16]. In the pharmaceutical industry, the biofilms are used as drug encapsulants, films, and patches [17]. On the other hand, in mining industry, they are utilized as flocculating agents and precipitants of heavy metals [18].

The publications of biofilms are framed in the first place in the development of new materials from polysaccharides of great abundance and wood and agroindustrial production such as cellulose acetate [19] and starch [20]. Other polysaccharides available, but at high cost, are alginates [21], chitosan [22], and guar gum [23]. From an original point of view, the development of new films with biopolymers of various types such as arabinoxylans [24], soybean polysaccharides [25], and watercress polysaccharides [26] is worth highlighting. To clarify the latter, the latest advances in these films are described below.

1.5.1 Regarding pectin films

Pectin due to its biodegradability, biocompatibility, edibility, and versatile chemical and physical properties (such as gelling, selective gas permeability, etc.) is a polymer matrix suitable for the production of edible films intended for the packaging of active foods. It is understood by an edible film as a packaging material, which is a thin layer of edible material placed on or between food components. Active packaging is a system that has basic barrier functions and others that are achieved by incorporating active ingredients in the packaging material and/or by

using functionally active polymers. For example, if the packaging system has antimicrobial activity, packaging limits microbial growth by extending the latency period. Espitia et al. [27] performed an interesting review that describes the main methods for manufacturing edible pectin films, the main characterization techniques to determine their physico-mechanical properties, and the applications of edible pectin films as antimicrobial food packaging.

Parris et al. [28] evaluated the water vapor permeability of hydrophilic films of alginate and pectin. Their results suggested that these diminished the mechanical properties by incorporating whole milk, sodium caseinate, skimmed milk powder, or whey in the film. In addition, this work evaluated the appropriate choice of plasticizer. Sodium alginate films exhibited lower water vapor permeability values than films prepared using low or high methoxylation pectin. The author found that sodium lactate was an effective plasticizer and alginate films containing 50% by weight or more of sodium lactate had an elongation greater than 13%. Films prepared with sorbitol as a plasticizer had the best water vapor permeability values but tended to be stiff and in some cases too fragile for tensile measurements. The addition of mixtures of whole milk to film effectively reduced water vapor permeability values by up to 35%.

On the other hand, Pasini Cabello et al. [29] studied the effect of two plasticizers, glycerol (GLY) and polyethylene glycol (PEG), on the structure of the pectin films. The results revealed that glycerol acted as an internal plasticizer. Meanwhile, glycerin increased the predominant amorphous character of the plasticized films due to the decrease in intermolecular attraction, which resulted in degradation at low temperature and allowed the conformational transformation of the film to galacturonan ring through a can conformation. Glycerol produced more deformable and weaker films. In addition, glycerol produced films with a higher swelling index (SI) and a water vapor permeability value (WVP). When PEG was used as a plasticizer, a lower Young's modulus was obtained than the pure pectin film. However, by increasing the molecular weight of polyethylene glycol, more compact and less deformable films were obtained. WAXD spectra and DSC thermograms indicate that PEG works as a separate phase in the pectin matrix, more compact and less permeable to water vapor as the molecular weight of PEG increases. These results show that PEG acts as an external plasticizer.

Azeredo et al. [30] conducted the investigation of pomegranate juice into pectin films, giving it a bright red color, and also acted as a plasticizer. The increase in the pomegranate juice/water ratio from 0/100 to 100/0 resulted in an improved elongation (from 2 to 20%), a decrease in strength (from 10 to 2 MPa) and a Young's modulus (from 93 to <10 MPa), an increase in water vapor permeability (WVP, from 3 to 9 g mm kPa^{-1} h^{-1} m^{-2}), and a decrease in insoluble matter (IM, of 35–24%). Although an effect of cross-linking (cross-linking) by citric acid was not confirmed, it is demonstrated by its effects on the films. Citric acid markedly increased MI (from <10% to almost 40%); in addition, when measured on a dry film basis, the effects of citric acid showed a notable tendency to increase resistance and modulus and to decrease WVP. The citric acid decreased the density of the red color, which suggests a destabilization of the anthocyanins.

Different characterization techniques allow to determine the properties of biopolymers. Some techniques are used in solution and others in film. The data in solution demonstrate the tendency of the biopolymer to interact with the solvent and the conformation it acquires, besides the possible implications of its molecular weight with the ability to form gels. The techniques that provide this important information are gel permeation chromatography [31–34], ultracentrifugation-sedimentation, polarization to light, refractive index, light scattering, osmometry, diffusometry [24], viscosimetry, densimetry [26], and rheology [25, 35]. When the film is already formed, the most common analyses are water adsorption, either as

steam or pure water, water vapor permeation, and swelling index, data that provide information about the affinity of water with the biopolymer. The mechanical tests (Young's modulus) help to elucidate the ductility of the material under study. The structural characteristics are determined by FTIR-ATR, diffraction of RX, SEM, and AFM [36], techniques that help to interpret parameters of the intimate nature of the film and the displacements in its signals caused by its chemical structure. The TGA-DTG and DSC data show the stability of the material against thermal changes during synthesis. In short, all these techniques give us information on the possible applications of each biopolymer and select what type of hydrolysis should be performed. Once the biopolymer has been obtained and characterized, permeoselectivity tests are carried out, such as the controlled release of drugs [37], coagulation or heavy metal precipitation [38], adsorption studies [39], gas permeation [40], and food packaging [41, 42].

In present work, pectin extraction from orange peel was carried out through the hydrolysis process; several extraction conditions were evaluated, specifically the pH in aqueous solution performing acid and basic hydrolyses at different concentrations and times but at constant temperature. Then the pectin obtained was characterized by a comparative analysis with commercial pectin with different techniques described below.

2. Pectin extraction fundament

The citriculture in the international field is important not only for its nutrient and vitamin characteristics; it has also become a valuable source of raw materials to obtain the pectin, since it is found in the internal and external parts of the citrus peel. In our country there are several varieties of orange, from which you can obtain other by-products besides juice, such as essential oils, fertilizers, concentrates, and pectin.

The purpose of this work is to exploit the waste of criolla orange peels, which in some cases causes pollution problems in the ecosystem as it is an adequate source for the proliferation of insects and microorganisms that are harmful to human health, in particular the by-products of citrus fruits used in the preparation of juices and marmalade. On the other hand, the industry generates an expense for the elimination of citrus waste if it is not used. Also, the lack of national raw materials for the pharmaceutical and food industry allows us to search for the natural resources that could be exploited. All these cases motivate to investigate the benefits obtained from citrus waste, such as oranges, which is used as an input for the agro-industry in the production of juices mainly, whose process involves a considerable generation of waste such as husks, pulp, and seeds. This has two benefits; on the one hand, it seeks to increase its "added value" with the process of agro industrialization and, on the other, reduce the environmental impact they produce.

In present work, the pectin extraction was carried out from the orange peel of the variety *Citrus sinensis* and through acidic or basic hydrolysis, by means of pH changes and extraction times at a constant temperature, with which it is expected to obtain a higher yield (**Figure 3**).

There are many processes for the extraction of pectin. In general, the raw material undergoes pretreatment that involves cleaning to remove foreign particles, washing to remove sugar and acid, drying, crushing, and storage. The substrate material is heated at reflux for several hours, with stirring, with an acid of known concentration (sulfur, sulfuric, nitric or hydrochloric acid) [43].

The objective of this work was to compare the different reagents used during hydrolysis and compare performance as well as mechanical, physical, and chemical behaviors. Also, the characterization techniques of pectin were in order to determine its quality.

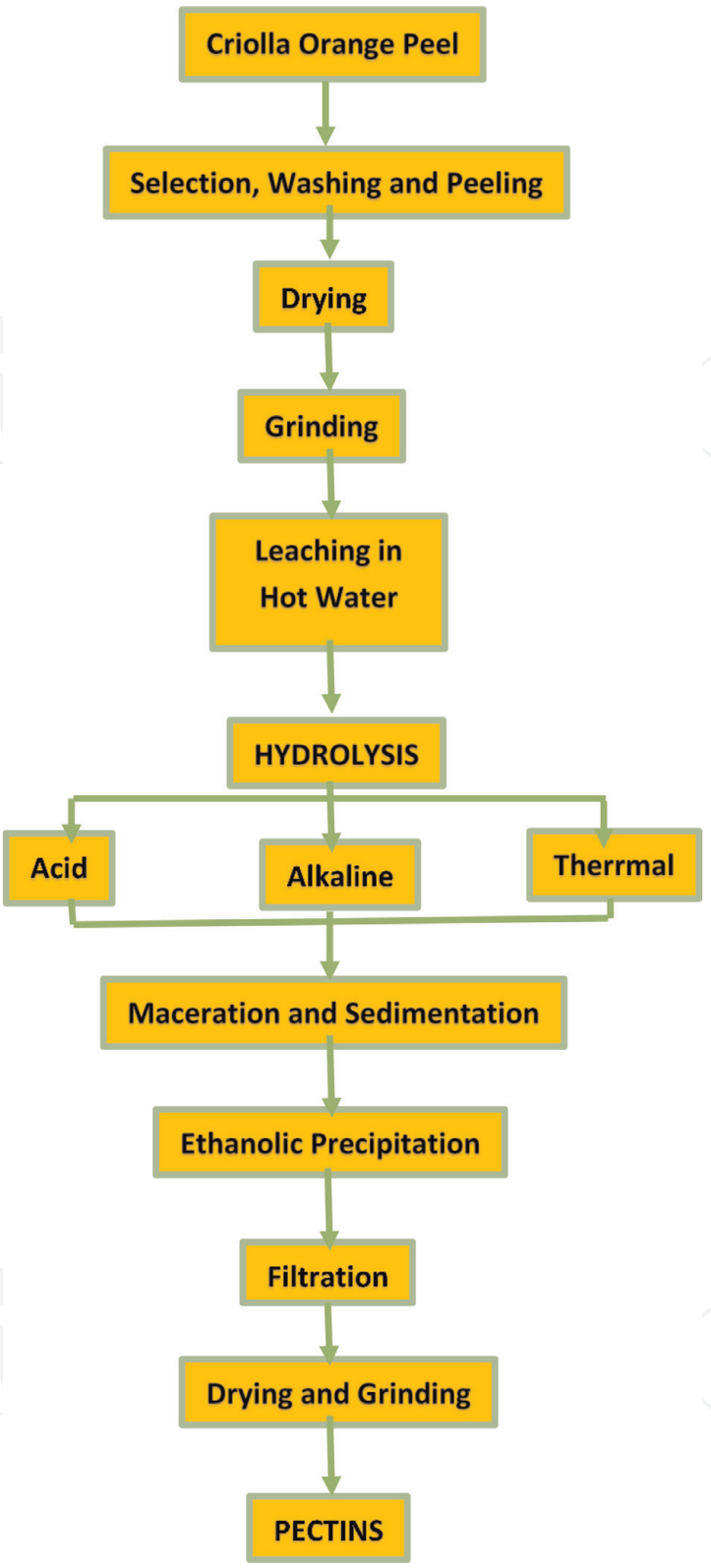

Figure 3.
Scheme of pectin production in this work.

2.1 Pectin extraction from *Citrus sinensis*

Many raw materials and products of the chemical and food Industry require preparation and conditioning. The process begins with the formation of flour from the raw material used, in this case the orange peel. Later, it is treated to achieve the necessary conditions that are required in the subsequent steps to obtaining the final

product, the unit operations necessary to carry out the pectin formation process (see **Figures 3–5**).

Selection, washing, and peeling of *Citrus sinensis*: In a first step, the oranges were manually selected. The quantity and quality of useful pectin obtained depend on the species of the fruit, the quantity that the fruit contains naturally, the state of maturation, the management conditions, and the enzymatic activity after harvesting and of the extraction process. They also depend on the part of the fruit that is used. For example, in unripened fruits the greater the amount of pectic material is insoluble in water, the quantity and the solubility increase with maturity [44].

It was decided to investigate as raw material the "criolla," orange which is a very common and easy-to-obtain species, observing that they do not present any type of microorganism or cuts and/or blows that may affect its safety or the development of

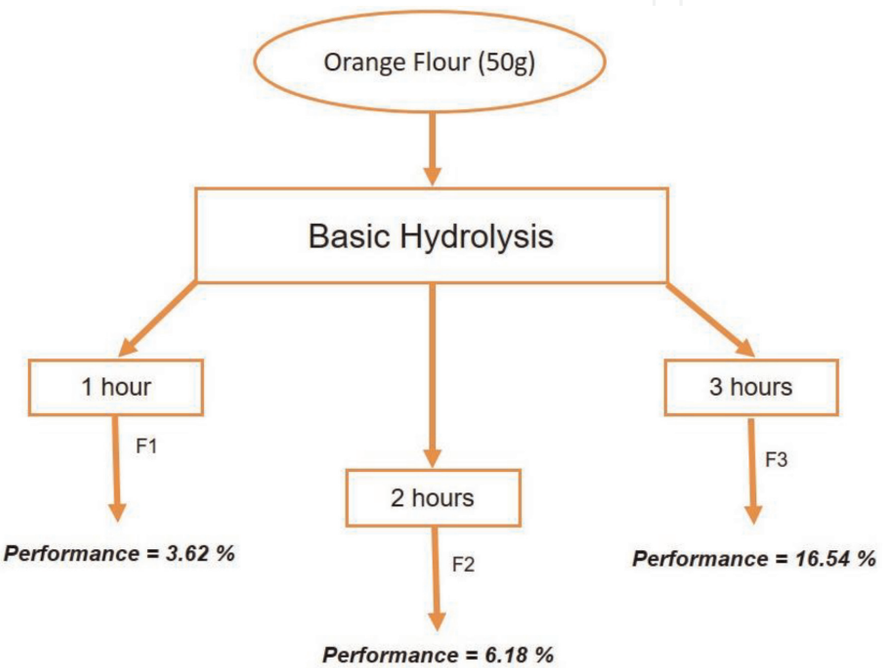

Figure 4.
Representative diagram of F pectins, with their respective performance.

