coatings

Review

State of the Art in the Development and Properties of Protein-Based Films and Coatings and Their Applicability to Cellulose Based Products: An Extensive Review

Maria-Beatrice Coltelli [1,*], Florian Wild [2,*], Elodie Bugnicourt [3,*], Patrizia Cinelli [1], Martina Lindner [2], Markus Schmid [2,4], Verena Weckel [2], Kerstin Müller [2], Pablo Rodriguez [2], Andreas Staebler [2], Laura Rodríguez-Turienzo [3] and Andrea Lazzeri [1]

Received: 16 September 2015; Accepted: 30 November 2015; Published: 31 December 2015
Academic Editor: Rafael L. Quirino

[1] Department of Civil and Industrial Engineering, University of Pisa, Largo Lucio Lazzarino 1, Pisa 56122, Italy; patrizia.cinelli@diccism.unipi.it (P.C.); a.lazzeri@diccism.unipi.it (A.L.)
[2] Fraunhofer-Institute for Process Engineering and Packaging IVV, Giggenhauser Strasse 35, Freising 85354, Germany; martina.lindner@ivv.fraunhofer.de (M.L.); markus.schmid@ivv.fraunhofer.de (M.S.); verena-weckel@gmx.de (V.W.); kerstin.muller@ivv.fraunhofer.de (K.M.); prodriguez@iris.cat (P.R.); andreas.staebler@ivv.fraunhofer.de (A.S.)
[3] Innovació i Recerca Industrial i Sostenible (IRIS), Parc Mediterrani de la Tecnologia, Avda. Carl Friedrich Gauss No. 11, Castelldefels 08860, Spain; lrodriguez@iris.cat
[4] Chair of Food Packaging Technology, Technische Universität München, Weihenstephaner Steig 22, Freising 85354, Germany
* Correspondence: mb.coltelli@ing.unipi.it (M.-B.C.); florianwild@web.de (F.W.); ebugnicourt@iris.cat (E.B.); Tel.: +39-050-2217-856 (M.-B.C.); Fax: +39-050-2218-065 (M.-B.C.)

Abstract: There is increasing research towards the substitution of petrochemicals by sustainable components. Biopolymers such as proteins, polysaccharides, and lipids derive from a variety of crop sources and most promisingly from waste streams generated during their processing by the agro food industry. Among those, proteins of different types such as whey, casein, gelatin, wheat gluten, soy protein or zein present a potential beyond the food and feed industry for the application in packaging. The general protein hydrophilicity promotes a good compatibility to polar surfaces, such as paper, and a good barrier to apolar gases, such as oxygen and carbon dioxide. The present review deals with the development of protein-based coatings and films. It includes relevant discussion for application in paper or board products, as well as an outlook on its future industrial potential. Proteins with suitable functionalities as food packaging materials are described as well as the different technologies for processing the coatings and the current state of the art about the coating formulations for selectively modulating barrier, mechanical, surface and end of life properties. Some insights onto regulations about packaging use, end of life and perspectives of such natural coating for decreasing the environmental impact of packages are given.

Keywords: biopolymer; protein; film; coating; packaging; paper; paperboard; cellulose; renewable

1. Introduction

Paper and board are the oldest and most versatile packaging materials available on the market today [1]. They consist of microfibrils of long-chain cellulose molecules in a crystalline state with regularly disrupting amorphous regions [2]. Paper is the material of choice in many food and non-food packaging applications due to its low weight, printability, affordability, stiffness, use of natural resources, recyclability and biodegradability leading to its general green image [3,4].

However, paper is hydrophilic, hygroscopic, porous and has poor barrier properties. Therefore waxes or conventional synthetic polymer-based films are coated or laminated in order to provide paper with specific properties [1], such as barrier to gases, vapors, aroma, grease and water or to modify thermo-mechanical properties like sealability. In current commercial applications, synthetic coatings of different origin are used for that purpose such as ethylene vinyl alcohol (EVOH), polyvinylidene chloride (PVDC), polyethylene (PE), acrylic, latex, or fluorocarbon [3,5]. Inorganic layers are also sometimes laminated such as aluminum foil or deposited by metallization. Also combinations of several of the previous approaches are often employed [6]. However, such coated and laminated paper solutions are often not recyclable anymore.

Therefore, some efforts have been made to develop paper coated with bioplastics to substitute non-biodegradable polymers [7–13] to benefit from specific properties of the coating and to maintain at the same time the biodegradability of cellulosic materials. Although the use of polylactic acid (PLA), thermoplastic starch and regenerated cellulose to coat paper has been tried in industrial applications, the penetration in the market is limited to now. Legislations on end of life packaging and consumer awareness drive the need to replace the use of depleting fossil resources by renewable ones and to reduce the environmental impacts of packaging [14–17].

Bio-based polymers have a very diverse chemistry and architecture of the side chains that provides unique possibilities to tailor the properties of the derived materials [18]. They can be derived from a range of resources (from proteins, lipids, polysaccharides to some biogenic polyesters such as PHA naturally accumulated in microbial cultures) including by-products and industrial waste. Their production generally requires less energy than petrochemical counterparts.

Among those, due to excellent functional properties, there is a huge amount of academic work on protein-based coatings which could fill the need for high-performance renewable materials in the packaging industry. Proteins have a unique structure which confers a wide range of functional properties, especially a high intermolecular binding potential often allowing protein-based films to exceed mechanical properties of polysaccharide and fat-based films [19]. They have been studied in combination with different substrates (plastic, paper, leather, *etc.*) or as edible coating for food.

A great number of agro-resources are rich in proteins (e.g., soybeans (38%–44%), peas (22%–28%), cereal grain (8%–15%), sunflower seeds (28%–42%), liquid whey (1%) and some of those can also be recovered from under-used industrial by-products [20]). The protein ingredient market is principally focused on food supplementation and dominated by wheat gluten, milk and soy protein as well as gelatin. In addition, several proteins with limited commercial availability and promising properties as paper coating are discussed here.

Two main families of converting processes, whose applicability to protein coatings will be subsequently described in this paper, can be used to make materials based on proteins: a wet process based on dispersion or solubilisation of proteins, followed by drying, and a dry process based on the thermoplastic properties of proteins when they are extruded [21]. Additional procedures such as chemical modification and cross-linking of proteins or their blending with other polymers are also often used for properties optimization [6,22–25]. Also specific protein fractions from raw materials obtained through the protein extraction process and the different amino acidic sequences may be more or less prone to a given post-process.

The intrinsic hydrophilicity of protein films results in good adhesion to polar surfaces such as paper and a good barrier to apolar gases such as oxygen and carbon dioxide, but scarce to water vapor [26]. Due to their good oxygen barriers at low relative humidity, various types of protein have been tested and used as edible coatings/films such as those from gelatin, casein, whey, corn zein, wheat gluten, soy, mung bean and peanut [27]. In addition, some high molecular weight proteins are insoluble or only weakly soluble in water and may be used to obtain water resistant films [28,29]. Applied as coatings on substrates used in the packaging sector, proteins are aimed to improve a wide range of properties such as gas and water vapor barrier [30] and sealability [31]. Whey protein coatings have been shown as good barriers when applied on paper [32] by increasing oil resistance and reducing water vapor permeability [8,33], on LDPE [34] and on PP [35]. In addition they exhibited excellent visual and good mechanical properties on their substrates. Gelatin-based coatings on PE, PET, and PP improved barrier properties of substrate against oxygen and UV radiation [36]. Zein coated paper [37] had oxygen barrier highly over performing those of PE (barrier improvement factor up to 73) that may be usable on paper boxes as an alternative to paraffin. Plasticized corn-zein coating (thickness of 3–4 μm) on PP showed three orders of reduction in oxygen permeability [38]. When combined with a protein-based coating, biodegradable substrate materials maintain their ability to be organically recycled. They even catalyze the biodegradation of the substrate [39].

Besides, the main object of the present review dealing with coating and film, the use of proteins as adhesive can also be relevant for the paper industry. Various proteins have been used for adhesive production during both ancient and modern ages, including blood protein [40], casein [41], and soybean protein [42,43]. More recently also whey protein, coming from cheese industry waste, blended with sucrose, was used for formulating adhesives to be employed in the paper industries [44]. Using more polar and well aqueous suspendable proteins is fundamental to improve recycling of paper products. In fact protein-based adhesives can be washed away from the fibers during the recycling process and successive pulping. Traditional glues mostly stick onto the fibers making them less suitable for being employed in new paper based products.

This review paper will first of all review the relevant processing, properties and functionality of proteins as well as the characteristic of several options that have been used as coating or extruded films with potential for the packaging sector. Then the application technologies that can be used to combine these protein coatings with paper and the properties of derived materials are described. The present work provides a summary of the relevant properties of protein coatings for the paper-based packaging application which encompasses specific data with this substrate as opposed to previous reviews which were also less comprehensive in terms of protein coating and films in general. This will help to a certain extent comparing the different solutions although the individual literature use variable conditions, processes and tests. The paper finally focuses briefly on some other constrains generally lacking in previous related papers for their use in the packaging sector such as food safety and environmental regulations applied to end of life materials. The described protein coated papers have industrial potential to replace synthetic coated paper and even, in some applications, plastic films.

2. Proteins with Suitable Functionalities as Food Packaging Materials

Proteins have in nature a wide range of structures and functions such as the promoting of biochemical reactions and the building of tissues. Further they appear in storage formations that serve as nutrient and building block for seedlings and offspring. The different proteins chemical structures correspond to much specific chemical and physical-chemical behaviors. In the packaging sector, the application of proteins in barrier layers demands film-forming properties. Proteins derived from animal or microorganism tissues show a filament structure, whereas storage proteins form globules. An overview of proteins with film-forming properties that were tested for food packages, stand-alone films and edible coatings in the past is illustrated in Table 1 [16,21,45–49].

From the analysis of the Table 1 and the related literature survey, it is possible to conclude that, even though gelatin—coming from highly available collagen waste—is widely employed for obtaining edible or non-edible films, usually globular protein were most investigated and used for applications in the field of film production. This probably depends on the usually good suspendability in water thanks to their amphiphilic behavior or good interaction with polar plasticizers. These properties are necessary for obtaining a water based coating or a protein material processable as cast or melt extruded films respectively.

Table 1. Overview of proteins with film-forming properties tested for food packages, stand-alone films or edible coatings.

Origin	Plant Protein	Animal Protein	Fungi
Globular proteins	Wheat gluten Rice (bran) protein Corn zein Soy protein Pea protein Phaseolin Peanut protein Winged beans protein Pistachios protein Lupin protein Sunflower protein Cottonseed protein Amaranth protein Sorghum kafirin Potato protein	Whey protein Egg albumin	Hydrophobins
Fibrillar proteins		Casein Gelatin/collagen Keratin Fish myofibrillar protein	

3. Protein Processing and Purification

Most of the globular proteins are soluble in water or can be solved in aqueous solutions. The solubility of these ampholytic molecules largely depends on the pH-value and salt concentration. Purification processes can exploit this property. By shifting the pH-value or the ionic strength of the aqueous solution proteins can remain soluble or precipitate. In the former case, it is possible to separate insoluble components such as fiber and starch, whereas in the latter case, it is possible to separate soluble components such as sugars and lipids from the proteins [50,51]. Further mild purification processes can use size exclusion methods such as micro- and ultrafiltration. It allows separating smaller and larger molecules than the target protein fraction. Chromatographic and adsorption processes are used for purification of single protein fractions that require a high pureness of specific proteins as in nutraceutical and pharmaceutical applications. Furthermore the addition of organic solvents or multivalent ions into aqueous protein solutions provokes the precipitation of proteins to separate soluble compounds. Proteins that are insoluble in water such as corn zein or wheat gluten are processed otherwise. Zein solubilization requires alcoholic solvents such as ethanol or propanol. In the case of wheat gluten, that forms a water insoluble network already when sheared at room temperature, a washing out procedure is applied to remove non-gluten components. Further heat induced denaturation and subsequent flocculation of proteins allow separating proteins cost-efficiently from low concentrated aqueous streams such as in waste water treatments. However, in such like processes, proteins often lose their film-forming properties due to thermal denaturation.

Fibrous proteins in contrast show rather inhomogeneous properties due to their nature. Several of these proteins such as keratin and gelatin require a long extraction procedure and strong acidic or alkaline conditions in order to cleave the strong network of crosslinks between proteins, minerals and other components within cellular, skin and cartilage tissues. The relevant characteristic of the fibrous proteins is the dense association in parallel structures stabilized by hydrogen bonding in order to form fibers. These dense structures are insoluble in water. However, several proteins can be dissolved, when high temperature, neutral to alkaline pH or high ionic strength contribute to swelling and breaking up of bonding within such agglomerates. As can be observed in Table 1, the source of fibrous proteins are typically found in by-products or waste of animal origin.

4. Protein Characterization and Functional Properties

Proteins can be characterized and grouped according to their amino acid composition, geometrical conformation, solubility in various solvents, molecular weight, sedimentation behavior, their surface polarity and distribution or whether they maintain their native molecular configuration. Their application potential depends on bio- and techno-functional properties. Bio-functional properties refer to nutritious or pharmaceutical properties whereas techno-functional properties relate to the ability to build up physical structures in foods or technical applications. With respect to the application in packaging, most relevant are the techno-functional properties such as solubility, viscosity and network formation.

Protein solubility—at least partial—and protein swelling is a prerequisite for a stable protein dispersion to be applied as coating layer and for inducing viscosity and network-formation. It contributes to loosen the molecular structure of the protein thus allowing interactions with both the solvent and further protein molecules. Functional groups of the protein molecule need to be accessible to build up highly viscous solutions or dispersions and to form networks.

Most proteins are soluble in water or other polar solvents, whereas few proteins become soluble in moderate polar solvents like alcohols. Protein solubility is highly dependent on pH of the solution and the ionic strength. At pH levels close to their respective isoelectric point (pI), the protein molecules have a maximum of total anionic and cationic charges, whereas the net charge is zero. In consequence electrostatic repulsion is minimized at the pI-value and the proteins aggregates densely to each other. Thus typically proteins show an unwanted strong aggregation at the pI that leads to protein precipitation and to rough surfaces of the coating. Further protein solubility and swelling is influenced by salts. Low salt concentrations typically increase protein solubility and swelling by reducing electrostatic protein-protein interactions.

5. Protein Film-Formation

Proteins unfold and dissociate in subunits when treated with heat, acid, base and various solvents [52]. In consequence the proteins irreversibly loose their native geometric conformation and display initially covered free reactive groups. This is the initial step to form new intermolecular bonding between protein molecules and thus networks or films. Once unfolded, protein chains interact through hydrogen, ionic, hydrophobic and covalent bonding [53]. Bonding formation is affected by the degree of denaturation and the nature and concentration in amino acid able to form those bonds. Increased interactions and cross-linking result in stiffer films that are also less permeable to gases, vapors and liquids [25,54]. High molecular weight and fibrous proteins are in general more prone to form film with good cohesion than globular proteins, although they are difficult to solubilize or suspend in water, as already explained.

Proteins layer can be combined with paper in various ways as will be further defined in the Section 11 dealing with processes. On the one hand, lacquering processes allow preheating the protein solutions to provoke a high degree of protein unfolding. Thus the proteins are able to form a dense and stable protein film when applied on the substrate. However, due to viscosity increase, dry matter concentration of the coating formulation is rather limited. In consequence, the drying costs are high. Schmid *et al.* [55,56] showed it is possible for whey protein to use high concentrated aqueous protein solutions. In this case the proteins are still in their native state prior to the lacquering stage. Subsequently, partial denaturation and film-formation occurs during the drying stage following a patented process [57]. On the other hand, formulations with very low levels of solvent can be used in hot melt systems. Thereby, proteins are plasticized during extrusion at high temperature and are directly applied on the substrate. Plasticization temperature for the protein melt is significantly higher than denaturation temperature for protein solutions and requires typically the addition of plasticizers to improve processability and film properties. Standalone extruded protein-based layers can then be laminated with paper using adhesives.

While incorporation of plasticizers increases in general the flexibility of protein films by loosening the protein network, it generally increases the permeation of gases. The higher the plasticizer level, the higher the water vapor permeability (WVP), since plasticizers reduce internal hydrogen bonding and increase intermolecular spacing [58,59]. Incorporation of lipids, waxes or long chain saturated fatty acids in protein films, either in an emulsion [60] or as a coating [61,62] reduces the WVP. The barrier efficiency of such composite films strongly depends on the polarity of the components and the uniform or dispersed distribution of hydrophobic substances [63–65]. When the lipid forms a layer on the hydrophilic film, the barrier against water vapor transfer was higher than when a stable emulsion film was formed [63].

6. Protein Modification to Control and Optimize Film-Formation

Chemical, physical and enzymatic modifications of proteins as well as the combination with hydrophobic material or other polymers can improve the mechanical strength of protein films and their poor water vapor resistance. Protein agglomeration is one of the most critical threats for a successful coating process. By setting pH-value and selecting kind and level of dissociated salts, electrostatic and ionic conformation of the proteins can be controlled. Further modification can be used to adapt the original protein properties to a desired functionality and therefore also preventing the agglomeration and obtain a suitable film building behavior. Mechanical treatments such as dynamic high pressure, ultrasonic treatment [66] or ionizing radiation [67] are relatively mild processes leading to dissociation of protein aggregates and partial unfolding of the molecules. Partial enzymatic hydrolysis is used if a reduction of molecular weight represents an advantage or if a higher solubility is required. Specific modifications of the properties can be carried out by introducing chemical groups into the protein molecules by using for example anhydrides (acylation) [68]. Both steric and electrostatic repulsion lead to a partial unfolding and improves forming of a homogeneous network.

Acetylation with acetic anhydride inserts covalently bound neutral acetyl groups to the protein amino group. The result is a partial unfolding of the protein backbone because of reduced electrostatic attraction between oppositely charged amino acid side chains. Practical effects of acetylation are slight increase of aqueous solubility, reduced isoelectric point, and decreased tendency to gel upon heating [69]. Reaction with succinic anhydride introduces anionic succinate groups covalently linked to the amino groups of lysine. Succinylation generally has greater effects upon protein conformation and functional behavior than acetylation. The electrostatic repulsive forces, resulting from the enhanced negative charge, lead to more extensive unfolding of the polypeptide chain. Alterations of functionality commonly associated with succinylation include increase in water solubility, enhanced hydration, and modified surfactant properties. Consequently the preparation of highly homogenous films is possible.

Strength of protein networks can be increased by an actively improved cross-linking of proteins. The presence of reactive functional groups in the amino acid side chain of proteins allows cross-linking via chemical reactions with, e.g., acid, alkali or formaldehyde, glutaraldehyde, glyoxal or diisocyanate [70–72]. In addition some enzymes like transglutaminase can crosslink protein by catalyzing acyl-transfer reactions between α-carboxyamide groups of glutaminase residues (acyl donor) and ε-amino groups of lysine residues (acyl acceptor), resulting in the formation of ε-(α-glutaminyl) lysine intra and intermolecular bonds [73]. Porta *et al.* [74,75] deeply studied this process to reticulate protein component of edible films and bioplastic.

Other physical approaches such as irradiation have also been reported to modify protein conformation. Gamma-irradiation of the protein solution caused the disruption of the ordered structure of protein macromolecules as well as degradation, cross-linking and aggregation of polypeptide chains [76]. Similar results were also obtained by UV or X-rays irradiation [77]. However these reactions are difficult to control and not industrially employed for improving protein film formation.

7. Different Technologies for Processing the Coating

As mentioned regarding film forming behavior of proteins, the protein layer can be obtained via different processes whose two main types with potential for later industrial use of the protein coating in the paper-based industry are here classified in two families: wet and dry (mainly extrusion) processes. Depending on the target structure, mono- or multilayer, a technique may be favored *vs.* the other.

7.1. Wet Coating

The wet process consists of the formation of a thin layer solution which can be a stand-alone film (casting) or a coating on a different substrate. Casting is the most common method used in academic research for initial evaluation of protein solutions. In terms of coating, different techniques can be applied such as deposition of polymer films from solution (spray, dip-, roll-, spin-coatings), physical vapor deposition, *etc.* Wet coatings from polymer solutions or suspensions are mostly performed through lacquering or spraying techniques, while the application depends mainly on rheological properties of the coating formulation [78]. Especially for research, solvent casting displays the main role in protein polymer films since equipment varies from simple casting plates to batch or continuous lab coaters, making it a cost-efficient method [79]. For the drying process, different methods are applicable. The most common technique is drying under ambient conditions, while relative humidity highly influences the film properties. Other techniques are convectional drying with hot air, infrared energy or microwave energy. Each method influences film properties, e.g., morphology, appearance, barrier and mechanical properties [79,80].

Aqueous protein solutions can be used for solvent casting to obtain stand-alone films as well as for coatings. Here, the proteins are usually denatured by heat to induce an intermolecular cross-linking of disulphide bonds. Heat treatment can be applied either before or after casting or coating of the films, according to the method used. A formation of native protein films is also possible [81].

Each protein requires different processing conditions for film formation, also depending on their degree of purification. When formed out of water-borne solutions, the resulting films become brittle when dry, due to the cross-linked network. To achieve desirable properties, the use of plasticizers is indispensable [82]. Therefore film formulations comprise a polymer with high molecular weight, in this case a protein, a plasticizer and a solvent. Compatibility of all components directly influences film properties [83,84].

For film formation via solvent casting the solution is spread out evenly on surfaces before its later release to form a stand alone sample. Coatings are mostly applied via spraying or lacquering. When food products are coated, enrobing or panning the food with edible coating formulations is also possible. Film formation in both cases requires a removal of the solvent from the formulation by drying after its application [49].

Drying may limit the thickness of the coating obtained via this route. Film formation is based on removal of proteins from the solvent by precipitation or phase separation due to solvent drying in general, but changes in solvent polarity or in pH were typical of this process; other thermal treatments can also be considered. The solvents used to prepare protein film-forming solutions are generally based on water, alcohol or mixture of water and alcohol or a mixture of other solvents. Most commonly, the solvent removal is generally achieved by hot air or a combination of techniques as it may lead to a differential protein structuring and therefore variable protein films properties [85]. Indeed, as previously mentioned, during film formation, covalent bonds leading to a three-dimensional network forming may occur which have an important effect on resulting properties. As such, the functional properties of materials obtained by the wet process depend on protein concentration in solution, pH, additives, solvent polarity, drying rate and temperature [27,86].

Drying conditions are highly dependent on the drying rate and temperature. High drying rates, caused by high temperatures or air flow rates or low relative humidity can cause inhomogeneous drying which results in flaws or even film fracture. However, drying rates that are too low can easily result in uneconomic processing. Therefore, the evaluation of suitable drying conditions should be determined in a laboratory scale to indicate parameters for process up-scaling [82].

The effect of solvent polarity is quite important in film formation and properties. For film homogeneity, high protein solubility is required. Hydrophilic water-ethanol mixtures favor unfolding, which is important for the subsequent hydrogel formation. Reducing agents such as sulfites also contribute unfolding by reduction of disulphide bonds. However, protein solubility and unfolding highly depends on the adjusted pH [82].

Another important parameter to control is pH. Since proteins have a zwitterionic character, their behavior in solutions for film formation, such as unfolding, aggregation and solution viscosity is related to pH conditions. Therefore it is important to choose conditions away from the isoelectric points of the used proteins to achieve desirable viscosity of solutions and homogeneity of final films [82]. With regard to protein solutions, glycerol and sorbitol are the most common and compatible plasticizers. However, to obtain desirable barrier properties, the plasticizer content should be adjusted as low as possible and as high as necessary for film formation [49].

The temperature is also very important. As mentioned before, films can be formed out of native or heat-denatured formulations. Since heat treatment favors cross-linking of disulphide bonds, the cohesion in native protein films is mainly attributed to hydrogen bondings, resulting in water soluble, weaker film properties. Heat-denatured films show stronger networks due to cross-linking and more hydrophobic interactions caused by unfolding of the protein globular structure and therewith exposure of hydrophobic groups [81].

7.2. Extrusion

Many different proteins were investigated for manufacturing into plastics and thus processed in extruder as this is the conventional industrial method for preparing plastic granules [87]. Both plants and animal proteins were extruded such as soy proteins, corn zein, WPI, sunflower protein isolate [88], fish, beef and pork gelatin [89–91]. Proteins tend to denature and cross-link with temperature, leading to a thermoset behavior. In order to extrude the proteins, they need to be modified to display a thermoplastic behavior as studied in the extensive related work on whey protein [92–95]. However, in certain cases, a coloring of the films was observed at extrusion temperature that may be an issue in packaging film applications [96].

Process parameters such as temperature, plasticizer concentration, and residence time influence properties. Under low-moisture, proteins can exhibit a thermoplastic behavior, through a temperature increase or plasticizer addition and modification. Such protein-based materials can be shaped by extrusion, calendaring, molding and thermoforming.

Extrusion is one of the most important polymer processing techniques with key assets *vs.* other polymer converting technologies and is therefore an attractive option for biopolymers such as those derived from proteins. An extruder allows melting the polymer at high-temperature during relatively short time. With the mechanical action of the screw and temperature, the material is melted, conveyed, compressed, sheared, mixed, undergoes variation of its amorphous content, optionally reacts, and is finally shaped through a die of a desired shape. The mixing of polymers, especially filled ones, is more efficient when using twin-screw extruders often leading to superior films in terms of functional properties also depending of the main process parameters such as barrel temperature profile, screw speed, configuration (order and dimensions of the different screw segments such as conveying elements and kneading blocks along the shaft) and length-to-diameter ratio (L/D); die size/shape. Process parameters used in proteins extrusion in the literature are described in Table 2.

Table 2. Typical ranges of extrusion parameters in the processing of some proteins.

Protein	*L*/*D* Ratio	Speed Screw Range (rpm)	Temperature Profile (°C)	Reference
Whey protein	30:1	250	20–130	[93,95]
Soy protein	–	20–25	120–160	[97]
Gelatin	25:1	100–200	65–105	[90]
Keratin	25:1	25	120	[98]
Sunflower protein	25:1	20–200	85–160	[88]

Product temperature, residence time distribution, and eventual addition of moisture, torque, specific mechanical energy (SME), pressure at the die, and degree of screw fill are other important parameters. The SME is the ratio between the product of torque and screws rate and the mass flow rate. A high SME affects the structure and level of interactions among proteins, promoting cross-linking, so the suitable process of protein should keep SME low during extrusion working at low screws speed and high mass flow rate. SME depends on the rheological properties of the melt, the extent of macromolecular transformations, and interactions among components. For example an investigation about extrusion of gluten/glycerol blends revealed that temperature and mechanical energy input strongly affected the structural characteristics of the final product. In particular, extensive gluten cross-linking was observed when high SME input was used during extrusion [99].

Extrusion and other thermal processing techniques led to structure rearrangements and new interactions, which can be adjusted with the use of plasticizers and chemical additives. In general, efficient plasticizers will increase the flexibility of a molded product, imparting greater ductility. On the other hand, increasing molecular interactions, through the addition of cross-linkers or fillers, will result in a material with higher tensile strength and stiffness.

Temperature appears to be the critical parameter of protein processing. As for many polymers, the mobility of polypeptides is increased with the increase of temperature but their movement is restricted because of branched structure, of hydrophobic interactions becoming stronger and of coagulation which follows protein denaturation [88]. High Temperature and low moisture can result in protein degradation during thermoplastic processing. As the protein is fed in the extruder and comes in contact with plasticizer and moisture at increasing temperatures, their mobility increases to bridge the glass-to-rubber transition resulting in a different rheological/viscoelastic behavior. The mixture further undergoes a rubbery to free-flow transition increasing the temperature or moisture content.

