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Review

Pectin and Alginate Functional Biopolymers: Factors Influencing Structural Composition, Functional Characteristics and Biofilm Development

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Abstract: Various natural polymers have been explored for their specific and desirable functional properties. Biopolymers have been found to hold the potential to satisfy many current environmental and health needs from a natural food packaging perspective. With a drive towards a more sustainable and plastic-free future, polymers like pectin and alginate have been considered key to reducing traditional plastic packaging usage in the food industry. These well-established, commercially available biopolymers display unique functional properties that can be manipulated in the development of biofilms that possess specific physical–mechanical properties. Pectin and alginate have also proven successful in the biomedical applications of encapsulation, drug delivery, wound healing and tissue engineering, greatly due to their ability to form biofilms and coatings. The structural and consequent functional properties of pectin and alginate have been investigated, although rarely concurrently with one another, focusing on biofilm development. Research has specifically identified and highlighted the importance of pectin and alginate in developing biofilms due to their versatile and charged structural nature. This review article discusses and highlights factors responsible for the specific properties displayed by pectin and alginate biofilms from a chemical and film development perspective.

Keywords: biopolymer film; natural packaging; pectin; alginate; biofilm; food packaging; functional properties; biofilm development

Citation: Van Rooyen, B.; De Wit, M.; Van Niekerk, J. Pectin and Alginate Functional Biopolymers: Factors Influencing Structural Composition, Functional Characteristics and Biofilm Development. *Coatings* **2024**, *14*, 987. https://doi.org/10.3390/coatings14080987

Academic Editors: Roniérik Pioli Vieira and Luís Marangoni Júnior

Received: 30 May 2024
Revised: 16 July 2024
Accepted: 17 July 2024
Published: 5 August 2024

1. Introduction

A specific area of interest regarding the application of functional biopolymers is in developing biofilms that can find applications as naturally biodegradable packaging [1,2]. With research focused on addressing environmental concerns over the use and disposal of non-biodegradable, petroleum-based plastics, together with consumers' demands for improved food safety and extended shelf-life, there has been a driving force aimed at identifying and investigating natural polymers to be used in the development of natural packaging [3–5]. More recently, the COVID-19 pandemic has further highlighted the need for more naturally biodegradable packaging displaying antimicrobial properties. The need to enhance food safety and prevent extensive environmental damage from the extensive use of non-biodegradable packaging and related personal protective equipment was a major consideration for the investigation of a variety of polymeric materials by many researchers [6,7].

The developments of naturally biodegradable packaging types have not only been limited to polysaccharides, but proteins and lipids have also been considered [1,3,8]. However, polysaccharides are often considered for these specific developments due to their availability, sustainability, low-cost functionality and health benefits. Polysaccharides such as pectin, alginate, guar, carrageenan, chitosan and locust bean gum, as well as emerging biopolymers, have been gaining much attention in the development of biofilms with signs of being used as natural polysaccharide-based packaging [1,2,4,8–13]. In addition, these

polymers have proven successful in a variety of coating applications. Biopolymers such as pectin, alginate and chitosan have been found to have successful medical applications due to their encapsulation properties. Chitosan coating has specifically been found to have additional antibacterial, antifungal and anti-inflammatory properties, proving beneficial for specific medical applications [13].

These alternative forms of packaging are often referred to as 'edible packaging' as they are primarily composed of edible ingredients applied directly to the food via a thin layer. Alternatively, pre-formed films can also be applied as edible packaging, which is applied without altering the manufacturing process of the final products [14,15]. It is, therefore, important to note that although edible packaging is composed of natural edible materials that are non-toxic for human and animal consumption, the name more accurately describes the natural biodegradability of biofilms and possible packaging [8]. Therefore, biopolymer-based packaging can be divided into two main derivatives that usually differ based on the application method [8,15]. A biofilm is defined as a thin layer placed as a barrier between food and the surrounding environment, and it can be consumed as is [16]. These films are usually pre-formed separately as laminates and thereafter placed on or between the food product [16]. A second type of application is the development of edible coatings, which are predominantly formed by coating the food product directly with a thin layer of natural biopolymer material, which is usually in liquid form, by either spraying, coating or immersion of the product [8,15,16].

As there is an ongoing development in the field of natural packaging developed from polysaccharides, certain functional film characteristics are investigated when considering the viability and application of specific natural packaging [3,17]. Biofilms produced from polysaccharides usually display good gas barrier properties against oxygen and carbon dioxide due to their tightly packed structure. Negatively, due to the hydrophilic nature of hydrocolloids, they display poor water vapour barrier properties. As biofilms produced from hydrocolloids have generally been accepted to display successful gas, aroma and lipid barrier properties, increased focus has been placed on the specific mechanical properties displayed by different polymers used in biofilms [3,18,19]. The mechanical properties of biofilms can often be considered the main functional property in their development as these properties will largely highlight the specific biopolymers' viability to form a biofilm that can find potential applications in natural packaging [8,20]. The mechanical properties of biofilms are of utmost importance, as they directly describe a film's potential to withstand stress and strain during transportation, handling and storage while ensuring that the food remains structurally uncompromised and protected from the outside environment. As films' specific mechanical needs vary between different products and their applications, the capabilities, as well as limitations, of different polymers used in the development of biofilms have been considered by multiple researchers [2,4,10,11,17,21,22]. These factors specifically influencing the mechanical properties of biofilms will further be explored and presented holistically.

It is generally well accepted that most polysaccharides perform exceptionally well at producing highly viscous solutions, even at low application concentrations. However, some polysaccharides have an additional functional property, enabling them to form a hydrogel, which can ultimately be exploited in the development of functional biofilms [23–25].

The presence of charged groups in a polymer molecule is considered the primary reason for the formation of true polymer hydrogels. These charged groups are specifically responsible for the functional properties displayed by a polymer [26]. The most common process describing the gelation of functional biopolymers involved in the development of biofilms is the aggregation of primary inter-linkages between polymer chains, which results in the formation of junction zones, providing the basic three-dimensional structure of a gel [24,27]. Various factors have been shown to influence the primary structure of these junction zones within the gel network and the resultant film's mechanical properties. Specifically, pH, the presence and concentration of charged ions and the inherent structure of the polymer molecule have all been shown to alter the mechanical properties of a biofilm [11].

The gelation of polymers through this process is unique to polymers possessing a charge and not applicable to more natural polymers. Specifically, polymers that have the presence of carboxyl groups are generally associated with a negative charge. Due to these negative charges, polymer repulsion is observed between the polymer chains, resulting in an extended polymer configuration. This extended configuration, with increased chain length, results in increased polymer volume in the solution, allowing for increased functionality. These charged groups also allow for the formation of more stable film-forming solutions as the negative charges aid in preventing the polymer chains from aggregating due to repulsion by the similarly charged groups [26,28].