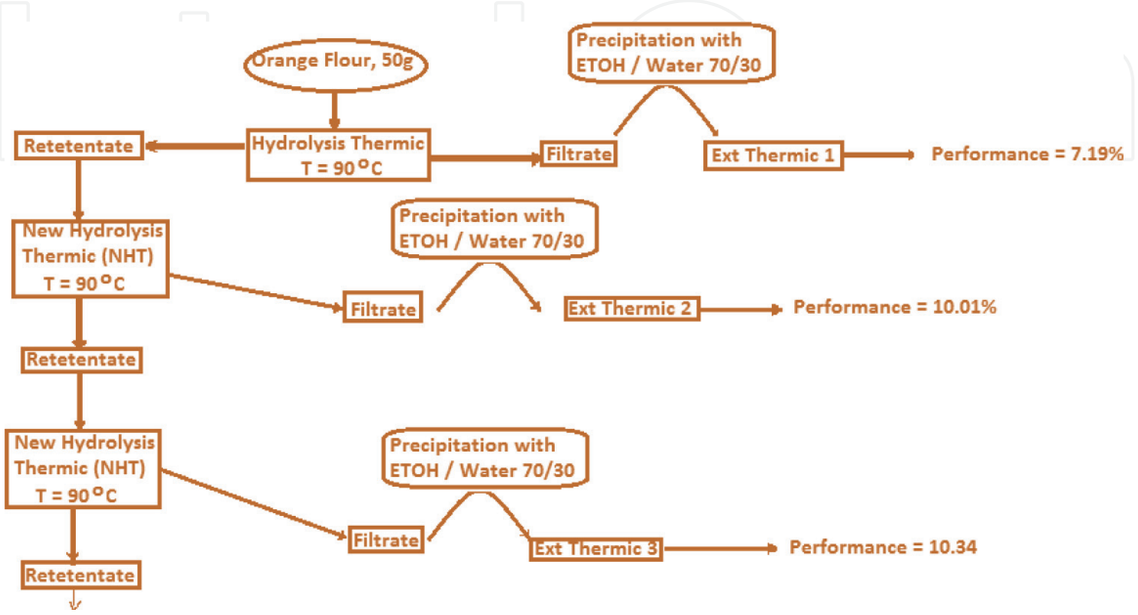

Figure 5.
Representative diagram for thermal hydrolysis with its respective performance.

bacteria or fungi, in addition to being in their commercial maturity. Once selected, the oranges are washed for later use. They are peeled manually without removing the albedo, since it is rich in pectic substances.

2.2 Drying of orange peels

In general, the drying of solids consists in separating small amounts of water or other liquid from a solid material in order to reduce the residual liquid content to an acceptably low value. The liquid to be vaporized can increase on the surface of the solid, as in the drying of saline crystals; inside the solid, as in the case of solvent removal of a sheet of a polymer; or part on the outside and part inside. The feeding of some dryers is a liquid in which the solid is suspended in the form of particles or in solution. The product that dries can withstand high temperatures or requires gentle treatment at low or moderate temperatures. This leads to the existence of a large number of types of commercial dryers in the market [45].

2.3 Pectin obtainment

2.3.1 Hydrolysis

The degradation of the polysaccharides begins, in general, at the reducing end of the molecule and proceeds step by step through the anhydroglucose chain [46].

The degradation of the polysaccharides proceeds by a peeling process in which the reducing end group is released from a chain by removing the remainder of the chain as a glycoxyl anion. The elimination takes place when the chain is in the beta position of a carbonyl group of the final reducing unit [47].

The extraction of pectin can be by acid or aqueous base. The basic extraction process produces a pectin of low degree of esterification (low methoxyl pectin) as a result of the saponification of the ester groups, while the acid extraction process generally produces a pectin with a high degree of esterification (high methoxyl pectin), approximately equal to the naturally occurring degree of esterification (DE). The high pectin has an esterification degree of 50% or more. Low DE and high pectin generally have different uses in food products, since they gel by different mechanisms. Both are sold commercially [48].

In the extraction process with acid and base, the plant material was treated with acid or base at temperatures between 70 and 90°C for a sufficient time to eliminate the desired quantities and the quality of the pectin from the cellulose plant material. The pectin was separated from the reaction mixture by filtration. The pectin is precipitated from the extract juice by specific means, either precipitation with alcohol (ethyl or isopropyl can be used) or salifying with aluminum chloride. The precipitated pectin was separated from the precipitating solution by filtration. The extract obtained consists of those molecules that are soluble under the conditions of pH, time, and temperature used during extraction. The extract was composed of a mixture of pectins of different molecular weights and degrees of esterification. Molecular weights can vary from 100,000 to 200,000, but average molecular weights are more typically 140,000 g/mol [48].

Hydrolyzed pectins were compared against commercial pectin from citrus peel by Sigma (galacturonic acid ≥74.0%, methoxy groups 6.7%).

2.4 Maceration and sedimentation

This mechanism is frequently used in the food industry for the separation of solid particles contained in liquids as well as for the separation of two immiscible liquid phases. The driving force is the difference in density between the two phases [45].

The evolution of the sedimentation of a typical flocculated suspension is as described below. **Figure 6** shows a suspension uniformly distributed in the liquid and ready to sediment. The total depth of the suspension is Z. If there are no sands in the mixture, the first appearance of solids in the bottom of the settler is due to the flocs that originate in the lower part of the mixture. As **Figure 6** shows, these solids, consisting of flocs resting gently on each other, form a layer called zone D. Above area D, another layer is formed, called zone C, which was a transition layer whose content in solids varies from that of the original pulp to that of zone D. Above zone C was zone B, which consisted of a homogeneous suspension of the same concentration as the original pulp. Above zone B is zone A which, if the particles have been totally flocculated, is a clear liquid. In well-flocculated pulps the boundary between zones A and B is clear. If particles remain unglued, zone A is cloudy, and the boundary between zones A and B is unclear. **Figure 6c** shows that as sedimentation progresses, the thicknesses of zones D and A increase, and that of zone C remains constant, while that of zone B decreases.

The whole process represented in **Figure 6** is called sedimentation. Subsequently, as shown in **Figure 6d**, zones B and C disappear, and all the solids are in zone D, beginning then a new effect called compression. The moment in which the compression is evident for the first time is called a critical point. In the compression part of the liquid that accompanied the flocs in the compression, zone D is expelled when the weight of the deposited solids breaks the structure of the flocs. During compression, a part of the liquid contained in the flocs is projected out of zone D forming small jets, and the thickness of this area decreases. Finally, as shown in **Figure 6e**, when the weight of solids reaches the mechanical equilibrium with the resistance to compression of the flocs, the sedimentation process is stopped. At this time the silt reaches its final height [45].

In this stage, the mixture was left to marinate for 24 h so that the unusable solids would settle. The separation of the phases was observed until obtaining the solid at the bottom of the crystallizer and in this way to be able to more efficiently separate the soluble pectin found in the supernatant, illustratively in the area A of **Figure 6e**.

2.5 Filtration

Filtration consists in the separation of the solids contained in a suspension by means of a perforated plate (filtering medium), which allows the passage of the

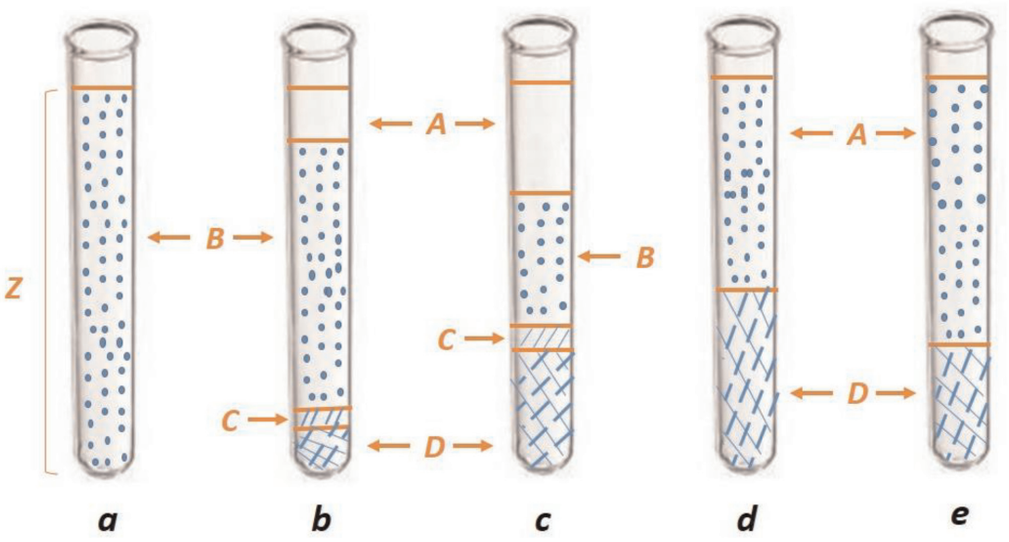

Figure 6.
Evolution of the sedimentation process.

liquid and retains the solid particles. The solid-liquid suspension that is fed to the filter is called syrup; the liquid stream that passes through the filter medium and which is obtained as a product is known as the filtrate. The retained solids form a bed or cake, whose porosity depends on the characteristics of the product to be filtered and the operating conditions, whose thickness increases throughout the filtration process. During the formation of the first layers of the cake, particle bridges are formed which block the perforations of the filter material. In order to overcome the resistance of the cake and the filter medium to the circulation of the liquid phase, a pressure difference must be established between both sides of the filter medium [45].

After the maceration, the previous step was gently filtered with sieve and canvas cloth, manually applying pressure until obtaining liquid rich in pectic substances. The remaining solid is discarded.

2.6 Precipitation with ethanol

The pectin was precipitated from the extract juice by specific means, either precipitation with ethanol or salifying with aluminum chloride [46, 47]. In this work ethanol was added to 96% vol., in a ratio of 70/30 (filtrate/alcohol), to precipitate the pectin. The precipitated pectin was separated from the precipitating solution by filtration. In this case, a canvas cloth filter was used again.

2.7 Drying

The pectin, obtained in the filtrate, was placed in plastic trays and placed in an oven at a constant temperature of 60°C for 48 h to eliminate the excess moisture. Then, they were kept in a sealed container until they were used. The flour final humidity cannot be greater than 5% by weight.

3. Characterization of pectin flour

3.1 Thermal analysis

The generally accepted definition of thermal analysis covers the group of techniques in which a physical property of a substance or a material is measured as a function of temperature while it is subjected to a controlled temperature program. More than a dozen thermal methods that differ in the measured properties and in the temperature programs can be distinguished. The effects of heat on the materials are numerous among which loss of mass, structural changes, conformational changes, changes of state, chemical reaction, degradation, etc. can be mentioned. In thermal analysis, weight changes form the basis of thermogravimetry (TGA), while the measurement of energy changes is the basis of differential thermal analysis (DTA, for its acronym in English) and differential scanning calorimetry (DSC, for its acronym in English). Thus, for example, thermogravimetry gives information about the loss or gain of weight of a sample, while the DTA and the DSC provide information on the variation of heat in a process such as a reaction or physical change, indicating whether it is endothermic or exothermic.

Thermal analysis is the series of techniques where the physical properties of a material are measured as a function of time, while the material is subject to a temperature program. Physical properties are also measured as a function of temperature, when it is variable. In all calorimetric techniques, the measured property is heat.

These methods find broad application in both quality control and research of pharmaceuticals, clays and minerals, metals and alloys, and polymers and plastics [48, 49].

Then, the thermoanalytical methods used in the characterization of the compound of interest of this work were defined and referenced to measurement principles of the instruments occupied.

3.1.1 Differential scanning calorimetry (DSC)

This technique allows the study of those processes in which enthalpic variation takes place, determining the temperatures where physical or chemical changes take place, points of crystallization and boiling, enthalpies of reaction, and determination of other transitions of first and second order [49].

It is a technique in which the difference in the rate of heat flow (or power) to the sample and to the reference sample is monitored against time while the samples are exposed to a temperature program [50].