Plasticizers consist of low molecular weight, low volatility substances that interact with the polymer chains producing swelling. Without water addition, the temperature region of thermal degradation would be easily reached before films could be formed [100]. However, an excessive amount of water during protein extrusion would decrease melt viscosity, which, in turn, would lead to low motor torque and specific mechanical energy input, resulting in low product temperature that could reduce the degree of protein transformation and interactions.

Water is the most effective plasticizer in biopolymer materials facilitating deformation, and processability of the biopolymer matrix. Some other types of external plasticizers used in protein-based films are monosaccharides, oligosaccharides, polyols, lipids, fatty acids and derivatives [101,102]. Glycerol, sorbitol, sucrose are examples of common plasticizers used in edible films.

Protein plastics manufactured through extrusion allowed the preparation of materials with reasonable mechanical properties. Further research into extrusion of proteins is required to produce bioplastics with less water sensitivity, high tensile strength and good ductility. Thermal processing and additives will reduce protein/protein interactions, therefore reducing the decomposition temperature [87]. In general, protein modifiers that reduce intermolecular interactions, such as plasticizers or reducing reagents, usually slightly reduce the thermal decomposition temperature of bioplastics whereas cross-linking agents and additives that increase intermolecular interactions, usually slightly increase the thermal decomposition temperature. However these effects are generally counterbalanced by the different rheological properties, as in the former case the plasticization results in a decrease in melt viscosity allowing to reduce local temperature, whereas in the latter case, in the presence of cross-linking, the increase in viscosity boosts the local increase of temperature. The study of a proper balance in melt viscosity and profile temperature is particularly important in protein processing.

Proteins, especially keratins, are defined by the large amount of the amino acid cysteine compared with other proteins. Cysteine (C) is a sulfur-containing amino acid and can form sulfur–sulfur (S–S) cystine bonds with other intra- or intermolecular cysteine molecules. Proper agents are often used to break the cysteine bonds [103]. As unfolding agents urea or guanidine/HCl are frequently used. For S–S bonds reduction Mercaptoethanol and Dithiothreitol are the most used agents. Sulfite reacts with a disulfide bond of proteins cleaving them and giving one equivaleny of S-sulfonate and a sulfhydryl group. Sodium sulfite is approved for food and drug use and is often used in low quantities as a preservative in food [54].

Some authors have studied other different reducing agents based on thiol and/or disulfide functionalized additives showing that both additives affected the cross-linking during processing and demonstrated that the degree of cross-linking is not the only factor that determines the strength of rigid, glassy gluten materials. The effect of the additives depends on the system used to mix the additives with gluten. Mechanical properties were improved when additives acted as a reducing agent during mixing [104].

The process occurring during extrusion is considered an equilibrium reaction between temperature induced cross-linking and shear induced chain scission/degradation. High temperatures and high SME input could induce excessive cross-linking and/or degradation of protein chains. At high temperatures and low moisture or plasticizer content, viscous heat dissipation could increase the likelihood of protein degradation. Optimal processing temperature is highly dependent on internal and external factors such as the amino-acid sequence and amount and type of plasticizer used.

The blending of proteins with thermoplastic polymers was widely reported as an interesting strategy to overcome the processing issues associated with almost uncontrollable reactivity of proteins in the melt extrusion. The possibility of better controlling the processing properties of whey protein by blending it with poly(ethylene-*co*-vinyl acetate) (EVA) was evidenced by Schmid *et al.* [105]. In fact they succeeded in preparing flexible flat die continuously extruded films from plasticized whey protein blended with EVA. The processing of the plasticized protein could not feed the same process, hence the blending with EVA was found essential for producing films. However, in the case where biodegradability of the blend can be essential, some biodegradable polyesters with a low processing temperature, compatible with protein low thermal decomposition temperature can fulfill this aim. As an example blends of poly(ε-caprolactone) (PCL) and corn gluten meal (CGM) were prepared by Aithani *et al.* [106]. CGM, was plasticized using glycerol/ethanol mixture, denatured by the addition of guanidine hydrochloride (GHCl), and then blended with PCL. Extrusion followed by injection molding were adopted for producing specimens and items. The processing conditions were observed to affect the thermo-mechanical performances of the blends. Mariani *et al.* [107] prepared and characterized the thermal, mechanical and morphological properties of blends based on PCL and modified corn starch, with added soy protein isolate (SPI) and sorbitol. The insertion of soy protein isolate in the formulations was done with the aim of balancing the C/N ratio of the blend, which plays a key role in the biodegradation process of these materials. Gosh *et al.* [108] employed whey protein isolate or WPI (highly aggregated) and its component lactalbumin (less aggregated) melt blended with PCL. The effects of structural morphologies of the proteins on the mechanical, morphological and *in vitro* enzymatic degradation were examined. In general, the tensile strength and modulus of the biomaterials decreased with increasing protein content, but the processability was improved and it is possible to obtain easily injection molded objects or films. As often protein wastes are employed, a high content of protein in these blends should be attained. Interestingly, the processability and properties of these blends are correlated with the phase morphology developed during extrusion which can be controlled by acting on processing parameters or on compatibilization techniques.

8. Coating Formulations Based on Widely Available Proteins

Only few of the proteins presented in Table 1 are widely commercially available as dried and concentrated or isolated protein fractions with protein content between 65% and 95% by weight [79,96]. The poor commercial availability of protein ingredients from many raw materials, high costs and unfavorable technical properties of some protein ingredients limited the interest for packaging applications to only some protein sources. Extensive studies in applications such as coatings on packaging films and paper or stand-alone films were carried out with the following six proteins: casein and caseinates, whey, gelatin, wheat gluten, soy and corn zein. These proteins origin, structures and properties are briefly described in the following.

8.1. Casein and Caseinates

Dairy protein powders are the dominant protein ingredient and are used ubiquitously in food industry. Thereby casein is the principal protein fraction in cow milk that accounts for 80% of its total proteins. It appears as self-assembled casein micelles with 50–300 nm diameter in milk [109]. Caseins include the fractions αS1-, αS2-, β-, κ-casein ranging in size from 12 to 25 kDa. In its micellar state, casein is relatively heat stable, capable to bear pasteurisation temperature of 65–72 °C. The casein structure is amphiphilic and with presence of phosphoseryl groups able to bind calcium ions [110]. In contrast to whey proteins, caseins are lacking sulfhydryl-groups and do not exhibit a tertiary molecular structure, thus they do not show the typical denaturation behavior of proteins. Centrifugation is applied to separate fat globules as well as membrane based processes to separate lactose and minerals in order to process concentrated or purified caseins that maintain their native colloidal state. A common practice to purify caseins and to alter their functional properties is based on acidic or rennet induced precipitation. So-called acid casein is produced by shifting the pH towards the isoelectric point of casein at pH 4.6 resulting in the formation of a coarse curd fraction high in casein. However, as sour casein tends to aggregate and to a low rehydration rate once dried, the potential functionality of the casein has to be released by re-neutralisation. Therefore sodium hydroxide, calcium hydroxide or other alkali are added. The formed caseinates show high functionality that depends highly on the used alkali. Sodium caseinates (NaCAS) is soluble in water with the ability to act as emulsifiers for fat and oil. Based on aqueous solutions NaCAS can easily form films. This property is based on the random coil nature of this protein and the ability to create extensive intermolecular bonds through hydrogen, electrostatic and hydrophopic interactions [111–113]. In contrast, adding calcium hydroxide forms small aggregates because of the ability of calcium, as bivalent ion, to form cross-links between two casein macromolecules.

8.1.1. Barrier Properties

In general casein-based films have a hydrophilic character. According to this, the films are sensitive to water and show high water vapor permeability [65,114]. However, in some industrial applications casein-based coatings enhance for example the water vapor barrier and the mechanical properties of paper. Khwaldia *et al.* [2,115] for example reported that NaCAS has a higher oxygen barrier than nonionic polysaccharide films. A reason for this behavior could be the more polar nature and more linear structure than noionic polysaccharides, which results in a higher cohesive energy density and lower free volume [116]. Moreover, Aloui *et al.* compared sodium alginate, chitosan and hydroxypropylmethylcellulose (HPMC) and sodium caseinate (NaCAS) as paper coating and showed that the latter exhibited the lowest WVP values [3].

The effect of plasticizers on barrier properties was investigated by different authors. Pereda *et al.* [117] used sodium caseinate blended with tung oil. Although surface tension and water absorption evidence a decrease in hydrophilicity, the tung oil did not have an impact on the water vapor barrier. Fabra *et al.* [118] also used lipids (beeswax and oleic acid) to blend with sodium caseinate and polysaccharides (k-carrageenan and alginate). In films only containing polysaccharides, water vapor barriers were reduced compared to the pure caseinate. After adding lipids the permeation decreased. Moreover the same authors [119] observed that the addition of beeswax supports the effectiveness of oleic acid in reducing water vapor permeability. Besides they stated that the substitution of sodium caseinate by calcium caseinate led to a higher water vapor barrier. However, when calcium casein substitution and beeswax and oleic acid addition were combined, the effect of calcium caseinate was hindered. Aliheidari *et al.* [120] also confirmed that oleic acid reduces water vapor permeability. Although, when compared to stearic acid and Matricaria recutita essential oil applied in sodium caseinate composite films, the latter showed the highest impact. Khwaldia *et al.* [121] compared the impact of mica, carnauba wax, glycerol and sodium caseinate concentrations on water vapor permeabilities. They reported that carnauba wax and glycerol concentrations had the highest impact on water vapor permeability (WVP). While glycerol increased the permeability, carnauba wax and mica reduced permeability. Thus in general the water vapor barrier increased when increasing the content of lipids in casein based films. Chick *et al.* [122] describe higher barrier properties for sorbitol than for glycerol plasticized casein films. Moreover they proposed that if the cross-linking of rennet casein was more extensive, this could lead to a lower oxygen permeability.

Different authors evidenced that the coating thickness significantly influenced barrier properties [2] showing that an increase of the coating weight resulted in a decrease in the water vapor permeability. This statement resulted from the comparison with different biopolymers of casein based as paper coatings.

Although no information was found about the effect of irradiation on barrier properties, it was reported that ultrasound treatment did not show any effect on the water vapor permeability [123].

The effect of cross-linking was studied by many authors, but the results were not all in agreement. In fact, cross-linking treatments with peroxidase increased the water vapor permeability of casein films [124]. However, Bruno *et al.* [125] observed that water vapor barrier increased for not cross-linked films when transglutaminase was added. Oh *et al.* enzymatically cross-linked casein films with zein hydrolysates by transglutaminases but they did not observe an impact on barrier properties [14]. The correlation between a high cross-linking degree and high barrier properties was not confirmed by these authors. Water vapor permeability can be influenced by molecular weight and developed crystallinity. Hence, the different chemical structures formed as a consequence of cross-linking, as well as the dimensions of cross-linked macromolecules network, can influence barrier properties significantly.

8.1.2. Mechanical Properties

Fabra *et al.* [118] observed that the mechanical characteristics were strongly dependent on film composition. The presence of plasticizers affects mechanical properties to a large extent. Pereda *et al.* [117] reported that tung oil content in sodium caseinate films increased tensile strength and Young Modulus but reduced deformability. Others described similar results with oleic acid, stearic acid and Matricaria recutita essential oil [120]. Sodium caseinate films containing these plasticizers had a lower tensile strength and elastic modulus but a higher elongation at break. Likewise, Khwaldia *et al.* [121] stated that higher contents of mica, carnauba wax, glycerol and sodium caseinate reduced tensile strength but increased elongation of coated papers. The effect of glycerol and sorbitol was compared on rennet and lactic acid casein films [122]. Similar results were later reported [121]. Chick *et al.* [122] found that glycerol addition resulted in more extensible films, whereas sorbitol in stronger films. However, the percentage of elongation increased for a lower plasticizer content for rennet casein films, whereas it was reduced for lactic acid casein films. In fact while k-carrageenan and alginate improved tensile properties of caseinate films, the positive effect of alginate was deteriorated when oleic acid and beeswax were added. Successively they described that elongation at break and tensile strength increased by replacing sodium caseinate by calcium caseinate [119]. However a dependence on lipid content was also observed.

The effect of cross-linking onto mechanical properties was also investigated. Brault *et al.* [112] investigated the effect of γ-Irradiation on mechanical properties of calcium caseinate and sodium caseinate film. The latter showed less cross-links and less mechanical strength than the former. Glycerol supported cross-linking and its content largely influenced mechanical properties. Likewise, Mezgheni *et al.* [126] used γ-Irradiation to create edible caseinate films. Again the irradiation enhanced puncture strength with plasticizers (propylene glycol or triethyleneglycol) thanks to cross-linking and consequent improvement of mechanical resistance. Triethyleneglycol led to films with a higher extensibility. Elevated irradiation dose induced lower breaking strength of gels, most probably due to damage in protein structure. Similar results were reported by Ressouany *et al.* [127]. Apart from γ-Irradiation, as already evidenced, Banerjee *at al.* [123] reached higher tensile and puncture strength after ultrasound treatment of NaCAS films. Nevertheless, no impact on the elongation at break was observed.

Another treatment investigated to improve the properties of casein films is the enzymatic cross-linking of proteins with itself or with other proteins (for example zein hydrolysates) by transglutaminases. Resulting films showed higher mechanical strength and flexibility [14].

8.1.3. Surface Properties

The presence of a plasticizer also influenced the surface properties of casein and caseinate films. After adding tung oil to sodium caseinate films Pereda *et al.* [117] observed more opaque and milky surfaces. Fabra *et al.* [128] investigated gloss and transparency of sodium caseinate films as a function of glycerol and oleic acid/beeswax mixture content. As a function of the increasing content of each component, gloss and transparency decreased and roughness increases, with beeswax having the strongest effect. Moreover it was found that the substitution of sodium caseinate by calcium caseinate and the addition of lipids leads to less transparent and glossy films. Conversely, pure calcium caseinate films are the glossiest.

Gastaldi *et al.* [1] calculated the penetration of wheat gluten, calcium caseinate and corn gluten meal coatings into paper. The penetration increased in the following order: calcium caseinate < corn gluten meal < wheat gluten.

8.1.4. End of Life

The effect of cross-linking onto end life was investigated as the chemical modification due to cross-linking can affect the biodegradation behavior. Mezgheni *et al.* [126] tested the effect of γ-irradiation on biodegradability. They found that irradiation increased the amount of cross-links and consequently affected biodegradability. It was described that degradation was delayed for 8 days after irradiation with 64 kGy in comparison to an irradiation dose of 4 kGy. Successively, the same authors [129] made a different observation for the same irradiation doses: for 64 kGy degradation raised significantly after 3 days. During the next 60 days, degradation went on continuously.

8.2. Whey Proteins

Whey is the soluble constituent of milk and represents about 20% of the proteins in cow milk. Being the by-product of cheese and casein manufacturing, it is highly available and still underused due to its high water content and respective high transportation and processing costs [114,130]. Only from the mid-eighties, the interest in whey has increased due to its content of nutritious and highly functional proteins. The main part of whey protein is β-lactoglobulin followed by α-lactalbumin. Depending on the manufacturing process for whey proteins, other proteins such as immunoglobulin, bovin serum albumin and specific polypeptides are included in various proportions [13,83,131]. Purified whey protein isolate powders (WPI) are processed by membrane and ion exchange chromatography techniques followed by spray drying. WPI contains above 90% protein based on dry matter and comprises α-lactalbumin and β-lactoglobulin in a ratio of 1:4 when manufactured in the membrane process and 1:7 when the chromatography process is applied [132,133].

β-lactoglobulin has a molecular weight of 18.4 kDa and contains two disulphide bonds and one free thiol group, which can interact and form new disulphide bridges once the molecule is unfolded. The rate of interaction between the thiol groups increases significantly above a pH-value of 6.8 [134]. At room temperature and at pH-values between 5 and 7 β-lactoglobulin is present as a dimer, consisting of two identical subunits that dissociate into monomers at temperatures above 40 °C.

α-lactalbumin is, with a molecular weight of 14.2 kDa, smaller than β-lactoglobulin and does, in its native form, not contain free reactive groups. It is the most heat resistant molecule among whey proteins [135,136].

Native whey proteins are globular proteins with most of the hydrophobic and sulfhydryl groups in the interior of the molecule. The formation of whey protein films usually consists of heat denaturation of whey proteins in aqueous solutions. In fact heating modifies the 3-dimensional structure of the protein, by exposing internal thiol and hydrophobic groups [137]. This allows the molecules, in particular β-lactoglobulin, to interact and to form networks. Depending on factors such as pH-values and ionic strength fine-stranded or particulate gels are formed [138]. Smooth fine stranded networks are formed at neutral to alkaline as well as strong acidic pH values, when the repulsion in the system is high.

Beside the casein fraction, whey proteins make about 20% of the total protein in milk [139]. For film formation, protein denaturation followed by intermolecular interactions is required. Denaturation of globular proteins leads to unfolding of the molecule, thereby exposing reactive functional groups that are able to form new chemical bondings such as disulfide bridges or physical linkages including van der Waals interactions, hydrogen bonding, electrostatic and hydrophobic interactions [140]. During extrusion processing, the denaturation is caused by heat treatment as well as shear stress. Whey proteins are able to form transparent, flexible, water-insoluble films and coatings out of aqueous solutions with across-linked structure [81,113]. Films were generally formed from whey protein isolate, whey protein concentrate or β-lactoglobulin in aqueous solution with different plasticizers, among which glycerol is the most common. Film properties of the formulations can be influenced or modified by the addition of lipids, salts and different plasticizers but also by chemical, biochemical or physical modifications. Whey proteins for film productions are kept into account in several recent research activities due to their good industrial potential especially as plastic lacquer and paper extruded coating [39,55,56,85,141–146]. Rossi *et al.*, prepared and investigated transglutaminase-crosslinked whey protein/pectin films [147].

8.2.1. Barrier Properties

Due to cross-linking and the high content of intermolecular hydrogen bonds, whey protein films provide aroma, oil and excellent oxygen barrier properties [13,116]. At low humidity conditions, oxygen permeability is that the range of EVOH polymer's and can therefore be used for coatings to improve oxygen barrier property of food packaging [2]. Due to their hydrophilic character, whey protein films show only poor water vapor barrier also implying a humidity dependent barrier performance for oxygen [81]. However, water vapor barrier properties can be enhanced by incorporation of lipids [113,148,149]. Used for paper coatings, whey proteins improved the packaging material properties by increasing oil resistance, reducing water vapor permeability as well as oxygen permeability compared to uncoated paper [8,33].

The type of plasticizer used for film formation highly influences the resulting barrier properties of whey protein films and coatings. Whey protein isolate coated paperboard showed a good grease barrier using glycerol as a plasticizer. However, the high migration potential of glycerol caused cracking of the coating resulting in minor grease resistance [150]. Coatings consisting of sucrose-plasticized whey protein concentrate paperboard coatings showed similar grease resistance to glycerol-plasticized films. Even after ambient storage, grease resistance remained excellent due to minor migration into paperboard when compared to low molecular weight plasticizers [151]. Therefore it was hypothesized that large plasticizer molecules may prevent plasticizer migration into paperboard during storage [150].

Sothornvit *et al.* [101] investigated the effect of a plasticizer on oxygen permeability of β-lactoglobulin films. Best oxygen permeability values were obtained using sucrose ($<0.05\ \text{cm}^3\cdot\ \mu\text{m}/\text{m}^2\cdot \text{d}\cdot \text{kPa}$), followed by sorbitol and glycerol. Polyethylene glycol (molecular weight 200 and 400) plasticized films had the poorest oxygen barrier properties. Comparing glycerol, sorbitol and xylitol as plasticizers for whey protein isolate films, Shaw *et al.* [152] evidenced that glycerol showed the highest water vapor permeability at each protein-plasticizer ratio. Increasing sorbitol and glycerol contents led to increased WVP values, while the WVP values for xylitol did not change significantly when increasing the xylitol content. WPI/xylitol (2:1) formulations showed the lowest water vapor permeability ($0.97 \times 10^{-6}\ \text{g}\cdot \text{m}/\text{m}^2\cdot \text{sPa}$).

The effect of cross-linking on barrier properties was also investigated. Ustunol *et al.* [153] used different cross-linking agents for glycerol plasticized whey protein isolate films causing an increase of water vapor permeability values and a decrease of oxygen permeability. Highest WVP was determined with glutaraldehyde followed by carbonyldiimidazole and formaldehyde. Cross-linking with dialdehyde starch showed both lowest oxygen permeability as well as water vapor permeability. In contrast to WVP, incorporation of cross-linking agents to whey protein film forming solutions can reduce oxygen permeability of whey protein films.

8.2.2. Mechanical Properties

The use different plasticizers showed negative exponential dependence on their concentration in β-lactoglobulin films for elastic modulus and tensile strength, and a linear dependence for elongation. Incorporation of the used plasticizers increased elongation and decreased tensile strength in the following order: glycerol, polyethylene glycol (200), sucrose and polyethylene glycol (400). The most efficient plasticizers for desirable mechanical properties were glycerol and PEG 200 [102]. In glycerol plasticized whey protein films, increasing plasticizer content decreased the mechanical resistance and Young's modulus [154]. Using sodium dodecyl sulfate as a co-plasticizer with sorbitol or glycerol as main plasticizer, [155] produced more extensible films. In xylitol and sorbitol plasticized WPI-films, tensile strength was significantly lower compared to glycerol-plasticized films [152]. In general, increasing plasticizer content decreased tensile strength while film elongation was increased [156].

The usage of cross-linkers (glutaraldehyde, carbonyldiimidazole, formaldehyde and dialdehyde starch) significantly increased tensile strength, while elongation is not affected in glycerol plasticized WPI films. The results are in agreement with previous ones [154] who showed that the cross-linking effect of formaldehyde enhanced mechanical properties while calcium chloride in low concentrations had no impact on functional properties of glycerol plasticized whey protein films.

8.2.3. Surface

Lyn and Krochta used whey protein coatings on peanuts, suggesting a concentration of surfactant higher than the critical micelle concentration in the coating solution to significantly increase hydrophilicity and thus coating properties [151]. Surface hydrophobicity of whey protein concentrate films could also be significantly increased by treatment with microbial transglutaminase, suggesting that moderate cross-linking can result in exposure of hydrophobic groups within the protein structure [157].

8.2.4. End of Life

Li and Chen compared the biodegradability of whey protein isolate and whey protein concentrate films from glycerol plasticized aqueous solutions using *Pseudomonas aeruginosa* under commercial composting conditions. Whey protein concentrate films degraded faster than those obtained from whey protein isolate, which might show dependence to film composition and extent of cross-links. At commercial compost conditions, whey protein isolate films lost more than 80% of total solids in 7 days [158].

8.3. Gelatin

Gelatin refers to the purified and modified collagen protein. Collagen is the principal protein component in animal connective tissues such as skins, cartilages and bones. Within collagen the protein appears in a fibrous triple helical structure consisting of three protein molecules with a total weight of around 30 kDa. These so called tropocollagen forms straight alignments with a dense, elastic and insoluble structure. Whereas the proteins inside the helical structure form almost no covalent intermolecular crosslinks, they do at the loose ends of the helical structure. Breaking down the covalent bondings under strong acidic (Type A) or alkaline (Type B) conditions with subsequent protein separation from other bone and skin materials results in gelatin. Gelatin loses its triple helical structure in aqueous solution at elevated temperatures. For fish-based gelatin, a temperature above 45 °C is required [159], whereas gelatin from pork and bovine demands a temperature above 60–65 °C. Type A and type B gelatin differ in their isoelectric point (IEP) being around pH 8.0–9.0 for type A and around pH 4.8–5.4 for type B gelatin. Consequently, type A is well suited for application in acid to neutral conditions, whereas type B is typically applied at strong acid or neutral to alkaline conditions.

Furthermore gelatin has the ability to form cold–set thermo-reversible networks when chilled. At elevated temperatures the proteins in this network become water soluble again. Network formation depends further on the cooling rate. At low cooling rate, single molecules form alignments and helical structures similar to the origin material, whereas for high cooling rates short alignments and disordered areas alternate. The latter network type has the ability to immobilize high quantities of water and to form elastic gels.

Gelatin has excellent film forming properties but its films are brittle and plasticization or toughening is needed for most practical applications. Solutions based on gelatin and polyvinyl alcohol (PVA) revealed Newtonian behavior at 30 °C and were successfully used for preparing films. PVA and plasticizer concentration did not change the viscosity. Optimal film-forming conditions were found at a pH of 10.54 while adding 55.18%, w/w corn oil [160]. Also chitosan can be used for preparing gelatin based films [161]. In both cases (PVA and chitosan), the formation of hydrogen bonding is fundamental for attaining a good miscibility.

8.3.1. Barrier Properties

Concerning barrier properties, oxygen permeability values of mammalian gelatin films are higher than for cold-water fish gelatin films thus showing the effect of different aminoacidic sequences on barrier properties [162].

Arvanotoyannis *et al.* [163] observed that a higher amount of renaturated gelatin initiated an improvement in gas and water barrier. Other authors [58] reported that formaldehyde increased cross-linking but decreased water vapor barrier. Also the addition of polyols (except sorbitol) led to a lower barrier, as the free volume increased. Sorbitol improved barrier properties.

According to different authors [164,165], UV treatment reduced the swelling of collagen films during hydration. In transglutaminase cross-linked gelatin films containing glycerol, the cross-linking led to a decreasing in oxygen barrier at relative humidities >50% [166]. Other authors [167] used chemical and enzymatic modifications of gelatin and found that each of them led to a decrease in solubility. The best water vapor barrier occurred for enzymatic treatments. They suggested that, due to the hydrophobicity of the protein itself, the influence of cross-linking is not significant on oxygen barrier.

By adding bergamot and lemongrass oil improved barrier properties were observed for gelatin films [168]. Other authors [169] had similar results by using 25%–30% of palm oil. Moreover both water vapor permeability and oxygen permeability were shown to be affected by corn oil content and pH value [160]. Furthermore, other authors [170] found that increasing the content of citric acid and soy lecithin as emulsifiers in films reduced the barrier towards water vapor because of increased hydrophilicity of the films.

In films obtained by flat die extrusion, extruder speed and temperature did not have a major effect on barrier properties [89,90]. A higher screws speed determined a higher solubility of the films in water, while a higher extrusion temperature reduced water vapor permeation.

8.3.2. Mechanical Properties

Mechanical properties of gelatin from different sources were compared. Tensile strength, elongation percentage at break and puncture deformation declined in the following order: gelatin from mammals, from warm- and from cold-water fish. It was reported that at 70 °C heated squid gelatin films showed increased tensile strength but reduced elongation [171]. Temperatures above 70 °C lead to an opposite behavior.

Hanani *et al.* [89] tested the effects of speed and temperature in a twin screw extruder. Whereas higher temperatures increased the elongation at break and decreased the tensile strength, higher screw speeds increased both tensile strength and puncture strength. The results were in agreement with those of Rubin *et al.* [164] about collagen films. It was reported that these films reached a higher burst strength after a UV treatment carried out on wet films.

In order to create a biodegradable film with improved barrier properties, films made of gelatin and polyvinyl alcohol cross-linked with glutaraldehyde were produced [172]. No major improvements in mechanical properties were observed. Gelatin cross-linked with transglutaminase showed greater elongation but lower tensile strength with water and glycerol as plasticizers than non-cross-linked ones. Various authors [166,173] tried different edible casein–gelatin blends cross-linked with transglutaminase. Blends showed better elongation properties than pure casein or gelatin, but no effect was observed in terms of tensile strength.

Other authors [170] discovered that higher contents of acetyltributyl citrate and tributyl citrate decreased tensile strength. It did not show an effect on the elongation. Moreover different glycerol concentrations in PVA gelatin films were used [174]. Films containing PVA Celvol 418 (DH = 91.8%) showed very good tensile resistance (tensile strength = 38 MPa), but after adding glycerol, the quality drastically decreased. Other authors [175] added catechin-lysozyme to fish gelatin. An addition of catechin-lysozyme of 0.5% showed the highest increase in elongation at break and water vapor barrier.