When decreasing the pH of a film-forming solution, a decreased resultant ionisation of the carboxylic groups occurs (acting similarly to more neutral polysaccharides), resulting in less likelihood of the system forming a gel with ionic molecules. However, when increasing the pH, the carboxyl groups can be ionised, forming more carboxylic acid groups, which display an increased likelihood of reacting with ionic molecules, which can form stable solutions and hydrogels. Additionally, the presence of more carboxylic acid groups will result in increased polymer chain repulsion [8,29].

For polymers associated with a charged structure, the presence of a crosslinking agent is another important factor to consider when manufacturing biopolymer films. Charged metal ions, such as calcium and magnesium, are often considered as crosslinking agents. Although the presence of a crosslinker is not set as a standard in the successful development of biofilms, sufficient amounts of polymers must then be available for film formation [4,30,31].

Lastly, different methods used in forming biofilms have been shown to have consequential effects on the mechanical properties of the films. Biofilms can specifically be differentiated based on 'dry' and 'wet' variants. Due to the advantages offered by low-moisture films, 'dry' biofilms have specifically been a focus area to satisfy industry requirements, as they more strongly represent a viable alternative to conventional plastic packaging [15]. Only pre-formed, 'dry' biopolymer films will, therefore, be further considered. In addition to the moisture content of biofilms, the presence of additional polymeric and non-polymeric materials in biofilms must also be considered to influence their properties. Specifically, blending more than one biopolymer together into the formation of a single biofilm and the addition of a plasticiser are two main factors that have been shown to alter biofilm mechanical properties [1,2].

As already highlighted, various hydrocolloids are used for their specific and desirable functional properties in the food industry [1,8,32]. However, some well-established commercially available biopolymers can be considered essential for their functional properties of altering a food system's rheological properties and in the development of biofilms. Specifically, due to their charged nature, pectin and alginate have been well researched for their functionality, resulting in their extensive use in the food industry for a range of diverse applications [33–35]. Both pectin and alginate are considered for their excellent thickening and gelling properties, resulting in their specific application in the development of biofilms for naturally biodegradable packaging applications [28,35–37]. It is important to note that determining the viscosity of biopolymers is important in understanding the rheological behaviour of polymer solutions. Consequently, the rheological behaviour of a polymer in solution further provides insights into the functional behaviour of that specific polymer and, ultimately, its biofilm formation potential [30,38–40]. Therefore, the rheology of a polymer in solution is often used to describe its functional behaviour.

Multiple researchers have specifically identified and highlighted the importance of pectin and alginate in developing biofilms due to their versatile chemical and physical properties and functional behaviour in a solution. Pectin and alginate have, therefore, specifically been identified as excellent biofilm-forming polymers [2,10,35,41].

Native pectin is a polysaccharide typically found in the plant cell wall and middle lamella of fruit and vegetables and often finds application as a functional food ingredient used during food processing [33,34,41]. Commercially available pectin is usually extracted from citrus peels, with lemon and lime peels preferred, as well as apple pomace. Pectin is often listed as a food ingredient with the EU code E440 [41]. The pectin chemical structure is primarily

represented by a negatively charged galacturonic acid backbone, displaying varying degrees of methylation and many side chains. The nature of a charged Pectin is primarily linked to its desirable functional properties. However, various factors can alter its functionality to different degrees [28,41]. In addition, pectin has also been found to have a variety of applications.

Alginate is a well-researched polysaccharide extracted from the cell wall and intercellular spaces of brown seaweeds. Alginate is considered a glycuronan of high commercial importance due to its widely used food applications as it exhibits excellent viscosity enhancement potential, gelling properties and stabilising functions and finds specific applications as edible biopolymer films and coatings [38,42–44]. The presence of uronic acid associated with the alginate polymer structures is of specific importance, as these charged uronic acids are one of the main bio-functional properties linked to alginate functionality and, thus, have vast applications in the food industry [38,42,45].

Although pectin and alginate have shown to differ on a molecular level, they have been explored as excellent polymers used in the development of biofilms. Interestingly, these biofilms have not only been limited to the food industry but also found new applications as sensors/biosensors. Specifically, work conducted by Safitri et al. [46] showed the success of using pectin–chitosan blend matrix membranes in developing sensors. These sensors could accurately detect salivary pH, further highlighting the diversity of these natural polymers and their successful applications [46]. However, this current review article focuses on the mechanical properties of pectin and alginate films, specifically as these biofilms generally display satisfactory mechanical properties [2,17]. The tensile test has specifically been applied to accurately measure the mechanical properties of biofilms [2,10,35]. Bierhalz [35] investigated the mechanical properties of pectin and alginate polysaccharides used to develop biopolymer packaging. The authors noted physical differences between the two polymer films. These physical differences were quantified by the completion of mechanical tests on the various films, specifically for the tensile strength (TS) and elongation at break percentage (%E) [35]. Polysaccharide-based films' mechanical properties were also investigated by Galus and Lenart [17], who found alginate biopolymer films to produce a higher film strength and elasticity than pectin films. However, both alginate and pectin biofilms were considered to have satisfactory mechanical properties. In general, a film's TS is a measure of its mechanical resistance accounted to chain–chain cohesion forces. At the same time, %E refers to the film's ability to extend before rupture and is indicative of its 'brittleness' and/or plasticity [17,36].

In addition to the tensile measurements (TS and %E), the puncture tests have also been employed to measure the mechanical properties of biofilms. These tests include measuring the film's puncture force (PF) and distance to puncture (DTP) measurements [2,10,11]. Many authors have specifically evaluated variations in the mechanical properties of films by using both the tensile and puncture tests [10,11,47]. Both these mechanical tests are important, as the tensile tests account for accurate differentiation between the specific films related to film strength and elasticity [17,36], and the puncture test is used to accurately evaluate a film's potential commercial application. Both the TS and PF are related to film strength, and %E and DTP are related to film elasticity and consequential brittleness [10].

An important application of polysaccharides that display gel-like behaviour is in the development of biofilms and coatings, ultimately aimed at expanding alternative naturally biodegradable packaging. Given the current global concerns regarding the environmental impacts of synthetic packaging, the development of biopolymers-based films and coatings is a major field of interest in the application of functional biopolymers. Pectin and alginate are two biopolymers that have specifically been explored for their ability to form biofilms and coatings, with various factors influencing these film properties [1–3,8]. Although abundant knowledge is available on specific factors influencing these polymers' functional behaviour and resultant film mechanical properties, a holistic view of biofilm development related to pectin and alginate could still be explored. Consequently, this research further provides essential insights into pectin and alginate coating formation potential, further promoting the investigation and application of pectin and alginate in coatings as the functional behaviour

remains greatly similar between film and coating applications of these polymers investigated. Therefore, this current review is synonymous with the 'Coatings' journal chosen for its publication. This review, therefore, aims to provide a concise and comparative approach to pectin and alginate biofilm development, encompassing important factors related to these specific polymers' functional behaviour from a molecular to a film development level to further promote developments in the field of natural packaging.