The instrument is a differential scanning calorimeter, and the main components of any DSC instrument are shown in **Figure** 7.

The purpose of differential scanning calorimetry is to record the difference in the enthalpy change that takes place between the sample and an inert reference material as a function of temperature or time, when both are subjected to a controlled temperature program. The sample and the reference standard are housed in two identical wells that are heated by independent resistances. This makes it possible to use the principle of "zero balance" of temperature. When a thermal transition occurs in the sample (a physical or chemical change that results in a release or absorption of heat), thermal energy is added to either the sample or the reference, in order to maintain both at the same temperature. Because the thermal energy is exactly equivalent in magnitude to the energy absorbed or released in the transition, the energy balance provides a direct calorimetric measurement of the energy of the transition. The energy difference required to maintain the two sample cells at the programmed temperature is the quantity that is represented as a function of

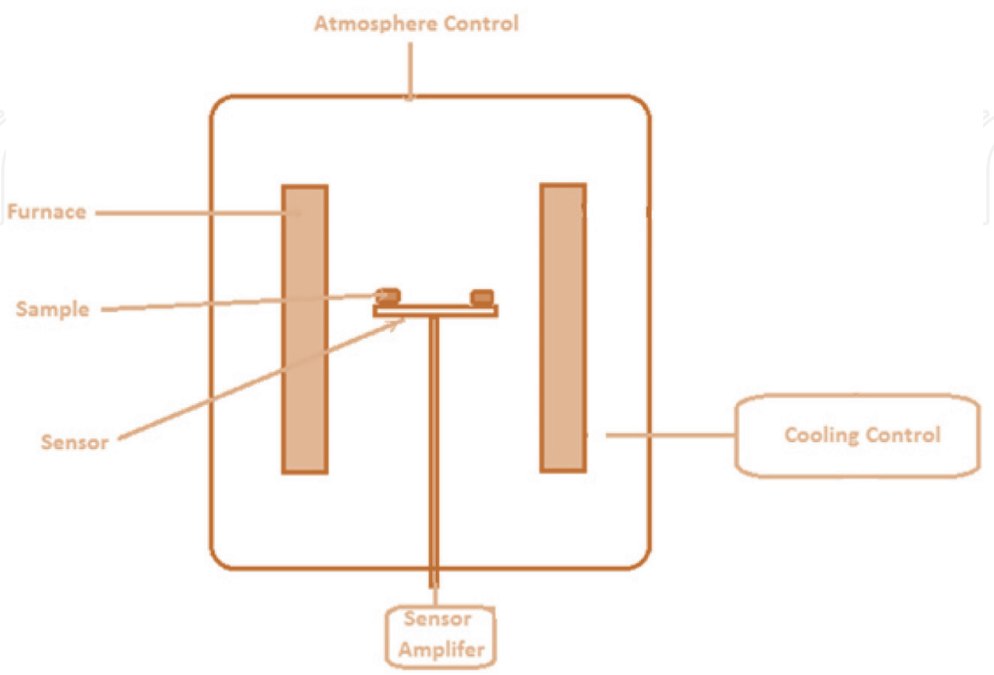

Figure 7.
DSC instrument.

temperature or as a function of time at constant temperature. These two representations are called thermograms [50, 51].

Figure 8 shows the most important events that occur when a synthetic polymer is measured by DSC. These are often characteristic of a substance and serve as a fingerprint, allowing them to be used for quality control [51]. The typical first heating curve of polymers as it shows is the glass transition, cold crystallization, and fusion. The vitreous transition exhibits enthalpy relaxation, which is shown by the overlapping endothermic peak. The latter occurs when the sample has been stored for a long time at a temperature below the glass transition. Cold crystallization occurs when the sample cools rapidly and does not have time to crystallize during the cooling phase. The DSC curve can also be used to determine the specific heat capacity, Cp. A general scheme of a thermogram is shown for a typical semicrystalline polymer, which has been rapidly cooled down to a temperature below its T_g (glassy temperature), the thermogram then being obtained at a certain rate of heating [51].

3.1.1.1 Process in this work

In the differential scanning calorimetry technique, two capsules are available. One of them contains the sample to be analyzed, and the other one is left empty and is the so-called reference capsule. 5 ± 0.5 mg of powdered pectin is weighed, previously ground with glass mortar, and it is placed in one of the aluminum crucibles which is inert. To both capsules an aluminum lid is added, so as not to contaminate the sample. The sweep gas is opened, which in this case is nitrogen with a flow rate of 50 ml/min, and the software is programmed at a sweep temperature with a progressive increase of 5°C/min until it reaches 400°C, while the team records the results for the subsequent analysis.

The measurement was made for each pectin that was obtained and compared to the analysis of a commercial pectin (see **Figure 9**).

According to DSC analysis, the endothermic property of pectin was affected by extraction temperature, while the exothermic property of pectin was only affected by its constituents and raw material [52] (**Table 1**).

The effects of extraction temperature and raw material on the thermodynamic properties of pectin were examined by DSC between 20 and 400°C. As shown in **Figure 9**, an endothermic peak and three exothermic peaks were observed in the DSC thermograms of all pectin samples [52]. The parameters of the peaks were listed in **Table 2**, such as glassy temperature (T_g), melting temperature (T_M),

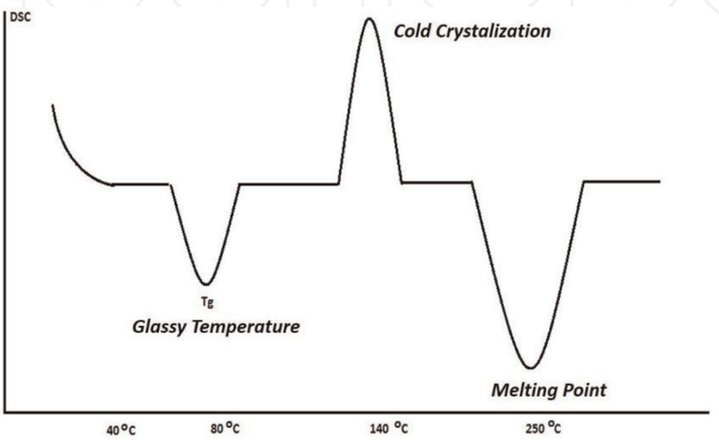

Figure 8.
Main effects measured in DSC using the polyester sample. Temperature range 30–300°C; heating rate 20 K/min; purge nitrogen gas at 50 ml/min.

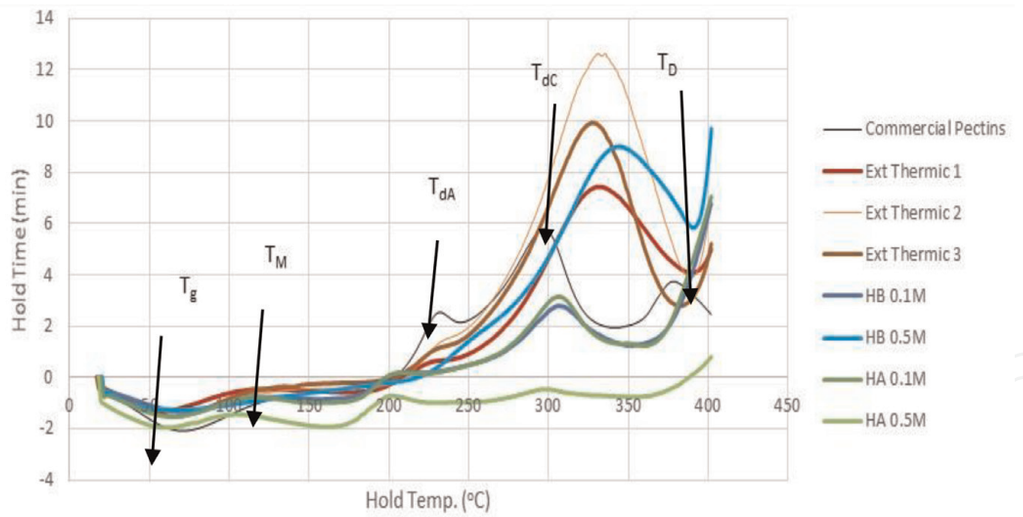

Figure 9.
DSC of pectin produced in this work.

Pectin type	T_g (°C)	T_M (°C)	T_{dA} (°C)	T_{dC} (°C)	T_D (°C)
Commercial pectin	70.81	119.5	234.73	299.63	380.89
HA 0.1 M	62.54	101.3	199.73	309.10	—
HA 0.5 M	58.90	101.8	200.00	300.00	—
HB 0.1 M	69.83	100.7	205.20	308.11	—
HB 0.5 M	69.85	101.6	183.27	342.48	—
Ext. thermic 1	59.74	124.0	223.48	334.17	—
Ext. thermic 2	68.81	133.1	236.45	336.66	—
Ext. thermic 3	68.89	128.7	230.85	332.81	—

Table 1.
Data of DCS of pectin obtained produced in this work.

Group	Band (cm^{-1})	Type	Mode
O-H	3600–3100	Stretching	—
C-H	3000–2800	Stretching	—
Pyranose	1200–950 1149, 1104, 1076, 1052, 1019, and 1016	Resonant absorption energy	Cycle vibrations
COO^-	1617 and 1384	Stretching	Asymmetrical and symmetrical stretching vibrations
COOH	1750	Stretching	Mode non-ionized methylated or protonated carboxyl
COOMe	1750	Stretching	Idem
R-O-R	1200–1100	Absorption ether and pyranosic ring	—
C-C	1200–1100	Stretching	Idem
Galacturonic acid	1120–990		Typical pectin group

Table 2.
FTIR signals of pectin.

deacetylation temperature (T_{dA}), decarboxylation temperature (T_{dC}), and degradation temperature (T_D).

As can be observed in both the figure and the table, the different hydrolyses affect the thermal parameters of the obtained pectin. Analyzing the glass transition temperature data, the T_g of the obtained pectins was less than the T_g of commercial pectin, the T_g for HA 0.5M decreases substantially due to a strong deacetylation of the pectin, on the other hand for the basic hydrolysis it approaches to the value of commercial pectin, this is due to the incorporation of sodium cation as pectinate salt. T_M was very similar for basic and acid hydrolyses but less than the commercial pectin. Thermal hydrolysis resembles the T_M value with respect to commercial pectin. The deacetylation temperature varies from 183.27 to 236.45°C; these values depend on many experimental variables during the hydrolysis, which ultimately results in less or more acetyl groups released. Decarboxylation is another thermal variable intimately related to the previous one and varies from 299.63 to 336.66°C. DSC thermal studies of pectin with other components can be found in the following references [53–58].

3.1.2 Thermogravimetric analysis

TGA is a technique in which the change in the mass of the sample is analyzed while the sample is subject to a change in temperature. The usual thing is that there is a loss of weight; however, it is also possible that there is a gain in weight in some cases. It is controlled in a specific atmosphere. The atmosphere can be static or dynamic with a given flow rate (reduced pressure conditions are also used), and the most common gases are nitrogen, air, argon, and carbon dioxide. Hydrogen, chlorine, and azure dioxide are also used. A fundamental characteristic of the TGA technique is that it only allows to detect processes in which a weight variation occurs such as decompositions, sublimations, reduction, desorption, and absorption while not allowing to study processes such as mergers and phase transitions [49].

As a result of the thermogravimetric analysis, the mass change data are obtained with respect to the temperature or time and a thermogram, which graphically represents the percentage variations of the mass. It should be clarified that this method does not allow to know the chemical composition of the material under study or to identify the thermal changes that are not associated with mass variations such as crystallization or glass transition [50].

Most TGA curves are produced by weight loss, whose main reason is usually [49]:

- Chemical reactions (decomposition and separation of water of crystallization, combustion, reduction of metal oxides)

- Physical transformations (evaporation, vaporization, sublimation, desorption, desiccation)

3.1.2.1 Process in this work

A TG 2950 equipment was used, TA Instruments, Inc., New Castle, USA. The samples are loaded in aluminum cells sealed with a lid of the same material that prevents contamination of the oven in case of dilatation or decomposition of the sample. Each of the pectin obtained was ground with a glass mortar until it reached a fine powder. Here 5 ± 0.5 mg of sample was used and placed in a platinum crucible, as in the DSC technique (of aluminum or platinum). Then, the equipment was started. The scavenging gas was also nitrogen at 50 ml/min, and previously a purge gas was also used, which was allowed to flow before the start of the experiment. The latter was used to remove gases that may damage the sample and also

nitrogen, with a flow of 30 ml/min. As in DSC, the software is programmed at a sweep temperature with a progressive increase of 5°C/min up to 400°C. This experience is also performed for each obtained pectin and commercial pectin, with which it will be compared.

All of the samples presented a characteristic three-step thermal degradation, typical of pectin. In pure pectin, the first step, occurring at about 80°C, corresponds to the water loss; then, it is followed by the second step, between 200 and 400°C (see **Figures 10–12**). In this temperature range, it has been reported that the degradation is primarily derived from pyrolytic decomposition. It consists in a primary and secondary decarboxylation involving the acid side group and a carbon in the ring [59].