In order to replace glycerol, bergamot and lemongrass oil into gelatin films were used but lower tensile strength and elongation at break were obtained. The addition of palm oil [169] resulted in increased elongation at break but lower tensile strength. On the other hand, corn oil content linearly enhanced tensile strength and puncture strength, whereas pH had a stronger effect on elongation values [160].

For modulating mechanical properties, the applicability of different plasticizers for gelatin films (sucrose, oleic acid, citric acid, tartaric acid, malic acid) was studied [176]. Only malic acid enhanced the ductility. The comparison of polyethylene glycols of different molecular weights illustrated that higher molecular weights led to minor plasticization. Other authors [177] used glycerol, propylene glycol, di-ethylene glycol and ethylene glycol in different concentrations. Di-ethylene glycol showed improvements in puncture deformation whereas glycerol showed the best plasticizing effect. No influence on water vapor barrier was detected.

Arvanitoyannis *et al.*, described that higher amounts of plasticizers (water, glycerol, sorbitol) led to lower elastic modulus and tensile strength in their gelatin/starch edible films but they did not observe an increase in elongation at break [163]. Interestingly the addition of KNO_3 and microbial transglutaminase (for enzymatic cross-linking) in gelatin films obtained from water solutions was found to improve elongation at break of films thanks to the combined effect of cross-linking and KNO_3 crystallization [178].

8.3.3. Surface Properties

Some studies were reported about functionalisation of polymeric surfaces [179] or paper [180] with gelatin, especially for improving biocompatibility in biomedical applications and tissue engineering.

Gelatin is often used as additive in several water based formulations for imparting anti-microbial or adhesive properties to paper. As an example in the field of packaging some authors [31] evaluated sealing properties of gelatin based coatings on oriented polypropylene (OPP) surfaces. Up to 1 wt % of a hydrophobic component improved sealing properties. Glycerol as a plasticizer gave the best results. A pH value beyond the isoelectric point also improved sealing properties.

8.3.4. End of Life

Gelatin, as a protein, is subject to a fast biodegradation when the contamination and the environmental conditions are adequate. Bacterial contamination can be present at different stages of the gelatin production process and quality control of gelatin producing factories pointed out that thermotrophic, aerobic, proteolytic endospore forming bacteria might be present during the production process. The presence of peculiar bacteria such as Bacillus *B. amyloliquefaciens* and *B. subtilis* can accelerate the biodegradation rate of gelatin based films used in the photographic sector [181].

8.4. Wheat Gluten

Wheat gluten is known to form a unique visco-elastic network that gives integrity to wheat dough and is used for a wide range of bakery and other food products for centuries. In recent years, it became the most available plant based protein ingredient, manufactured as co-product of starch and ethanol production from wheat.

Wheat gluten is a general term for more than 50 different salt water-insoluble wheat flour proteins of different classes and represents about 85% of total proteins. It contains the glutelin and prolamine fractions of wheat flour proteins, typically referred to as glutenin and gliadin, respectively. Gliadin is soluble in 70% ethanol, but glutenin is not [182–184]. Although insoluble in natural water, wheat gluten dissolves in aqueous solutions of high or low pH at low ionic strength. Gliadins consist mainly of monomeric chains with molecular weights in the range from 28 to 55 kDa. Having only very few disulphide and sulfhydryl groups, gliadins associate while forming non-covalent bonds and thus contribute to increase both in viscosity and extensibility of the gluten network [185]. In contrast, glutenin refers to a polymeric, high molecular weight protein fraction with a molecular weight that range from 500 to 10,000 kDa. However, after reduction of disulphide bonds, the resulting glutenin subunits with molecular weight of 32–88 kDa show a solubility in aqueous alcohols similar to gliadins [186]. The ability of glutenin to form intermolecular covalent disulphide bonds and, to a minor degree, covalent bonds at the tyrosine side chain account for the elastic properties of the gluten network. Léon *et al.* [187] measured thermally induced denaturation by DSC of wheat glutenins at 60 °C for dry (12.7% moisture) and 41 °C for moist (52.1% moisture) protein preparations.

The unique property to form an insoluble protein network while shearing a wheat flour with water enables separation and purification of aggregated wheat gluten in industrial scale by washing out starch, fibers and further components with water [188]. Gentle drying at to a low moisture level and short temperature exposure allows the production of the so-called vital wheat gluten powder that maintains the typical properties of purified wheat gluten. This powder forms again a rubber like, cohesive and turbid network when rehydrated.

Wheat Gluten films are typically prepared from aqueous alcoholic protein solutions. Since solvent casting is the method widely used for gluten film formation, homogeneous film solutions are required, aided by mechanical mixing, heating as well as the adjustment to alkaline or acidic conditions [97]. During heating, dissociation of native disulphide bonds takes place, resulting in free thiol groups that can form new bonds during drying. Other influences on film formation are hydrogen and hydrophobic interactions [182]. When pH is high, the cleavage of disulphide bonds is favored, allowing a higher stretchability and resulting in weaker films compared to films prepared from acidic conditions [189]. To achieve flexible wheat gluten films, the addition of plasticizers like glycerol or sorbitol is indispensable [15] and also for molding wheat gluten based materials [190]. Wheat gluten is also used as a binder for paper coating dispersions, showing good film-forming properties. These paper coatings possess good adhesion to various substrates [191].

8.4.1. Barrier Properties

Generally wheat gluten films show selective oxygen and carbon dioxide barriers, while, due to their hydrophilic nature, water vapor barrier properties are low [192]. Films prepared from glutenin fraction showed higher water vapor permeability values than those obtained from gliadin, which can be attributed to the extended random coil conformation of glutenin [97]. Carbon dioxide sorption and diffusion was found to increase by increasing relative humidity, leading to higher carbon dioxide permeation values [193].

The presence of plasticizers strongly influences the poor water vapor barrier of wheat gluten films [192]. When the plasticizer content is increased, water vapor permeability also increases, resulting in a reduced water vapor barrier [189].

Concerning the effect of cross-linking, it was observed that differences in heat treatment and formaldehyde treatment, both influencing the cross-linked state of wheat gluten films, did not affect water vapor permeability [192], while calcium cations used for the promoting of cross-links in the protein network structure decreased water vapor permeability [27].

8.4.2. Mechanical Properties

The effect of plasticizers on mechanical properties was widely investigated. Mechanical properties of wheat gluten films are generally dependent on the water content, having even larger influence than the processing conditions. Therefore, storage conditions and water contents in film formulations must be considered and controlled [194].

Pommet *et al.* [195] stated that a low melting point and a moderate hydrophobicity were the critical characteristics to consider when choosing a wheat gluten plasticizer. They compared water, glycerol, 1,4-butanediol, lactic and octanoic acids on their plasticizing properties. Lactic acid as well as water resulted in improved elongation at break while tensile strength properties were found to be closely related to the plasticizer content in general [195]. In an alkaline film casting process of gliadin films, different plasticizer concentrations also led to a wide range of elongation values. Tetra-ethylene glycol was found more convenient than glycerol when comparing different polyols of the polyethylene glycol series [196]. In fact less volatile plasticizer allow a better durability in mechanical properties.

Concerning the effect of cross-linking on mechanical properties, Micard *et al.* [192] obtained cross-linking of gluten films by thermal treatment in the presence of formaldehyde. Both treatments improved mechanical resistance of films, leading to an increase in tensile strength and Young's modulus but a decrease in elongation at break. The addition of sodium sulfite used as a reducing agent for intermolecular disulphide bonds resulted in a slight increase in tensile strength (14%). A similar result was achieved by using calcium cations as cross-linking agents [197].

8.4.3. Surface Properties

Sanchez and others studied the effect of different plasticizers (five polyols of the ethylene glycol series and glycerol) on the surface properties of wheat gliadin films, showing high hydrophobicity but no influence of the plasticizers used [157,196] significantly increased ($p < 0.01$) the contact angle values and therewith surface hydrophilicity of wheat gluten films by microbial transglutaminase treatment.

Using a wheat gluten solution for paper coating, coated paper showed a smooth surface the porous structure of paper was covered [1].

8.4.4. End of Life

In a simulated farm and soil mix (topsoil, sand, sunshine compost, vermiculite; 59:6:25:10 wt %) the biodegradable behavior of films containing wheat gluten and soy protein (S:G 4:1; prepared at pH 7) was evaluated. The films showed rapid biodegradation with a weight loss of 50% in 10 days and up to 95% in 30 days and a significant decrease of tensile strength in only 3 days. After 20 days the films were disintegrated [10].

8.5. Soy Protein

Proteins in grain legumes such as soy, pea, bean and peanut, consist of two principal fractions. About 10%–20% of the proteins are water soluble and small (10–20 kDa) albumins, whereas the main protein fraction exists as large, globular storage proteins. These globulins are described as vicilin- and legumin-like proteins, according to respective proteins fractions in pea. In soy, these globular components are named β-conglycinin (7S) and glycinin (11S). Both of them have a quaternary (subunit) structure which can be disrupted by high ionic strength, acidic and base pH-values and heat treatment. β-conglycinin, a trimer, consists of three subunits, each with a molecular mass of 52–72 kDa. Two out of three known subunit structures possess one cysteine residue (–SH), whereas no cystine residue (–SS–) exist in any of the subgroups [198,199]. In contrast, glycinin is a hexamer with six subgroups, each consists of an acidic (~35 kDa) and a basic (~20 kDa) polypeptide which are linked together by a disulphide bond [200]. A total of ten different subunits are described for glycinin. Each subunit contains two to three cysteine and cystine residues. Those depending on the subunit composition of β-conglycinin and glycinin is genetically determined, so soy protein can possess various gelling properties. Overall, gels from β-conglycinin proteins are described as transparent, soft and rather elastic, whereas gels high in glycinin appear more turbid and hard. These gelling properties are further depending on ionic strength and pH-value that control aggregation and dissociation of the proteins and their subunits. In particular, solubility of both globulin fractions depends highly from these conditions, with minimal value in the pH-range of 4.0–4.5 and high solubility at neutral to alkali pH-value. Consequently, aqueous extractions procedures with alternating increases and decreases in pH-values are used to produce soy protein isolates (SPI). SPIs are commercialized as spray dried, stable powders of creamy color [201].

Soy protein isolate (SPI) is usually a by-product of the soybean oil industry. Like other globular proteins, SPI shows good film-forming properties and is therefore suitable for edible films and coatings [202]. By modification of SPI formulations, mechanical properties as well as water resistance can be improved [203]. Soy proteins are widely used for cast film production out of aqueous solutions. To avoid brittleness, a plasticizer must be added for film formation [24]. For soy protein based films, glycerol is the plasticizer most often used [97]. Film qualities can be improved by alkali treatment, different types of plasticizers like glycerol or sorbitol, via chemical modification or addition of hydrophobic substances such as waxes or lipids [202,204]. By the use of plasticizers and chemical modification (e.g., by sodium sulfite), dry processes like extrusion processing of soy protein films are also possible [87].

Especially in wet processes, protein concentration and pH of solutions are decisive factors. Common protein concentrations are 4%–5% in wet processes and about 80% in dry processes [205]. Films can be formed in both acidic and alkaline conditions [97]. The cross-linking of pectin-soy by enzymatic treatment with transglutaminase was also investigated for preparing edible films. The presence of pectin facilitated the formation of high molecular weight species due to electrostatic interactions occurring between soy proteins and pectins, resulting in a suitable conformation for acting as transgluminase support [206,207].

8.5.1. Barrier Properties

Concerning the effect of plasticizers on barrier properties, Wan and others compared soy protein films plasticized with glycerol and propylene glycol, sorbitol or sucrose at a ratio 25:75, 50:50, 75:25, and 0:100. Plasticizer type and ratio both affected water vapor permeability. The substitution of glycerol by only 25% of the less hydrophobic plasticizers in the mixture resulted in a significant reduction of water vapor permeability [208]. Kim and others compared sorbitol and glycerin plasticized films as well as sorghum wax composites films based on SPI. With increasing sorbitol and glycerol content, WVP increased. However, sorbitol addition gave lower WVP compared to glycerol plasticized films, while the addition of sorghum wax lowered WVP in all samples [209].

Concerning the effect of cross-linking on barrier properties, it was observed that thermal induced cross-linking by heat-treatment decreased water vapor permeability in soy protein films, while enzymatic treatment (horseradish peroxidase) provided no further improvement of barrier properties [210]. Ghorpade *et al.* [211] chemically modified glycerol-plasticized soy protein films with the addition of acetic anhydride, succinic anhydride, calcium cations, and formaldehyde. While formaldehyde treated films showed higher oxygen permeabilities, water vapor permeability was reduced. All other used cross-linkers did not affect film barrier properties. Using microbial transglutaminase as cross-linking agent, [212] also found no significantly differences ($p > 0.05$) in water vapor transmission rates by enzymatic treatment.

Although it was suggested that UV irradiation induces cross-linking in glycerol plasticized soy protein cast films, irradiation (13.0, 25.9, 38.9, 51.8, 77.8 or 103.7 $\mathrm{J/m^2}$) had no influence on water vapor permeation [165].

Rhim *et al.* [20] determined the barrier properties of SPI coatings on paperboard with and without cross-linking treatment via formaldehyde. The barrier properties for water vapor were increased by both coatings.

Concerning the effect of pH on barrier properties, films obtained from different pH conditions of the soy protein isolate solutions show different barrier properties. Acidic films showed higher WVP than alkaline films (pH 8 and pH 11). Furthermore, films formed at pH above the isoelectric point of the proteins showed lower WVP than those prepared in acidic conditions (pH 1–3). This effect might be attributed to different intermolecular cross-linking behavior as well as the hydrophilic character which is correlated to the water binding of films which allow faster permeation [213].

8.5.2. Mechanical Properties

Concerning the effect of plasticizers on mechanical properties, Wan *et al.* [208] compared films plasticized with mixtures of glycerol and one of the plasticizers propylene glycol, polyethylene glycol, sorbitol or sucrose. To increase tensile strength significantly, at least 50% glycerol had to be present in the mixture. Films with a plasticizer mixture of glycerol and sorbitol (50:50) showed relatively high flexibility and strength. Pure glycerol plasticized films presented a tensile strength of 7.8 MPa and elongation values of 155%, while using polyethylene glycol 400 for plasticization lowered tensile strength to 5.8 MPa [208]. Kim and others compared the mechanical properties of soy protein films with addition of sorghum wax, sorbitol, glycerol. All independent components significantly ($p < 0.05$) had an effect on tensile strength and elongation at break of films. With increasing sorbitol content, tensile strength values also increased [209].

Within the study about the effect of cross-linking on mechanical properties, Gennadios *et al.* [165] compared the mechanical properties of glycerol plasticized soy protein cast films irradiated with different UV radiation values (13.0, 25.9, 38.9, 51.8, 77.8 or 103.7 $\mathrm{J/m^2}$). They detected a linear increase of tensile strength and a linear decrease of elongation with increasing UV dosage due to suggested induced cross-linking in films [165].

Heat induced cross-linking of SPI edible films (heat treatment with 85 °C) increased elongation at break and tensile strength. Further enzymatic cross-linking treatment (horseradish peroxidase) increased Young's modulus while elongation at break and also tensile strength decreased.

Cross-linking of glycerin-plasticized soy protein cast films (alkaline protein solution at pH 8.5) induced via acetic anhydride, succinic anhydride, calcium cations, and formaldehyde also affected the mechanical properties. Tensile strength was slightly increased with calcium cations treatment and more than two-fold increased by formaldehyde treatment [211].

Tang and others used microbial transglutaminase as an enzymatic cross-linking agent for glycerol and sorbitol (0.6 g plasticizer per g SPI) plasticized SPI cast films, but a slightly increased tensile strength and a highly reduced elongation at break were observed [212].

Using SPI with further cross-linking treatment as a coating for paperboard, the material's tensile strength was reduced by 12.5%–37.5% compared to the uncoated paperboards [214].

Gonzalez *et al.* [215] used different amount of naturally occurring genipin as cross-linking agent for SPI cast films from heated and alkaline aqueous solutions plasticized with glycerol. Mechanical properties were improved with only small amounts of genipin due to linearly increasing cross-linking. Tensile strength and elongation were both increased although addition of larger amounts of genipin were followed by a decrease of elongation values.

Concerning the effect of pH on mechanical properties, Mauri and others studied SPI cast films at different pH. Showing a higher amount of disulphide bonds, films obtained from solutions at pH 2 and 11 presented a higher tensile strength and Young's modulus of about 1.05 MPa and 0.15 MPa, respectively, compared to films obtained at other pH. Alkaline films (pH 8 and 11) showed a 70% higher deformation than films at the acidic pH of 2 due to native protein fractions that are able to unfold under mechanical load and therewith enabling greater deformation. This is consistent with a study by Brandenburg *et al.* [24], showing that alkali treated films also showed higher elongation. By comparing film properties at pH 6, 8, 10 and 12, a lower tensile strength and elongation at break was detected at pH 6 while at higher pH an increase in tensile strength was observed.

8.5.3. Surface Properties

Enzymatic cross-linking via 4 units microbial transglutaminase per g SPI in glycerol and/or sorbitol plasticized films increased surface hydrophobicity by 17%–56% depending on the plasticizer used. The films displayed a rougher surface and a more compact cross section than untreated formulations, suggesting a modification of microstructure by enzymatic treatment [212].

8.5.4. End of Life

Biodegradation studies on glycerol plasticized SPI films showed a biodegradation time of at least 14 to 33 days, depending on the film composition [215]. Park *et al.* [10] used a simulated farmland soil mix (topsoil/sand/sunshine compost/vermiculite, 59:6:25:10, wt %) for biodegradation of soy protein-wheat gluten films. After 20 days, films were disintegrated into the soil mix environment.

8.6. Corn Zein

In corn all four Osborne fractions (albumins, globulins, prolamin and glutelin) are present. As in wheat, the prolamin fraction named corn zein or corn gluten, is the principal protein fraction in corn endosperm, where it represents about 50% of the protein followed by glutelin. However, protein composition varies widely depending on corn variety and includes also proteins that cannot be classified into the four Osborne fractions. Malumba *et al.* [216] found reduced protein solubility once corn kernels were dried at a temperature of 80 °C. Since kernel moisture content is rather low, protein denaturation temperature under moist conditions may even be lower.

Native zein consists of a polypeptide with a molecular weight of about 85 kDa or larger. Under reducing conditions that break up disulphide bonds, four major substructures with molecular weights ranging from 9 to 50 kDa can be defined [217]. Zein dissolves in 70%–80% aqueous ethanol or pure propanol and is insoluble in water at room temperature [218]. Sessa *et al.* [219] reported that zein aggregates in water/glycol solution and becomes more viscous when exposed to heat and shear that allows film formation in extrusion processes. Lawton found, that zein can form dough like structures with water and starch when processed above glass transition temperature at 60 °C [220]. Commercially available zein products extracted with aqueous-alcoholic solutions contain a zein ratio greater than 90% [48]. Compared to other plants, zein protein isolates processing is more sophisticated and thus zein is relatively costly [221].

Sessa *et al.* [219] investigated the torque of zein. Zein aggregates and becomes more viscous when exposed to heat and shear, what may considerably affect manufacturing processes like e.g., extrusion. In fact, Selling *et al.* [222] had examined the effect of water and triethylene glycol on zein. The torque increased at temperatures of 90 °C and contents higher than 10% of water. Moreover the torque linearly increased with time. Only at 120 °C a quick torque rise was observed. The same authors [219] added different amounts of triethylene glycol, sodium dodecyl sulfate and water. It is reported that the addition of sodium dodecyl sulfate and triethylene glycol delayed the viscosity rise and the effect was more pronounced by increasing the amount of sodium dodecyl sulfate.

Other authors [223] already carried out experiments extruding zein through a twin screw-extruder. They reported that zein proteins flowed easily through the extruder barrel and melted homogeneously. Moreover films produced by extrusion with were compared with films produced by casting [224]. Only the latter one showed phase separation during heating and brittleness. Film processing was thus shown to have a strong influence on film properties. Moreover, Wang *et al.* [225] stated that the film forming process is easier when using oleic acid.

8.6.1. Barrier Properties

Within the studies about the effect of plasticizers on barrier properties, some authors [226] investigated oxygen permeability of zein films containing a mixture of sorbitol/glycerol/mannitol. Glycerol and sorbitol decreased oxygen permeation (sorbitol more significantly). In contrast, mannitol increased oxygen permeability.

Instead of blending zein with oils, other authors [227] coated the zein film surface with tung oil, linseed oil and a mixture of tung and soybean oil. Reduced water vapor permeability was observed. In the successive step Rakotonirainy *et al.* [228], validated the usability of coated zein films for modified atmosphere packaging (MAP). Similarly, the same authors [229] coated zein films with flax oil and tung oil respectively. Likewise, water vapor permeability and liquid water transmission was reduced, most probably thanks to hydrophobicity and peculiar microstructure, as the microstructure of flax oil samples showed a good surface distribution. Moreover a high resistance of zein films containing oleic acid towards water could be observed. However, films absorbed water after a longer time and were therefore not suggested to be used for wet products. Similarly, Santosa *et al.* used oleic acid and linoleic acid as plasticizers [230]. Moreover, additional heating in fatty acid of oleic acid zein samples led to a replastification. The result was the achievement of a lower water absorption of zein sheets.

Padgett *et al.* [231] described the influence of lauric acid on zein films. For microbiological protection, the films contained a few milligrams of nisin per gram of film. Moreover thanks to the addition of lauric acid water permeability was considerably decreased.

Other authors [37] added glycerol to zein paper coating and report increased water vapor permeation values. Parris *et al.* [232] reported good water vapor properties for unplasticized zein films cross-linked with 20% polymeric dialdehyde starch. Similar to Trezza *et al.* [37] the addition of a mixture of glycerol:poly(propylene glycol) having a ratio of 1:3 nearly increased water vapor permeation by a factor 2.

As also water acts as a plasticizer for zein films, increasing relative humidity leads to more flexible films. As the water content increases, oxygen permeability coefficients increases accordingly. Ghanbarzadeh *et al.* [233] used fructose, galactose and glucose as plasticizers and observed reduced water vapor permeability, especially by using galactose.

The effect of coating weight was investigated by Trezza *et al.* [234] as they coated paper with zein coating at 7.9, 15.8 and 23.7 g/m^2 respectively. Although grease resistance was lowest for the lowest coating weight, there was no significant difference in grease resistance between 7.9 and 15.8 g/m^2. Other authors [235] observed, that grease permeation through zein isolate levels above a specific value is independent on coating weight. However, water vapor permeation correlated with coating thickness. Likewise, Trezza *et al.* [37] stated that although the water vapor permeation is reduced for higher coating weights, no change appears between 16 and 24 g/m^2. Also oxygen permeation was reduced by higher coating weights.

Concerning the effect of cross-linking on barrier properties, Soliman *et al.* [236] irradiated zein film-forming solution and found that this treatment could increase water barrier properties. Other authors [229] found that zein films covered with flax oil/tung oil and treated with UV/γ-irradiation showed better water vapor barrier depending on the combination of treatments and testing conditions.

Concerning the effect of processing onto mechanical properties, according to Parris *et al.*, grease resistance of kraft paper mainly increased when coated with a composition of 80%–85% protein, 15%–20% lipid and <0.25% starch. They showed that spraying the coating led to better results than brushing it: the grease stained area was about 13.8% ± 10.8% for brushed coating whereas it was 3.0% ± 2.8% for spray coatings. Ha *et al.* reported that also extrusion temperatures affected water resistance of zein sheets [224].

8.6.2. Mechanical Properties

Some authors studied the effect of plasticizers onto mechanical properties of corn based films. As an example zein was plasticized with different contents of sorbitol, glycerol and mannitol [237]. At low plasticizer concentration, the strain at break is similar for sorbitol and glycerol. At higher plasticizer concentration, sorbitol had higher strain at break values than glycerol and mannitol. At low plasticizer concentration, sorbitol led to a higher ultimate tensile strength than glycerol and mannitol. Gillgren *et al.* [238] added polyethylene glycol, glycerol and lactic acid in different concentrations to kafirin, zein and avenin. In particular, zein was more stretchable at higher plasticizer contents [232] compared to zein films prepared in aqueous ethanol or acetone containing no plasticizer, glycerol or glycerol/poly(propylene glycol) (1:3 ratio). Films made with acetone were stronger but films with ethanol were more flexible. The addition of glycerol and especially of glycerol:poly(propylene glycol) 1:3 enhanced tensile properties. Ghanbarzadeh *et al.* compared glycerol and olive oil as plasticizer in single and laminated films of whey and zein protein [239]. The elongation at break was 260% for whey–zein–glycerol laminate and 200% for the whey–zein–olive oil laminate.

Some authors [227] applied coatings of tung oil, linseed oil, or a mixture of tung and soybean oils on zein films containing oleic acid. Higher tensile strength, elongation and film strength were observed. Other authors [229] found that also the coverage of zein films by flax oil or tung oil increased elongation values. The reason for that might be the filling of pinholes and cracks in the films [237].

The effect of cross-linking was investigated by Soliman *et al.* [236] who observed the effects of irradiation on mechanical properties. Nevertheless, the results did not lead to a definite conclusion.

Lai *et al.* [224] related film processing (conventional casting from ethanol solutions *vs.* stretching of plasticized resins) to material properties. Cast films were stiff and brittle, whereas films obtained by material blended in a discontinuous mixer showed more flexibility and toughness. Ha *et al.* extruded zein films and changed extrusion temperatures, which lead to altered mechanical properties [223]. Wang *et al.* [225] compared solution casting, stretching of moldable resins and blown film extrusion of zein. Based on the X-ray analysis, it was possible to deduct that supramolecular arrangements vary with processing methods, whereas the macromolecular structure withstands processing.

8.6.3. Surface Properties

In terms of surface properties the effect of a plasticizer was investigated by Ghanbarzadeh *et al.* [226] who compared surface morphology and roughness and found that glycerol favors the achievement of smoother surfaces with lower roughness than sorbitol and mannitol. Wang *et al.* [229] found, that flax oil coatings lead to a homogeneous coverage and finish. In comparison to that, tung oil coatings with UV light treatment showed darkened regions. Ghanbarzadeh, *et al.* report that not plasticized zein films have holes and cracks, whilst fructose addition produced smoother, loose structures with a lot of cavities and voids [233]. The films plasticized by fructose had smoother surfaces. Santosa *et al.* [230] reached sheets of high clarity after re-plastification through reheating.

Trezza *et al.* [234] compared different coating levels of 2.2 kg, 4.4 kg and 6.6 kg per ream and found surface discontinuities only in the lowest coating level.

Within processing influence on surface properties, Lai *et al.* [224] discovered that conventional casting of zein films from ethanol solutions led to more stiff and brittle films than the film obtained by the solution precipitated in water, dried and processed in a discontinuous mixer. The stretching of the latter led to more flexible and tough films. Moreover a more distinct structure development and orientation is visible in stretched films than in cast films.

8.6.4. End of Life

According to Trezza *et al.* [234], corn zein coatings do not hinder paper recycling and separation of protein and paper layers is not necessary. On the other hand Khwaldia [7] reports that more investigation about biodegradability, compostability and recyclability are necessary.

Parris *et al.* [235] evaluated the hydrolysis of zein by R-chymotrypsin, pancreatin 8× and pancreatin 1×. After 10 and 30 min, no additional growth in the hydrolysate peak arose for R-chymotrypsin and pancreatin 8×. Nevertheless, 1 h after hydrolysis by pancreatin 1×, zein and lipid could still be detected.

In a previous work, Parris *et al.* [240] tried to hydrolyse zein-wax-coated paper enzymatically. It is reported that 97% of the zein-wax coating was successfully separated from the substrate after adding Tris buffer pH 8.5, containing 0.1% SDS and R-chymotrypsin with and enzyme-to-substrate ratio 1:100 for 0.5 h.