2. Structural Composition of Pectin and Alginate

2.1. Pectin Molecular Structure

Generally, pectin is represented by a heterogeneous chemical structure consisting of a (1→4)-α-D-galacturonic acid (GalA) residue backbone, displaying various degrees of methyl esterification, distinguished by different pectin domains. The most prevalent domains (or regions) are linear homogalacturonan (HG) and branched rhamnogalacturonan (RGI) regions, which can vary depending on their sugar composition [28,34,41,48]. Pectin's chemical composition can vary considerably and is, therefore, more accurately described as a collective family of pectic biopolymers possessing common characteristics. It has been well established that for a polymer to form part of the pectin family, about 60%–65% of the molecule must consist of galacturonic acid molecules [33,49]. These GalA residues form part of the 'smooth' HG region of the pectin molecule, with some of the carboxyl groups esterified with methanol [28,48–50].

2.1.1. Proposed Pectin Side Chains

The pectin molecule is also presented by a 'hairy' region. The 'hairy' regions are mostly composed of branched, neutral saccharide side chains, typically associated with the RGI regions of the polymer structure, as presented in Figure 1 [28,48,50,51]. The RGI typically consists of a backbone of L-rhamnose and D-galacturonic acid. These disaccharides are repeated in chains of about 20 residues long. The origin of the pectin will determine the amount of rhamnose residues, constituting anything between 20 and 80% of the backbone [49,51]. At the C_4 of the rhamnose residues, attachment of the side chains of arabinose and galactose is observed. The side chains are composed of singular residues, or, depending on the source of the pectin, other side chains may be present, such as varying amounts of galactose, arabinose or arabinogalactans [33,48,50]. Some pectin types have also been shown to contain side chains consisting of varying amounts of D-xylose and/or arabinogalactan, as well as other neutral sugars, forming part of an additional rhamnogalacturonan region, often referred to as the second rhamnogalacturonan region (RGII). These sugars are also attached to a backbone of GalA, generally at C_2 and C_3 [48,49].

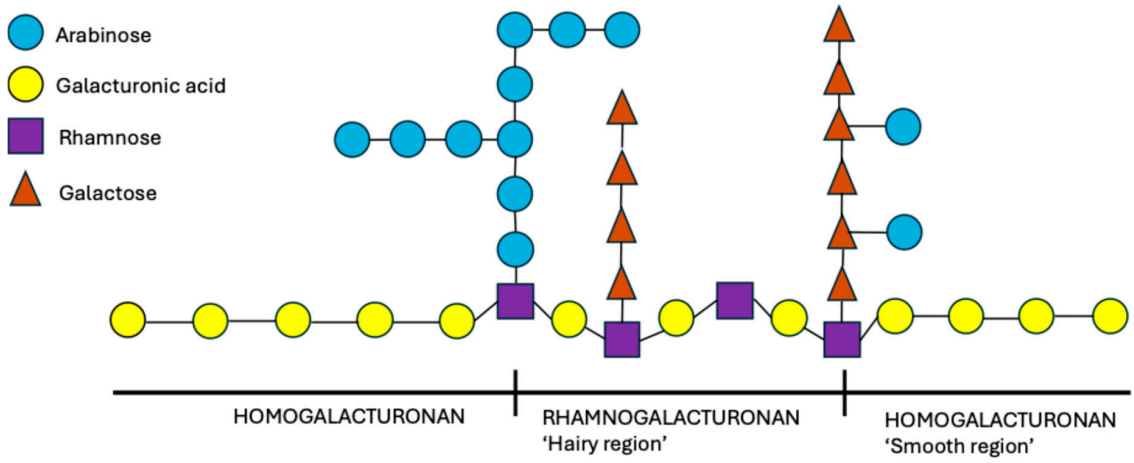

Figure 1. Basic schematic representation of the typical chemical structure of pectin, including the galacturonic acid backbone and the 'hairy' side chains associated with the rhamnogalacturonan region, based on [52].

2.1.2. Degree of Methylation of Pectin

Methylation is an important characteristic when it comes to key aspects that are relative to pectin's functional properties. The degree of methylation (DM) of pectin is the term used to describe the number of methyl-esterified carboxyl groups in relation to the total number of galacturonic acid molecules [53]. The DM of pectin is important as commercially available pectin is extracted from the by-products of fruit juice production, comprising mainly citrus peel and apple pomace. Acid extraction is usually used to extract the native pectin polymers, often resulting in the methyl-esterification of the galacturonic acid's carboxyl groups [34]. Pectin available for commercial applications can generally be classified into distinguishable groups according to the degree of methylation of their carboxyl groups: low methoxy (LM) and high methoxy (HM). The amount of galacturonic acid and its DM will, therefore, greatly determine the functionality of pectin and the factors that influence pectin solutions [54,55]. LM pectin is present in less than half of the carboxyl groups in the methyl-esterified form, whereas HM pectin is considered to have more than half of its carboxyl groups in the methyl-esterified form. Pectin with less than 5% methyl-esterification is defined as pectic acid [56–58].

2.2. Alginate Molecular Structural Composition

Alginate must be considered as a collective term to describe a diverse group of biopolymers that vary in composition and structure. The main structural component of alginate is considered a linear polysaccharide consisting of (1→4) β-D-mannuronic acid and α-L-guluronic acid residues [25,38,44]. Alginate is a binary copolymer as it is composed of unbranched β-mannuronic acid (M blocks) and α-guluronic acid (G blocks) [42,45,59]. These M and G blocks are found in homopolymeric (GG and MM) as well as heteropolymeric (GM/MG) sequences with varying ratios of M to G blocks, as presented in Figure 2 [44,60]. The sequence of M and G blocks significantly impacts the functional properties displayed by the alginate polymer. A ratio of 2:1 for M to G blocks is usually expected, but the ratios of M to G blocks are greatly dependent on the source and extraction methods used to obtain the alginate [42,45,59,61]. The spatial conformation and ratio of the M and G blocks substantially affect the functionality displayed by alginate [25]. The M blocks are presented by an extended ribbon shape, allowing the more linear chain to be flexible. The G blocks have a strong structure, presented by a buckled shape (Figure 2) [42,44,60].

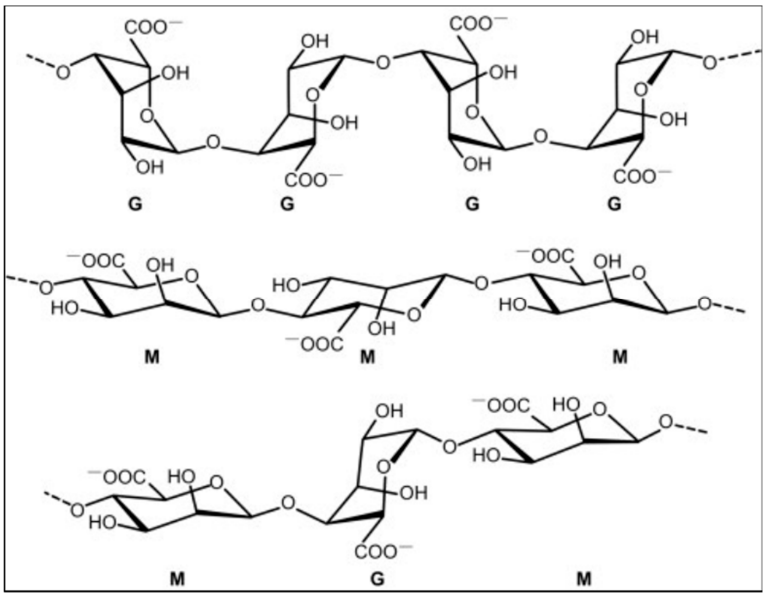

Figure 2. Proposed heteropolymeric (M-G-M) and homopolymeric (G-G-G/M-M-M) structural representation of G and M blocks of alginate, displaying the linear, flexible M blocks and the more ridged, buckled G blocks [60].