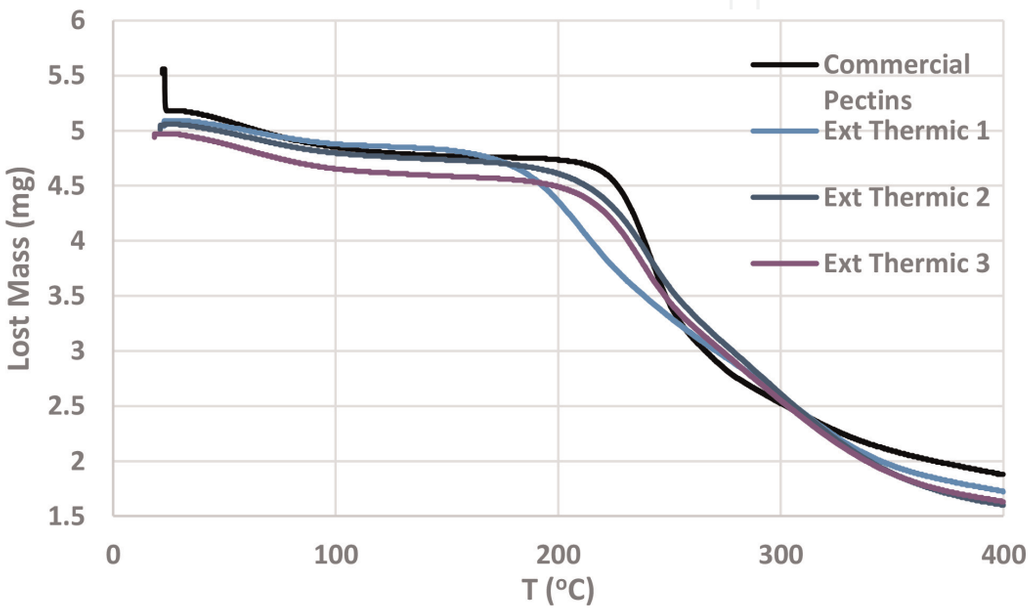

Figure 10.
TGA of commercial pectin and pectin obtained by thermic extraction.

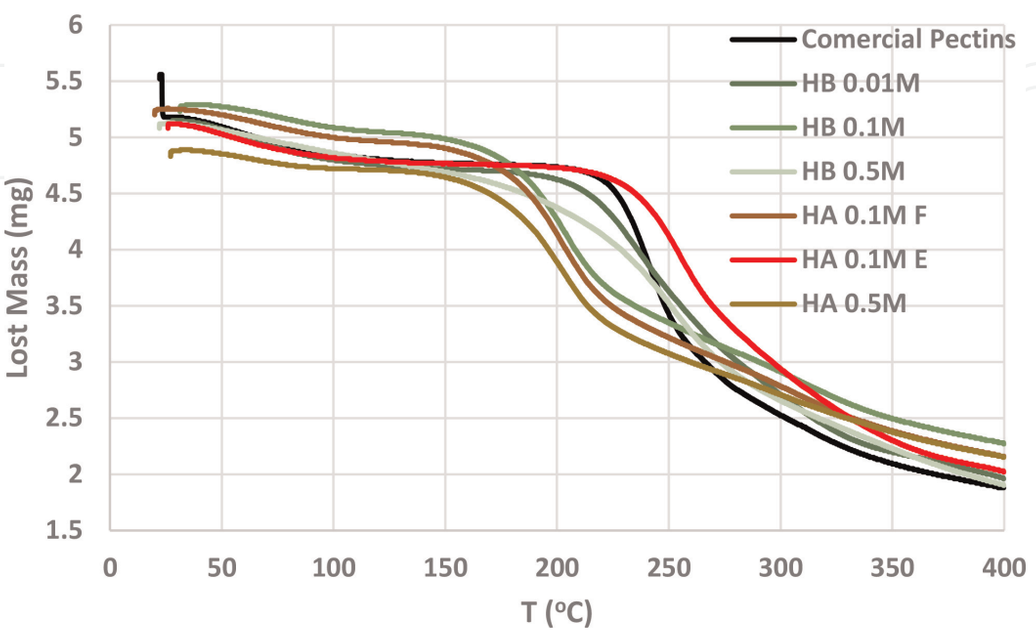

Figure 11.
TGA of commercial pectin and pectin obtained by acid/basic hydrolysis.

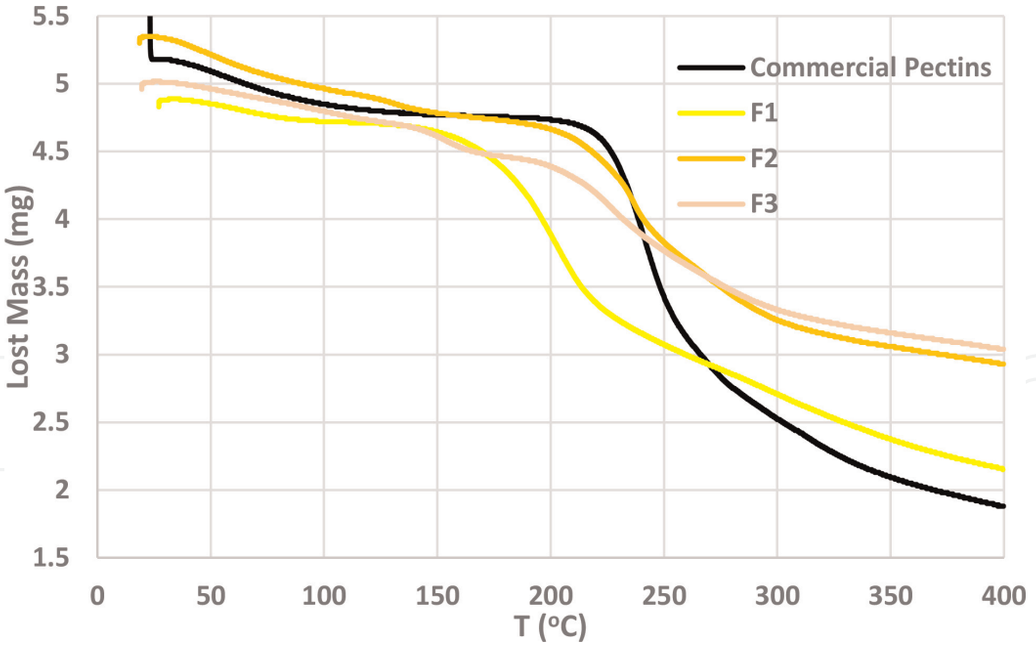

Figure 12.
TGA of commercial pectin and pectin obtained by acid hydrolysis in different fractions.

Moreover, the hydrolysis produces an increase in the evaporation temperature, going from 80°C for commercial pectin to higher values for all the hydrolyzed pectins. The water evaporation step was greatly reduced in these samples, indicating that a very small quantity of water was absorbed. The second degradation stage was very similar for either the pure or the modified pectin samples, although a slightly lower mass loss and a lower midpoint temperature were observed for the modified samples. The third stage, due to oxidative reactions, was postponed, particularly for linoleic residues, as expected for the oxygen scavenging effect of the double bonds [59]. To know more about different thermogravimetric studies of pectins with other compounds, the following references [60–69] can be read.

3.2 Infrared spectroscopy (FTIR)

Infrared spectroscopy is a technique of chemical and structural analysis since it allows the rapid identification of functional groups that are part of an organic and inorganic molecule, being able to perform not only qualitative but also quantitative determinations. This procedure is based on the Michelson interferometer (initially developed to accurately determine the speed of light) and the method of the French mathematician Fourier that allows to convert the obtained information (interferogram) into a spectrum. The infrared region of the electromagnetic spectrum extends between the visible zone and the microwave zone.

The most useful practical section of the extensive IR region is that between 4000 and 650 cm^{-1} called the middle infrared region. The use of the far-infrared (NIR) region, between 650 and 200 cm^{-1}, has expanded considerably in recent decades, especially for the study of organometallic or inorganic compounds (heavy atoms, weak bonds). The near-infrared (NIR) region, between 12,500 and 4000 cm^{-1}, accessible to quartz optics, has been used for quantitative determinations rather than structural purposes. The existence of these characteristic bands of functional groups allows a wide use of IR spectroscopy in the structural determination as well as in the identification of molecular interactions, formation of new bonds, and monitoring of reactions, among others [70, 71].

The most relevant characteristics of this spectroscopy are the following [71]:

1. If two molecules are constituted by different atoms or have different isotopic distributions or configurations or are in different environments, the infrared spectra will be different.

2. A defined substance can be identified by its infrared spectrum. These spectra can be considered as the fingerprints of said substance.

3. The spectra show bands that are typical of particular functional groups and that have specific locations and intensities within the infrared spectra.

4. Molecular structures can be inferred from the spectra. For this a model is required on which to base the calculations.

5. The intensities in the spectrum bands of a mixture are generally proportional to the concentrations of the individual components. Therefore, it is possible to determine the concentration of a substance and perform analysis of samples with several components.

3.2.1 The Fourier-transform infrared spectrometer (FTIR)

A Fourier-transform spectrometer consists of three basic elements: a light source, a Michelson interferometer, and a detector [71].

Its operation is as follows: a collimated beam, coming from a source that emits throughout the infrared region, hits a beam splitter. The incident beam is divided into two perpendicular beams of equal energy, one of which falls on the moving mirror and the other on the fixed mirror. The beams are reflected by both mirrors and recombine upon reaching the beam splitter. This results in an interference, which can be constructive or destructive depending on the relative position of the moving mirror with respect to the fixed mirror. The resulting beam passes through the sample, where a selective absorption of wavelengths occurs and finally reaches the detector. The information collected by the detector is used to obtain the interferogram, which is digitized. A computer develops the approximate calculation of the Fourier transform of the interferogram [71].

Solid materials generally exhibit too much absorption to allow direct transmission of infrared radiation. Only in some cases can very thin films of the material be obtained that allow, without mixing it with others, to obtain a spectrum by transmission. In solids in the form of dust, in addition to the problem of absorption, another occurs: much of the radiation transmitted is scattered. Since the dispersion is proportional to the difference between the refractive indices, some improvement is obtained by placing the finely pulverized solid in a liquid medium whose refractive index coincides with that of the substance. The medium serves at the same time as a diluent. Often hydrocarbon oil (nujol), or oil from a fluorocarbon polymer (fluorolube), is used for this purpose; both have their own absorption bands. To prepare this sample sprayed in oil, milligrams of the powder are ground in a drop of oil until a very fine paste is obtained, which then spreads like a thin film between two layers of sodium chloride (NaCl). Another technique for preparing diluted solid samples is the potassium bromide tablet (KBr) method. The sample is milled with powdered potassium bromide and then compressed in a die, at a pressure of 700 kg cm^{-2} ($\sim 7 \times 10^7$ Pa), in a hydraulic press. The thin disk thus formed is sufficiently transparent and only shows appreciable dispersion at wavelengths less than 10 μm. Since potassium bromide is hygroscopic, and it is almost impossible to

eliminate atmospheric water, in the samples prepared by this method, the OH band is always observed. Other materials than potassium bromide can be used; sometimes polyethylene powder offers advantages [71].

3.2.1.1 Process

The technique used was the preparation of the sample with potassium bromide. A pinch of pectin, primarily ground in a glass mortar, was mixed with potassium bromide. The resulting mixture was compressed through a hydraulic press, and a thin pellet was formed, which is placed in the spectrometer to perform the corresponding analyses.

FTIR spectra provided structural information of pectin in the region between 4000 and 400 cm^{-1}, where the major chemical groups in the pectin were identified (see **Figures 13–15** and **Table 2**).

For the pyranose cycle vibration region, one should note almost identical spectral parts with bands at 1149, 1104, 1076, 1052, 1019, and 1016 cm^{-1} characteristic for peptic substances. The band at 1750 cm^{-1} is assigned to stretching C=O mode non-ionized methylated or protonated carboxyl. Ionization, i.e., the formation of

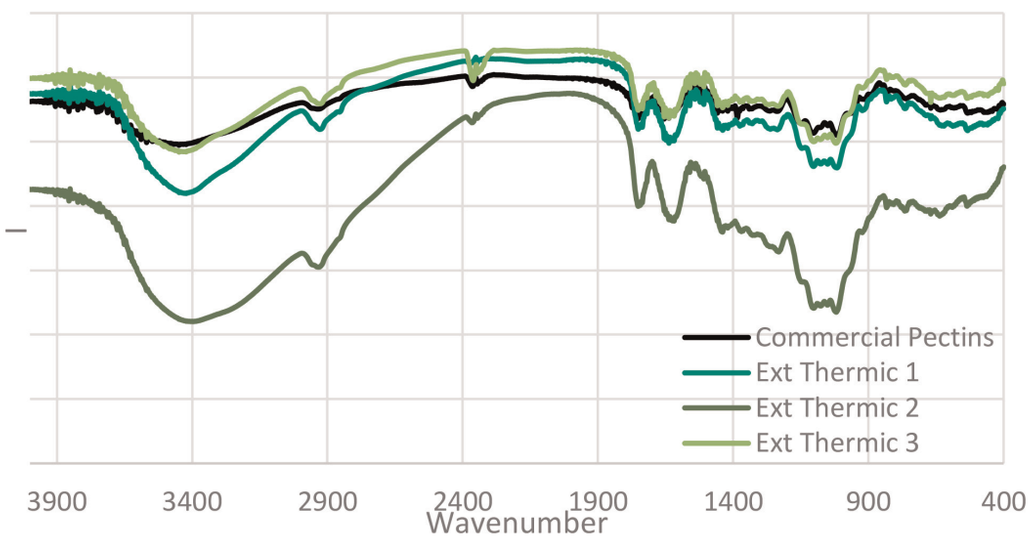

Figure 13.
FTIR of pectin thermic extractions.