9. Proteins with Low Availability or Not yet Developed

Many proteins were investigated as sources of materials for making films or coatings. For some of them the investigations are still in a phase of evaluation of availability and possibility to extract them through a sustainable industrial process. The investigated proteins can be classified in those coming from the animal, plant or fungi Kingdom.

9.1. Emerging Proteins from Animal Kingdom

Most investigated proteins form animal Kingdom are those coming from the food industry that produces high amount of waste not easy to manage. Hence the use of waste for producing structural and/or functional materials can have a strong relevance.

Keratin protein, for instance, can be abundantly obtained from poultry feathers. Barone *et al.* [98] extruded it at 120 °C using a combination of glycerol, water, and sodium sulfite as processing aids. Rheological properties were assessed as a function of additives content as well as processing parameters such as extruder die temperature. The blends showing the lowest viscosity at a constant feather keratin concentration of 60 wt % were found at glycerol concentrations that were higher than the water concentration and the optimum sodium sulfite concentrations was of 3–4 wt % of the feather keratin fraction. Out of this latter range, an increased viscosity was observed. In the solid state, it was observed that processing induced macromolecular orientation that increased the tensile properties of the extrudates. However, keratins contain large amount of the amino acid cysteine with respect to other proteins, hence the control of their processing can be in general more difficult and for this reason a high content of sulfite is necessary for processing. In fact up to now films were prepared mainly by solvent casting [241] using cross-linker such as dialdehyde starch (DAS). The addition of DAS increased the transparency value and moisture content and decreased the solubility, indicating the cross-linking between the keratin and DAS. The films with 2% DAS showed increased tensile elongation and water vapor permeability compared to the control films, whereas the tensile strength of the film decreased, most probably because the cross-linking effect was counterbalanced by the plasticization of DAS. Thanks to the addition of DAS, an increase in the water resistance of the feather keratin films was obtained, thus allowing the possible application in food packaging.

However, currently, the attention of researchers is mainly devoted to proteins from sea species. Shiku *et al.* [242] investigated the preparation and properties of edible transparent and flexible films made of fish myofibrillar proteins. They improved the previous work [19] by studying more in detail the effect of pH of film-forming solutions on film formation, on mechanical, barrier and optical properties. Myofibrillar protein-based films were prepared within pH 2–3 and 7–12. They were not prepared between pH 4 and 6 because of the poor protein dispersion around the isoelectric point. The tensile strength of the films was higher when prepared in acidic (pH 2, 3) and alkaline (pH 11, 12) conditions than in neutral, whereas the elongation at break was almost independent of pH. Overall, the pH of film-forming solutions had no effect on water vapor permation, light transmission, film solubility and enzymatic hydrolysis. Water vapor permeation of myofibrillar protein films [242] were slightly lower than those of other protein-based edible films, such as whey, gluten and zein (Table 6) and higher by one to three orders of magnitude than those of synthetic films. The films prepared at lower or higher pH possessed superior transparency, similar to synthetic films. Successively Sobral *et al.* [243] extended the work to the muscle proteins of the Thai Tilapia fish, finding the correct conditions for obtaining a film similar to general myofibrillar protein films but with different optical properties with marked color and less transparency.

Hake fish proteins were also employed for preparing edible films by Pires *et al.* [244] that studied their mechanical, antioxidant and antimicrobial properties, also when citronella, coriander, tarragon and thyme oils were added as plasticizers Dried hake proteins were solubilized at pH 11.0 and glycerol and 0.25 mL of each essential oil per gram of protein was added. The obtained films were homogeneous and transparent with a yellowish color. The addition of the different essential oils increased the solubility of films in water and reduced the water vapor permeability. Among all essential oils plasticized films, the thyme oil films exhibited the lowest mechanical properties but the highest inhibition against *Shewanella putrefaciens*. The antioxidant activity films based on hake protein generally increased with the incorporation of essential oils.

A strong interest was devoted in the last decade to mussel proteins because of the high adhesive strength that they can develop on the rock, which was attributed to the proteins containing the unusual amino acid 3,4-dihydroxy-L-phenylalanine (DOPA) [245]. Although the adhesion mechanism is not completely known, glue proteins secreted by marine mussels bind strongly to all inorganic and organic surfaces in aqueous environments. Hence several papers were published in order to explain the peculiar properties of this kind of proteins and develop biomimetic adhesives or coatings by modifying synthetic polymers using moieties similar to DOPA, suitable for making films [246]. However, in this approach, natural proteins were not used up to now due to lack of protein availability, and for this reason recombinant approaches are currently under study.

In the field of the proteins not coming from sea animals, it is worth noting the work developed onto egg albumin protein by Gennadios *et al.* [247] that prepared films from heated alkaline solutions of egg white at pH 11.25. They added also polyethylene glycol (molecular weight 400 g/mol), yolk solids and dialdehyde starch (DAS) in different concentrations. It was demonstrated that the addition of DAS increased the film tensile strength but also yellowness. It reduced the solubility of protein in buffered water but, by increasing the amount of DAS, some aggregates were observed, probably because of the extensive cross-linking derived from this additive.

9.2. Emerging Proteins from Plant Kingdom

Potato protein contains approximately 71% patatin/tuberin, 7.6% glutein, 6.6% albumin, 3% globulin, 1.7% prolamin, and 8.8% other proteins and is highly available from potato starch industry. The use of potato protein for biopolymer manufacturing was recently studied by Du *et al.* [248] who investigated the thermal processing of two potato protein isolates (PPIs) with glycerol as a plasticizer. The PPIs were pretreated by alkali or alkali under reducing conditions. The films produced by compression molding showed highest tensile strengths in the range 20–25 MPa and it was observed that the material can be brittle or ductile depending on the plasticizer content. The molecular weight and protein structure of the PPIs markedly affected the resultant static and dynamic mechanical properties of the films.

Rice protein concentrate can be obtained on an industrial scale by the alkaline extraction of rice flour or of the broken rice kernels that are by-products in the rice mills. Rice protein concentrate is underutilized as compared with other plant protein products, so Shih [249] investigated the possibility of preparing edible films by combining this protein with a polysaccharide, pullulan. The protein-pullulan mixture with up to 50% protein concentrate could be cast on a glass plate into films with tensile strength of about 18 MPa. Film strength and water vapor resistance were improved by the addition of small amounts of propylene glycol alginate, which interacts with both protein and carbohydrates, under alkaline condition. Oils were added into the film and an improved water vapor resistance was achieved.

Amaranth (Amaranthus hypochondriacus) is a crop whose seeds have a protein content of about 14%. This content is higher than that of other cereals. Moreover amaranth has a well-balanced composition in essential amino-acids and an important content of sulfur-containing amino-acids. The main protein fractions present in the amaranth grain are albumins, 11S-globulin, P-globulin, and glutelins [250]. To date, no work on the use of these amaranth proteins for edible films preparation has been published. Denavi *et al.* [251] have produced amaranth flour films with interesting mechanical and water vapor barrier features. These features were later assigned to the interactions between polymers (starch and proteins) and lipids, to the natural concentrations of each component in the film and to the distribution of these interactions within the film matrix. Avanza *et al.* [252] have studied the gel-forming properties of amaranth proteins in different protein concentrations and thermal conditions. They reported that minimum conditions for gelation were a temperature of 70 °C and 7% w/v of protein isolates. They evidenced that a rapid denaturation of globulins followed by sulfhydryl/disulphide interchange reactions between protein molecules led to a phenomenon of gelation enhanced by protein aggregation. Gels prepared under these critical conditions presented a strong gel-like behavior. Although these gels presented low adhesiveness, they were elastic in nature and they had high hardness, fracturability and cohesiveness. The values of tensile strength, elongation at break and elastic modulus of the films resulted quite modest, probably due to the fact that the protein molecules present in the film mainly maintained their native structure The results were similar to those reported by Denavi *et al.* [251] for films prepared with native soy protein films, but resulted much lower than the values of elastic modulus and tensile strength reported by other authors working with films prepared with soy and sunflower proteins partially or totally denatured following the same process as the one employed herein [253]. The greater surface hydrophobicity of amaranth protein films gave them better potential to overcome the general limitation of hygroscopic features of proteins.

Many studies concerned the legume proteins (peas, chickpeas, faba beans, lupines).

Peas are generally composed of carbohydrates (35%), proteins (27%), fibers (27%) and a very small amount of lipids. While the fiber and starch fraction of peas are highly utilized by bakery and meat industry, the protein fraction has been underutilized. *Pea protein* consists primarily of globulins (80% of total proteins) mainly and a small fraction of albumins. The globulins consist of legumin, the most abundant, vicilin and convicilin. Albumins, which compose 13%–14% of the total proteins, are cytoplasmic proteins consisting of many kinds of subunits and contain more sulfur amino acid residues than the globulins. Edible films produced from denatured pea protein concentrate solution were prepared by Choi *et al.* [254]. Increasing the concentration of the plasticizer (glycerol) in the film decreased tensile strength and elastic modulus, and increased elongation and water vapor permeability (WVP). Very strong and stretchable films were obtained from 70/30 and 60/40 of pea protein concentrate/glycerol composition, respectively. The physical and mechanical properties of the PPC films were demonstrated to be comparable with those of soy protein and whey protein films.

Another interesting protein is phaseolin [255–259], a globular protein from *Phaseulus Vulgaris L.* and proteins purified from Vicia Ervilia. Many papers have been published recently using as crosslinking agent the enzyme transglutaminase, and as plasticizers glycerol or trehalose with or without different polysaccharides to obtain composite films. Barrier, mechanical, surface and also biodegradability properties have been demonstrated to be influenced by the composition. In particular edible films made of pectin and phaseolin and cross-linked with transgluminase were demonstrated to have lower CO_2 and oxigen permeability than LDPE and MATER-BI.

Chickpea is considered a good source of carbohydrates and proteins. Like other legumes, chickpea globulins and albumins represent the two major fractions found in beans. Chickpea protein concentrate displayed good water and oil absorption capacities and protein solubility. These properties are highly dependent on the pH values. The lowest protein solubility and emulsifying capacity was observed at pH 4.5 which is the isoelectric pH of chickpea albumins and globulins. Chickpea proteins do not have good foaming properties, but can lead to highly stable foams [260].

Faba bean (*Vicia faba L.*) is a rich source of food quality protein and, depending on ist cultivar, may contain up to 35% protein (weight percent of dry beans). Saremnezhad *et al.* [22] investigated the potential of faba bean protein isolate (FPI) as a new protein source for preparation of an edible film. They determined the effects of solution pH and plasticizer (glycerol) concentration on the film properties. The plasticizer content did not affect WVP over the studied range. Results showed that increasing the pH improved tensile strength, elongation at break and solubility of the films, but caused the water vapor permeability (WVP) and trasparency of the films to decrease. Moreover, elongation and solubility increased and the tensile strength decreased by increasing plasticizer content of the film forming solutions. The lowest WVP and the highest tensile strength were observed at pH 12 and 40% by weight (with respect to FPI) of glycerol concentration.

Lupin seeds contain up to 42% of protein, whereas lupin protein concentrate, prepared by alkali solubilization method, contains up to 73% of protein [46]. Lupin protein isolate was prepared by extraction and spray drying [261]. Chango *et al.* [262] investigated the optimum pH value for lupin protein film formation at slighly alkaline conditions (pH 8.5). In particular, lupin seeds soluble extract (adjusted to pH 8.5) was heated at 85 °C for 1 h to obtain the solution for preparing edible films. Moreover lupin seeds, for instance, after soaking in water for 5 h to remove the lupin alkaloids, were homogenized in water to produce a lupin milk suitable for human consumption [263]. The "milk" had 5%–6% solid and produced a film on the surface containing about 60% protein and 9% lipid when heated to 80–85 °C at pH 7–8.

Plants usually cultivated for producing oils were also explored.

Pistachio oil cake, which is waste of oil production, is rich in protein and minerals and is commonly used as an animal feed. Pistachio protein consists of four different fractions: globulin (66%), albumin (25%), glutelin (7.3%), and prolamin (2%). The globulin is soluble in 0.5 M· NaCl. Its extraction is economically favorable. The use of pistachio isolate protein to produce edible films was investigated and researchers investigated the effects of adding pistachio protein isolate to a cellulose-based edible film [264]. However the resulting composite edible film had an increased water vapor permeability (WVP) due to the hydrophilic nature of protein, which contains many hydrophilic amino acids such as aspartic acid, glutamic acid, and arginine. Zahedi *et al.* [265] prepared edible films from pistachio globulin protein, saturated fatty acids, and glycerol. The WVP of the emulsified films was reduced by approximately 37%–43% thanks to fatty acid addition. The mechanical properties of films were influenced by fatty acid addition: the ultimate tensile strength decreased, and elongation at break increased considerably (35%–70%) as a function of fatty acids content. Furthermore, the incorporation of fatty acid decreased the transparency of the films.

Peanut protein were classified in albumins, arachin and nonarachin proteins. The two latter proteins consist of large molecular weight globulins. Peanut protein concentrate can be prepared by alkaline extraction and acid precipitation from defatted peanut flour. Jangchud *et al.* [266] determined the effect of type and content of plasticizer on film properties and their sorption isotherms. Peanut protein films were formed by using four types of plasticizers (glycerol, sorbitol, polyethylene glycol and propylene glycol) at three concentration (0.67, 1.17, 1.67 g per g of protein). Glycerol showed the best plasticizing performance compared to the others and its concentration did not affect the water permeability and oxygen permeability. On the other hand, tensile strength decreased (from 5.14 to 4.10 MPa), with an increase in glycerol content from 1.17 to 1.67 g per g of protein and elongation percentage increased (from 105% to 164%) correspondingly. Peanut proteins are very hydrophilic. The moisture content of film at equilibrium increased sharply at pH 7.5 and 9.0 with an increase in relative humidity (RH) from 75% to 93% RH. Film thickness increased when increasing the relative humidity, and film at pH 6.0 showed the greatest increase in film thickness.

Sunflower proteins can be extracted from the residual pellet of oil industry through the use of low cost simple procedures. The resulting protein concentrate and isolate products retain a residual content of phenolic compounds, consisting mainly in chlorogenic and caffeic acids that are naturally present in sunflower seeds and cannot be completely eliminated because of their interaction with proteins. The preparation of sunflower protein films containing clove essential oil and the application of the resulting materials on the preservation of fish were studied by Salgado *et al.* [253]. The addition of clove essential oil to formulations based on sunflower protein concentrates allowed to prepare biodegradable and edible films with increased antioxidant properties and important *in vitro* antimicrobial properties. The presence of clove essential oil modified protein inter-macromolecular interactions reducing water solubility and glass transition temperature of the films, but it did not markedly modify their moisture content, color, thickness, water vapor permeability, opacity and mechanical properties. When applied to the preservation of fish these films allowed to retard lipid auto-oxidation and to slightly delay the growth of total mesophiles.

In cotton seeds, proteins represent 30%–40% w/w of the cottonseed kernels and are composed of globulin (60%), albumin (30%), prolin (0.5%), and glutelin (8.5%). Cottonseed protein isolate can be obtained from glandless delipidated flour after oil extraction by hexane. *Cottonseed proteins* have good film-forming properties. The preparation of biodegradable materials from cottonseed by casting has been demonstrated on a laboratory scale [267]. Successively, Grevellec *et al.* [268], in order to obtain homogeneous isolate/glycerol mixtures, mixed the isolate with water to obtain a paste. Glycerol was then added and mixed with this protein paste in a mixer. The paste was subsequently lyophilized to remove water, crushed in a mortar and sieved to obtain a thin dry powder. Films 1 mm thick and 30 mm in diameter were then prepared by compression molding. The results indicated that cottonseed proteins were thermoplastics with a glass transition temperature ranging from 80 to 200 °C when the glycerol content varied from 40% to 0% (w/w, dry basis). The proteins thermal denaturation temperature increased from 141 (without glycerol) to 195 °C in the presence of 40% (w/w) glycerol. Protein degradation occurred at 230 °C irrespective of glycerol content.

Sorghum proteins were divided into albumins (water-soluble), globulins (salt-soluble), kafirins (prolamins, aqueous alcohol-soluble), cross-linked kafirins (aqueous alcohol + reducing agent-soluble), and glutelins (detergent + reducing agent + alkaline pH-soluble). Kafirins represent storage proteins. Sorghum was compared to maize in that it contained a higher cross-linked kafirins to kafirins ratio. This suggested a higher propensity towards intermolecular disulfide cross-linking, despite a similar high degree of sequence homology between the storage proteins of the two cereals [269]. Buffo *et al.* [270] evidenced that films from laboratory-extracted sorghum kafirin containing glycerol and polyethylenene glycol 400 and prepared by solution casting showed tensile and water vapor barrier properties similar to films from commercial corn zein. Kafirin films appeared more intensely colored than zein films. The authors suggested that yields of extracted kafirin could be increased by use of sulfites to break down protein disulfide linkages and by exhaustive removal of oil. This study indicated that sorghum kafirin has potential to be used as a biopolymer for edible or nonedible film and coating applications.

9.3. Emerging Proteins from Fungi Kingdom

Hydrophobins are nano-metric surface active proteins having low molecular weight (7–9 kDa) produced by filamentous fungi having a role in fungal growth as structural components and in the interaction of fungi with their environment.

There is little amino acid sequence similarity between different hydrophobins, except for a characteristic pattern of eight cysteine residues that form four intramolecular disulfide bonds. Their structures are not yet completely known [271]. These proteins function by self-assembling into amphipathic polymeric films at the interface between hydrophobic and hydrophilic surfaces.

The amphipathic properties of hydrophobins facilitate the formation of aerial structures such as hyphae, spores and fruiting bodies, necessary for the reproduction of fungi. Two classes of hydrophobins have been identified. For class I hydrophobins, the polymer film comprises cylindrical rodlets with dimensions of 10–250 nm and their outward-facing hydrophobic surface has extremely low wettability.

These films are very robust; they are resistant to boiling in detergents or strong alkalis. Class II hydrophobin films are significantly less robust and lack the rodlet morphology of class I hydrophobins [272,273]. The biophysical properties of the isolated proteins are remarkable, such as strong adhesion, high surface activity and the formation of various self-assembled structures. The strong and stable assembly of class I hydrophobins make them attractive candidate molecules for surface coating of biomaterials, for biomedical application and also for nano-coating applications [274]. Their production also from bacteria or form plant was attempted [275]. Although hydrophobins need to be better explored in the future, interestingly some patents were filled about the use of hydrophobin in cellulose products. In fact by treatment with hydrophobins, cellulosic materials such as paper, cardboard, cotton or, especially, wood-based materials, such as solid wood, glued wood products or particle board, can be effectively protected against absorption and attack by water and/or moisture [276]. Cellulose materials are treated by bringing the surface of the material into contact with a hydrophobin solution. Different techniques can be adopted such as spraying, coating or roller application, or alternatively by immersing the entire article in the formulation.

In Table 3, the proteins listed in the literature survey about proteins with low availability or not yet developed are reported with the main reference about film-forming and the potential applications described in the reference.

Table 3. Scarcely or recently investigated proteins, references and applications.

Origin	Proteins	References and Applications	Preparation/Applications
Animal	feathers	Dou, Huang, Zhang, He, Yin and Cui, 2015 [241]	Films by solution casting (cross-linker)
	egg albumin	Gennadios, Handa, Froning, Weller and Hanna, 1998 [247]	Films by solution casting alkaline solutions (plasticizer)
	fish myofibrills	Cuq, Aymard, Cuq and Guilbert, 1995 [19]; Shiku, Hamaguchi, and Tanaka, 2003 [242]; Sobral, Santos and Garcia, 2005 [243];	Edible and transparent films by solution casting from acidic or alkaline solutions (plasticizer)
	hake	Pires, Ramos, Teixeira, Batista, Nunes and Marques, 2013 [244]	Edible but yellowish films by solution casting from alkaline solutions (plasticizer)
	mussel	Lee, Scherer and Messersmith, 2006; Meredith and Wilker, 2015 [245]	Under study the industrial production of protein by recombinant approaches
Plant	potato	Du, Chen, Zhang, Rempel, Thomson and Liu, 2015 [248]	Compression molded films
	rice	Shih, 1996 [249]	Edible films containing pullulan
	amaranth	Denavi, Tapia-Placido, Anon, Sobral, Mauri and Menegalli, 2005 [251]	Films by solution casting (plasticizer)
	pea	Choi and Han, 2001 [254]	Films by solution casting (plasticizer), properties similar to soy and whey films
	phaseolin	Giosafatto, Di Pierro, Gunning, Mackie, Porta and Mariniello, 2014 [257]	Edible films by solution casting (plasticizer) containing also polysaccharides
	chickpea	Ionescu, Aprodu, Daraba, Gurau, Baciu and Nichita, 2009 [260]	Edible films by solution casting (plasticizer)
	faba bean	Saremnezhad, Azizi, Barzegar, Abbasi and Ahmadi, 2011 [22]	Edible films by solution casting of alkaline solutions (plasticizer)

Table 3. *Cont.*

Origin	Proteins	References and Applications	Preparation/Applications
Plant	lupine	Chango, Villaume, Bau, Nicolas and Mejean, 1995 [262]	Edible films by solution casting of alkaline solutions
	pistachio	Zahedi, Ghanbarzadeh and Sedaghat, 2010 [265]	Edible films by solution casting of solutions (in cellulose based edible films)
	peanut	Jangchud and Chinnan, 1999 [266]	Edible films and coating by solution casting
	sunflower	Salgado, Lopez-Caballero, Gomez-Guillen, Mauri and Montero, 2013 [253]	Edible films by solution casting with antioxidant properties and antimicrobial properties
	cotton seed	Grevellec, Marquié, Ferry, Crespy and Vialettes, 2001 [268]	Films for packaging by solution casting or compression molding (plasticizer)
	sorghum	Buffo, Weller and Gennadios, 1997 [270]	Films by solution casting with properties similar to corn zein films but more colored
Fungi	hydrophobin	Gabor, Buthe, Eck, Kaufmann, Koch and Meurer, 2007 [276]	Nano or molecular films by solution casting on cellulose products

10. Combinations of Properties of Protein Coatings and Films

The knowledge of the combination of properties of the different proteins based films can promote their use in paper or board based multilayer systems or also in other flexible packaging applications to obtain packages properly designed for specific foods/beverages or products.

For this reason a comparative study of proteins films or coating is reported in Table 4. The different scores (–, 0, 1, 2, 3) were assigned on the basis of literature results reported in References section and described in the text in section 8 and considering the maximum performance obtainable from a protein, plasticizing it or modifying it. From the analysis of this table it is possible to deduct that among the proteins having high availability and yet consolidated in production, the soy, whey and casein proteins show significant structural, barrier and adhesive properties that make them most promising for application in the cellulose products field.

However the latter proteins are the most studied and investigated up to now, which means that many investigations were carried out to optimize their properties. Hence the results of this speculation can change in the future also for the other proteins as a result of further investigations and deeper knowledge. In particular the blending with biodegradable polymers, the preparation of composites using natural fillers or the use of nano-fillers can significantly affect the results.

In Table 4, the barrier and tensile properties of low density polyethylene (LDPE) are considered for comparison, and the barrier to oxygen properties of several proteins resulted significantly improved with respect to it. However it is necessary to consider that poly(ethylene terephthalate) (PET) shows better oxygen barrier properties than LDPE and is actually the main market competitor of proteins as paper coating, but has mechanical properties completely different from them. Also a chlorinated polymer such as poly(vinylidene chloride) (PVDC) shows very good oxygen barrier properties. However the latter is banned in many countries because it can release chloride acid in the operations occurring at its end life (recycling or thermal treatments). Poly(Ethylene-co-vinyl alcohol) (EVOH) is considered up to now the best oxygen barrier polymer.

In all cases, the barrier to humidity of proteins is low and can be improved by using hydrophobic additives or designing a multilayer system in which a biodegradable polymer is employed. As biodegradable polyesters show significant barrier properties, only slightly lower than those of PET, the production of multilayer packaging containing both proteins and biodegradable polyester has attracted the attention of some researchers.

Among the proteins available only on a research scale, the most interesting seem those from legumes and cereals for their high availability and properties not very different from the properties of the most investigated proteins. In particular, it should be noticed that amaranth based films show very promising barrier and mechanical properties, although the tensile strength should be improved. Also sorghum and faba bean results are promising, although more research work is needed to complete the knowledge of these proteins and improve the properties of films. Interestingly, the latter proteins represent the crops of three different world continents (South America for amaranth, Africa for sorghum and Middle East-Mediterranean countries for faba bean) and can represent sources for driving the economies of these countries.

Table 4. Comparative table for protein films properties.

Protein	Barrier Properties		Mechanical Properties		Adhesion
	Oxygen	Water Vapor	Tensile Strength	Elongation at Break	
Casein/caseinates	3	1	1	2	3
Whey	3	1	1	2	–
Gelatin	1	1	2	1	3
Wheat	2	2	1	1	2
Soy	3	1	1	2	2
Corn zein	3	1	1	1	2
Feathers	–	2	2	1	–
Egg albumin	–	–	2	2	–
Fish myofibrills	–	2	2	1	–
Hake	–	2	1	2	–
Mussel	–	–	–	–	3
Potato	–	–	2	1	2
Rice	–	1	2	0	–
Amaranth	3	2	0	2	–
Pea	–	1	1	1	–
Phaseolin	3	1	1	1	–
Chickpea	–	–	–	–	–
Faba bean	–	1	1	2	–
Lupine	–	–	–	–	–
Pistachio	–	0	2	1	–
Peanut	0	0	1	2	–
Sunflower	–	1	0	1	–
Cotton seed	–	–	–	–	–
Sorghum	–	1	1	2	–
Hydrophobins	–	–	–	-	–

0, very low; 1, moderate; 2, high (comparable with petrochemical option -LDPE-); 3, very high (better than petrochemical option -LDPE-).

The proteins from feathers seem very promising, but the high content of sulfur in the primary structure make difficult their processing. In the case of such kind of proteins solution casting techniques are probably more convenient. In fact the addition of sulfite during high temperature processing to avoid cross-linking, causing the emission of SO_2, can be an issue.

Fish based proteins (myofibrillar and hake) films show in general interesting mechanical/barrier properties, although more research is needed (no data about oxygen barrier are available) to have a better comparison with other proteins films properties.

The research onto mussel proteins and hydrophobins is still in a chemical-structural/biotechnology characterization phase, however these proteins must be cited in this review for their promising adhesive and hydrophobic properties respectively.

Moreover for each kind of protein it is demonstrated that the condition of processing, the kind and amount of additives can allow properties modulation in a wide range. Hence a promising protein for application in paper or board coating can be selected also on the basis of its availability in the paper plant region, thus optimizing costs and environmental impacts.

The study of new additives for modulating structure and properties of proteins based films is an important topic. The preparation of specific composites can be very promising for properties modulation. In particular the addition of natural micro-fillers such as dried potato pulp to whey blends [145] or nano-particles to whey protein isolate films [277] allowed improving mechanical properties thanks to the interactions between the functional –OH group on the fillers and the functional groups of proteins. Hence the dispersed filler domains act as cross-linking points. In the case of nano-particles the very high surface area of the fillers allow obtaining significant changes in properties also by using a few percent of nano-fillers.

The properties of adhesion to cellulose fibers of the different proteins are not completely known and more work should be made to assess the possibility of developing new adhesives and glues based on proteins, especially if they are recovered as by-products of industrial processes.

11. Application to Cellulose Based Products

Protein coatings are used until now mainly for the direct protection of food as edible coating. In research reports mainly self-standing films were investigated, because the results can be adapted to different substrates. When applied as coatings, more results deal with plastic coatings than with paper and board coatings. In consequence to this, the application on paper has been studied only for a few proteins such as whey proteins [8,9,32,33], isolated soy proteins [214,278–282], caseinates [1,7,115,121], wheat gluten [32,280,283–285] and corn zein [1,37,234,235]. As could be observed so far, the literature is countless and it is of course also expected that some of the results on standalone films and on coatings on different substrates can be transferred to paper at least as trends.