3. Pectin and Alginate Functional Properties

3.1. Pectin Functional Properties

Pectin is a highly diverse polysaccharide often used to alter and enhance the rheological properties of a food system. In general, it is well accepted that increasing the concentration of pectin in a solution is likely to result in an increase in the viscosity as a higher concentration of pectin may promote intermolecular interactions between polymers, allowing for hydrogen bonding [62,63]. In a study completed by De Oliveira [64], the author described the different phases of thickening with pectin. Firstly, applying pectin at a concentration of ≤1.0% (*w/v*) was unable to alter the resultant viscosity, typical of a dilute polymer solution phase. A semi-dilute secondary phase was observed when pectin was applied at a concentration range of 1.0%–2.5% (*w/v*). The higher concentration resulted in a reduced distance between the pectin molecules and stronger interactions, increasing viscosity. A final concentrated phase was observed when pectin was applied at a concentration range of 2.5%–3.5% (*w/v*), resulting in the aggregation of pectin polymer chains, a large increase in viscosity and possible network formation [62,64].

Pectin application in the food industry greatly depends on its functional properties, although various factors have been shown to influence its functionality. Therefore, Understanding these factors is essential, as they directly influence pectin thickening and gel formation potential [37,49,62]. Specifically, the degree of methylation, concentration, pH and the presence of gelling ions are well-established factors that directly influence the functional properties of charged polymers, such as pectin. The source used to obtain the pectin and their extraction method will also influence pectin's functional properties. These factors consequently are responsible for alternations of a pectin solution's viscosity, gel strength, gelation time, degree of hydrophobic behaviour and hydrogel biofilm formation [48,62,65].

3.2. Functional Properties Associated with Alginate

Alginate has found various applications in the food industry, which can primarily be attributed to an abundance of functional groups distributed throughout the polymer. These functional groups can be modified to alter various rheological, biochemical and mechanical properties displayed by the alginate when applied to a food system [38,44,66].

Research has shown alginate to display typical polyelectrolyte behaviour [67]. This non-Newtonian, shear-thinning, rheological behaviour leads to chain extension and results in an increase in reduced viscosity upon dilution, often considered a consequence of a polymer displaying polyelectrolyte properties [43,67,68]. Therefore, determining the viscosity of alginate is important in understanding the rheological behaviour of alginate in a solution and, ultimately, its biofilm formation potential [38–40]. Viscosity greatly depends on the molecular weight, chain rigidity and/or conformation and electrostatic charge carried by the polymer [68].

Mancini et al. [68] found that an increase in viscosity was observed with increasing molecular weight. Furthermore, increasing the alginate concentration in a solution resulted in a proportional increase in solution viscosity [68]. These increases in viscosity are dependent on intra-chain electrostatic repulsion forces between similarly charged groups, including the unreacted functional groups found along the sodium alginate chain [67]. Therefore, the degree of charge carried by the polymer will determine whether a molecule prevents self-association within a solution, having a direct impact on the solution's viscosity.

The functionality of alginate is typically closely related to the ratio of the M to G block, as well as their specific molecular weight [25,68]. However, alginate's functional properties are dependent on a variety of parameters. Such as with pectin, the functionality of alginate can be altered by various factors, which will ultimately determine its application in the food industry. Factors influencing the behaviour of alginate in a system will further be discussed in more detail.

3.3. Effect of a Crosslinker

3.3.1. The Effect of Crosslinkers on Pectin's Functional Properties

Pectin crosslinking is the typical mechanism used to describe the formation of low-methoxy (LM) pectin gel formation. Calcium is a commonly used cation in the crosslinking of pectin. Other than calcium (Ca^{2+}), certain divalent and trivalent ions have also been shown to alter a pectin solution's rheological properties [69–71].

Mierczyńska, et al. [37] compared different cations' ability to alter the viscosity of a pectin solution. The authors reported that (iron) Fe^{2+} and Ca^{2+} cations were both able to increase the viscosity of the LM pectin in a solution to a similar extent. It was also found that by increasing concentrations of Fe^{2+} or Ca^{2+} cations, a resultant increase in viscosity could be expected for a pectin solution. However, when comparing these increases in viscosity initiated by Fe^{2+} and Ca^{2+} to the viscosity increases brought about by magnesium (Mg^{2+}) ions, the authors reported Mg^{2+} to be less effective. The authors concluded that Fe^{2+} is likely to interact similarly to Ca^{2+} to increase the pectin solution's viscosity, with Mg^{2+} showing the least effective viscosity enhancement potential [37]. Similar results were reported by Huynh et al. [70], as the authors also observed a poor binding of Mg^{2+} to pectin. Therefore, it can be expected that the use of different ions may result in varying rheological properties displayed by a pectin solution, thus allowing pectin to be used for many diverse applications in food processing and production industries [59,70].

Although charged ions have the ability to alter the rheology of a pectin solution, the main consequence of pectin crosslinking is the formation of a physical gel. It is important to note that although both high-methoxy (HM) and LM pectin can undergo gelation, LM pectin gelation is specifically initiated by the inclusion of charged ions, following a completely different mechanism than observed for HM pectin gelation [62,71]. Therefore, the likelihood of pectin reacting with charged ions will greatly depend on the degree of methylation (DM) displayed by the pectin. Pectin with low amounts of charged group esterification can easily react with ions to form true gels.

Typically, the "egg-box" model is often used to explain the formation of LM pectin gels, similar to that used to describe the formation of calcium–alginate gels [59]. However, there are differences in the gel formation mechanisms between pectin and alginate gel formation [59,72]. The "egg-box" model describes the formation of junction zones when the coordination of Ca^{2+} and carboxyl groups (non-esterified) between GalA molecules takes place [72]. Gel formation, described by the coordination and possible formation of junction zones, is similar to what is described in the "egg-box" model, as presented in Figure 3 [62].