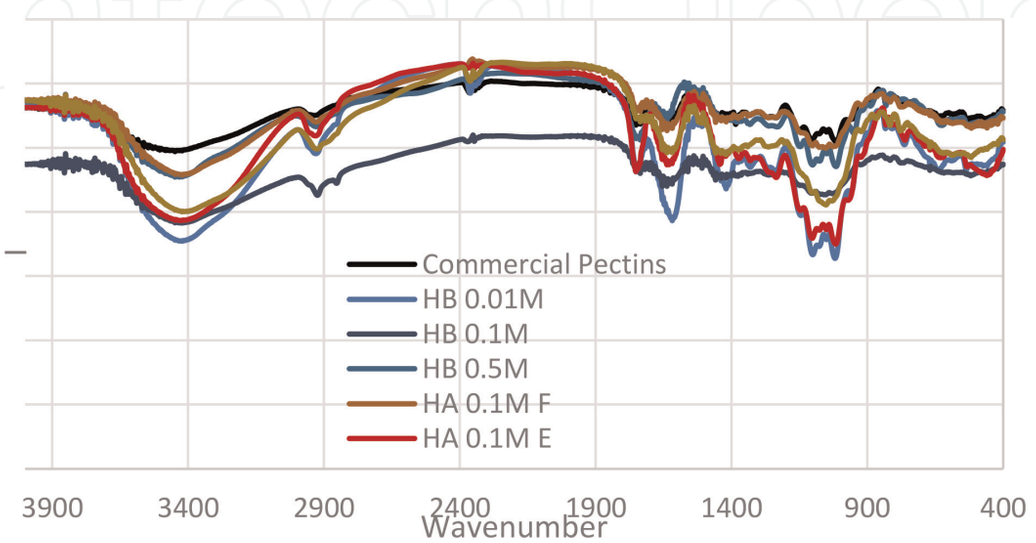

Figure 14.
FTIR of pectin obtained by acid/basic hydrolysis extractions.

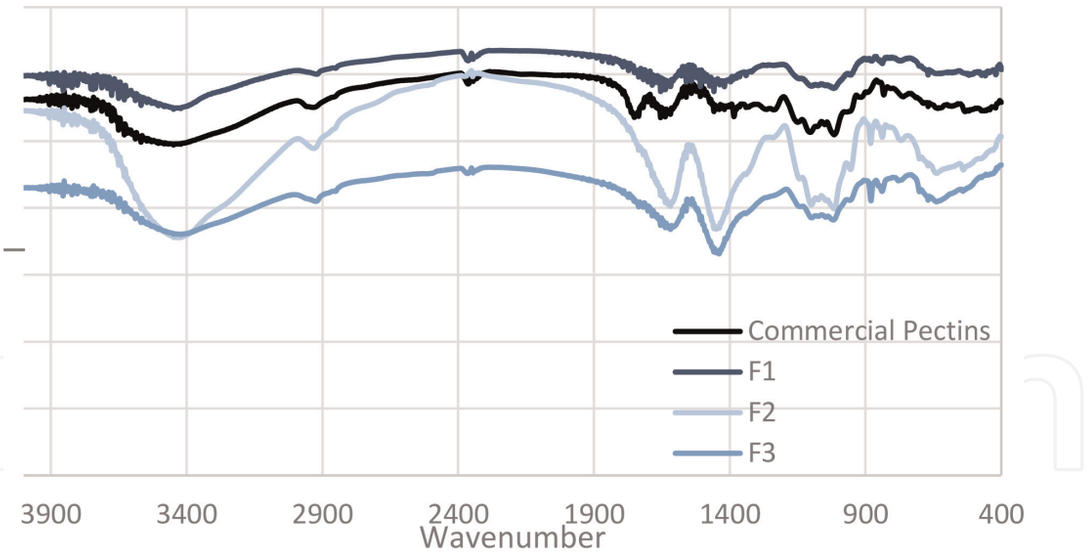

Figure 15.
FTIR of pectin fractions.

salts, leads to its disappearance, and two new bands appear due to asymmetric and symmetric stretching modes of COO^- at 1650–1600 and 1450–1400 cm^{-1}, respectively. The absorption bands between 1200 and 1100 cm^{-1} were from ether (R-O-R) and ring C–C bonds in pectin molecules. The selection of the most important wave numbers, by two independent chemometric techniques, allowed to define the region between 1120 and 990 cm^{-1} as the range for the spectral identification of galacturonic acid in peptic polysaccharides. There are two bands in the quince pectin spectrum within this region, a major one centered at 1617 cm^{-1} and a less intense one at 1384 cm^{-1}. These two bands correspond, respectively, to asymmetrical and symmetrical stretching vibrations due to the COO^- group of polygalacturonic acid. The absorbances at 1104 and 1000 cm^{-1} are the galacturonic acid, because all peptic polysaccharides are characterized mainly by these peaks (see **Table 2**) [72–76].

In general traits, hydrolyzed pectins have a greater degree of affinity for water due to the -OH groups that increase greatly; this may also be due to the fact that the flours were not dried correctly, but even so a slight and positive tendency can be observed. Another positive aspect is that the hydrolyzed pectins have an increase in the C=O bands because they possess a more marked degree of acetylation, although it cannot be calculated analytically. The typical galacturonic acid signal in 1618, 1388, and between 1000 and 1105 cm^{-1} is observed and specifically characterizes the pectin. On the other hand, there are no substantial differences between commercial pectin and the different hydrolyzates obtained, neither between these and other pectins reported in the literature [77–79] (see **Figures 13–15**).

4. Characterization of pectin solutions

4.1 Densimetry

The measurement of the density is a complementary measure to the viscosity and allows to calculate the partial specific volume and together with the viscosimetry allows to determine the hydration value of the macromolecule in solution.

The density data of the hydrolyzed pectin solutions have a $\partial\rho/\partial c$ ratio similar to that of pectin commercial but with different order at the origin. This phenomenon

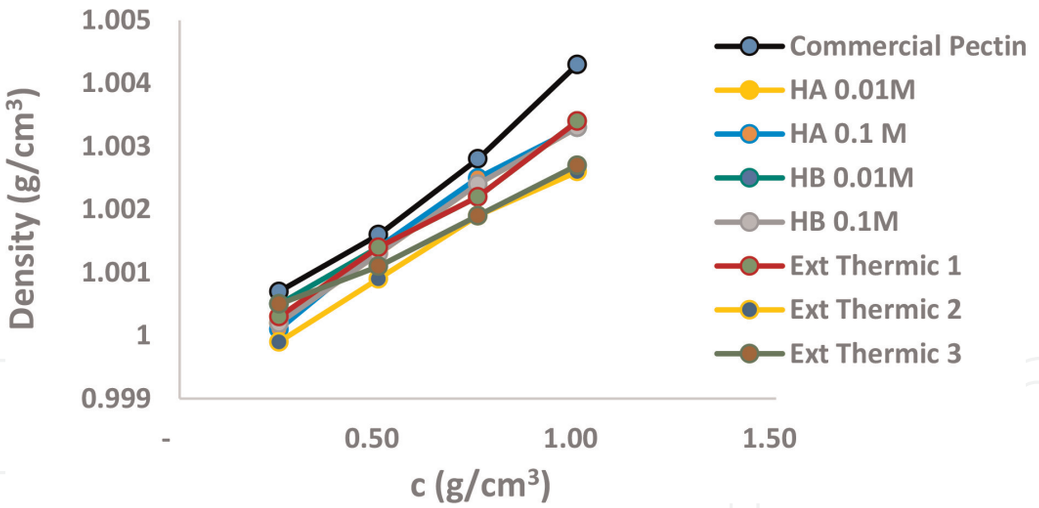

Figure 16.
Density data of pectin solutions.

is attributed to the fact that the commercial pectin has a molecular weight superior to that of the different hydrolysates, which ultimately translates into a greater capacity to incorporate water in its polymeric structure, that is to say it is more hydrophilic than the rest (see **Figure 16**).

4.2 Viscosimetry

Viscosity is one of the most important properties of polymer solutions. The viscosity depends on the chemical structure of the polymer, the interactions with the solvent, and the molecular weight. Normally, a molecule of high molecular weight in a good solvent acquires a large hydrodynamic volume, and the viscosity of the solution increases [80].

The viscosimetry of diluted solutions is related to the measurement of the intrinsic ability of a polymer to increase the viscosity of a solvent at a given temperature and is useful for obtaining information related to the size and shape of the polymer molecules in solution and the polymer-solvent interactions. In the diluted regime, the viscosity of a polymer solution (for very low polymer concentrations) is determined relative to the viscosity of the solvent. The following terms are defined in these cases:

$$\text{Relative viscosity, } \eta_r = \frac{\eta}{\eta_0} \tag{1}$$

where η is the viscosity of the polymer solution and ηo is the viscosity of the pure solvent.

$$\text{Specific viscosity, } \eta_{sp} = \eta_r - 1 = \frac{\eta - \eta_0}{\eta_0} \tag{2}$$

$$\text{Reduced viscosity, } \eta_{red} = \frac{\eta_{sp}}{C} \tag{3}$$

where C is the concentration of the polymer. This is a measure of the ability of the polymer to increase the viscosity of a solvent.

$$\text{Inherent viscosity, } \eta_{inh} = \frac{\ln \eta_{red}}{C} \tag{4}$$

Even in highly diluted solutions, polymer molecules are capable of forming intermolecular interactions. The two contributions to reduced viscosity are the movement of the isolated molecules in the solvent and the interaction between the polymer molecules in the solution. To eliminate the interactions, it is necessary to extrapolate to zero concentration to obtain the inherent and reduced viscosities commonly known as intrinsic viscosity:

$$[\eta] = (\eta_{red})_{c\to 0} = (\eta_{inh})_{c\to 0} \tag{5}$$

The intrinsic viscosity has the units of mass / volume, it is a measure of the size of a molecule in solution, based on the ability of a polymer molecule to increase the viscosity of a solvent in the absence of intermolecular interactions [80].

The most common equations for evaluating the intrinsic viscosity are the Huggins and Kraemer equations, given by Eqs. (6) and (7), respectively. The most usual procedure for determining the intrinsic viscosity is to determine the relative viscosity for different concentrations of polymer and to represent the data using Eqs. (4) and (5) and then calculate the value at zero concentration. **Figure 15** shows a typical plot of this type of data [80]:

$$\frac{\eta_{sp}}{c} = [\eta]_H + k_H\,[\eta]_H{}^2 c \tag{6}$$

$$\frac{\ln \eta_r}{c} = [\eta]_k + k_k\,[\eta]_k{}^2 c \tag{7}$$

Mark and Houwink, M-H, independently correlated the intrinsic viscosity with molecular weight, and this equation is applicable to many polymers and biopolymers and is used to determine molecular weight. The *k* and *a* parameters both vary with the nature of the polymer, temperature, and solvents. The calculation of M-H parameters is carried out by the plot representation of the following equation:

$$\ln [\eta] = lnk + alnMW_v \tag{8}$$

The exponent *a* is a function of polymer geometry and varies from 0.5 to 2.0. These constants can be determined experimentally by measuring the intrinsic viscosity of several polymer samples for which the molecular weight has been determined by an independent method (e.g., diffusion, osmotic pressure, sedimentation equilibrium, and light scattering).

4.2.1 Process

A capillary viscometer Ubbelohde IVA 1C of glass was used (**Figure 17**) where the time required by the two fluids to run between two marks in a capillary was recorded. Therefore, a digital chronometer was also required to take the runoff time, and an Anton Paar DMA 35 M density meter was used to determine the density of water and solutions.

Diluted solutions of the different pectins were prepared with distilled water at 0.25, 0.50, 0.75, and 1% wt. Each of them was stirred for a few minutes, under temperature conditions (50°C), to form a homogeneous solution. And before starting the measurements, it was allowed to cool to approximately room temperature.

The runoff time of each solution was taken by loading the viscometer, starting with the most diluted and continuing with the most concentrated. Previously the runoff time of the water that was used was taken. All measurements were made in triplicate to calculate an average. In addition to the times, density and temperature

Figure 17.
Ubbelohde capillary viscometer.

are measured for each solution and for the solvent, to then perform the corresponding calculations, taking into account these variables.