Although some authors already made this exercise at least on a partial basis independently on the film type or as plastic coatings to measure the mechanical performance and in general coated on plastic to measure oxygen barrier properties [285–288], in the following sections, if possible, the properties of paper coatings made of different proteins are discussed and summarized. One of the difficulties to derive conclusions and reliable comparisons between the developed protein-based materials lie in the variability of substrates, of coating thickness, of the conditions for applying them, of properties characterized and of test methods.

11.1. Barrier Properties

The permeation process relates to the selective mass transfer rate of given substances through a film. Paper and board are very permeable materials limiting their application for packaging or justifying the use of coatings. The application of a protein based layer onto a cellulose product can effectively improve its barrier properties towards many compounds, especially polar, as evidenced by Andersson [13]. Cellulose based products are generally highly porous and synthetic polymers are currently used to improve their barrier properties. Petrochemical coating or films are applied, mainly by extrusion coating or lamination, on cellulose based products or multilayer sheets, containing paper or board based layers and polymeric layer are industrially produced. The multilayer products often contain also a layer of aluminum, granting a total barrier effect for easily perishable foods or beverages.

Coatings' efficiency depends on the coating composition, thickness, and different layers used. Generally, as shown in number of academic studies reported so far, standalone protein derived biopolymers have shown to be excellent gas barriers in dry conditions but limited humidity barrier, as already mentioned, in view of general intrinsic protein hydrophilicity. The coating permeability depend on several factors such as film integrity, crystallinity, hydrophilic-hydrophobic ratio, chain mobility, interface between the coating and films and the presence of a plasticizer or other additives. For example, plasticizer addition leads to an increase in water vapor transmission rate (WVTR) while incorporation of fat acts inversely. In all subsequent references, thickness may vary and as such values are to be considered as indicative.

Number of publications give data of oxygen barrier properties of protein coatings, but usually they deal with films obtained using a wet coating technique rather than extrusion. Also it should be noted that the conditions of the measurements in terms of analysis technique used, unit reported, *etc.*, prevents the direct comparison between sources. The relative humidity (RH) used for the measurement also has a very large impact on the OTR value and not all results were obtained at the same humidity. Similarly with certain synthetic materials, a large RH dependence of the results can be noticed.

The available data about barrier properties of paper coated with proteins films are not many (Table 5) and the results are often not in agreement. The reported data are mainly in the range 0.25–100 × 10^{-11} g·m/m^2·sPa for the Water vapor transmission (WVTR) and in the range 2–70 × 10^{-15} g·m/m^2·s·Pa for the Oxygen transmission rate (OTR). The high variability of data is also due to the fact that the thickness of the paper or board sheets can also affect the results.

Table 5. Reported barrier performances for coatings applied on paper based on the commonly used proteins (best value reported when several samples were tested).

Protein Used	Process (Wet/Extrusion)	WVTR	OTR	Reference
whey	wet	134×10^{-11} g·m/m^2·s·Pa	–	[8]
soy	wet	6.6×10^{-14} kg·m/m^2·s·Pa	2.4×10^{-18} m^3·m/m^2·s·Pa	[214]
caseinates	wet	5.4 g·mm/m^2·d·kPa	3.2 cm^3·μm/m^2·d·kPa	[115]
	wet	938 g·m/m^2·d·kPa	–	[121]
	wet	0.25 g·mm/m^2·d·kPa	–	[2]
wheat gluten	wet or compr. molded	–	0.38 mL/mm·day·m^2·atm	[32]
	wet	3.27×10^{-11} mol·m^{-1}·s^{-1}·Pa^{-1}	$49{,}689 \times 10^{-18}$ mol·m^{-1}·s^{-1}·Pa^{-1}	[283]
corn zein	wet	881 g/m^2·d	–	[235]
	wet	70 g/100 in^2·d	16.000 cm^3/100 in^2·day	[37]

Zein was reported as a coating on waxed paper boxes as an alternative to paraffin [289]. The oxygen barrier properties of zein coated on paper have been reported in the range of 15.94 cm^3/100 in 2 day with 15 lb/ream [37] which mean an important improvement when compared to those of PE (450–1150 cm^3/100 in 2 day). No values where found for gelatin coated paper.

Not many data are available about the barrier properties of paper or board treated with proteins, but many data are available about the properties of protein based films. In these papers comparisons between films made of proteins, polysaccharides or traditional petrochemical polymers are reported [115,286]. As the results are not easily comparable only the data in the paper of Lin *et al.* [288] are reported (Table 6). In this paper zein, gluten, whey and soy protein based films were reported to have good oxygen barrier properties, especially zein and soy. The comparative results are in good agreement with other data sets.

Calcium caseinate, not present in Table 6, was also reported to have low OTR [285,290].

Table 6. Comparison of permeability (at 25 ± 2 °C, 50% to 70% relative humidity (RH)) of some synthetic polymers with edible films/coatings [288].

Material	O_2(g·m/m^2·s·Pa)	H_2O Vapor (g·m/m^2·s·Pa)
Synthetic polymer		
Polyester	0.38×10^{-15}	3.6×10^{-13}
Polypropylene (PP)	0.38×10^{-19}	6.5×10^{-13}
Polyvinyl chloride (PVC)	0.36×10^{-21}	2.16×10^{-11}
Polyethylene terephthalate (PET)	0.15×10^{-21}	–
Low-density polyethylene (LDPE)	0.16×10^{-19}	8.1×10^{-13}
High-density polyethylene (HDPE)	0.35×10^{-20}	2.52×10^{-13}
Edible coating material		
Methylcellulose (MC)	0.27×10^{-8}	9.35×10^{-11}
Hydroxypropyl cellulose (HPC)	0.22×10^{-8}	5.55×10^{-70}
Sucrose polyester	0.15×10^{-20}	4.2×10^{-13}
Zein	0.5×10^{-21}	1.17×10^{-10}
Chitosan	0.98×10^{-24}	4.9×10^{-10}
Wheat gluten	0.21×10^{-19}	9.18×10^{-11}
Whey protein isolate (WPI)	0.79×10^{-21}	1.1×10^{-9}
Soy protein	0.22×10^{-21}	3.49×10^{-10}

It is evident that the oxygen barrier properties of gelatin based films are poor [291]. The reported value for tuna gelatin films was 5.3 $\text{cm}^3/\text{m}^2\cdot$ day, while that of porcine gelatin films was 7.4 $\text{cm}^3/\text{m}^2\cdot$ day. No values where found for gelatin coated paper but the effects are expected to be moderated in view of results on other substrates or as films [89,162,292]. Gelatin tends to swell or dissolve in contact with moisture content, due to its hygroscopic nature [293]. As explained before bi-layer or composite systems are already used to overcome this drawback. Some studies have reported improvements on the water vapor barrier when combining chitosan and gelatin [294]. Overall, it is reported that films and coatings that were heterogeneous in nature, consisting of a blend of polysaccharides, protein, and/or lipids gave good results. In most other publications, gelatin seems to be used together with other proteins such as soy or whey or even with polysaccharides such as starch [295] or chitosan [296].

Furthermore, in a similar manner to polysaccharide films, protein films exhibit relatively high water vapor permeability values, *i.e.*, approximately two to four orders of magnitude greater than those of conventional polymeric packaging materials such as poly(ethylene), poly(propylene), polyester, and poly(vinylidene chloride) as it can be appreciated from Table 6.

As previously stated, in spite of great OTR values, due to their hydrophilic nature, biopolymers and especially those based on proteins, often exhibit a low water vapor barrier. Paper coated with zein-lipid mixture exhibit good grease proofing and water vapor properties [289]. Some studies report corn-zein nanocomposites coated poly(propylene) films as an effective water vapor and oxygen barrier, capable to lower the permeability of oxygen up to 25% and the one of water vapor up to 30% [297]. On the other hand, the addition of plasticizers to zein can negatively affect to water vapor barrier properties because of the increase in the free volume between polymer chains [38].

Interestingly Martelli *et al.* [298] showed that the WVP properties of plasticized films of keratin from feathers obtained by solution casting was lower than that of fish water-soluble proteins, peanut proteins and meat myofibrillar proteins. The reported values (from 0.1 to 1 $\text{g}/\text{m}\cdot\text{s}\cdot\text{Pa}$) are lower than those observed for soy and wheat gluten [288].

Similarly to what was pointed out for the oxygen barrier properties, water vapor barrier of cold-water fish is reported as lower than the one for both mammalian and warm-water fish, because of the higher amounts of hydrophobic amino acids and lower levels of hydroxiproline from the first source [293].

To enhance water vapor barrier, several fat types (natural waxes, fatty acids) can be used in combination with biopolymers, e.g., beeswax was reported to reach WVTR values of 6×10^{-13} $\text{g}/\text{m}\cdot\text{s}\cdot\text{Pa}$ and candelilla wax 2×10^{-13} $\text{g}/\text{m}\cdot\text{s}\cdot\text{Pa}$ and can thus compete with PE [79]. Nevertheless, although lipids indeed provide very good WVTR, when used as standalone films, they have a very poor integrity and thermomechanical properties, as well as poorer oxygen barrier than protein based films. Therefore a composite or bi-layer approach is often considered.

Even if most research deals with barrier gases and especially oxygen as previously described, the aroma barrier, which is the barrier to volatile organic compounds, is also of importance for the packaging application of such coatings. In that respect, it was for instance reported that the permeability of D-limonene (one of the main compounds of citrus flavor) in whey protein-based films is lower than that of EVOH or PVC, proving thereby an interesting aroma barrier [49].

Guazzotti *et al.* [299] applied bio-based coatings as potential barriers to chemical contaminants from recycled paper and board for food packaging. They investigated water-based, renewable biopolymers, such as modified starches (cationic starch and cationic waxy starch), plant and animal proteins (gluten and gelatin), poured onto paper with an automatic applicator. All the bio-polymers used in this study were previously studied for food contact applications because of their food-grade nature and their ability to increase gas barrier properties. Partition and diffusion results demonstrated the better performances of starch-based coatings than proteins both for a polar and a non polar compound (diisobutyl phthalate and benzophenone), often present in recycled paper packaging. In particular, proteins based coatings were not effective in limiting the migration of diisobutyl phthalate, the non-polar compound. Hence also this study reveals the necessity of adopting not only protein based but also carbohydrates based coatings to modulate functional barrier performances of natural and renewable coatings.

The use of two dimensional nano-fillers, such as phillosilicates, was recently investigated for improving the barrier properties of polymer films [300,301]. Sothornvit *et al.* [302] prepared whey protein isolate (WPI)-based composite films using a solution-casting method with three different types of nanoclays (Cloisite Na+, Cloisite 20A and Cloisite 30B). The WPI/nanoclay composite films showed a decrease in water vapor permeability, although only significant in the WPI/Cloisite Na^+ composite, that is the most polar clay based product. Probably in this case, a better dispersion of the clay in the protein matrix is achieved. However, no significant improvement in mechanical properties was observed. Interestingly, the incorporation of Cloisite 30B into WPI films showed significant bacteriostatic effect against *L. monocytogenes*, suggesting application for enhancing safety of food packaged with such film composites.

Kumar *et al.* [303] prepared a bio-nanocomposite powder of SPI and montmorillonite (MMT) via melt extrusion method, and then made SPI/MMT nanocomposite films via solution-casting method. Their results showed significant improvements by using montmorillonite in tensile strength, elongation at break and processing conditions (temperature profile and screws rate) were selected to minimize the water vapor permeability of the obtained films. Thus the value of water vapor permeability was reduced by at least 25% with respect to soy based films.

11.2. Mechanical Properties

Tensile strength and Elongation at break of several protein based films applied to paper compared to traditional polymers are reported in Table 7.

Table 7. Reported mechanical performances for coatings applied on paper based on the commonly used proteins (best value reported when number of samples were tested).

Protein Used	Process (Wet/Extrusion)	Young Modulus (MPa)	Tensile Strength (MPa)	Elongation at Break (%)	Reference
whey	wet	140	–	7	[8]
	wet	4200	0.08	4.2	[32]
soy	wet	–	17 *	177 *	[214]
	wet	–	40 Nm/g	–	[304]
caseinates	wet	–	16 **	30 **	[115]
	wet	–	5 kN/m	4	[2]
wheat gluten	compression molded	121	106	6	[32]

* multilayer with PLA; ** coating properties.

The data about Modulus and Tensile Strength are not easily comparable, because the grammage of the cellulosic sheets employed was quite different in the different papers. The elongation at break is in the range 4%–7%. The value of the elongation is typical of the paper and board based products. The higher value obtained for a whey coating (177%) is related to a multilayer containing poly(lactic acid) (PLA) layers.

Data about protein coatings or film mechanical properties are reported in Table 8 [285].

First of all, the primary structure (and also secondary if the denaturation is not complete) influences the mechanical properties of protein based films. For instance whey protein films show a higher tensile strength and lower elongation at break with respect to many casein or caseinates based films, but among casein based films α-casein and β-casein result in quite different mechanical properties. This fact is very important, but natural proteins are often a mixture of different poly(aminoacidic) macromolecules, in some cases the structures are not completely known, hence more investigations are necessary to correlate amino-acidic sequences and assembly properties to the final mechanical properties. Moreover the identification of a reliable and constant source is quite important for the industrial exploitation of processes based on proteins processing that require the achievement of constant properties, especially if the protein based product is a film having structural functions in the final cellulose based products.

Table 8. Mechanical properties of various plasticized proteins films.

Film	Tensile Strength (MPa)	Elongation (%)
UF-TMP [a]:Gly [b] (4:1)	10.1	5.2
β-casein:Gly (2:1)	6–9	250
αs1-casein:Gly (49:1)	4.1	38
αs1-casein:Gly (49:1), Tgase [c]	10.6	77
β-Lg [d]:Gly (2.6:1), Tgase	141.2	–
NaCas [e]:Gly (4:1)	17.4–26.7	10.5
NaCas:Gly (2:1)	2.98	29.9
NaCas:PEG [f] (4:1)	10.9–16.35	5.3
NaCas:PEG [f] (4:1)	10.9–16.35	5.3
NaCas [g]:Gly (2:1)	4.25	1.4
KCas [h]:Gly (2:1)	2.97	42.8
WPI [i]:Gly (2:1)	5.76	22.7
WPI:Gly (2.3:1)	13.9	30.8
WPI:Gly (5.7:1)	29.1	4.1
WPI:Sor [j] (1:1)	14.7	8.7
WPI:Sor (2.3:1)	14.0	1.6
WPC [k]:Gly (2:1)	3.49	20.8
NaCas:AM [l]:Gly (2:2:1)	1.32	27.4
CaCas:AM:Gly (2:2:1)	2.14	13.4
KCas:AM:Gly (2:2:1)	1.66	17.7
WPI:AM:Gly (2:2:1)	3.14	10.8
WPC:AM:Gly (2:2:1)	1.08	13.6
LDPE [m]	13	500
LDPE [n]	26	300

[a] Milk protein obtained by ultrafiltration; [b] Glycerol; [c] Ttransglutaminase; [d] β-lactoglobulin; [e] Sodium caseinate; [f] Pholyethylene glycol; [g] Calcium caseinate; [h] Potassium caseinate; [i] Whey protein isolate; [j] Sorbitol; [k] Whey protein concentrate; [l] Acetylated monogliceride ; [m] Low density polyrthylene; [n] High density Pollyethylene.

The highest elongation at break values were reported for wheat gluten, casein and soy protein isolate based formulations but the corresponding tensile strength are in all the cases lower than 8 MPa [285].

It is however evident that the mechanical properties of protein based films much vary on the basis of the presence of plasticizers (Table 8), their chemical structure and molecular weight, their concentration, as evidenced in Sections 9 and 10 for many proteins formulations. In general a higher concentration of plasticizer is related to a lower tensile strength and elastic modulus and a correspondent increase in elongation at break. The best proteins films are those with a tensile strength of at least 10 MPa and an elongation at break higher than 100%. In this case, we can consider that the properties of LDPE (tensile strength of about 8–13 MPa and elongation at break > 300), the traditional petrochemical polymer employed for flexible films, are almost achieved, although, especially in elongation at break, the values for protein based films are lower.

The cross-linking of proteins usually determines an increase in tensile strength and elastic modulus, but correspondingly it usually results in a decrease in elongation at break. However this trend is strongly dependent on the extent of chemical modification: for low and controlled cross-linker concentration usually all the properties can be improved [153], including gas barrier properties. If the cross-linking is very extensive, some heterogeneities can be formed due to the presence of non processable aggregates. These aggregates represent defects in the film structure and worsen the tensile and barrier properties of films.

In cast films obtained from water based solutions, also the pH plays an important role, as evidenced in Section 7.1. Generally acid or alkali conditions allow the formation of neat positive or negative electric charges onto the proteins chains and determine the achievement of a more extended structure. In condition close to isoelectric point the lower solubility of proteins usually determines difficulties in film forming and consequent worsening of film tensile properties.

In film obtained from flat die extrusion and calendaring, the effect of pH is present to a lower extent as the content of water in the system is reduced. However it has an effect onto denaturation occurring during the extrusion, hence it must be controlled. In the case of flat die extrusion, as evidenced in Section 7.2, the content and reactivity of agents limiting or favoring cross-linking and the processing viscosity influence significantly mechanical properties. The example about keratin processing [98] is the most representative of this issue.

The process by which the protein based treatment is applied on a cellulose based products is also of importance. If applied on paper sheets by roll, the water protein solution (industrial version of solution casting) can penetrate in the cellulose porous structure and the paper and the proteins can consist in a unique interpenetrated system, obviously depending on the thickness of the cellulose based sheet. If the thickness is high we can assume that the protein treatment affect mainly the superficial properties on one side of the cellulose based product.

On the contrary if a protein based film is applied through lamination, it is important that the adhesion of the protein based film (obtained industrially by extrusion) with the cellulose based layer is sufficient for granting good mechanical properties avoiding delamination. Usually in the plastic sector, the heating at high temperature of plastic film results in a good adhesive properties of them. However, if they are not enough thermal resistant or the adhesion properties are not sufficient, glues can be applied between cellulose based layer and protein based film in order to obtain enough resistant multilayer packages. Hence, for applications in the paper sector adhesion properties of the protein coating are quite important.

11.3. Binding and Adhesion Properties

The cellulose material has abundant amount of polar hydroxyl groups which can develop hydrogen bonds with oxygen and nitrogen atoms of protein adhesives. The interaction between cellulose and proteins can occur through penetration of protein adhesive molecules into the fiber cells through crevices via capillary paths followed by anchoring due to in-place curing of adhesive. The curing of the adhesive often implies the occurrence of cross-linking reactions and cross-linking additives, chemical or enzymatic, are often used in protein based adhesives.

In the attempt of developing renewable binders for cellulose based products, Flory *et al.* [305] compared the adhesive properties of several carbohydrates, proteins and lignins in order to find cheap and highly available binders. Interestingly soy protein, hydroxyproline-rich glycoprotein (HRGP), obtained from corn silk, gelatins from different sources and zein were also compared. The results showed that the proteins exhibit the best binding properties. In particular it was found that gelatin at 9% concentration combined with horseradish peroxidase enzyme, improving the tendency to cross-linking of the protein, provided significantly higher tear strength than the current commercial binder. Also other formulations based on gelatin, soy or zein based binder showed good properties, equivalent to the properties of current commercial binders as well as whey based glue [199]. Potato pulp, the by-product of potato starch manufacturing, was also employed as adhesive for fiberboard by Mayer [306].

More recently [304] the potential of soy protein isolate (SPI) and its hydrolysates to provide interfiber bonding enhancements in lignocellulosic furnishes was investigated. Soy protein is currently the most used protein based adhesives in cellulosic products, such as wood. However these authors set up a new process. Soy protein isolate was extracted from soy flour and hydrolyzed to provide a series of hydrolysates. The soy protein isolate and its hydrolysis products were later cross-linked with ethylendiaminetetraacetic acid (EDTA) in the presence of sodium hypophosphite (SPH). Then they were complexed to chitosan to improve their incorporation into old corrugated container (OCC) matrix and thus increase inter-fiber bonding. Approximately 2% SPI-EDTA-chitosan and hydrolysed SPI-EDTA-chitosan additives by mass (OCC-based slurry) were thoroughly mixed in order to prepare a sheet for physical testing. The tensile indices of the SPI-EDTA-chitosan additive-treated OCC pulp sheet increased 46.3%, while the inter fiber bonding of SPI-EDTA-chitosan additive-treated OCC pulp sheet increased by 74.86% compared to the control. The treatment significantly increased gloss (100%), but decreased roughness (70%) and tear strength.

Pervaiz [307] investigated the possibility of recovering proteins from the secondary paper sludge of paper-making mills. Secondary sludge typically consists of polysaccharides, nucleic acids, enzymes and proteins. Alkali treatment allowed the release the intracellular proteins into the aqueous phase. Thus the recovered sludge protein (RSP) containing 30% crude protein or soy protein Isolate (SPI) were modified with 1.0 M urea solution. The RSP was used as a wood adhesive and its adhesion performance was compared with soy protein isolate (SPI) and phenol formaldehyde (PF) resins, both employed traditionally in wood adhesives. The testing of plywood lap joints has shown up to 41% shear strength level of RSP adhesive compared to PF. This work demonstrated the technical feasibility and potential of secondary sludge as a biomass resource to develop eco-friendly adhesives for wood composite applications.

The adhesion properties were not investigated for many proteins, especially those not yet developed at industrial scale, however the possibility of using proteins of different sources for this application is quite promising and can support also the production of multilayer packaging containing biodegradable layers by lamination.

12. Food Safety Aspects, Migration, Legal and Regulatory Aspects

When it comes to the regulation of protein films and coatings, either applied directly or applied over a substrate, they can be classified as food contact materials, food additives, ingredients or even food products [12]. There is no specific legislation on biopolymer coatings yet, so conventional regulations must be applied in each case. In scientific literature, none or the revised papers for this review deals with safety legislation issues as these works are more focused on the characterization and the study of their effects on food products than in legislation compliance. As a result, test related with regulation compliance, such as migration tests, are not usually carried out within the scope of these works.

When applied directly as edible coatings, protein coating formulations contain food grade components usually regulated as additives; in that case the added amount should comply with legislation (Regulation (EC) No. 1333/2008). It is worth mentioning another important regulatory requirement in case a known allergen (as is the case of some proteins) has been employed within the coating formulation; it must be clearly labeled [308].

In the case of the protein coatings applied on paper- or board-based materials, food contact materials within the European Union, need to comply with Framework Regulation 1935/2004 (EC), among other relevant regulations. According to this regulation, food contact materials need to be manufactured in compliance with good manufacturing practice so that, under normal and foreseeable conditions of use, they shall not transfer their components into the food in quantities that could endanger human health or bring about an unacceptable change in the composition of the food or a deterioration in its organoleptic properties.

All things considered, safety regulatory requirements of protein coatings and films remain an issue that must still be addressed in order to scale-up their use to industry despite the promising results reported in this review, especially in view of their absence from the main list of materials and additives approved for food contact (EC 10_2011 in case of plastics) in spite of their food nature and inherent safety.

13. Biodegradation, Environmental Degradation and Other Environmental Aspects

13.1. Biodegradation and Composting of Protein Based Films

Biodegradation is a chemical process during which micro-organisms that are available in the environment convert materials into natural substances such as water, carbon dioxide, and compost (artificial additives are not needed) [309]. The biodegradability of a material is related to the specific environment and is frequently very different in compost, soil, anaerobic conditions, and particularly in water. This is related to the different microorganism present in the different substrates and also to differences in temperature, pHs, *etc.* Thus, specific standards have been made for the evaluation of the biodegradability in different environments.

In the literature survey often not comparable results are proposed about biodegradability of protein based films or coating but however the main conclusions that can arise from the analysis carried out in Section 8, are the following:

The presence of nitrogen in proteins make the material more suitable than polysaccharides or polyester based materials, containing only C and O atoms, for composting. Hence, usually the presence of proteins in natural based formulates, such as products with cellulose, has a positive effect on the whole biodegradation process.

The degradation of a protein usually starts by hydrolysis at one or more of its peptide bonds producing acid and amine groups. Protein can degrade by covalent modification with chemical, no enzymatic reaction, or reaction with protease enzymes.

The biodegradation rate may decrease in biopolymers containing covalent bondings [310]. Thus covalent cross-linking, *i.e.*, the formation of chemical bonds between individual macromolecules, improves physicochemical properties of multi-component films from polysaccharide and protein since it leads to an increase in molecular size and, at higher conversions, to certain kinds of superstructure (spatial networks) with characteristic physical properties but, when the degree of cross-linking is high, it slows degradation [311] (Schnabel, 1981). For example a higher number of bi-tyrosine cross-links induced by γ- irradiation slows bacterial degradation of calcium caseinate films [127,129]. On the other hand disulfide cross-linked soy protein–wheat gluten films still presented excellent biodegradability under simulated composting conditions [10]. Tests in whey protein isolate and whey protein concentrate based films evidenced bio-degradation in compost for raw materials and also for films based on protein cross-linked through disulfide bonds [158].

Protein coatings [234] do not hinder paper recycling and separation of protein and paper layers is not necessary. On the other hand Khwaldia *et al.* [7] reported that more investigation about biodegradablily, compostability and recyclability are necessary. The thickness and the composition of the coating in fact much influence the effect onto paper fibers recycling by pulping.

The use of proteolytic enzymes (R-chymotrypsin, pancreatin) can affect the rate of protein degradation [235].

Very important is the use of protein based coatings or films in the packaging sector. The Packaging Directive 94/62/EC, and most recent amendments, set minimum recovery and recycling targets for each type of packaging product. This Directive concerns the amounts of biodegradable waste land-filled or incinerated. The following European standards provide a framework within the different requirements must be integrated:

- EN 13431:2000 Packaging. Requirements for Packaging Recoverable in the Form of Energy Recovery Including Specification of Minimum Inferior Calorific Value.
- EN 13432:2000 Packaging. Requirements for Packaging Recoverable Through Composting and Biodegradation. Test Scheme and Evaluation Criteria for the Final Acceptance of Packaging.
- CR 13695-1 Packaging. Requirements for Measuring and Verifying the Four Heavy Metals (Cr, Rd, Hg, Pb) and Their Release into the Environment, and Other Dangerous Substances Present in Packaging.

- EN 13427:2000 Packaging. Requirements for the Use of European Standards in the Field of Packaging Waste ("Umbrella Norm").
- EN 13428:2000 Packaging. Requirements Specific to Manufacturing and Composition. Prevention by Source Reduction.
- EN 13429:2000 Packaging. Reuse.
- EN 13430:2000 Packaging. Requirements for Packaging Recoverable by Material Recycling.

The norm EN 13432 has been endorsed by the European Commission and therefore is a harmonized EU standard with a high juridical for defining a product as compostable.

A product can be defined as compostable as required by EN 13432 (2000) if it respects specific characteristics:

- The product must contain at least 50% organic matter and may not exceed the heavy metal limits specified in the standard.
- The products should mineralize for at least 90%, adopting the mineralization of a similar sample of cellulose as 100% positive control, within 6 months under controlled composting conditions, where mineralization is defined as the conversion of the organic C to CO_2 and biomass, this characteristic is linked to the chemical composition of the sample.
- The product, in the form which enters the market, should, within a timeframe of 12 weeks, fragment in parts smaller than 2 mm under controlled composting conditions. It has to be outlined that this requirement refers to the physical form of the product instead of to the chemical composition. Consequently, this characteristic is linked to the thickness and the physical construction (e.g., laminate, coating, *etc.*) of the sample, and can result tricky to be met also for packaging based on biodegradable materials.
- The compost obtained at the end of the composting trial, that can also contain some no degraded residuals from the product, must not have any negative effect to the germination and growth of plants.