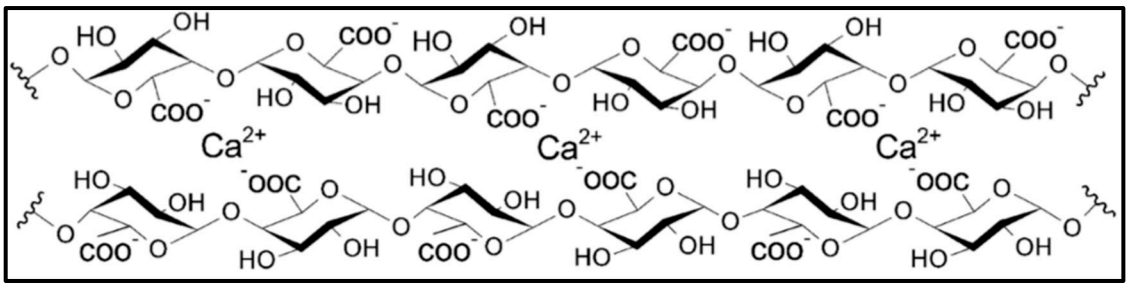

Figure 3. Schematic representation of the "egg-box" model showing the formation of junction zones of calcium (Ca^{2+}) crosslinking with carboxyl groups [62].

Other than the coordination of Ca^{2+} to the oxygen atoms in the carboxyl groups of GalA, Ca^{2+} has also shown potential to bind to the oxygen molecules within the ring structure of the glycosidic bonds and to hydroxyl group binding sites [54,73].

The neutral sugar side chains of pectin (rhamnogalacturonan and/or the proposed rhamnogalacturonan II) are responsible for the non-linear conformation with irregularities in the formation of "egg-box" dimers. These curves are referred to as the 'pectic elbow', adding a degree of flexibility to the three-dimensional gel matrix. Subsequently, this may hinder the

lateral association of the dimers when compared to calcium–alginate gel formation [17,35,36]. As a result, alginate–calcium gels could display increased strength compared to that of pectin–calcium gels, although similar gelation mechanisms are employed [59,70,74].

3.3.2. The Effect of a Crosslinker on the Functional Properties of Alginate

The addition of a crosslinker is well known for its ability to alter the functionality of alginate. Crosslinkers have specifically been shown to increase a solution's viscosity without increasing the alginate concentration in the solution. At a constant concentration, the viscosity of an alginate solution may be increased by the addition of gelling cations [74,75]. The strong interactions between the carboxyl groups of guluronic acid and gelling cations, such as Ca^{2+}, are used to describe the basic process of alginate gel formation. Generally, alginate displays a high affinity to react with cations, forming insoluble, thermo-irreversible hydrogels, allowing for a water-holding capacity of up to 95% [76–78]. This process is often implemented in the formation of calcium–alginate gels, making use of Ca [75]. The functional groups undergo chemical modifications to create a stable gel with alginate. These modifications result in a decrease in internal electrostatic repulsion forces between polymers. Alginate can undergo gelation in the presence of a variety of metal cations. Calcium is generally used, although zinc, copper, manganese, barium, cadmium and other ions have also been investigated for the gelling potential with alginate [40,75]. It has been noted that the type of ion used in alginate gel formation will significantly determine the gel properties, as not all ions produce gels of similar properties [25,76]. Ca^{2+} is predominantly used to crosslink alginate as it binds preferably to the G blocks found along the polymer chains of alginate. The binding of Ca^{2+} and alginate has been well described, often referred to as the "egg-box" model [76,79–82].

When a polymer chain is linked with other polymer chains, a three-dimensional (3-D) gel network is formed [77]. This model is similar to that used to describe pectin–calcium gelation [24,37]. Studies have described the "egg-box" model as the process whereby alginate–calcium gels are formed following a three-step process [59,77]. Initially, Ca^{2+} forms mono-complexes with single G block residues. The mono-complexes then aggregate, resulting in the formation of junction zones that aid in holding the Ca^{2+} firmly in place. This process has been termed 'cooperative binding' and results in the formation of dimers. Lastly, the "egg-box" dimers form multimers by lateral association (Figure 4) [76,77]. Importantly, the physical binding points or junction zones are formed by the aggregation of multiple Ca^{2+}.

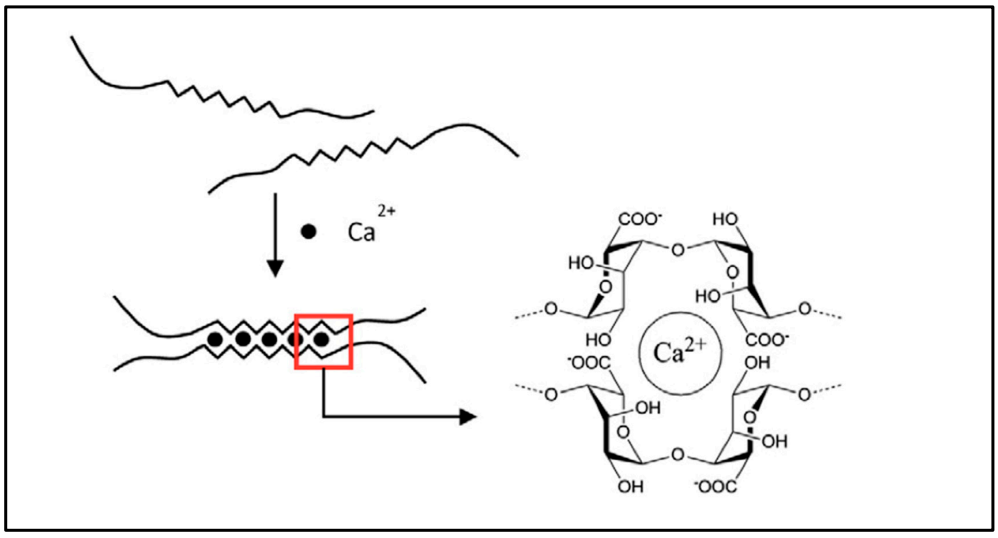

Figure 4. Schematic representation of the step formation involved in the alginate–calcium gel formation displaying the typical "egg-box" structure together with the formation of junction zones. The 'red box' zone represents the specific area evaluated, indicated by the flow of the arrow [77].

The "egg-box" model is only used to describe the mechanism of gel formation and does not highlight the limitations thereof. There are parameters impacting gel quality and, ultimately, the application of the hydrogels formed [39,76,78]. Gel strength can be considerably variable and inconsistent, resulting in highly unpredictable gel quality. The arrangement of M and G blocks in the polymer chain and their ratios (which can vary considerably from one species of seaweed to another) is not to be overlooked [25,68]. Therefore, the strength of gels can vary significantly from one source of alginate to another [38]. As the "egg-box" model describes, cations have a higher affinity for G blocks than for M blocks. Consequently, alginates with higher ratios of G blocks are more likely to form stronger gels and are referred to as alginates that have low M-to-G ratios [25]. Ca^{2+} does not necessarily bind preferably to the G block section of the polymeric chain, but the conformation of the G blocks allows for a more favoured aggregation of Ca^{2+} and, consequently, the formation of junction zones [77]. The more linear, flexible conformation of M blocks does not favour the aggregation of Ca^{2+}. Thus, gel formation, as described by the "egg-box" model, is not just crosslinking, although calcium does play a role in maintaining the structure.