The intrinsic viscosity is obtained at the intersection of the line with the axis of the ordinates, that is, when the concentration tends to zero.

The pectin commercial has an intrinsic viscosity of 284.14 cm^3/g, which is lower than other data reported in previous studies [81, 82]; this is due to the fact that there are different hydrolyses and pectin purification treatments.

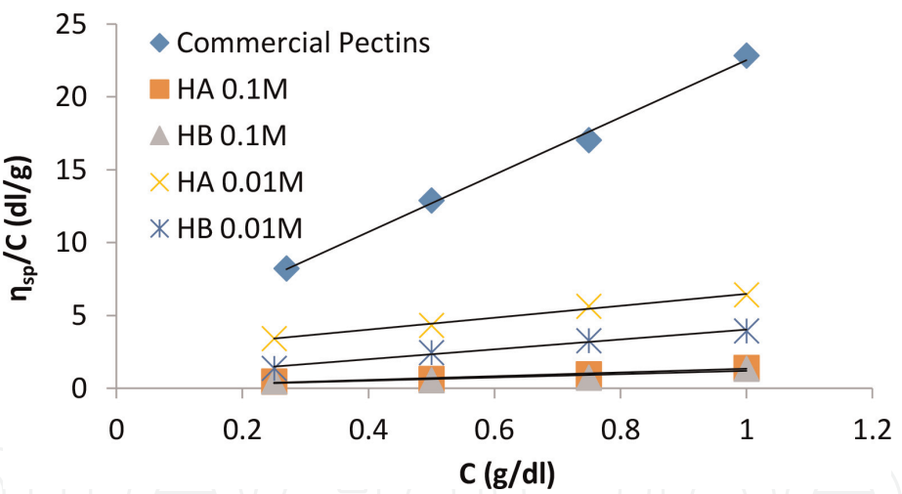

Figure 18.
Evaluation of the intrinsic viscosity for pectin solutions at 25°C.

Hydrolysis	c (mol/L)	[n] (cm^3/g)	k_H	M (g/mol)	R_H (nm)	ṽ	δ (g/g)	h
Commercial pectin		284.14	19.69	92928.83	12.12	0.51	26.48	3.75
Acid	0.01	237.93	4.10	74883.61	10.63	0.57	22.03	3.41
	0.1	144.88	3.01	40956.73	7.37	0.64	13.12	2.78
	0.5	7.79	1.25	1169.89	1.85	0.73	0.03	1.01
Basic	0.01	187.34	2.05	55988.91	8.91	0.58	17.22	3.13
	0.1	62.86	3.39	14832.63	3.98	0.51	5.46	2.27
	0.5	7.25	1.11	1072.00	1.25	0.49	0.20	1.12

Table 3.
Hydrodynamic data of hydrolyzed pectins (for data, read references [81, 82]).

As for the different hydrolysates, both basic and acid, the intrinsic viscosity data are lower than the data of commercial pectin, and in turn these decrease with the increase of acid or base added to the hydrolysis. The hydrodynamic radius (R_H) and the hydration value (δ) decrease for the same reason in addition to the fact that the molecular weight also decreases. Another aspect not less is that for the same hydrolysis, but different reagent, be it hydrochloric acid or sodium hydroxide, the salt formed in the latter is more soluble in water. A consistent phenomenon is the increase of the partial specific volume ($\tilde{v}$) and is reasonable due to the increase of the hydration value. Finally, it can be said that K_H values show that water is a good solvent for pectins (see **Figure 18** and **Table 3**).

5. Film synthesis and characterization

5.1 Film synthesis

The technique used for the preparation of the membranes is the "casting" method. This consists of depositing a polymer solution on a mold or support of a given material, allowing it to evaporate at room temperature, or under some air flow or N_2, to obtain a membrane with a certain thickness and a surface area. The polymer is first dissolved in a solvent to form the solution, and then some other compounds, such as plasticizers, antioxidants, stabilizers, etc., are added to the polymer solution and dispersed by agitation. The size of the container and the polymer concentration will determine the thickness and length of the membrane; it is necessary that in the molding there are no bubbles and no bubbles are generated during the solvent evaporation process. Another important operative aspect for this procedure is the evaporation rate of the solvent and a strict control of the temperature. Subsequently, the membrane is molded by removing the solvent.

This method is the most used in the preparation of membranes at laboratory scale in order to study the influence of the composition and morphology of the materials on the behavior of the films. This technique has a great operational ease and is suitable for all types of soluble materials in a particular solvent. The concentrations of polymer and other added components can also be easily controlled.

5.1.1 Technique

Once the pectin flour was obtained in the previous step, a 2% by weight aqueous solution was prepared with 0.5% by volume of glycerin, stirring until total dissolution. The solution is then placed in a flat mold 10 cm in diameter and put into an oven at a temperature of 50°C for 24 h. Slow evaporation of the solvent is preferable in order to obtain a dense membrane. It is then demolded and placed between two glass plates for 48 h to avoid deformation of the surface of the membranes. In this last period, the solvent is finished evaporating.

Because the packages have multiple functions to which they are used to pack a great diversity of foods, their characteristics and properties are very varied. The materials used to make them are as different as paper, cardboard, plastics, glass, tin, aluminum, and combinations thereof. As for its shape, they are found as boxes, cans, bags, bottles, and movies, among others. The latter are defined as continuous, thin matrices that are structured around the food they protect. Most commercially used films are made with different types of plastics, which, besides being obtained from hydrocarbons, have the disadvantage of not being biodegradable [83–86].

A biodegradable packaging is defined by the ASTM as one that is capable of decomposing into carbon dioxide, methane, water, inorganic compounds, or

biomass, the dominant mechanism of decomposition being the enzymatic action of the microorganisms, and the resulting products can be obtained and measured in a given period of time [87].

The materials used for the production of biodegradable packaging can be polymers of natural origin (proteins, starch, lipids, and chitosan, among others) or of synthetic origin (i.e., polyhydroxyalkanoates and polylactic acid). Among the polymers of natural origin that are being used for the production of biodegradable films are pectins [87].

Plasticizers are normally used to facilitate the processing and/or increase the flexibility of the film. Water, some oligosaccharides, polyols, and lipids are different types of plasticizers widely used in films based on hydrocolloids. Their combination could lead to synergistic effects between the components improving the properties of the films. Various theories have been raised about how these compounds work. The theory of lubrication postulates that plasticizers intermix and act as internal lubricants by reducing the frictional forces between the polymer chains. The theory of the gel postulates that the rigidity of the polymeric network comes from its three-dimensional structure; then the plasticizers would act by breaking polymer-polymer interactions that are interposed between them. The theory of free volume postulates that the addition of plasticizers is a way to increase the free volume, reducing the interactions between the chains. All the proposed theories agree that the effects generated by the addition of glycerol are due to these small molecules that are located between the polymer chains [87].

The technique used for the formation of the films was the following: A sample of 1 g was mixed with 50 ml of distilled water and 0.5 ml of glycerin. It was stirred with mechanical agitator for half an hour at 40°C. The identified petri dish solutions were overturned, so that only the surface of the petri dish was covered. It was taken to the stove at 80°C for 48 h. The formed films were demolded and kept in sealed envelopes. After obtaining the films, tests were conducted to evaluate their properties against a commercial pectin.

5.2 Scanning electron microscopy (SEM)

This technique is used to inspect in a relatively easy way the morphological features of the membranes, especially since this technique is very suitable to evaluate if the film is dense or porous and the pore size distribution and elemental analysis (EDS) are a standard procedure to identify and quantify the elemental composition of sample areas of up to several cubic micrometers. The sample material is bombarded with electrons in a scanning electron microscope, and the X-rays produced are measured with an X-ray spectroscope. Each element has a characteristic wavelength through which it can be identified.

The images of an electron microscope are obtained by the detection, processing, and visualization of the signals resulting from the interactions between a high-energy electron beam and matter. These interactions can provide information on topography, composition, and crystallographic structure [87].

In a scanning electron microscope, the image is obtained from the signals emitted by the sample, and it is formed as the electron beam moves on a portion of its surface. This scan is performed line by line on a small area of rectangular shape (raster). This zone is the one that is visualized and amplified in the final image. As the beam explores the sample in this way, the intensity of the generated signal varies according to the particular point analyzed at each instant. You can thus obtain images of all types of structural materials or biological material with a minimum of previous preparation and directly observe all types of surfaces with a great depth of focus. This is a unique feature of electronic instruments that allows

obtaining micrographs in focus of irregular surfaces as a fracture surface. For this it is only necessary to ensure that the samples are clean, dry, resistant to high vacuum of the instrument, and good electrical conductors. If it is a question of observing a non-conductive material, the samples are usually coated with a thin metallic layer, as gold, or the samples that have high water vapor contents are previously dried [87].

The composition of the sample affects both the depth and the shape of the interaction volume. Denser samples (composed of heavy elements) tend to reduce the penetration of the beam and also reduce the distance that the signals generated can pass without being reabsorbed by the sample. The volume of interaction tends to be more squashed and similar to a hemisphere. On the contrary, in less dense samples, composed of light elements, the volume takes on the characteristic shape of a drop [87].

5.2.1 Equipment

All scanning electron microscopes consist of an electronic cannon in a high vacuum column, in the order of 10^{-5} mmHg, in which a high-energy electron beam (5–30 kV) is generated. This beam is collimated by a series of electronic lenses and focused on the sample analyzed. The detectors register the signals originated by the interaction between the electron beam and the sample, which are processed and visualized in the final observation system (monitor or computer screen). The electron gun is the first component of the microscope column and is the one that produces the electron beam. It consists of filament emitting electrons that are then accelerated by an anode positively polarized to a variable voltage between about 5 and 30 kV [87].

Interpretation of the images: the contrast of a micrograph in the secondary electron mode (emissive mode) comes from the variations in the topography of the sample. In effect, the voltage applied between the grid of the detector and the sample favors the collection of secondary electrons on sharp edges since the electric field is more intense there. More secondary electrons can be collected from a projection or step of the sample than from a depression or cavity. The projections then appear brighter than the depressions, which makes the interpretation of the micrographs immediate [87]. The equipment used to obtain the SEM images was a scanning electron microscope JOEL 1450VP.

The superficial images of the selected pectins (**Figures 19** and **20**) show that the commercial pectin (**Figure 21**) is more homogeneous with a morphology similar to

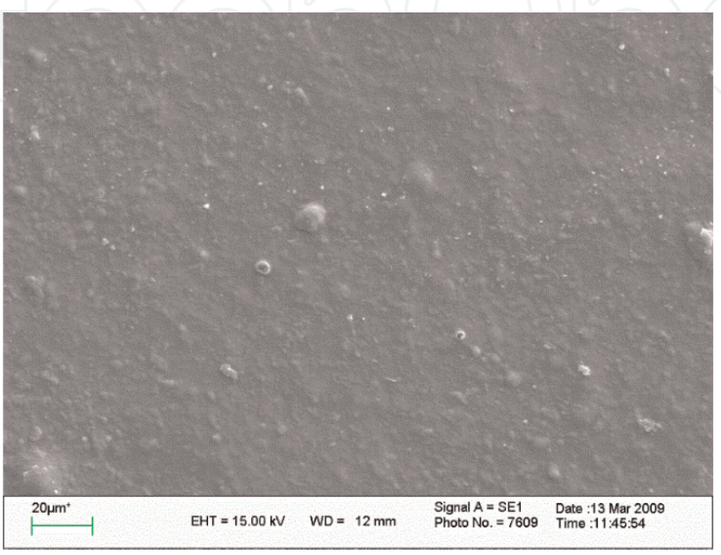

Figure 19.
SEM surface image 500× of HA 0.1 M membrane.

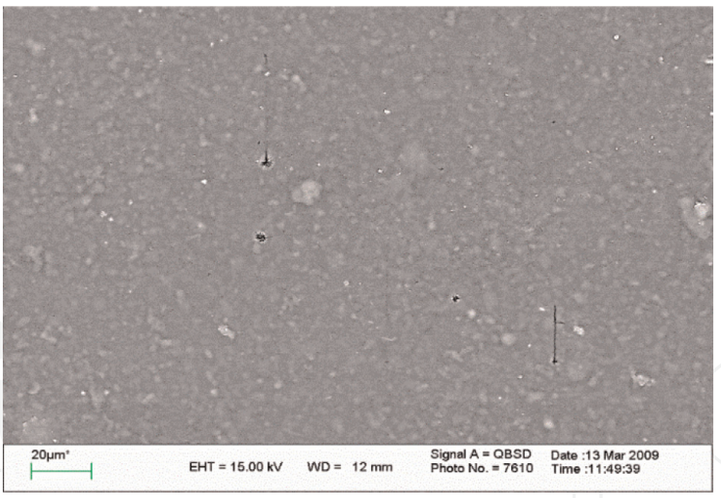

Figure 20.
SEM surface image 500× of HB 0.1 M membrane.