Thus we can say that compostability comprises more than just biodegradability. A packaging which is compostable is always biodegradable, while a packaging which is biodegradable may not be compostable (as it can be too thick for disintegration or can contain toxic components). In order to avoid confusion on terminology several authorities prefer (or even mandate) the use of the term "compostable" instead of "biodegradable". In Europe there are several certification logos used by institutes for industrial compostability.

The goal of using biodegradable plastic materials is to replace slowly degrading synthetic plastics with rapidly degrading biopolymers to minimize harmful effects on the environment [312]. The challenge for the successful use of biodegradable polymer products is achieving a controlled lifetime. Products must remain stable and function during storage and intended use but then biodegrade efficiently later [16]. Edible films based on natural bio-polymers are assumed to be biodegradable.

Whey proteins are easily degraded not just in compost but also in soil due to easy degradability of proteins in several environments. When applied as coating on compostable materials such as poly(lactic acid) the presence of whey protein do not hinder compostability but promotes the disintegration supporting the achievement of requirements for composting that very often face an issue in disintegration in the requested time frames for materials having some thickness.

13.2. Integration of Protein Based Technologies in Current Waste Management System

The current method for recycling cellulose based post-consumer packages is the pulping of the grinded packages in dedicated plants. Cellulose fibers are thus suspended in water and, after filtration, they can be reused in the production of cellulose packages. The water containing suspended fibers is filtrated in the plant and residual fraction are thus separated, often consisting in petrochemical plastic, mainly poly(ethylene), and also aluminum.

The quality of the fibers is suited for recycling if they are not contaminated from substances that hinder their orientation during the formation of paper sheet. The contamination can lead to sheet irregularities and not reliable surficial properties. These impurities are generally indicated as "glues" as often they consists in the glues present in laminated based products. A good protein based coating should not act as a glue in the recycling of the cellulose fibers.

Moreover the residual fraction is generally recyclable for obtaining, by mincing and extrusion, plastic materials containing aluminum platelets derived from aluminum foil. The dimension of this particles is of some tenths of microns, hence they represent defects in the polymeric matrix composite resulting in poor impact resistance. The possibility of recovering separated fractions from this residue, separating plastic from aluminum, could be much convenient.

In this context, the use of protein based coatings or film in cellulose products could facilitate the current waste management system by two different approaches: (1) favoring the separation of the different materials for improving recycling; (2) allowing the composting of a significant fraction of materials employed in packaging or the production of a fully compostable packaging.

For the first approach it is necessary to refer to the investigations related to the European project WHEYLAYER (European Community's Seventh Framework Programme (FP7/2007–2013) Grant agreement No. 218340-2 WHEYLAYER "Whey protein-coated plastic films to replace expensive polymers and increase recyclability" (http://www.wheylayer.eu/); and WHEYLAYER 2 "Barrier properties for sustainable packaging" Seventh Framework Programme (FP7/2007-2013) grant agreement No. 315743) where the presence of coating containing whey protein between a PET and PE sheets allowed separating the two sheets in an enzymatic water bath. As the two polymers have a different density the separation by flotation is particularly easy. The process is described by Bugnicourt *et al.* [85].

For the second approach, as paper is a biodegradable material and it is usually compostable, coating treatment or multilayer packages in which petrochemical polymers are used make the package not compostable. Hence replacing these treatments or films with compostable ones would allow the composting of the full package, or at least the composting of a high fraction of packages in the case of multilayer high barrier system.

14. Conclusions and Industrial Perspectives

The present review represents an insight in the different raw materials and processing options that would allow producing protein-based coatings or film to be applied on cellulose (paper or board)-based products.

Many possibilities were illustrated on the basis of scientific literature, but more research work is needed to extend the knowledge of the structure-morphology-properties correlations of the different proteinaceous raw materials, taking also into account their regional availability. This link is particularly important as in a specific territory peculiar crops are developed and consequently the related industries of processing of natural products are present. Hence, the study of this kind of processes promotes a better knowledge of the specific industrial development of a territory, thus valorizing also its industrial waste with an industrial ecology approach. Both economic (because usually the management of waste represent a cost for companies or municipalities), and ecologic (because the saving of resources will allow reducing the environmental impact) benefits would result from this action.

The application of protein based coatings or film to cellulose based products would allow a better modulation of barrier and mechanical properties of the full product. Hence these coatings can be applied to many cellulosic products such as cardboard for boxes, board based containers for food (for instance pasta) or stationery products.

In food and beverages packaging the multilayer cellulose packages are widely (worldwide) employed. In Figure 1 the current scenario about multilayer paper or board based products and the future scenario, on the basis of environmental issues discussed on Section 13 is reported. In order to facilitate the recycling, efforts are also made to decrease the number of layers and use of adhesives whereby, provided sufficient properties for the defined application, protein could display a multifunctional role by providing tightness to liquid, barrier to gases, adhesiveness of layers to resulting paper and board packages.

As previously explained, paper or board are present in multilayer system in which also a polyethylene layer, granting the suitable water barrier properties, is present. For not dried food, creams or liquids, also an aluminum foil layer is present to grant a total barrier effect for the packaging. The recycling of cellulose is actually carried out in paper plants, by re-pulping the grinded recovered packaging material. A residual fraction (about the 30% by weight of the material) consists in aluminum and polyethylene, which can be employed in the injection molding sector. The recycling process should be optimized, for example by separating the Al from the PE or replacing the PE with a compostable polymer.

In the perspective of having a compostable option for future packaging, the possible scenario can be the one described in Figure 1 (when no aluminum layer is involved). The multilayer paper based flexible packaging systems need to be modified by replacing the PE layer with compostable ones. In the case of lamination, the employed glues should be natural, and, on the basis of literature surveys, they can consist of protein formulations. Overall, the packaging has to grant the same properties in terms of content protection as the synthetic polymer based options.

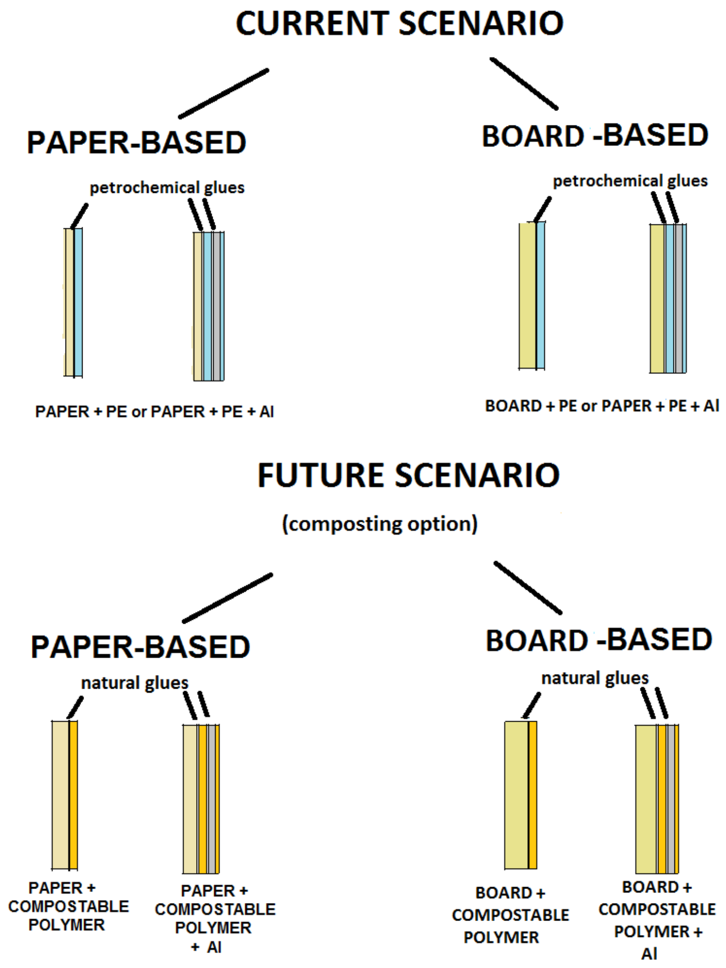

Figure 1. Current and future scenario for multilayer packaging based on cellulose (paper or board) on the literature survey basis.

These possible options for multilayer paper based packaging are the object of the BIOBOARD European project (www.bioboard.eu) SEVENTH FRAMEWORK PROGRAMME (FP7/2007-2013) under grant agreement No. 315313. Bio-Board research activities were aimed at designing a new multilayer system exploiting the properties of whey proteins and potato pulp based films to replace polymer barrier layer currently used (generally PE in beverage cartons and dehydrated food pouches, this latter also using several other alternatives such as EVOH, PVDC, *etc.*).

Innovations to current multilayer systems can be proposed following two different routes: by replacing the two polymers with compostable ones or by replacing the adhesive, always present within multilayer plastic or paper laminates to grant adhesion, using a protein based material, which can be removed by allowing the separation of the different layers. While substituting the EVOH barrier layer, such approach was investigated by Bugnicourt *et al.* [85] was shown to allow an optimized recycling approach. Interestingly the presence of the protein based layer in the system would allow improving the barrier properties of the packages, replacing petrochemical layers with natural ones and also improving their recycling, by separating the different material in post-consumer packages and introducing composting for the bio-based fraction.

Besides the promising environmental advantages envisaged from the use of protein based coatings in packaging, especially based on paper and board, in terms of industry perspectives, and besides the already reported need for large availability and organized supply chain, work on the scaling up the processes involved need to be continued. Indeed, as clearly evidenced by this review the film forming behavior of protein based coatings is significantly different from those of polymer coatings, so the industry cannot use them as drop in solutions. Besides, the often insufficient water resistance, inherent color, or even odor, of such coatings can prevent reaching selected packaging applications. Moreover the solid content is often more limited in terms of wet processes and the difficulties in thermoplastic processing of proteins limiting converting speeds are to be overcome. Recent advances towards the industrialization of whey protein-based barrier layer applied as wet coatings, and in terms of extrudable ones have been made respectively in the two previously quoted Wheylayer and BioBoard projects.

Paper industry already widely uses proteins, e.g., gelatin binders, casein adhesives, *etc.* Nevertheless, while several recent market available options have emerged from the polysaccharide side in terms of barrier coatings for paper (thermoplastic starch such as Novamont's among others, zein with US commercial sources covering several sectors including paper application, paper coating based on polysaccharide from husk from the Swedish company Xylophane, *etc.*), no market available protein based solution with wide availability was returned. In fact, while the bio sourcing is one of the main market driver for packaging innovation, other materials like sugar-based PE, PLA, regenerated cellulose and PHA are already commercially used as paper coatings. Nevertheless, as indicated by a recent market study from Smithers Pira indicate that the market for functional and barrier coatings in paper and board is rapidly increasing (it was worth $4.2 billion in 2012, and will increase by almost 420,000 tons by 2018) with food and beverage packaging representing 85% of the demand. Hence there is an increasing need and a possible market space for multifunctional solutions based on proteins. Prices that industry most time demands to be aligned with current solutions is also often an issue for the uptake of emerging protein solutions, although their sourcing from agro-wastes, in a holistic valorization approach, can also help reducing costs.

Acknowledgments: The authors wish to acknowledge the funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under Grant agreement No. 315313, BioBoard project (http://www.bioboard.eu/), as well as all the consortium partners, especially Gascogne Laminates and TetraPak for their invaluable insights on paper and board industries as well as Lucense (Lucca, Italy), the authors' colleagues Felicia Hammann and Carola Herbst for their instructive contributions when discussing the data included in this review.

Author Contributions: Maria-Beatrice Coltelli was the editor of the manuscript, main reviewer of content and formatting, contributing also to writing some sections, to integration of the different parts and to conclusions; Florian Wild contributed to the definition of the review structure based on available literature survey and contributed also in writing some sections on proteins and their specific properties; Elodie Bugnicourt contributed to the address of the review towards real industrial exigence, contributing much to introduction, perspectives and conclusions and revising all the manuscript; Patrizia Cinelli contributed in describing biodegradation aspects of

proteins film and coatings; Martina Lindner contributed in writing some sections about preparation of specific protein coatings; Markus Schmid contributed to the definition of the review structure and in writing some sections about processing and barrier properties; Verena Weckel contributed in writing some sections about preparation of specific protein coatings; Kerstin Muller contributed in writing some sections about preparation of specific protein coatings; Pablo Rodriguez contributed in writing some sections about protein processing; Andreas Staebler revised the documents regarding protein relevant aspects; Laura Rodriguez contributed to the description of food safety aspects, migration, legal and regulatory aspects; Andrea Lazzeri revised the document on the point of view of material science and technology aspects, in particular mechanical properties.

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

Acronyms

CGM	corn gluten meal
DAS	dialdehyde starch
EDTA	ethylendiaminetetraacetic acid
EVA	poly(ethylene-*co*-vinyl acetate)
EVOH	poly(ethylene-*co*-vinyl alcohol)
GHCl	guanidine hydrochloride
HPMC	hydroxypropylmethylcellulose
LDPE	low density poly(ethylene)
MAP	modified atmosphere packaging
MMT	montmorillonite
NaCAS	sodium caseinate
OCC	old corrugated containers
OPP	oriented poly(propylene)
OTR	oxygen transmission rate
PCL	poly(e-caprolactone)
PE	poly(ethylene)
PLA	poly(lactic acid)
PVA	poly(vinyl alcohol)
PVC	poly(vinyl chloride)
PVDC	poly(vinylidene chloride)
RH	relative humidity
SME	specific mechanical energy
SPH	sodium hypophosphite
SPI	soy protein isolate
WPI	whey protein isolate
WVP	water vapor permeability
WVTR	water vapor transmission

References

1. Gastaldi, E.; Chalier, P.; Guillemin, A.; Gontard, N. Microstructure of protein-coated paper as affected by physico-chemical properties of coating solutions. *Colloids Surf. A Physicochem. Eng. Aspects* **2007**, *301*, 301–310. [CrossRef]
2. Khwaldia, K. Water vapor barrier and mechanical properties of paper-sodium caseinate and paper-sodium caseinate-paraffin wax films. *J. Food Biochem.* **2010**, *34*, 998–1013. [CrossRef]
3. Aloui, H.; Khwaldia, K.; Ben Slama, M.; Hamdi, M. Effect of glycerol and coating weight on functional properties of biopolymer-coated paper. *Carbohydr. Polym.* **2011**, *86*, 1063–1072. [CrossRef]
4. Ottenio, D.; Escabasse, J.-Y.; Podd, B. *Packaging Materials Vol. 6. Paper and Board for Food Packaging Applications*; ILSI: Brussels, Belgium, 2004; pp. 1–28.
5. Dixon, J. *Packaging Materials Vol. 9. Multilayer Packaging for Food and Beverages*; ILSI: Brussels, Belgium, 2011; pp. 1–43.

6. Farris, S.; Schaich, K.M.; Liu, L.; Piergiovanni, L.; Yam, K.L. Development of polyion-complex hydrogels as an alternative approach for the production of bio-based polymers for food packaging applications: A review. *Trends Food Sci. Technol.* **2009**, *20*, 316–332. [CrossRef]
7. Khwaldia, K.; Arab-Tehrany, E.; Desobry, S. Biopolymer coatings on paper packaging materials. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 82–91. [CrossRef]
8. Han, J.H.; Krochta, J.M. Physical properties and oil absorption of whey-protein-coated paper. *J. Food Sci.* **2001**, *66*, 294–299. [CrossRef]
9. Lin, S.Y.; Krochta, J.M. Plasticizer effect on grease barrier and color properties of whey-protein coatings on paperboard. *J. Food Sci.* **2003**, *68*, 229–233. [CrossRef]
10. Park, S.K.; Hettiarachchy, N.S.; Were, L. Degradation behavior of soy protein-wheat gluten films in simulated soil conditions. *J. Agric. Food Chem.* **2000**, *48*, 3027–3031. [CrossRef] [PubMed]
11. Peelman, N.; Ragaert, P.; de Meulenaer, B.; Adons, D.; Peeters, R.; Cardon, L.; Devlieghere, F. Application of bioplastics for food packaging. *Trends Food Sci. Technol.* **2013**, *32*, 128–141. [CrossRef]
12. Debeaufort, F.; Quezada-Gallo, J.A.; Voilley, A. Edible films and coatings: Tomorrow's packagings: A review. *Crit. Rev. Food Sci. Nutr.* **1998**, *38*, 299–313. [CrossRef] [PubMed]
13. Andersson, C. New ways to enhance the functionality of paperboard by surface treatment—A review. *Packag. Technol. Sci.* **2008**, *21*, 339–373. [CrossRef]
14. Oh, J.H.; Wang, B.; Field, P.D.; Aglan, H.A. Characteristics of edible films made from dairy proteins and zein hydrolysate cross-linked with transglutaminase. *Int. J. Food Sci. Technol.* **2004**, *39*, 287–294. [CrossRef]
15. Gennadios, A.; Weller, C.L.; Gooding, C.H. Measurement errors in water-vapor permeability of highly permeable, hydrophilic edible films. *J. Food Eng.* **1994**, *21*, 395–409. [CrossRef]
16. Krochta, J.M.; DeMulder-Johnston, C. Edible and biodegradable polymer films: Challenges and opportunities. *Food Technol.* **1997**, *51*, 61–74.
17. Pol, H.; Dawson, P.; Acton, J.; Ogale, A. Soy protein isolate/corn-zein laminated films: Transport and mechanical properties. *J. Food Sci.* **2002**, *67*, 212–217. [CrossRef]
18. Weber, C.J. *Biobased Packaging Materials for the Food Industry: Status and Perspectives*; KVL: Frederiksberg, Denmark, 2000.
19. Cuq, B.; Aymard, C.; Cuq, J.L.; Guilbert, S. Edible packaging films based on fish myofibrillar proteins: Formulation and functional properties. *J. Food Sci.* **1995**, *60*, 1369–1374. [CrossRef]
20. Rhim, J.-W. Potential use of biopolymer-based nanocomposite films in food packaging applications. *Food Sci. Biotechnol.* **2007**, *16*, 691–709.
21. Cuq, B.; Gontard, N.; Guilbert, S. Proteins as agricultural polymers for packaging production. *Cereal Chem.* **1998**, *75*, 1–9. [CrossRef]
22. Saremnezhad, S.; Azizi, M.H.; Barzegar, M.; Abbasi, S.; Ahmadi, E. Properties of a new edible film made of faba bean protein isolate. *J. Agric. Sci. Technol.* **2011**, *13*, 181–192.
23. Viroben, G.; Barbot, J.; Mouloungui, Z.; Gueguen, J. Preparation and characterization of films from pea protein. *J. Agric. Food Chem.* **2000**, *48*, 1064–1069. [CrossRef] [PubMed]
24. Brandenburg, A.H.; Weller, C.L.; Testin, R.F. Edible films and coatings from soy protein. *J. Food Sci.* **1993**, *58*, 1086–1089. [CrossRef]
25. Bamdad, F.; Goli, A.H.; Kadivar, M. Preparation and characterization of proteinous film from lentil (*Lens culinaris*): Edible film from lentil (*Lens culinaris*). *Food Res. Int.* **2006**, *39*, 106–111. [CrossRef]
26. Cutter, C.N.; Susan, S.S. Application of Edible Coatings on Muscle Foods. In *Protein Based Films and Coatings*; Gennadios, A., Ed.; CRC Press: Boca Raton, FL, USA, 2002.
27. Gennadios, A.; Weller, C.L.; Testin, R.F. Modification of physical and barrier properties of edible wheat gluten-based films. *Cereal Chem.* **1993**, *70*, 426–429.
28. Tang, C.-H.; Xiao, M.-L.; Chen, Z.; Yang, X.-Q. Influence of succinylation on the properties of cast films from red bean protein isolate at various plasticizer levels. *J. Appl. Polym. Sci.* **2011**, *120*, 1934–1941. [CrossRef]
29. Tsoukala, A.; Papalamprou, E.; Makri, E.; Doxastakis, G.; Braudo, E.E. Adsorption at the air-water interface and emulsification properties of grain legume protein derivatives from pea and broad bean. *Coll. Surf. B Biointerfaces* **2006**, *53*, 203–208. [CrossRef] [PubMed]
30. Lange, J.; Wyser, Y. Recent innovations in barrier technologies for plastic packaging—A review. *Packag. Technol. Sci.* **2003**, *16*, 149–158. [CrossRef]

31. Farris, S.; Cozzolino, C.A.; Introzzi, L.; Piergiovanni, L. Development and characterization of a gelatin-based coating with unique sealing properties. *J. Appl. Polym. Sci.* **2010**, *118*, 2969–2975. [CrossRef]
32. Gallstedt, M.; Brottman, A.; Hedenqvist, M.S. Packagin-related properties of protein- and chitosan-coated paper. *Packag. Technol. Sci.* **2005**, *18*, 161–170. [CrossRef]
33. Han, J.H.; Krochta, J.M. Wetting properties and water vapor permeability of whey-protein-coated paper. *Trans. ASAE* **1999**, *42*, 1375–1382. [CrossRef]
34. Hong, S.I.; Krochta, J.M. Oxygen barrier properties of whey protein isolate coatings on polypropylene films. *J. Food Sci.* **2003**, *68*, 224–228. [CrossRef]
35. Hong, S.I.; Krochta, J.M. Whey protein isolate coating on LDPE film as a novel oxygen barrier in the composite structure. *Packag. Technol. Sci.* **2004**, *17*, 13–21. [CrossRef]
36. Farris, S.; Introzzi, L.; Piergiovanni, L. Evaluation of a bio-coating as a solution to improve barrier, friction and optical properties of plastic films. *Packag. Technol. Sci.* **2009**, *22*, 69–83. [CrossRef]
37. Trezza, T.A.; Wiles, J.L.; Vergano, P.J. Water vapor and oxygen barrier properties of corn zein coated paper. *Tappi J.* **1998**, *81*, 171–176.
38. Tihminlioglu, F.; Atik, I.D.; Ozen, B. Water vapor and oxygen-barrier performance of corn-zein coated polypropylene films. *J. Food Eng.* **2010**, *96*, 342–347. [CrossRef]
39. Schmid, M.; Cinelli, P.; Wildner, J.; Bazzichi, A.; Lazzeri, A. Whey protein based barrier layers to enhance the barrier properties in polylactic acid films while maintaining biodegradability. In Proceeding of the Bipoco 2012 International Conference on Bio-Based Polymers and Composites, Siofok, Hungary, 27–31 May 2012.
40. Fuss, C.; Palmaz, J.; Sprague, E. Fibrinogen: Structure, function, and surface interactions. *J. Vasc. Int. Radiol.* **2001**, *12*, 677–682. [CrossRef]
41. Walter, B.K.; Bainbridge, N.Y. Casein Adhesive Composition and Laminated Structure Utilizing the Same. US Patent 2754240, 10 July 1956.
42. Cheng, E.; Sun, X.; Karr, G.S. Adhesive properties of modified soybean flour in wheat straw particle board. *Compos. Part A Appl. Sci. Manufac.* **2004**, *35*, 297–302. [CrossRef]
43. Kalapathy, U.; Hettiarachchy, N.; Myers, D.; Hanna, M. Modification of soy proteins and their adhesive properties on woods. *J. Am. Oil Chem. Soc.* **1995**, *72*, 507–510. [CrossRef]
44. Wang, G.; Guo, M. Property and storage stability of whey protein-sucrose based safe paper glue. *J. Appl. Polym. Sci.* **2014**, *131*, 39710:1–39710:8. [CrossRef]
45. Gennadios, A.; Hanna, M.A.; Kurth, L.B. Application of edible coatings on meats, poultry and seafoods: A review. *LWT-Food Sci. Technol.* **1997**, *30*, 337–350. [CrossRef]
46. Baldwin, E.A.; Hagenmaier, R.D.; Bai, J. *Edible Coatings and Films to Improve Food Quality*, 2nd Edition ed; CRC Press: Boca Raton, FL, USA, 2012.
47. Hettiarachchy, N.S.; Ziegler, G.R. *Protein Functionality in Food Systems*; Marcel Dekker: New York, NY, USA, 1994.
48. Shih, F.F. Film-forming properties and edible films of plant proteins. *Food/Nahrung* **1998**, *42*, 254–256. [CrossRef]
49. Gennadios, A. *Protein-Based Films and Coatings*; CRC Press: Boca Raton, FL, USA, 2002.
50. Bourtoom, T. Review Article. Edible protein films: Properties enhancement. *Int. Food Res. J.* **2009**, *16*, 1–9.
51. Scopes, R.K. *Protein Purification: Principles and Practice*; Springer-Verlag: New York, NY, USA, 1994.
52. Cheftel, J.C. Nutritional effects of extrusion-cooking. *Food Chem.* **1986**, *20*, 263–283. [CrossRef]
53. Damodaran, S.; Kinsella, J.E. Effect of conglycinin on the thermal aggregation of glycinin. *J. Agric. Food Chem.* **1982**, *30*, 812–817. [CrossRef]
54. Barone, J.R.; Schmidt, W.F. Nonfood applications of proteinaceous renewable materials. *J. Chem. Educ.* **2006**, *83*, 1003. [CrossRef]
55. Schmid, M. Properties of cast films made from different ratios of whey protein isolate, hydrolysed whey protein isolate and glycerol. *Materials* **2013**, *6*, 3254–3269. [CrossRef]
56. Schmid, M.; Dallmann, K.; Bugnicourt, E.; Cordoni, D.; Wild, F.; Lazzeri, A.; Noller, K. Properties of whey-protein-coated films and laminates as novel recyclable food packaging materials with excellent barrier properties. *Int. J. Polym. Sci.* **2012**, *2012*, 562381:1–562381:7. [CrossRef]
57. Schmid, M.; Noller, K.; Wild, F.; Bugnicourt, E. Whey Protein Coated Films. Pantent WO2013014493 A1, 2013.
58. Lieberma, E.R.; Gilbert, S.G. Gas permeation of collagen films as affected by crosslinkage, moisture, and plasticizer content. *J. Polym. Sci. Part C Polym. Symp.* **1973**, *41*, 33–43. [CrossRef]

59. Tanaka, M.; Iwata, K.; Sanguandeekul, R.; Handa, A.; Ishizaki, S. Influence of plasticizers on the properties of edible films prepared from fish water-soluble proteins. *Fish. Sci.* **2001**, *67*, 346–351. [CrossRef]
60. McHugh, T.H.; Krochta, J.M. Water-vapor permeability properties of edible whey protein-lipid emulsion films. *J. Am. Oil Chem. Soc.* **1994**, *71*, 307–312. [CrossRef]
61. Martin-Polo, M.; Mauguin, C.; Voilley, A. Hydrophobic films and their efficiency against moisture transfer. 1. Influence of the film preparation technique. *J. Agric. Food Chem.* **1992**, *40*, 407–412. [CrossRef]
62. Bosquez-Molina, E.; Guerrero-Legarreta, I.; Vernon-Carter, E.J. Moisture barrier properties and morphology of mesquite gum-candelilla wax based edible emulsion coatings. *Food Res. Int.* **2003**, *36*, 885–893. [CrossRef]
63. Kamper, S.L.; Fennema, O. Use of an edible film to maintain water vapor gradients in foods. *J. Food Sci.* **1985**, *50*, 382–384. [CrossRef]
64. Weller, C.L.; Gennadios, A.; Saraiva, R.A. Edible bilayer films from Zein and grain sorghum wax or carnauba wax. *LWT-Food Sci. Technol.* **1998**, *31*, 279–285. [CrossRef]
65. Chick, J.; Hernandez, R.J. Physical, thermal, and barrier characterization of casein-wax-based edible films. *J. Food Sci.* **2002**, *67*, 1073–1079. [CrossRef]
66. Rodriguez-Turienzo, L.; Cobos, A.; Diaz, O. Effects of microbial transglutaminase added edible coatings based on heated or ultrasound-treated whey proteins in physical and chemical parameters of frozen Atlantic salmon (Salmo salar). *J. Food Eng.* **2013**, *119*, 433–438. [CrossRef]
67. Vachon, C.; Yu, H.L.; Yefsah, R.; Alain, R.; St-Gelais, D.; Lacroix, M. Mechanical and structural properties of milk protein edible films cross-linked by heating and gamma-irradiation. *J. Agric. Food Chem.* **2000**, *48*, 3202–3209. [CrossRef] [PubMed]
68. Winkler, H.; Vorwerg, W.; Schmid, M. Synthesis of hydrophobic whey protein isolate by acylation with fatty acids. *Eur. Polym. J.* **2015**, *62*, 10–18. [CrossRef]
69. Santos, C.V.; Tomasula, P.M. Acylation and solubility of casein precipitated by carbon dioxide. *J. Food Sci.* **2000**, *65*, 227–230. [CrossRef]
70. Orliac, O.; Rouilly, A.; Silvestre, F.; Rigal, L. Effects of various plasticizers on the mechanical properties, water resistance and aging of thermo-moulded films made from sunflower proteins. *Ind. Crops Prod.* **2003**, *18*, 91–100. [CrossRef]
71. Bertoldo, M.; Bronco, S.; Gragnoli, T.; Ciardelli, F. Modification of Gelatin by Reaction with 1,6-Diisocyanatohexane. *Macromol. Biosci.* **2007**, *7*, 328–338. [CrossRef] [PubMed]
72. Hernández-Muñoz, P.; Villalobos, R.; Chiralt, A. Effect of cross-linking using aldehydes on properties of glutenin-rich films. *Food Hydrocoll.* **2004**, *18*, 403–411. [CrossRef]
73. DeJong, G.A.H.; Koppelman, S.J. Transglutaminase catalyzed reactions: Impact on food applications. *J. Food Sci.* **2002**, *67*, 2798–2806. [CrossRef]
74. Porta, R.; Mariniello, L.; di Pierro, P.; Sorrentino, A.; Giosafatto, C.V.L. Transglutaminase crosslinked pectin- and chitosan-based edible films: A review. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 223–238. [CrossRef] [PubMed]
75. Mariniello, L.; Porta, R. Transglutaminases as Biotechnological Tools. In *Transglutaminases: Family of Enzymes with Diverse Functions*; Mehta, K., Eckert, R., Eds.; Karger: Basel, Switzerland, 2003; pp. 174–191.
76. Gaber, M.H. Effect of gamma-irradiation on the molecular properties of bovine serum albumin. *J. Biosci. Bioeng.* **2005**, *100*, 203–206. [CrossRef] [PubMed]
77. Durchschlag, H.; Fochler, L.C.; Feser, I.B.; Hausmann, L.S.; Seroneit, T.; Swlentek, J.M.; Swoboda, L.E.; Wlnklmair, L.A.; WlEkL, L.C.; Zipper, P. Effects of X- and UV-irradiation on proteins. *Radiat. Phys. Chem.* **1996**, *47*, 501–505. [CrossRef]
78. Tracton, A.A. *Coatings Technology Handbook*; Taylor and Francis: Boca Raton, FL, USA, 2005.
79. Embuscado, M.E.; Huber, K.C. *Edible Films and Coatings for Food Applications*; Springer: Dordrecht, The Netherlands; New York, NY, USA, 2009.
80. Perez-Gago, M.B.; Krochta, J.M. Drying temperature effect on water vapor permeability and mechanical properties of whey protein-lipid emulsion films. *J. Agric. Food Chem.* **2000**, *48*, 2687–2692. [CrossRef] [PubMed]
81. Perez-Gago, M.B.; Krochta, J.M. Denaturation time and temperature effects on solubility, tensile properties, and oxygen permeability of whey protein edible films. *J. Food Sci.* **2001**, *66*, 705–710. [CrossRef]
82. Plackett, D. *Biopolymers: New Materials for Sustainable Films and Coatings*; Wiley: Chichester, West Sussex, UK; Hoboken, NJ, USA, 2011.