3.4. Effect of pH on Polymer Functionality

3.4.1. The Influence of pH on the Functionality of Pectin

LM pectin has specifically been shown to form gels with the addition of charged ions. HM pectin is also able to form gels but under different parameters than LM pectin, without the presence of a crosslinker. HM pectin gel formation results from hydrogen bonds and hydrophobic interactions [33]. An acidic medium of pH < 3.5 and high concentrations of neutral sugar side chains (about 55%) are required to form a gel network [28,65]. The HM pectin's gelling mechanism requires an acidic medium so that the carboxyl groups of GalA, which are esterified, are less likely to dissociate within an acidic environment, resulting in less repulsion between two pectin chains and an overall reduction in the energy of the system [33,65]. A large number of hydrogen bonds can be formed between pectin molecules, aiding in stabilising the structure [58]. For HM gel formation, besides an acidic environment, the polymer must comprise a 55%–60% concentration of neutral sugar side chains. The neutral sugars are responsible for hydrophobic behaviour in an aqueous medium, resulting in pectin strands being "forced" together [65]. In conclusion, HM pectin gels are formed by a reduction in the energy of the system, reducing the pectin molecules' contact with water and hydrophobic interactions [62]. The specific mechanisms that are used to result in gel formation are referred to as low-water-activity gels or sugar–acid–pectin gels.

Although the pH of the aqueous medium used to disperse pectin can have a consequential effect on the gel formation of HM pectin, it can, however, influence the formation of LM pectin gels [23,65,83]. At a pH $\leq$ 3, gel formation for LM calcium gels may not only be limited to the crosslinking by calcium but also result from acid-induced gelation [54]. However, HM pectin gel formation requires the availability of large amounts of neutral sugar side chains to form gels, which was not set as a requirement for LM pectin gel formation [28,58].

Debon and Tester [83] reported on the binding of Ca^{2+} and Zn^{2+} in both acidic and neutral mediums for LM pectin gels. The authors found that, at a low pH, cations could not bind to the pectin, as the carboxyl groups were protonated, preventing electrostatic interactions. At a neutral pH, the Ca^{2+} and Zn^{2+} bound to the pectin polymers, allowing electrostatic interactions between charged carboxyl groups and the cations [83]. The effect of low pH on gel formation for LM pectin–calcium was also studied by Yang et al. [84]. The authors concluded that when increasing the pH from 3.5 to 8.5, there was a consistent increase in gel hardness. However, at pH 9.5, a decrease in gel hardness was observed due to a decrease in molecular weight as a result of β-elimination reactions [84].

3.4.2. The Effect of pH on Alginate Functionality

As the charged nature of the alginate polymer is manipulated by gelling ions, changes in pH also have the potential to influence alginates' functional properties and, consequently,

their rheological properties. It has been reported that at pH 5–11, the alginate shows to be only minimally influenced, resulting in little changes in an alginate solution's viscosity. However, decreasing the pH of a solution to pH < 5 has been shown to induce a type of acid gelation between the alginate polymer chains. This acid gelation mechanism is similar to that described for LM pectin acid gelation [23,78].

Although alginate acid gelation has the potential to form gels, even under optimal conditions, alginate–calcium gels display superior gel properties, such as strength and water-binding capacity. Acid gelation of a polymer is generally induced at a lower pH as the polymer electrostatic repulsion is reduced between polymers, resulting in their aggregation. Specifically, the neutralisation of charged polymer chains is induced by the introduction of an acidifier, resulting in the protonation of the alginate charge groups. Gel strength can, therefore, loosely be related to the strength of the acidifier used. In contrast, incrementally increasing a solution's pH would deprotonate the polymer chains, with increased electrostatic repulsion observed between polymer chains, resulting in less polymer aggregation and, thus, gel formation [23]. Therefore, the formation of alginate acid gels is not dependent on the formation of junction zones, and therefore, the ratio of M to G blocks is less likely to affect gel quality than in the case of crosslinking. Alginate crosslinking and acid gelation must both be considered when determining the functional potential of alginate, as they can considerably alter the rheology of a food system.

4. Factors Influencing Biofilm Development

4.1. Biofilm Preparation Methods

Film preparation methods have also been shown to influence the properties of biofilms. Although most authors describe similar film formation processes, deviations thereof have been shown to have a consequential influence on the resultant film properties. The simplest of the film-forming procedures employed is the casting method, which has the advantage of being a batch procedure often used at laboratory scale [15,41,85]. These films are formed by evenly spreading the film-forming solution onto a non-stick, flat surface according to the desired size and thickness, which, once fixed/dried, is removed [41,86]. This casting method, used to describe film formation, is also referred to as solvent casting. The casting method has the advantage that it can also be upscaled into pilot-scaled film-forming operations.

The general procedure used to describe film formation using the casting method involves a three-step process. Firstly, the biopolymer is solubilised in an appropriate solvent at the desired concentration, which is usually distilled water in the case of hydrocolloids. The success of the casting method depends significantly on the solubility of the biopolymer used in film formation [15]. Secondly, the prepared solution is cast into a mould, Teflon-coated glass plates or even Petri dishes of an appropriate size and volume. The addition of a crosslinker must also be considered. Various authors reported on the efficacy of crosslinking for different biopolymers and accounted for differences in crosslinking potential to the specific procedure used to introduce a crosslinker into the solution [36,74]. Crosslinkers, such as calcium, have successfully been used in both the solution formation and casting steps. However, some authors have reported variations in the crosslinking efficacy at both solution formation and solution casting steps and found that selecting the appropriate crosslinking procedure will be dependent on the polymer used and the film properties required [36,74]. Lastly, the films are formed by the drying of the cast solution. The drying step is considered essential in the formation of the 'dry' films, as it is responsible for the intramolecular binding of polymer chains in the solution, which will ultimately determine the film's biochemical and mechanical properties displayed. Sufficient time must be allowed for the solvent to be evaporated and the specific film to be formed. The solvents are generally removed by various methods, which include drying at room temperature, hot oven drying, vacuum drying, microwave drying and tray drying. Importantly, some authors have found that rapid solvent evaporation has resulted in films of inferior biochemical and mechanical properties [15,17].

In order for cast films to be considered adequate, they should be free from defects and display homogeneous properties. Additionally, they must display certain functionality regarding their mechanical properties, as films displaying poor mechanical resistance to external forces would unlikely be deemed adequate for application as biodegradable packaging, regardless of their biochemical properties [15].