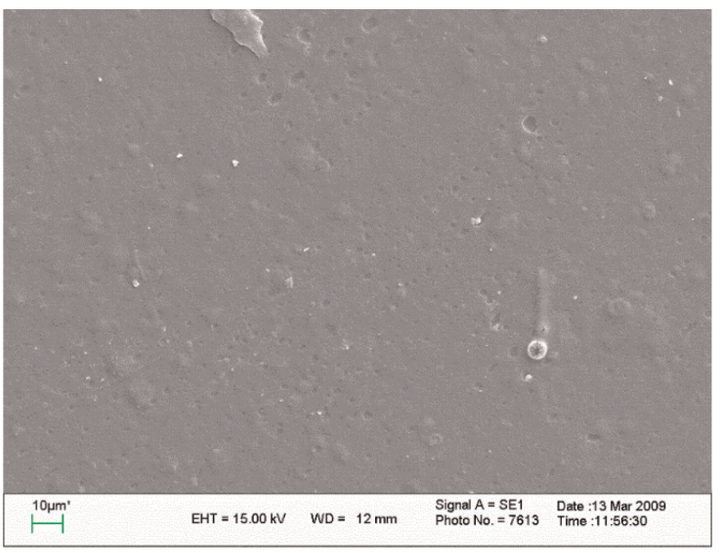

Figure 21.
SEM surface image 500× of commercial pectin membrane.

craters, valleys, and peaks (not acute). On the other hand, both acidic and basic hydrolysates are heterogeneous, where it should be noted that the pectin that the basic hydrolysis film has more rigidity which causes breakage on its surface due to the formation of the sodium pectinate salt.

5.3 Permeation

Unlike glass or metal packaging materials, packages made of plastic are permeable in different degrees to small molecules such as gases, water vapor and organic vapor, and other low molecular weight compounds such as flavorings and additives present in food. As a consequence of the barrier properties of the material, the transfer of this molecule varies from high to low. The knowledge of the behavior solution/diffusion/permeation of these molecules through the polymer film has become more and more important in recent years, especially for polymers used in the field of food packaging where external environmental contamination should be avoided and the shelf life of food controlled by the use of modified atmosphere packaging techniques (MAP). Many factors that can influence the performance of

polymer packages must be taken into consideration to design the correct package market solution [88].

Since contact with food can alter the performance of the polymer, it is important to study the characteristics of the material barrier under realistic conditions. For example, the absorption of vapor or ambient liquid can cause increased plasticization of the polymer, resulting in a decrease in mechanical properties. The purpose of this work is to give an overview of the state of the art of the permeability behavior of polymer packages used for food applications [88].

5.3.1 Theory of permeation

The diffusion of the permeate through a film is influenced by the structure of the film, the permeability of the film to specific gases or vapor, thickness, area, temperature, pressure difference, or concentration gradient through of the movie. Permeability, as reported in the literature, is defined as the quantification of permeate, gas, or vapor transmission, through a resistant material. Then, the concept of permeability is usually associated with the quantitative evaluation of the barrier properties of a plastic material. In a flawless material such as pores or cracks, the primary mechanism for the flow of gas and water vapor through a film or coating is an activated diffusion. This means that the permeate dissolves in the matrix of the film in the higher concentration part; diffuses through the film, driven by a concentration gradient; and evaporates from the other side of the surface. Differences in the solubility of specific gases can influence the diffusivity of the gases through the film. The second step, diffusion, depends on the size, shape and polarity of the penetrating molecule, and on the crystallinity, degree of cross-linking and segmental movement of the polymeric chain of the polymeric matrix. [88].

As for the theory, the penetration of gas through a polymer is described by a diffusion model, using the laws of Henry and Fick to obtain the expression that relates the rate of permeability with the area and the thickness of the film [88].

The mechanism can be described in a very simple way as in **Figure 22**, for a homogeneous polymer film with thickness l and permeable pressure p (with $p_1 > p_2$) and as the different concentration permeable through the film (with $c_1 > c_2$).

The flow of permeate (gas or vapor), indicated by J, is described by Fick's first law:

$$J = -D\,\Delta C \tag{9}$$

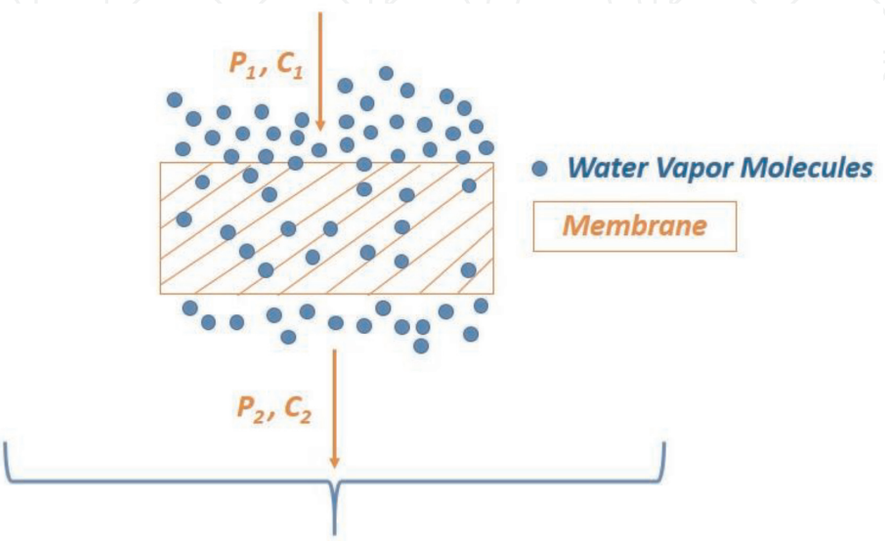

Figure 22.
General mechanism for water vapor permeation through a plastic film.

that, for the one-dimensional diffusion through a membrane polymer and in stationary conditions, it can be written as

$$J = -D\,\Delta C/l \tag{10}$$

where J is the diffusion flow (expressed in mol.cm^{-2} s^{-1}), D the diffusion or diffusivity coefficient (expressed in cm^2 s^{-1}), and ΔC the difference in concentration (expressed in mol cm^{-3}) through the thickness of the membrane l (expressed in cm). D reflects the rate at which the permeant diffuses through the polymer. When the mechanism of diffusion is in its stable state, the equilibrium of the concentration of gas c on the surface and the partial pressure of gas p obeys Henry's law. When the permeant is a gas, it is more convenient to measure the vapor pressure p (expressed in atm); then ΔC can be replaced by $S\,\Delta p$, where S (expressed in mol cm^{-3} atm^{-1}) is the solubility coefficient that reflects the amount of permeant in the polymer and Δp is the pressure difference in the entire film. Eq. (10) becomes

$$J = -D\,S\,\Delta p/l \tag{11}$$

The $D\,S$ product is indicated as a coefficient of permeability (or constant) or permeation coefficient or simply as permeability (PAG).

If S is independent of the concentration, that means a linear relationship between the concentration-distance through the polymer, the coefficient of permeability P can be defined as

$$P = -J\frac{l}{\Delta p} = -D\,S \tag{12}$$

The P/l ratio, indicated by q, is called permeance. The diffusion takes place only in one direction, through the film and not along or through it; in addition, the coefficients D and S are independent of the concentration of the permeate. This behavior of molecular diffusion in the polymer is indicated as Fick behavior. Obviously, as also reported by Robertson, there are many realistic cases where these hypotheses are not valid as, for example, when the steady state takes a long time to reach (as a glassy polymer) or when the coefficients D and S are correlated to the interaction between permeate and polymers, such as the interaction between water and hydrophilic film or similar solvent vapor that diffuses through polymer films. These cases are indicated as a non-Fick behavior [88].

5.3.2 Water vapor permeability

The concept of permeability is normally associated with the quantitative evaluation of the barrier properties of the material. If it does not have defects such as pores or cracks, the primary mechanism for the flow of gases or water vapor through the film is an activated diffusion. This means that the molecules enter the film matrix on the higher concentration side, diffuse through the film following the concentration gradient, and detach from the surface in the low concentration area. The second step of the phenomenon of permeability, diffusion, depends on the size, shape, and polarity of the penetrating molecule; it also depends on the crystallinity, degree of cross-linking, and movement of the polymer chains in the matrix of the film. The gaseous molecules are unable to permeate through the polymeric crystals since they are insoluble in them. Thus, gas permeation in semicrystalline polymers is confined to amorphous regions. The decrease in permeability in materials with a certain degree of crystallinity is due, then, to the smaller volume available for gas

penetration and to the long and tortuous trajectory that molecules must make between the crystals. The reduction in the permeability value is proportional to the volume fraction of the crystalline phase.

The permeation of a gas or vapor through the polymer is described by a diffusion model, using the Henry and Fick laws to obtain the expression representing the permeation rate taking into account the area and thickness of the film.

The mechanism can be described for a homogeneous polymer film of thickness λ, permeant pressure p (with $p_1 > p_2$), and permeant concentrations c through the film (with $c_1 > c_2$):

$$\tau = \frac{Q}{A * t}[=]\frac{ng}{m^2\, s} \tag{13}$$

$$P = \frac{\tau.\lambda}{\Delta P}[=]\frac{ng\ m}{m^2\, s\ Pa} \tag{14}$$

where τ is the transmission speed (ng/m^{2}s); Q, the permeating mass (ng); λ, the thickness of the film (m); A, the area of the cell (m^2); t, the measurement time (s); and ΔP, the pure water vapor pressure, in the case of calculating water vapor permeability (4238.605 Pa), or high pressure in the case of calculating gas permeation.

The pectin hydrolyzed with acid or base shows a lower permeability than the commercial pectin; the exception to the rule is the pectin extracted thermally (see **Figure 23**).

5.3.3 Water sorption

The properties of barrier to the steam of water for the packaging of alimentary products, in which the physical and chemical deterioration is related to his content of humidity in balance, are of big importance to maintain or extend his useful life. In fresh products, for example, it is important to avoid dehydration.

The ASTM E96 standard defines the water vapor permeability as the rate of water vapor transmission through a unit area of a flat material and per unit thickness induced by a unitary vapor pressure difference between the two surfaces of the material under study, under certain temperature and humidity conditions.

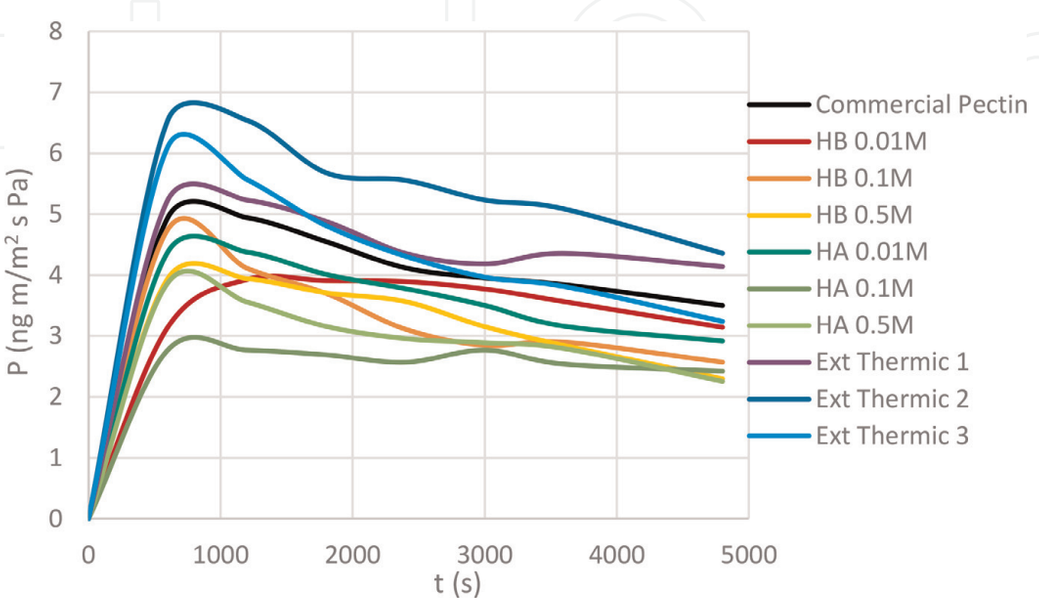

Figure 23.
Water vapor permeation of pectin membranes.

$$\%SW = 100\frac{w_{sw} - w_M}{w_M} \tag{15}$$

where %SW is the water sorbed percentage (% g/g), w_{sw} is the mass of water (g), and w_M is the mass of the membrane (g).

5.3.3.1 Instrument

The equipment has a hermetically closed chamber that inside has an automatic humidity and temperature controller, both variables controlled by sensors. The humidity range that this sensor can detect is from 0 to 100%, and the accuracy in the measurement is 2% when the relative humidity goes from 0 to 90% and 3% above 90% at 20°C. The temperature and humidity are regulated by turning on or off the controllers of both variables: a focus and a humidifier. In addition, a RADWAG balance with precision of 0.1 mg and a Kolve thickness meter with precision of 0.1 μm were used.