83. Lent, L.E.; Vanasupa, L.S.; Tong, P.S. Whey protein edible film structures determined by atomic force microscope. *J. Food Sci.* **1998**, *63*, 824–827. [CrossRef]
84. Mahmoud, R.; Savello, P.A. Solubility and hydrolyzability of films produced by transglutaminase catalytic cross-linking of whey-protein. *J. Dairy Sci.* **1993**, *76*, 29–35. [CrossRef]
85. Bugnicourt, E.; Schmid, M.; Mc Nerney, O.; Wildner, J.; Smykala, L.; Lazzeri, A.; Cinelli, P. Processing and validation of whey-protein-coated films and laminates at semi-industrial scale as novel recyclable food packaging materials with excellent barrier properties. *Adv. Mater. Sci. Eng.* **2013**, *2013*, 496207:1–496207:10. [CrossRef]
86. Kowalczyk, D.; Baraniak, B. Effects of plasticizers, pH and heating of film-forming solution on the properties of pea protein isolate films. *J. Food Eng.* **2011**, *105*, 295–305. [CrossRef]
87. Verbeek, C.J.R.; van den Berg, L.E. Extrusion processing and properties of protein-based thermoplastics. *Macromol. Mater. Eng.* **2010**, *295*, 10–21. [CrossRef]
88. Rouilly, A.; Mériaux, A.; Geneau, C.; Silvestre, F.; Rigal, L. Film extrusion of sunflower protein isolate. *Polym. Eng. Sci.* **2006**, *46*, 1635–1640. [CrossRef]
89. Hanani, Z.A.N.; Beatty, E.; Roos, Y.H.; Morris, M.A.; Kerry, J.P. Manufacture and characterization of gelatin films derived from beef, pork and fish sources using twin screw extrusion. *J. Food Eng.* **2012**, *113*, 606–614. [CrossRef]
90. Hanani, Z.A.N.; Beatty, E.; Roos, Y.H.; Kerry, J.P. Manufacture of Gelatin-Based Films Using Extrusion: Assessment of Extrusion Parameters on Film Properties. In Presented at the 11th International Congress on Engineering and Food, Athens, Greece, 22–26 May 2011.
91. Krishna, M.; Nindo, C.I.; Min, S.C. Development of fish gelatin edible films using extrusion and compression molding. *J. Food Eng.* **2012**, *108*, 337–344. [CrossRef]
92. Hernandez-Izquierdo, V.M. Thermal transitions, extrusion, and heat-sealing of whey protein edible films. Ph.D. Thesis, University of California, Davis, CA, USA, 2007.
93. Hernandez-Izquierdo, V.M.; Krochta, J.M. Thermoplastic processing of proteins for film formation—A review. *J. Food Sci.* **2008**, *73*, R30–R39. [CrossRef] [PubMed]
94. Hernandez-Izquierdo, V.M.; Krochta, J.M. Thermal transitions and heat-sealing of glycerol-plasticized whey protein films. *Packag. Technol. Sci.* **2009**, *22*, 255–260. [CrossRef]
95. Hernandez-Izquierdo, V.M.; Reid, D.S.; McHugh, T.H.; De J. Berrios, J.; Krochta, J.M. Thermal transitions and extrusion of glycerol-plasticized whey protein mixtures. *J. Food Sci.* **2008**, *73*, E169–E175. [CrossRef] [PubMed]
96. Dangaran, K.; Tomasula, P.M.; Qi, P. Structure and function of protein-based edible films and coatings. In *Edible Films and Coatings for Food Applications*; Embuscado, M.E., Kerry, C.H., Eds.; Springer: Dordrecht, The Netherlands; New York, NY, USA, 2009; pp. 25–56.
97. Zhang, H.; Mittal, G. Biodegradable protein-based films from plant resources: A review. *Environ. Prog. Sustain. Energy* **2010**, *29*, 203–220. [CrossRef]
98. Barone, J.R.; Schmidt, W.F.; Gregoire, N.T. Extrusion of feather keratin. *J. Appl. Polym. Sci.* **2006**, *100*, 1432–1442. [CrossRef]
99. Redl, A.; Morel, M.H.; Bonicel, J.; Guilbert, S.; Vergnes, B. Rheological properties of gluten plasticized with glycerol: Dependence on temperature, glycerol content and mixing conditions. *Rheol. Acta* **1999**, *38*, 311–320. [CrossRef]
100. Tostoguzov, V.B. Some physico-chemical aspects of protein processing in foods. Multicomponent gels. *Food Hydrocoll.* **1995**, *9*, 317–332. [CrossRef]
101. Sothornvit, R.; Krochta, J.M. Plasticizer effect on oxygen permeability of beta-lactoglobulin films. *J. Agric. Food Chem.* **2000**, *48*, 6298–6302. [CrossRef] [PubMed]
102. Sothornvit, R.; Krochta, J.M. Plasticizer effect on mechanical properties of beta-lactoglobulin films. *J. Food Eng.* **2001**, *50*, 149–155. [CrossRef]
103. Nguyen, H.A.; Wong, M.; Anema, S.G.; Havea, P.; Guyomarch, F. Effects of adding low levels of a disulfide reducing agent on the disulfide interactions of β-lactoglobulin and κ-casein in skim milk. *J. Agric. Food Chem.* **2012**, *60*, 2337–2342. [CrossRef] [PubMed]

104. Jansens, K.J.A.; Bruyninckx, K.; Redant, L.; Lagrain, B.; Brijs, K.; Goderis, B.; Smet, M.; Delcour, J.A. Importance of cross-linking and disulfide bridge reduction for the mechanical properties of rigid wheat gluten bioplastics compression molded with thiol and/or disulfide functionalized additives. *J. Appl. Polym. Sci.* **2014**, *131*. [CrossRef]
105. Schmid, M.; Müller, K.; Sängerlaub, S.; Stäbler, A.; Starck, V.; Ecker, F.; Noller, K. Mechanical and barrier properties of thermoplastic whey protein isolate/ethylene vinyl acetate blends. *J. Appl. Polym. Sci.* **2014**, *131*. [CrossRef]
106. Aithani, D.; Mohanty, A.K. Value-added new materials from byproduct of corn based ethanol industries: Blends of plasticized corn gluten meal and poly(β-caprolactone). *Ind. Eng. Chem. Res.* **2006**, *45*, 6147–6152. [CrossRef]
107. Mariani, P.D.S.C.; Allganer, K.; Oliveira, F.B.; Cardoso, E.J.B.N.; Innocentini-Mei, L.H. Effect of soy protein isolate on the thermal, mechanical andmorphological properties of poly (β-caprolactone) and corn starch blends. *Polym. Test.* **2009**, *28*, 824–829. [CrossRef]
108. Ghosh, A.; Azam Ali, M.; Selvanesan, L.; Dias, G.J. Structure–function characteristics of the biomaterials based on milk-derived proteins. *Int. J. Biol. Macromol.* **2010**, *46*, 404–411. [CrossRef] [PubMed]
109. Leman, J.; Kinsella, J.E. Surface-activity, film formation, and emulsifying properties of milk-proteins. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 115–138. [CrossRef] [PubMed]
110. Mark, H.F.; Kroschwitz, J.I. *Encyclopedia of Polymer Science and Engineering*; Wiley: New York, NY, USA, 1985.
111. Avena-Bustillos, R.J.; Krochta, J.M. Water-vapor permeability of caseinate-based edible films as affected by pH, calcium cross-linking and lipid-content. *J. Food Sci.* **1993**, *58*, 904–907. [CrossRef]
112. Brault, D.; Daprano, G.; Lacroix, M. Formation of free-standing sterilized edible films from irradiated caseinates. *J. Agric. Food Chem.* **1997**, *45*, 2964–2969. [CrossRef]
113. Mchugh, T.H.; Krochta, J.M. Permeability Properties of Edible Films. In *Edible Coatings and Films to Improve Food Quality*; Baldwin, E.A., Hegenmaier, R., Bai, J., Eds.; CRC Press: Boca Raton, FL, USA, 1994; pp. 139–187.
114. Guilbert, S.; Gontard, N.; Cuq, B. Technology and applications of edible protective films. *Packag. Technol. Sci.* **1995**, *8*, 339–346. [CrossRef]
115. Khwaldia, K.; Banon, S.; Desobry, S.; Hardy, J. Mechanical and barrier properties of sodium caseinate-anhydrous milk fat edible films. *Int. J. Food Sci. Technol.* **2004**, *39*, 403–411. [CrossRef]
116. Miller, K.S.; Krochta, J.M. Oxygen and aroma barrier properties of edible films: A review. *Trends Food Sci. Technol.* **1997**, *8*, 228–237. [CrossRef]
117. Pereda, M.; Aranguren, M.I.; Marcovich, N.E. Caseinate films modified with tung oil. *Food Hydrocoll.* **2010**, *24*, 800–808. [CrossRef]
118. Fabra, M.J.; Talens, P.; Chiralt, A. Effect of alginate and lambda-carrageenan on tensile properties and water vapor permeability of sodium caseinate-lipid based films. *Carbohydr. Polym.* **2008**, *74*, 419–426. [CrossRef]
119. Fabra, J.M.; Talens, P.; Chiralt, A. Influence of calcium on tensile, optical and water vapor permeability properties of sodium caseinate edible films. *J. Food Eng.* **2010**, *96*, 356–364. [CrossRef]
120. Aliheidari, N.; Fazaeli, M.; Ahmadi, R.; Ghasemlou, M.; Emam-Djomeh, Z. Comparative evaluation on fatty acid and Matricaria recutita essential oil incorporated into casein-based film. International. *J. Biol. Macromol.* **2013**, *56*, 69–75. [CrossRef] [PubMed]
121. Khwaldia, K.; Linder, M.; Banon, S.; Desobry, S. Effects of mica, carnauba wax, glycerol, and sodium caseinate concentrations on water vapor barrier and mechanical properties of coated paper. *J. Food Sci.* **2005**, *70*, E192–E197. [CrossRef]
122. Chick, J.; Ustunol, Z. Mechanical and barrier properties of lactic acid and rennet precipitated casein-based edible films. *J. Food Sci.* **1998**, *63*, 1024–1027. [CrossRef]
123. Banerjee, R.; Chen, H.; Wu, J. Milk protein-based edible film mechanical strength changes due to ultrasound process. *J. Food Sci.* **1996**, *61*, 824–828. [CrossRef]
124. Gennadios, A.; McHugh, T.H.; Weller, C.L.; Krochta, J.M. Edible Coatings and Films Based on Proteins. In *Edible Coating and Films to Improve Food Quality*; Krochta, J.M., Baldwin, E.A., Nisperos-Carriedo, M.O., Eds.; Technomic Publ. Co.: Lancaster, PA, USA, 1994; pp. 201–277.
125. Bruno, M.; Giancone, T.; Torrieri, E.; Masi, P.; Moresi, M. Engineering properties of edible transglutaminase cross-linked caseinate-based films. *Food Bioprocess Technol.* **2008**, *1*, 393–404. [CrossRef]
126. Mezgheni, E.; D'Aprano, G.; Lacroix, M. Formation of sterilized edible films based on caseinates: Effects of calcium and plasticizers. *J. Agric. Food Chem.* **1998**, *46*, 318–324. [CrossRef] [PubMed]

127. Ressouany, M.; Vachon, C.; Lacroix, M. Irradiation dose and calcium effect on the mechanical properties of cross-linked caseinate films. *J. Agric. Food Chem.* **1998**, *46*, 1618–1623. [CrossRef]
128. Fabra, M.J.; Talens, P.; Chiralt, A. Microstructure and optical properties of sodium caseinate films containing oleic acid-beeswax mixtures. *Food Hydrocoll.* **2009**, *23*, 676–683. [CrossRef]
129. Mezgheni, E.; Vachon, C.; Lacroix, M. Biodegradability behavior of cross-linked calcium caseinate films. *Biotechnol. Prog.* **1998**, *14*, 534–536. [CrossRef] [PubMed]
130. Seydim, A.C.; Sarikus, G. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food Res. Int.* **2006**, *39*, 639–644. [CrossRef]
131. Herrmann, P.S.D.; Yoshida, C.M.P.; Antunes, A.J.; Marcondes, J.A. Surface evaluation of whey protein films by atomic force microscopy and water vapor permeability analysis. *Packag. Technol. Sci.* **2004**, *17*, 267–273. [CrossRef]
132. Boland, M. Whey Proteins. In *Handbook of Food Proteins*; Phillips, G.O., Williams, P.A., Eds.; Woodhead Publishing: Cambridge, UK, 2011; pp. 30–55.
133. Elgar, D.F.; Norris, C.S.; Ayers, J.S.; Pritchard, M.; Otter, D.E.; Palmano, K.P. Simultaneous separation and quantitation of the major bovine whey proteins including proteose peptone and caseinomacropeptide by reversed-phase high-performance liquid chromatography on polystyrene–divinylbenzene. *J. Chromatogr. A* **2000**, *878*, 183–196. [CrossRef]
134. Dunnill, P.; Green, D.W. Sulphydryl groups and conformational change in β-lactoglobulin. *J. Mol. Biol.* **1966**, *15*, 147–151. [CrossRef]
135. Priestley, R.J. *Effects of Heating on Foodstuffs*; Applied Science Publishers: London, UK, 1979.
136. Dewit, J.N.; Klarenbeek, G. Effects of various heat-treatments on structure and solubility of whey proteins. *J. Dairy Sci.* **1984**, *67*, 2701–2710. [CrossRef]
137. Shimada, K.; Cheftel, J.C. Sulphydryl group/disulfide bond interchange reactions during heat-induced gelation of whey protein isolate. *J. Agric. Food Chem.* **1989**, *37*, 161–168. [CrossRef]
138. Langton, M.; Hermansson, A.M. Fine-stranded particulate gels of β-lactoglobulin and whey protein at varying pH. *Food Hydrocoll.* **1992**, *5*, 523–539. [CrossRef]
139. Belitz, H.D.; Grosch, W.; Schieberle, P. *Lehrbuch der Lebensmittelchemie*; Springer: Berlin, Germany, 2007. (In German)
140. Onwulata, C.; Huth, P. *Whey Processing, Functionality and Health Benefits*; Wiley-Blackwell: Ames, IA, USA, 2008.
141. Cinelli, P.; Schmid, M.; Bugnicourt, E.; Wildner, J.; Bazzichi, A.; Anguillesi, I.; Lazzeri, A. Whey protein layer applied on biodegradable packaging film to improve barrier properties while maintaining biodegradability. *Polym. Degrad. Stable* **2014**, *108*, 151–157. [CrossRef]
142. Hamman, F.; Schmid, M. Determination and quantification of molecular interactions in protein films: A review. *Materials* **2014**, *7*, 7975–7996. [CrossRef]
143. Schmid, M.; Eib, S.; Reinlet, M. Modeling, simulation, and experimental validation of drying and denaturation behavior of whey protein isolate-based coatings. *Dry. Technol.* **2015**, *33*, 1382–1395. [CrossRef]
144. Schmid, M.; Hinz, L.-V.; Wild, F.; Noller, K. Effects of hydrolysed whey proteins on the techno-functional characteristics of whey protein-based films. *Materials* **2013**, *6*, 927–940. [CrossRef]
145. Schmid, M.; Reichert, K.; Hammann, F.; Stäbler, A. Storage time-dependent alteration of molecular interaction—Property relationships of whey protein isolate-based films and coatings. *J. Mater. Sci.* **2015**, *12*, 4396–4404. [CrossRef]
146. Schmid, M.; Herbst, C.; Müller, K.; Stäbler, A.; Schlemmer, D.; Coltelli, M.B.; Lazzeri, A. Effect of potato pulp filler on the mechanical properties and water vapor transmission rate of thermoplastic WPI/PBS blends. *Polym.-Plast. Technol. Eng.* **2015**. [CrossRef]
147. Rossi Marquez, G.; di Pierro, P.; Esposito, M.; Mariniello, L.; Porta, R. Application of transglutaminase-crosslinked whey protein/pectin films as water barrier coatings in fried and baked foods. *Food Bioprocess Technol.* **2014**, *7*, 447–455. [CrossRef]
148. Perez-Gago, M.B.; Krochta, J.M. Water vapor permeability of whey protein emulsion films as affected by pH. *J. Food Sci.* **1999**, *64*, 695–698. [CrossRef]
149. Shellhammer, T.H.; Krochta, J.M. Whey protein emulsion film performance as affected by lipid type and amount. *J. Food Sci.* **1997**, *62*, 390–394. [CrossRef]

150. Chan, M.A.; Krochta, J.M. Grease and oxygen barrier properties of whey protein isolate coated paperboard. *TAPPI Solut.* **2001**, *84*, 1–11.
151. Lin, S.Y.D.; Krochta, J.M. Whey protein coating efficiency on surfactant-modified hydrophobic surfaces. *J. Agric. Food Chem.* **2005**, *53*, 5018–5023. [CrossRef] [PubMed]
152. Shaw, N.B.; Monahan, F.J.; O'Riordan, E.D.; O'Sullivan, M. Physical properties of WPI films plasticized with glycerol, xylitol, or sorbitol. *J. Food Sci.* **2002**, *67*, 164–167. [CrossRef]
153. Ustunol, Z.; Mert, B. Water solubility, mechanical, barrier, and thermal properties of cross-linked whey protein isolate-based films. *J. Food Sci.* **2004**, *69*, E129–E133. [CrossRef]
154. Galietta, G.; di Gioia, L.; Guilbert, S.; Cuq, B. Mechanical and thermomechanical properties of films based on whey proteins as affected by plasticizer and cross-linking agents. *J. Dairy Sci.* **1998**, *81*, 3123–3130. [CrossRef]
155. Fairley, P.; Monahan, F.J.; German, J.B.; Krochta, J.M. Mechanical properties and water vapor permeability of edible films from whey protein isolate and sodium dodecyl sulfate. *J. Agric. Food Chem.* **1996**, *44*, 438–443. [CrossRef]
156. Wihodo, M.; Moraru, C.I. Physical and chemical methods used to enhance the structure and mechanical properties of protein films: A review. *J. Food Eng.* **2013**, *114*, 292–302. [CrossRef]
157. Tang, C.-H.; Jiang, Y. Modulation of mechanical and surface hydrophobic properties of food protein films by transglutaminase treatment. *Food Res. Int.* **2007**, *40*, 504–509. [CrossRef]
158. Li, J.C.; Chen, H.D. Biodegradation of whey protein-based edible films. *J. Polym. Environ.* **2000**, *8*, 135–143. [CrossRef]
159. Gómez-Guillén, M.C.; Giménez, B.; López-Caballero, M.E.; Montero, M.P. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocoll.* **2011**, *25*, 1813–1827. [CrossRef]
160. Wang, L.; Auty, M.A.E.; Rau, A.; Kerry, J.F.; Kerry, J.P. Effect of pH and addition of corn oil on the properties of gelatin-based biopolymer films. *J. Food Eng.* **2009**, *90*, 11–19. [CrossRef]
161. Pulieri, E.; Chiono, V.; Ciardelli, G.; Vozzi, G.; Ahluwalia, A.; Domenici, C.; Vozzi, F.; Giusti, P. Chitosan/gelatin blends for biomedical applications. *J. Biomed. Mater. Res. Part A* **2008**, *86*, 311–322. [CrossRef] [PubMed]
162. Avena-Bustillos, R.J.; Chiou, B.; Olsen, C.W.; Bechtel, P.J.; Olson, D.A.; McHugh, T.H. Gelation, oxygen permeability, and mechanical properties of mammalian and fish gelatin films. *J. Food Sci.* **2011**, *76*, E519–E524. [CrossRef] [PubMed]
163. Arvanitoyannis, I.; Psomiadou, E.; Nakayama, A.; Aiba, S.; Yamamoto, N. Edible films made from gelatin, soluble starch and polyols, Part 3. *Food Chem.* **1997**, *60*, 593–604. [CrossRef]
164. Rubin, A.L.; Riggio, R.R.; Nachman, R.L.; Schwartz, G.H.; Miyata, T.; Stenzeel, K.H. Collagen materials in dialysis and implantation. *Trans. Am. Soc. Artif. Intern. Organs* **1968**, *14*, 169–175. [PubMed]
165. Gennadios, A.; Rhim, J.W.; Handa, A.; Weller, C.L.; Hanna, M.A. Ultraviolet radiation affects physical and molecular properties of soy protein films. *J. Food Sci.* **1998**, *63*, 225–228. [CrossRef]
166. Lim, L.T.; Mine, Y.; Tung, M.A. Barrier and tensile properties of transglutaminase cross-linked gelatin films as affected by relative humidity, temperature, and glycerol content. *J. Food Sci.* **1999**, *64*, 616–622. [CrossRef]
167. De Carvalho, R.A.; Grosso, C.R.F. Characterization of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. *Food Hydrocoll.* **2004**, *18*, 717–726. [CrossRef]
168. Ahmad, M.; Benjakul, S.; Prodpran, T.; Agustini, T.W. Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. *Food Hydrocoll.* **2012**, *28*, 189–199. [CrossRef]
169. Amiza, M.A.; Wong, Y.P. Effect of Palm Oil Incorporation on the Properties of Biodegradable Cobia (Rachycentron Canadum) Skin Gelatin Films. In *Gums and Stabilisers for the Food Industry 16*; Williams, P.A., Phillips, G.O., Eds.; RSC Publishing: Cambridge, UK, 2012; pp. 269–277.
170. Andreuccetti, C.; Carvalho, R.A.; Grosso, C.R.F. Effect of hydrophobic plasticizers on functional properties of gelatin-based films. *Food Res. Int.* **2009**, *42*, 1113–1121. [CrossRef]
171. Condés, M.C.; Añon, M.C.; Mauri, A.N. Amaranth protein films from thermally treated proteins. *J. Food Eng.* **2013**, *119*, 573–579. [CrossRef]
172. Alves, P.M.A.; Carvalho, R.A.; Moraes, I.C.F.; Luciano, C.G.; Bittante, A.M.Q.B.; Sobral, P.J.A. Development of films based on blends of gelatin and poly(vinyl alcohol) cross linked with glutaraldehyde. *Food Hydrocoll.* **2011**, *25*, 1751–1757. [CrossRef]