4.2. Influence of Natural Plasticisers on Biofilm Development

Once biopolymer films are dried, the film structures are generally always associated with brittleness, resulting in decreased mechanical properties and workability [15,19]. As research has been focusing on the development of biodegradable packaging from biopolymers due to their cost-effectiveness, availability and functionality, specifically regarding their mechanical properties, finding ways to combat film brittleness has also been actively pursued. Natural plasticisers have been investigated for their potential to improve the films' workability while maintaining their biodegradable characteristics [19,87]. Plasticisers are considered low-molecular-weight agents added to film-forming solutions to aid in their flexibility, allowing for easy handling and film processing [10,36]. In research by Gao et al. [87], glycerol and sorbitol were evaluated concurrently for their efficacy as a plasticiser in 'dry' alginate films. The authors reported that the addition of the plasticisers resulted in increased homogeneity of the films; however, there was a decrease in the films' TS values and increased %E values. The authors promoted the use of glycerol over sorbitol as it showed improved plasticisation efficacy, as glycerol-plasticised films had greater flexibility due to their smaller particle size and superior hydrophilic properties [87]. The increased flexibility of the films has been related to a decrease in the intermolecular forces, which act along the polymer chains and allow for increased chain mobility, which is of importance in 'dry' films that are characterised by strong, cohesive structures [19,36]. Work performed by Barbut and Harper [10], who formed 'dry' biopolymer films with alginate, showed that glycerol inclusions had little effect on the tensile and puncture measurement of the films. However, the authors indicated that increasing the glycerol in the film-forming solutions could result in increased mechanical differences between the plasticised films and those not plasticised [10]. Multiple researchers have thus highlighted the necessity of the inclusion of a natural plasticiser in the development of biodegradable packaging from biopolymers, often referring to them as essential in 'dry' film formation for both crosslinked and uncrosslinked films [10,19].

5. Pectin and Alginate Biofilm Development

5.1. Homopolymeric, Single-Polymer Biofilms

'Dry' biopolymer packaging is considered a pre-formed film that can be moulded into a specific shape and used to separate food from the direct environment. 'Dry' is explicitly used to describe the low moisture content of a diverse range of films, formed as laminates, that can be stored and used as required with no adverse effects regarding their structural and biochemical attributes [8,16]. However, variations in the processes of the development of 'dry' biofilms must be considered. In the work completed by Galus and Lenart [17], the authors described the formation of 'dry' biofilms. The films were formed by preparing film-forming solutions of biopolymers (specifically alginate or pectin) with the addition of calcium as a crosslinker. The same amounts (15 mL) of film-forming solutions were poured (cast) into 150 mm Petri dishes for all films formed. The moisture was removed from the films by drying them at room temperature (25 °C) for 24 h, forming the biofilms, which thereafter could further be evaluated for their physicochemical and mechanical properties [17]. Harper [47] described 'dry' biofilms, in the context of their research, as films that had the majority of their water removed and could thereafter be stored and used in that specific 'dry' form. Various authors agree with this definition of a 'dry' biofilm, with most authors reporting that 'dry' biofilms contain about 4%–7% moisture once dried [88]. The authors made use of an oven to remove the moisture from the films, usually set below 50 °C. However, some authors have let the films dry at room temperature with sufficient

ventilation. 'Dry' biofilms formed at room temperature usually require longer drying periods than oven-dried films [17,35,88,89].

Generally, 'dry' biofilms can be divided into two main categories: those that are crosslinked or gelled (often referred to as hydrogel films) and those that are uncrosslinked. The specific functional ability of polyelectrolyte hydrocolloids to crosslink with cations, such as calcium, has been exploited in the development of these biofilms, as crosslinking has been shown to structurally influence the properties of the resultant films [8,17,88]. These two types of 'dry' films are often investigated concurrently with each other, as both crosslinked and uncrosslinked films have shown potential biochemical and mechanical variations, influencing their specific applications [2,4].

Regarding this current review, pectin's influence on the rheology of a solution and its ability to form hydrogels, specifically harnessed in the development of biofilms, is most important. Pectin is also well known for its ability to form gels, which can be considered critical in the development of biopolymer films. Both HM and LM pectin types are available commercially, with both pectin types possessing gelling potential, each with unique factors influencing their gelation [33,62].

In research conducted by da Silva et al. [36], various properties of 'dry' pectin biofilms were investigated. It was found that pectin successfully produced biofilms displaying adequate strength and elasticity. Furthermore, the authors also suggested that biofilms require the addition of plasticisers and can display enhanced mechanical properties by adding calcium as a crosslinker. Kang et al. [90] investigated the TS and %E of pectin biofilms. The authors formed films by immersing the pectin films into 5 and 10% $CaCl_2$ solutions acting as a crosslinker. Films were prepared with no addition of calcium, which was considered the control film. The results confirmed that films formed using 5% $CaCl_2$ showed increased TS of 198 MPa higher than the control films. Furthermore, 5% $CaCl_2$ crosslinked films showed the lowest elongation at a break potential of 2.6% [90]. Due to pectin's ability to successfully form biofilms, multiple authors have investigated the development of pectin biofilms for various applications [41].

Over the years, alginate's functional ability to form hydrogels has been exploited in the development of biofilms. Specifically, 'dry' biofilms, developed from sodium alginate, have been extensively researched [4,10,36,91]. Although crosslinkers are not set as a requirement in the development of 'dry' alginate biopolymer films, much research has investigated the influence a crosslinker has on the functional properties of alginate films, as the alginate polymer possesses the unique ability to form hydrogels with the addition of gelling cations, such as Ca^{2+} and Mg^{2+}. Badita et al. [91] investigated the influence of calcium, used as a crosslinker, on 'dry' alginate biofilm properties. The authors found that alginate–calcium film properties were considerably influenced by both the crosslinker and the concentration of the crosslinker used. It was further confirmed that hydroxyl and carboxylic groups, associated with the alginate polymer chemical structure, were responsible for the hydrogel formation with the addition of calcium, highlighting the benefits of calcium as a crosslinker in biofilm formation [91]. Rhim [88] also investigated the influence of calcium, in the $CaCl_2$ form, on 'dry' alginate film physical properties. Comparing two different procedures used to treat the films with $CaCl_2$ showed that by immersing alginate films in a $CaCl_2$ solution, superior film strength was achieved compared to films with $CaCl_2$ mixed into the film-forming solution before they were cast. The authors further showed a strong link between increased film strength and the resulting decreased film elasticity. This phenomenon was specifically related to the crosslinking of alginate polymer chains with calcium, which was responsible for the development of a more structured film network. Van Rooyen et al. [2] also noted a similar phenomenon in work conducted on pectin and alginate biofilms reacted with calcium. The authors specifically suggested that an increase in film strength was often associated with a decrease in film elasticity and vice versa [2].

Lastly, increasing the calcium concentration further increased the' dry' alginate film strength [88].

As in the case of 'dry' pectin biofilms, the addition of a plasticiser has also been strongly recommended and, in some cases, deemed essential in the development of 'dry' alginate biofilms. Natural plasticisers, such as glycerol, have been recommended for their ability to improve alginate film properties [36,87].