5.3.3.2 Process

From the formed films, samples of 25 mm in diameter were cut, their thickness was taken, and they were placed in the perforated caps of bottles with 20 g of silica gel inside each one. Each bottle is identified by a number that corresponds to a particular pectin. Before starting the experiment, each bottle with silica gel was weighed and the lid placed. Then, they enter the equipment, which must be found at 30°C and 85% humidity, and every 10 min the weights of each one are taken, for 1 h. Then, they are weighed every 20 min. until the cell maintains a constant weight.

In a first stage (see **Figure 24**), which corresponds to a first-order kinetics for water sorption, all the membranes comply, but in the zone of zero-order plateau, they behave in a different way keeping the order. The exception to the rule is the HB 0.5M membrane that corresponds to a pseudo first order and in the plateau area the absorption of water keep increasing, this phenomenon is due to the excess of sodium cations in the pectin (as sodium pectinate) that increase the adsorption of water, and this happens in several stages. The same phenomenon can be observed in the rest of the basic hydrolysis but to a lesser degree.

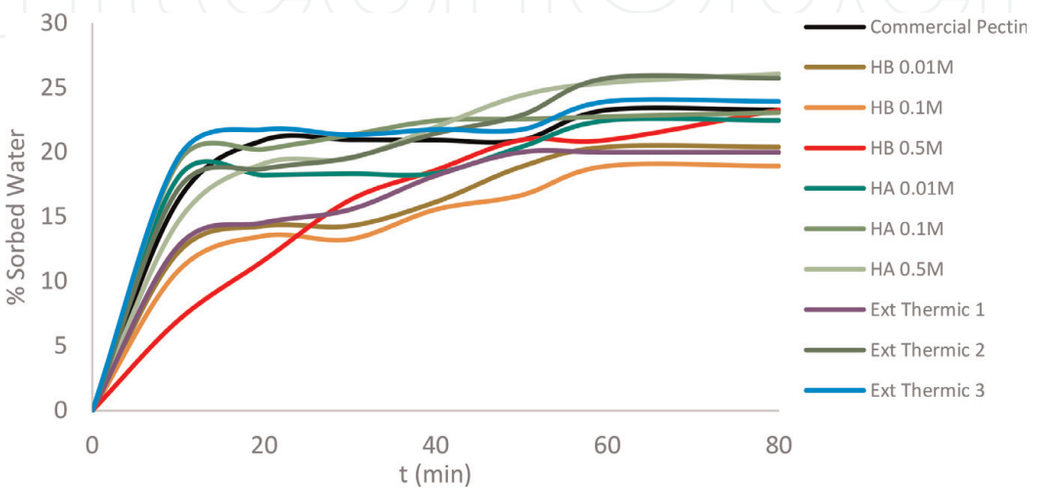

Figure 24.
Water sorption of pectin membranes.

5.4 Mechanical properties

When selecting a polymer to be used as a film, certain characteristics related to its mechanical properties must be considered to ensure that the material successfully fulfills its function. Some of them are tensile strength, elongation, impact resistance, sealing on the seals, resistance to tearing, easy opening of the container, etc.

Materials with the same chemical composition and other similar properties can have very different mechanical properties, depending on their microstructure. In addition, changes in temperature, the cyclical nature of the applied stresses, chemical changes caused by oxidation, corrosion or erosion, microstructural changes due to temperature, the effect of possible defects introduced during machining operations, or other factors can also have a great effect on the mechanical behavior of the materials.

The mechanical properties of a material describe the way in which it responds to the application of a force or load. The force, which acts on the unitary area, generates a deformation. In many materials, elastic stress and deformation follow a linear law. The slope in the linear portion of the strain versus strain curve defines the Young's modulus or elastic modulus of a material. The modulus of elasticity or Young's modulus (E) is the slope of the stress-strain curve in the elastic region (linear portion of the curve) and informs us about the fragility and ductility of the films made.

A material is selected by adapting its mechanical properties to the service conditions required for the component. The first step in the selection process requires that the application be analyzed, in order to determine the most important characteristics that the material must possess. Once the required properties are known, the appropriate material can be selected, using the information contained in the manuals [89]. Then, several tests that are used to measure the way in which a material resists an applied force will be studied. The results of these tests will be the mechanical properties of said material [89].

Tension test: the use of the stress-strain diagram is to measure the resistance of a material to a static or gradually applied force. A test device appears in **Figure 25**; a typical specimen has a diameter d and a calibrated length, l [89].

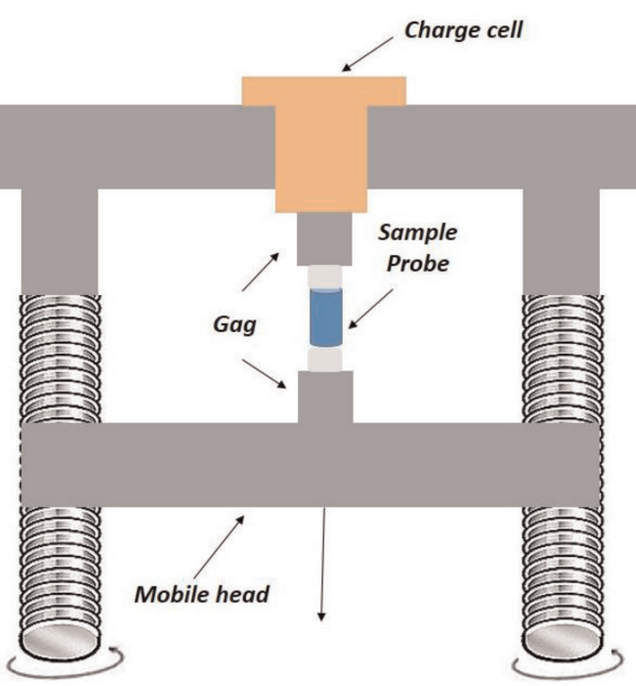

Figure 25.
Tensiometer model used in stress tests.

The test piece is placed between jaws and a force F is applied, which is known as load. To measure the elongation of the material caused by the application of force in the calibrated length, an extensometer is used [89] (see **Figure 25**).

1. Engineering effort and deformation: for a given material, the results of a single test are applicable to all sample sizes and shapes, if the force is converted into effort and the distance between calibrated marks in deformation. The stress σ and the engineering deformation ε are described by the following equations:

$$\sigma = \frac{F}{A} \tag{16}$$

$$\varepsilon = \frac{(l - l_0)}{l_0} \tag{17}$$

where A is the original cross-sectional area of the specimen before the start of the test, l_o is the original distance between calibrated marks, and L is the distance between them, after the force F has been applied [89].

2. Effort of yield: the effort or yield limit is the effort that divides the elastic and plastic behavior of the material. If you want to design a component that does not deform plastically, you must select a material with a high yield strength or make the component of sufficient size so that the applied force produces an effort that is below the yield strength.

3. Resistance to tension: the effort obtained from the highest force applied is the resistance to tension or maximum tension, which is the maximum stress on the engineering strain-deformation curve. In many ductile materials, the deformation does not remain uniform. At a certain moment, one region is deformed more than others, and a significant local reduction occurs in the straight section. This locally deformed region is known as a zone of stricture. Since the area of the cross section at this point becomes smaller, a smaller force is required to continue its deformation, and the engineering force, calculated from the original area A, is reduced. Tension resistance is the effort at which this stricture begins in ductile materials.

Elastic properties: the modulus of elasticity or Young's modulus, E, is the slope of the stress-strain curve in its elastic region. This relationship is Hooke's law:

$$E = \frac{\sigma}{\varepsilon} \tag{18}$$

This module is intimately related to the bonding energy of the atoms. A steep slope indicates that large forces are required to separate the atoms and cause the material to deform elastically. Therefore, the material has a high modulus of elasticity. The bonding forces and the modulus of elasticity are generally greater in materials with a high melting point (see **Figure 26**). The module is a measure of the rigidity of the material. A rigid material retains its size and shape [89].

The modulus of resistance (E_r), which is the area that appears under the elastic portion of the stress-strain curve, is the elastic energy that a material absorbs or releases during the application and release of the applied load, respectively.

1. Ductility: it measures the degree of deformation that a material can withstand without breaking. The distance between the calibrated marks in a test tube

before and after the test can be measured. The % elongation represents the distance that the test tube stretches plastically before the fracture:

$$\varepsilon = 100\frac{(l - l_0)}{l_0} \tag{19}$$

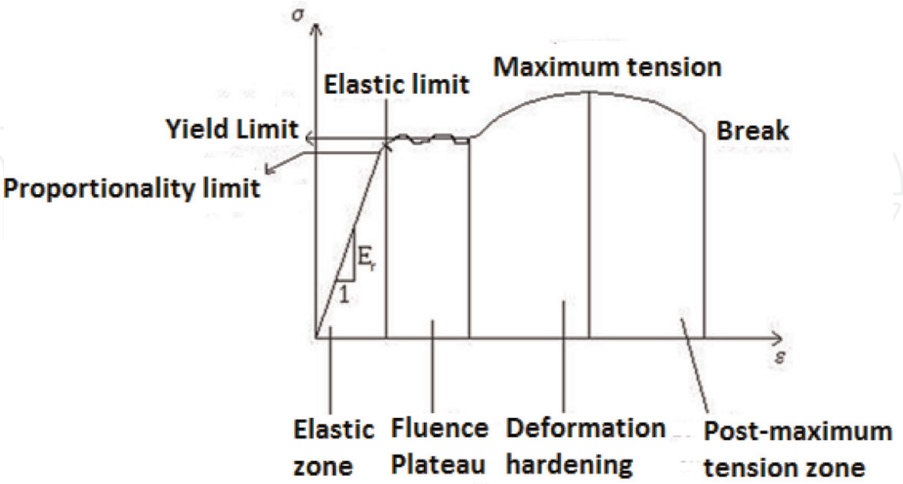

Figure 26.
Deformation diagram.

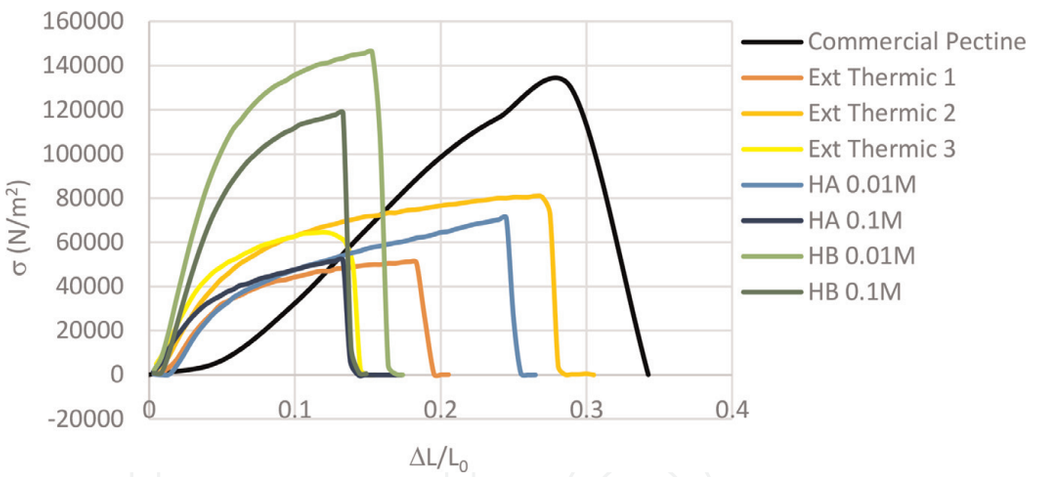

Figure 27.
Mechanical properties of pectin membranes.

Membrane	E (MPa)	ε_{max}	σ_{max} (MPa)
Commercial pectin	6.20	2.90	0.13
HA 0.01 M	0.94	0.24	0.07
HA 0.1 M	0.87	0.14	0.01
HB 0.01 M	2.54	0.15	0.15
HB 0.1 M	2.18	0.13	0.12
Ext. thermic 1	0.97	0.18	0.05
Ext. thermic 2	1.02	0.27	0.08
Ext. thermic 3	1.10	0.11	0.06

Table 4.
Data of mechanical properties of pectin membranes.

where l is the distance between the calibrated marks after the rupture of the material [89].

The commercial pectin membrane has a high value of Young's modulus, but it has good elasticity; this type of very positive characteristics when forming films is mainly due to the most exhaustive and purified purification processes, in which acetyl groups, proteins and nitrogen compounds, polyphenols, and finally sodium ion are eliminated. As for the pectin in which the different hydrolyses were made, the ones with greater rigidity are those made with sodium hydroxide, followed by the thermal extractions and finally the acid hydrolysis, which are the most elastic and flexible [90–94] (see **Figure 27** and **Table 4**).

6. Conclusions

It can be observed through this work that the hydrolyzed pectin obtained from *Citrus sinensis* has inferior properties to commercial pectin. Based on this it can be inferred that the selected procedure requires a greater degree of purification with the addition of pretreatment and posttreatment elimination of polyphenols and other undesired compounds such as proteins, etc. The hydrolyzed pectins that have the best qualities are HA 0.1 M and HA 0.5 M which can be applied to different industries such as pharmaceutical, food, and mining.

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