5.2. Blend/Composite Biofilms

In general, composite or blended biofilms are formed when two or more polymers interact. Composite matrixes have been successfully developed using a vast range of polysaccharides, including alginate, pectin, mucilage, gelatine and many other gelling and non-gelling macromolecules [17,36,92–94]. These mixtures have become ubiquitous, specifically for their application as naturally biodegradable packaging in the food industry [10,17,36,95]. The process of integrating two or more polymers in a matrix is considered advantageous, as these mixtures allow for enhanced functional potential, which would not be possible if only the individual polymer functionality were considered. These enhanced interactions between different polymers are often regarded as synergistic collaborations, resulting in the development of unique and desirable functional properties [1,2,16,17,95]. Enhanced functional properties include optimised viscosity and gel strength, increased stability of structures and additional unique synergistic effects between different polymers [95]. Importantly, components carrying a neutral charge have also been proven to enhance certain desirable properties of a specific system [10,93,94,96]. Research has shown that although there might be a complete lack of attracting interactions between the different polymers in a system, one polymer can still impart certain enhanced functional properties onto another polymer [95]. The functional properties displayed by the different biopolymers will depend on the type of interactions that are possible between the different components in the matrix. There are typically two types of interactions: firstly, the formation of disordered structures, which interact primarily on space occupancy, or, secondly, the formation of ordered structures, which allow for stable associations resulting in compact and structured networks [97].

Composite polymers find multiple applications, with extensive research published on their success at enhancing biopolymer films' biochemical and mechanical properties. These enhanced properties have resulted in broadening natural packaging's range of applications and improving its food protection properties from a structural and microbial perspective [2,12,14,17]. As with singular biopolymer film formation, certain properties are altered, allowing successful biofilm formation. These include the addition of crosslinking agents, pH alteration, polymer concentration and film preparation methods, as already discussed. These principles governing homopolymeric film formation must also be considered for composite and/or blend biofilms, as they have also been shown to influence their biochemical and mechanical properties [14,17]. Specifically, charged biopolymers have extensively been considered in the development of biofilms as they may enhance certain film properties due to their ability to form gels. Hydrocolloids are of specific importance due to their ability to form stable polymeric networks that display a high affinity to incorporate large amounts of water. Research has explored the abilities of natural polymers such as alginate, carrageenan and pectin in the development of natural films [17,98,99].

Other than single-polymer film formation, composite polymer matrixes may or may not co-exist to form a single phase as it has shown that they can also partially associate or completely separate in solution depending on the compatibility of the different components introduced into the system [47,100]. If more than one of the biopolymers in a composite matrix is allowed to act as a gelling agent, one of three types of gels can be formed, namely complex, mixed or filled gels. A complex gel is formed when the polymers interact, resulting in a gel formation [47]. If the two polymers form independent networks, a mixed gel is formed (synergistic), and filled gels are formed when one polymer is filled into the gel network of another. Filled gels can either be considered disruptive, resulting in a weakened gel structure or reinforcing, allowing for increased strength of the gel structure [101]. These

properties are important when understanding the interactions of polymers and the resultant functional properties displayed.

As mentioned, the degree of altered functionality of a biofilm (when compared to the homopolymeric biofilms) will be dependent on the compatibility, or lack of compatibility, between the different components used in the film matrix, ultimately affecting the microstructure of the films and thus their functional properties. More cohesive and homogeneous films are formed when there is a high degree of compatibility between different components within a film. When less compatible components are allowed to interact, it generally results in a more irregular film matrix due to the heterogeneous distribution of components, allowing for variations in the mechanical properties of the films, often resulting in phase separation between the different components [14,17,102]. A lack of specific attractive interactions between different polymers can result in poor mechanical properties displayed by the films. However, there are instances where a lack of attracting interactions does not indicate biocompatibility, allowing composite films to display enhanced or improved functional properties.

Pectin has, however, been used not only as an exclusive polymer in developing homopolymeric biofilms but also in combination with other biopolymers and macromolecules in forming composite/blend pectin biofilms. Work has been completed to understand the impact of composite pectin hydrogel films' functional properties on improving film biochemical and mechanical properties. Some authors have reported that the combination of pectin and alginate impacts the physio-chemical and mechanical properties displayed by this gel and film formation compared to single-polymer films [17,35,36]. These composite applications have been suggested to satisfy the limitations of the individual polysaccharides regarding biopolymer film formation and to allow for additional functionalities that would not usually be associated with the individual polymers used in composite pectin film development. The research has clearly highlighted the importance of these composite applications, especially regarding the role of pectin in functionality [2,17,36,41].

As with most commercial polysaccharides, alginate has also been considered in the development of composite biopolymer films [10,17]. Some authors have reported on a certain synergistic interaction between various polymers used in combination with alginate that is responsible for enhancing certain, and in some cases, multiple, physical properties of alginate-based 'dry' biopolymer films. Of specific interest is composite films' ability to enhance alginate films' mechanical properties, such as strength and elasticity [2]. Barbut and Harper [10] investigated the influence carrageenan and cellulose had on alginate-based 'dry' films. Although cellulose negatively influenced the alginate films' tensile strength and elasticity, carrageenan's superior synergy with alginate did not negatively impact the film TS and %E, highlighting various polymers' potential to interact differently with one another. The authors concluded that investigating composite films is essential in developing packaging that meets the needs of a specific application, which would be likely when considering only single-polymer film capabilities [10]. Paşcalau et al. [89] investigated the influence crosslinking with calcium had on composite alginate and carrageenan composite films with glycerol as a plasticiser. The authors found that in some instances, uncrosslinked alginate-based films' tensile strength was negatively influenced by carrageenan. However, crosslinking the films resulted in an unpredicted synergistic interaction between the two polymers, increasing the film strength from ±34 MPa to 51 MPa. These values were considered to be much higher than the sum of the two individual polymers' strengths. Although the phenomenon of increased film strength, linked to a decrease in film elasticity, was reported, carrageenan could still increase the alginate-based film's elasticity [89].

Similarly to 'dry' single-polymer alginate films, glycerol has been well researched and recommended as a plasticiser for composite alginate films. In research completed by Barbut and Harper [10], glycerol was recommended as a plasticiser as it was shown to have a noticeable impact on the mechanical properties of composite alginate films.

6. Conclusions

Pectin and alginate are biopolymers that have both shown excellent potential for use in the development of biofilms, finding potential applications in naturally biodegradable food packaging. However, displaying varying molecular structures and understanding the factors influencing their biofilms have shown to be essential when considering these biopolymers as potential natural packaging alternatives to conventional plastic-based packaging. It was also seen that various biofilm development methods must be evaluated concurrently with the polymer's functional potential to provide a holistic and optimised view of pectin and alginate biofilm development capabilities, which was provided in this review.

Author Contributions: Conceptualisation, B.V.R. and M.D.W.; methodology, B.V.R. and M.D.W.; formal analysis, B.V.R. and M.D.W.; investigation, B.V.R.; resources, M.D.W. and J.V.N.; data curation, B.V.R. and M.D.W.; writing—original draft preparation, B.V.R.; writing—review and editing, B.V.R. and J.V.N.; supervision, M.D.W. and J.V.N.; project administration, B.V.R., M.D.W. and J.V.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

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