173. Chambi, H.; Grosso, C. Edible films produced with gelatin and casein cross-linked with transglutaminase. *Food Res. Int.* **2006**, *39*, 458–466. [CrossRef]
174. Maria, T.M.C.; de Carvalho, R.A.; Sobral, P.J.A.; Habitante, A.M.B.Q.; Solorza-Feria, J. The effect of the degree of hydrolysis of the PVA and the plasticizer concentration on the color, opacity, and thermal and mechanical properties of films based on PVA and gelatin blends. *J. Food Eng.* **2008**, *87*, 191–199. [CrossRef]
175. Rawdkuen, S.; Suthiluk, P.; Kamhangwong, D.; enjakul, S. Mechanical, physico-chemical, and antimicrobial properties of gelatin-based film incorporated with catechin-lysozyme. *Chem. Cent. J.* **2012**, *6*. [CrossRef] [PubMed]
176. Cao, N.; Yang, X.; Fu, Y. Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films. *Food Hydrocoll.* **2009**, *23*, 729–735. [CrossRef]
177. Vanin, F.M.; Sobral, P.J.A.; Menegalli, F.C.; Carvalho, R.A.; Habitante, A. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. *Food Hydrocoll.* **2005**, *19*, 899–907. [CrossRef]
178. Frohberg, P.; Pietzsch, M.; Ulrich, J. Effect of crystalline substances in biodegradable films. *Chem. Eng. Res. Des.* **2010**, *88*, 1148–1152. [CrossRef]
179. Sartori, S.; Rechichi, A.; Vozzi, G.; D'Acunto, M.; Heine, E.; Giusti, P.; Ciardelli, G. Surface modification of a synthetic polyurethane by plasma glow discharge: Preparation and characterization of bioactive monolayers. *React. Funct. Polym.* **2008**, *68*, 809–821. [CrossRef]
180. Jabrane, T.; Dube, M.; Griffiths, M.; Mangin, P.J. Towards a commercial production of phage-based bioactive paper. *J. Sci. Technol. For. Prod. Process.* **2011**, *1*, 6–13.
181. Abrusci, C.; Martın-Gonzalez, A.; Del Amo, A.; Corrales, T.; Catalina, F. Biodegradation of type-B gelatine by bacteria isolated from cinematographic films. A viscometric study. *Polym. Degrad. Stab.* **2004**, *86*, 283–291. [CrossRef]
182. Gennadios, A.; Weller, C.L. Edible films and coatings from wheat and corn proteins. *Food Technol.* **1990**, *44*, 63–69.
183. Kieffer, R.; Schurer, F.; Köhler, P.; Wieser, H. Effect of hydrostatic pressure and temperature on the chemical and functional properties of wheat gluten: Studies on gluten, gliadin and glutenin. *J. Cereal Sci.* **2007**, *45*, 285–292. [CrossRef]
184. Reddy, N.; Yang, Y. Novel protein fibers from wheat gluten. *Biomacromolecules* **2007**, *8*, 638–643. [CrossRef] [PubMed]
185. Shewry, P.R.; Tatham, A.S. Disulphide Bonds in wheat gluten proteins. *J. Cereal Sci.* **1997**, *25*, 207–227. [CrossRef]
186. Wieser, H. Chemistry of gluten proteins. *Food Microbiol.* **2007**, *24*, 115–119. [CrossRef] [PubMed]
187. Leon, A.; Rosell, C.A.; de Barber, C.B. A differential scanning calorimetry study of wheat proteins. *Eur. Food Res. Technol.* **2003**, *217*, 13–16. [CrossRef]
188. Van Der Borght, A.; Goesaert, H.; Veraverbeke, W.S.; Delcour, J.A. Fractionation of wheat and wheat flour into starch and gluten: Overview of the main processes and the factors involved. *J. Cereal Sci.* **2005**, *41*, 221–237. [CrossRef]
189. Gontard, N.; Guilbert, S.; Cuq, J.L. Edible Wheat Gluten Films Influence of The Main Process And Environmental Conditions On Thermal, Mechanical And Barrier Properties. *Abstr. Pap. Am. Chem. Soc.* **1992**, *204*.
190. Sun, S.; Song, Y.; Zheng, Q. Morphologies and properties of thermo-molded biodegradable plastics based on glycerol-plasticized wheat gluten. *Food Hydrocoll.* **2007**, *21*, 1005–1013. [CrossRef]
191. Derksen, J.T.P.; Cuperus, F.P.; Kolster, P. Renewable resources in coatings technology: A review. *Prog. Org. Coat* **1996**, *27*, 45–53. [CrossRef]
192. Micard, V.; Belamri, R.; Morel, M.H.; Guilbert, S. Properties of chemically and physically treated wheat gluten films. *J. Agric. Food Chem.* **2000**, *48*, 2948–2953. [CrossRef] [PubMed]
193. Pochat-Bohatier, C.; Sanchez, J.; Gontard, N. Influence of relative humidity on carbon dioxide sorption in wheat gluten films. *J. Food Eng.* **2006**, *77*, 983–991. [CrossRef]
194. Lens, J.P.; de Graaf, L.A.; Stevels, W.M.; Dietz, C.; Verhelst, K.C.S.; Vereijken, J.M.; Kolster, P. Influence of processing and storage conditions on the mechanical and barrier properties of films cast from aqueous wheat gluten dispersions. *Ind. Crops Prod.* **2003**, *17*, 119–130. [CrossRef]

195. Pommet, M.; Redl, A.; Guilbert, S.; Morel, M.H. Intrinsic influence of various plasticizers on functional properties and reactivity of wheat gluten thermoplastic materials. *J. Cereal Sci.* **2005**, *42*, 81–91. [CrossRef]
196. Sanchez, A.C.; Popineau, Y.; Mangavel, C.; Larre, C.; Gueguen, J. Effect of different plasticizers on the mechanical and surface properties of wheat gliadin films. *J. Agric. Food Chem.* **1998**, *46*, 4539–4544. [CrossRef]
197. Gennadios, A.; Brandenburg, A.H.; Weller, C.L.; Testin, R.F. Effect of pH on properties of wheat gluten and soy protein isolate films. *J. Agric. Food Chem.* **1993**, *41*, 1835–1839. [CrossRef]
198. Utsumi, S.; Matsumura, Y.; Mori, T. Structure-Function Relationships of Soy Proteins. In *Food Proteins and Their Applications*; Damodaran, S., Paraf, A., Eds.; Marcel Dekker: New York, NY, USA, 1997; pp. 257–291.
199. Wang, G.; Zhang, T.; Ahmad, S.; Cheng, J.; Guo, M. Physicochemical and adhesive properties, microstructure and storage stability of whey protein-based paper glue. *Int. J. Adhes.* **2013**, *41*, 198–205. [CrossRef]
200. Staswick, P.E.; Hermodson, M.A.; Nielsen, N.C. Identification of the cystines which link the acidic and basic components of the glycinin subunits. *J. Biol. Chem.* **1984**, *259*, 13431–13435. [PubMed]
201. Renkema, J.M.S.; Knabben, J.H.M.; van Vliet, T. Gel formation by β-conglycinin and glycinin and their mixtures. *Food Hydrocoll.* **2001**, *15*, 407–414. [CrossRef]
202. Chao, Z.; Yue, M.; Xiaoyan, Z.; Dan, M. Development of soybean protein-isolate edible films incorporated with beeswax, Span 20, and glycerol. *J. Food Sci.* **2010**, *75*, 493–497. [CrossRef] [PubMed]
203. Song, F.; Tang, D.-L.; Wang, X.-L.; Wang, Y.-Z. Biodegradable soy protein isolate-based materials: A review. *Biomacromolecules* **2011**, *12*, 3369–3380. [CrossRef] [PubMed]
204. Kim, K.M.; Hwang, K.T.; Weller, C.L.; Hanna, M.A. Preparation and characterization of soy protein isolate films modified with sorghum wax. *J. Am. Oil Chem. Soc.* **2002**, *79*, 615–619. [CrossRef]
205. Ogale, A.A.; Cunningham, P.; Dawson, P.L.; Acton, J.C. Viscoelastic, thermal, and microstructural characterization of soy protein isolate films. *J. Food Sci.* **2000**, *65*, 672–679. [CrossRef]
206. Di Pierro, P.; Mariniello, L.; Giosafatto, C.V.L.; Masi, P.; Porta, R. Solubility and permeability properties of edible pectin-soy flour films obtained in the absence or presence of transglutaminase. *Food Biotechnol.* **2005**, *19*, 37–49. [CrossRef]
207. Mariniello, L.; di Pierro, P.; Esposito, C.; Sorrentino, A.; Masi, P.; Porta, R. Preparation and mechanical properties of edible pectin-soy flour films obtained in the absence or presence of transglutaminase. *J. Biotechnol.* **2003**, *102*, 191–198. [CrossRef]
208. Wan, V.C.H.; Kim, M.S.; Lee, S.Y. Water vapor permeability and mechanical properties of soy protein isolate edible films composed of different plasticizer combinations. *J. Food Sci.* **2005**, *70*, E387–E391. [CrossRef]
209. Kim, K.M.; Marx, D.B.; Weller, C.L.; Hanna, M.A. Influence of sorghum wax, glycerin, and sorbitol on physical properties of soy protein isolate films. *J. Am. Oil Chem. Soc.* **2003**, *80*, 71–76. [CrossRef]
210. Stuchell, Y.M.; Krochta, J.M. Enzymatic treatments and thermal effects on edible soy protein films. *J. Food Sci.* **1994**, *59*, 1332–1337. [CrossRef]
211. Ghorpade, V.M.; Li, H.; Gennadios, A.; Hanna, M.A. Chemically modified soy protein films. *Trans. Asae* **1995**, *38*, 1805–1808. [CrossRef]
212. Tang, C.H.; Jiang, Y.; Wen, Q.B.; Yang, X.Q. Effect of transglutaminase treatment on the properties of cast films of soy protein isolates. *J. Biotechnol.* **2005**, *120*, 296–307. [CrossRef] [PubMed]
213. Mauri, A.N.; Anon, M.C. Mechanical and physical properties of soy protein films with pH-modified microstructures. *Food Sci. Technol. Int.* **2008**, *14*, 119–125. [CrossRef]
214. Rhim, J.-W.; Lee, J.-H.; Hong, S.-I. Water resistance and mechanical properties of biopolymer (alginate and soy protein) coated paperboards. *LWT-Food Sci. Technol.* **2006**, *39*, 806–813. [CrossRef]
215. Gonzalez, A.; Strumia, M.; Alvarez Igarzabal, C.I. Cross-linked soy protein as material for biodegradable films: Synthesis, characterization and biodegradation. *J. Food Eng.* **2011**, *106*, 331–338. [CrossRef]
216. Malumba Kamba, P.; Vanderghem, C.; Deroanne, C.; Bera, F. Influence of drying temperature on the solubility, the purity of isolates and the electrophoretic patterns of corn proteins. *Food Chem.* **2008**, *101*, 564–572. [CrossRef]
217. Lawton, J.W. Plasticizers for zein: Their effect on tensile properties and water absorption of zein films. *Cereal Chem.* **2004**, *81*, 1–5. [CrossRef]
218. Dickey, L.C.; Parris, N.; Craig, J.C.; Kurantz, M.J. Ethanolic extraction of zein from maize. *Ind. Crops Prod.* **2001**, *13*, 67–76. [CrossRef]
219. Sessa, D.J.; Selling, G.W.; Willett, J.L.; Palmquist, D.E. Viscosity control of zein processing with sodium dodecyl sulfate. *Ind. Crops Prod.* **2006**, *23*, 15–22. [CrossRef]

220. Lawton, J.W. Viscoelasticity of zein-starch doughs. *Cereal Chem.* **1992**, *69*, 351–355.
221. Bai, J.; Alleyne, V.; Hagenmaier, R.D.; Mattheis, J.P.; Baldwin, E.A. Formulation of zein coatings for apples (Malus domestica Borkh). *Postharvest Biol. Technol.* **2003**, *28*, 259–268. [CrossRef]
222. Selling, G.W.; Sessa, D.J.; Palmquist, D.E. Effect of water and tri(ethylene) glycol on the rheological properties of zein. *Polymer* **2004**, *45*, 4249–4255. [CrossRef]
223. Ha, T.T.; Padua, G.W. Effect of extrusion processing on properties of zein-fatty acids sheets. *Trans. Asae* **2001**, *44*, 1223–1228.
224. Lai, H.M.; Padua, G.W. Properties and microstructure of plasticized zein films. *Cereal Chem.* **1997**, *74*, 771–775. [CrossRef]
225. Wang, Y.; Filho, F.L.; Geil, P.; Padua, G.W. Effects of processing on the structure of zein/oleic acid films investigated by X-ray diffraction. *Macromol. Biosci.* **2005**, *5*, 1200–1208. [CrossRef] [PubMed]
226. Ghanbarzadeh, B.; Oromiehie, A.R.; Musavi, M.; Falcone, P.M.; D-Jomeh, Z.E.; Rad, E.R. Study of mechanical properties, oxygen permeability and AFM topography of zein films plasticized by polyols. *Packag. Technol. Sci.* **2007**, *20*, 155–163. [CrossRef]
227. Rakotonirainy, A.M.; Padua, G.W. Effects of lamination and coating with drying oils on tensile and barrier properties of zein films. *J. Agric. Food Chem.* **2001**, *49*, 2860–2863. [CrossRef] [PubMed]
228. Rakotonirainy, A.M.; Wang, Q.; Padua, G.W. Evaluation of zein films as modified atmosphere packaging for fresh broccoli. *J. Food Sci.* **2001**, *66*, 1108–1111. [CrossRef]
229. Wang, Q.; Padua, G.W. Properties of zein films coated with drying oils. *J. Agric. Food Chem.* **2005**, *53*, 3444–3448. [CrossRef] [PubMed]
230. Santosa, F.X.B.; Padua, G.W. Tensile properties and water absorption of zein sheets plasticized with oleic and linoleic acids. *J. Agric. Food Chem.* **1999**, *47*, 2070–2074. [CrossRef]
231. Padgett, T.; Han, I.Y.; Dawson, P.L. Effect of lauric acid addition on the antimicrobial efficacy and water permeability of corn zein films containing nisin. *J. Food Process. Preserv.* **2000**, *24*, 423–432. [CrossRef]
232. Parris, N.; Coffin, D.R. Composition factors affecting the water vapor permeability and tensile properties of hydrophilic zein films. *J. Agric. Food Chem.* **1997**, *45*, 1596–1599. [CrossRef]
233. Ghanbarzadeh, B.; Musavi, M.; Oromiehie, A.R.; Rezayi, K.; Rad, E.R.; Milani, J. Effect of plasticizing sugars on water vapor permeability, surface energy and microstructure properties of zein films. *LWT-Food Sci. Technol.* **2007**, *40*, 1191–1197. [CrossRef]
234. Trezza, T.A.; Vergano, P.J. Grease resistance of corn zein coated paper. *J. Food Sci.* **1994**, *59*, 912–915. [CrossRef]
235. Parris, N.; Dickey, L.C.; Wiles, J.L.; Moreau, R.A.; Cooke, P.H. Enzymatic hydrolysis, grease permeation, and water barrier properties of zein isolate coated paper. *J. Agric. Food Chem.* **2000**, *48*, 890–894. [CrossRef] [PubMed]
236. Soliman, E.A.; Mohy Eldin, M.S.; Furuta, M. Biodegradable zein-based films: Influence of γ-irradiation on structural and functional properties. *J. Agric. Food Chem.* **2009**, *57*, 2529–2535. [CrossRef] [PubMed]
237. Ghanbarzadeh, B.; Oromiehie, A.R.; Musavi, M.; D-Jomeh, Z.E.; Rad, E.R.; Milani, W. Effect of plasticizing sugars on rheological and thermal properties of zein resins and mechanical properties of zein films. *Food Res. Int.* **2006**, *39*, 882–890. [CrossRef]
238. Gillgren, T.; Stading, M. Mechanical and barrier properties of avenin, kafirin, and zein films. *Food Biophys.* **2008**, *3*, 287–294. [CrossRef]
239. Ghanbarzadeh, B.; Oromiehi, A.R. Biodegradable biocomposite films based on whey protein and zein: Barrier, mechanical properties and AFM analysis. *Int. J. Biol. Macromol.* **2008**, *43*, 209–215. [CrossRef] [PubMed]
240. Parris, N.; Vergano, P.J.; Dickey, L.C.; Cooke, P.H.; Craig, J.C. Enzymatic hydrolysis of zein-wax-coated paper. *J. Agric. Food Chem.* **1998**, *46*, 4056–4059. [CrossRef]
241. Dou, Y.; Haung, X.; Zhang, B.; He, M.; Yin, G.; Cui, Y. Preparation and characterization of a dialdehyde starch crosslinked feather keratin film for food packaging application. *RSC Adv.* **2015**, *5*, 27168–27174. [CrossRef]
242. Shiku, Y.; Hamaguchi, P.Y.; Tanaka, M. Effect of pH on the preparation of edible films based on fish myofibrillar proteins. *Fish. Sci.* **2003**, *69*, 1026–1032. [CrossRef]
243. Sobral, P.J.A.; Dos Santos, J.S.; Garcia, F.T. Effect of protein and plasticizer concentrations in film forming solutions on physical properties of edible films based on muscle proteins of a Thai Tilapia. *J. Food Eng.* **2005**, *70*, 93–100. [CrossRef]

244. Pires, C.; Ramos, C.; Teixeira, B.; Batista, I.; Nunes, M.L.; Marques, A. Hake proteins edible films incorporated with essential oils: Physical, mechanical, antioxidant and antibacterial properties. *Food Hydrocoll.* **2013**, *30*, 224–231. [CrossRef]
245. Lee, H.; Scherer, N.F.; Messersmith, P.B. Single-molecule mechanics of mussel adhesion. *PNAS* **2006**, *103*, 12999–13003. [CrossRef] [PubMed]
246. Meredith, H.J.; Wilker, J.J. The interplay of modulus, strength, and ductility in adhesive design using biomimetic polymer chemistry. *Adv. Funct. Mater.* **2015**, *25*, 5057–5065. [CrossRef]
247. Gennadios, A.; Handa, A.; Froning, G.W.; Weller, C.L.; Hanna, M.A. Physical properties of egg white-dialdehyde starch films. *J. Agric. Food Chem.* **1998**, *46*, 1297–1302. [CrossRef]
248. Du, Y.; Chen, F.; Zhang, Y.; Rempel, C.; Thompson, M.R.; Liu, Q. Potato protein isolate-based biopolymers. *J. Appl. Polym. Sci.* **2015**, *132*. [CrossRef]
249. Shih, F.F. Edible films from rice protein concentrate and pullulan. *Cereal Chem.* **1996**, *73*, 406–409.
250. Scilingo, A.A.; Molina Ortiz, S.A.; Martinez, E.N.; Anon, M.C. Amaranth protein isolates modified by hydrolytic and thermal treatments. Relationship between structure and solubility. *Food Res. Int.* **2002**, *35*, 855–862. [CrossRef]
251. Denavi, G.; Tapia-Placido, D.R.; Anon, M.C.; Sobral, P.J.A.; Mauri, A.N.; Menegalli, F.C. Effects of drying conditions on some physical properties of soy protein films. *J. Food Eng.* **2009**, *90*, 341–349. [CrossRef]
252. Avanza, M.V.; Puppo, M.C.; Anon, M.C. Rheological characterization of amaranth protein gels. *Food Hydrocoll.* **2005**, *19*, 889–898. [CrossRef]
253. Salgado, P.R.; Lopez-Caballero, M.E.; Gomez-Guillen, M.C.; Mauri, A.N.; Montero, M.P. Sunflower protein films incorporated with clove essential oil have potential application for the preservation of fish patties. *Food Hydrocoll.* **2013**, *33*, 74–84. [CrossRef]
254. Choi, W.S.; Han, J.H. Physical and mechanical properties of pea-protein-based edible films. *J. Food Sci.* **2001**, *66*, 319–322. [CrossRef]
255. Mariniello, L.; Giosafatto, C.V.L.; Moschetti, G.; Aponte, M.; Masi, P.; Sorrentino, A.; Porta, R. Fennel waste-based films suitable for protecting cultivations. *Biomacromolecules* **2007**, *8*, 3008–3014. [CrossRef] [PubMed]
256. Mariniello, L.; Giosafatto, C.V.L.; di Pierro, P.; Sorrentino, A.; Porta, R. Swelling, mechanical and barrier properties of albedo-based films prepared in the presence of phaseolin crosslinked or not by transglutaminase. *Biomacromolecules* **2010**, *11*, 2394–2398. [CrossRef] [PubMed]
257. Giosafatto, C.V.L.; di Pierro, P.; Gunning, P.; Mackie, A.; Porta, R.; Mariniello, L. Characterization of *Citrus* pectin edible films containing transglutaminase-modified phaseolin. *Carbohydr. Polym.* **2014**, *106*, 200–208. [CrossRef] [PubMed]
258. Giosafatto, C.V.L.; di Pierro, P.; Gunning, P.; Mackie, A.; Porta, R.; Mariniello, L. Trehalose-containing hydrocolloid edible films prepared in the presence of transglutaminase. *Biopolymers* **2014**, *101*, 931–937. [CrossRef] [PubMed]
259. Porta, R.; di Pierro, P.; Rossi-Marquez, G.; Mariniello, L.; Kadivar, M.; Arabestani, A. Microstructure and properties of bitter vetch (*Vicia. ervilia*) protein films reinforced by microbial transglutaminase. *Food Hydrocoll.* **2015**, *50*, 102–107. [CrossRef]
260. Ionescu, A.; Aprodu, I.; Daraba, A.; Gurau, G.; Baciu, C.; Nichita, A. Chemical and Functional Characterization of Chickpea Protein Derivates. In *The Annals of the University Dunarea de Jos of Galati Fascicle VI—Food Technology, New Series Year III (XXXIII)*, Proceeding of the International Symposium Euro—Aliment 2009, Galati, Romania, 9–10 October 2009; pp. 16–27.
261. D'agostina, A.; Antonioni, C.; Resta, D.; Arnoldi, A.; Bez, J.; Kanuf, U.; Wasche, A. Optimization of a pilot-scale process for producing lupin protein isolates with valuable technological properties and minimum thermal damage. *J. Agric. Food Chem.* **2006**, *54*, 92–98. [CrossRef] [PubMed]
262. Chango, A.; Villaume, C.; Bau, H.M.; Nicolas, J.P.; Mejean, L. Fractionation by thermal coagulation of lupin proteins: Physicochemical characteristics. *Food Res. Int.* **1995**, *28*, 91–99. [CrossRef]
263. Schindler, S.; Wittig, M.; Zelena, K.; Krings, U.; Bez, J.; Eisner, P.; Berger, R.G. Lactic fermentation to improve the aroma of protein extracts of sweet lupin (Lupinus angustifolius). *Food Chem.* **2011**, *128*, 330–337. [CrossRef] [PubMed]
264. Ayranci, E.; Cetin, E. The effect of protein isolate of pistacia terebinthus L. on moisture transfer properties of cellulose-based edible films. *Lebensm. Wiss. Technol.* **1995**, *28*, 241–244. [CrossRef]

265. Zahedi, Y.; Ghanbarzadeh, B.; Sedaghat, N. Physical properties of edible emulsified films based on pistachio globulin protein and fatty acids. *J. Food Eng.* **2010**, *100*, 102–108. [CrossRef]
266. Jangchud, A.; Chinnan, M.S. Properties of peanut protein film: Sorption isotherm and plasticizer effect. *LWT-Food Sci. Technol.* **1999**, *32*, 89–94. [CrossRef]
267. Marquié, C.; Aymard, C.; Cuq, J.-L.; Guilbert, S. Biodegradable packaging made from cottonseed flour: Formation and improvement by chemical treatments with gossypol, formaldehyde and glutaraldehyde. *J. Agric. Food Chem.* **1995**, *43*, 2762–2767. [CrossRef]
268. Grevellec, J.; Marquié, C.; Ferry, L.; Crespy, A.; Vialettes, V. Processability of cottonseed proteins into biodegradable materials. *Biomacromolecules* **2001**, *2*, 1104–1109. [CrossRef] [PubMed]
269. Hamaker, B.R.; Bugusu, B.A. Overview: Sorghum Proteins and Food Quality. Available online: http://www.afripro.org.uk/papers/paper08hamaker.pdf (accessed on 4 December 2015).
270. Buffo, R.A.; Weller, C.L.; Gennadios, A. Films from laboratory extracted sorghum kafirin. *Cereal Chem.* **1997**, *74*, 473–475. [CrossRef]
271. Grunbacher, A.; Throm, T.; Seidel, C.; Gutt, B.; Rohrig, J.; Strunk, T.; Vincze, P.; Walheim, S.; Schimmel, T.; Wenzel, W.; *et al.* Six hydrophobins are involved in hydrophobin rodlet formation in *aspergillus nidulans* and contribute to hydrophobicity of the spore surface. *PLoS ONE* **2014**, *9*, e94546. [CrossRef] [PubMed]
272. Kwan, A.H.; Winefield, R.D.; Sunde, M.; Matthews, J.M.; Haverkamp, R.G.; Templeton, M.D. Structural basis for rodlet assembly in fungal hydrophobins. *PNAS* **2006**, *103*, 3621–3626. [CrossRef] [PubMed]
273. Magarkar, A.; Mele, N.; Abdel-Rahman, N.; Butcher, S.; Torkkeli, M.; Serimaa, R.; Paananen, A.; Linder, M.; Bunker, A. Hydrophobin film structure for HFBI and HFBII and mechanism for accelerated film formation. *PLoS Comput. Biol.* **2014**, *10*, e13745. [CrossRef] [PubMed]
274. Linder, M.B.; Szilvay, G.R.; Nakari-Setala, T.; Penttila, M.E. Hydrophobins: The protein-amphiphiles of filamentous fungi. *FEMS Microbiol. Rev.* **2005**, *29*, 877–896. [CrossRef] [PubMed]
275. Koivu, K. A Method for Hydrophobin Production in Plants and Methods to Produce Hydrophobin Multimers in Plants and Microbes. Patent EP 2631296 A1, 28 August 2013.
276. Gabor, E.; Buthe, A.; Eck, J.; Kaufmann, F.; Koch, H.J.; Meure, G. Method of Treating Cellulosic Materials with Hydrophobins. Patent CA 2679719 A1, 12 March 2007.
277. Zhou, J.J.; Wang, S.Y.; Gunasekaran, S. Preparation and characterization of whey protein film incorporated with TiO_2 nanoparticles. *J. Food Sci.* **2009**, *74*, N50–N56. [CrossRef] [PubMed]
278. Park, H.J.; Kim, S.H.; Lim, S.T.; Shin, D.H.; Choi, S.Y.; Hwang, K.T. Grease resistance and mechanical properties of isolated soy protein-coated paper. *J. Am. Oil Chem. Soc.* **2000**, *77*, 269–273. [CrossRef]
279. Ben Arfa, A.; Chrakabandhu, Y.; Preziosi-Belloy, L.; Chalier, P.; Gontard, N. Coating papers with soy protein isolates as inclusion matrix of carvacrol. *Food Res. Int.* **2006**, *40*, 22–32. [CrossRef]
280. Ben Arfa, A.; Preziosi-Belloy, L.; Chalier, P.; Gontard, N. Antimicrobial paper based on a soy protein isolate or modified starch coating including carvacrol and cinnamaldehyde. *J. Agric. Food Chem.* **2007**, *55*, 2155–2162. [CrossRef] [PubMed]
281. Chalier, P.; Ben Arfa, A.; Guillard, V.; Gontard, N. Moisture and temperature triggered release of a volatile active agent from soy protein coated paper: Effect of glass transition phenomena on carvacrol diffusion coefficient. *J. Agric. Food Chem.* **2009**, *57*, 658–665. [CrossRef] [PubMed]
282. Chalier, P.; Ben Arfa, A.; Preziosi-Belloy, L.; Gontard, N. Carvacrol losses from soy protein coated papers as a function of drying conditions. *J. Appl. Polym. Sci.* **2007**, *106*, 611–620. [CrossRef]
283. Guillaume, C.; Pinte, J.; Gontard, N.; Gastaldi, E. Wheat gluten-coated papers for bio-based food packaging: Structure, surface and transfer properties. *Food Res. Int.* **2010**, *43*, 1395–1401. [CrossRef]
284. Chalier, P.; Peyches-Bach, A.; Gastaldi, E.; Gontard, N. Effect of concentration and relative humidity on the transfer of alkan-2-ones through paper coated with wheat gluten. *J. Agric. Food Chem.* **2007**, *55*, 867–875. [CrossRef] [PubMed]
285. Khwaldia, K.; Perez, C.; Banon, S.; Desobry, S.; Hardy, J. Milk proteins for edible films and coatings. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 239–251. [CrossRef] [PubMed]
286. Bonilla, J.; Atarés, L.; Vargas, M.; Chiralt, A. Edible films and coatings to prevent the detrimental effect of oxygen on food quality: Possibilities and limitations. *J. Food Eng.* **2012**, *110*, 208–213. [CrossRef]
287. Hui, Y.H.; Guerrero Legarretta, I.; Lim, M.H.; Murrell, K.D.; Nip, W.K. *Handbook of Frozen Food*; CRC Press: Boca Raton, FL, USA, 2004.

288. Lin, D.; Zhao, Y. Innovations in the development and application of edible coatings for fresh and minimally processed fruits and vegetables. *Compr. Rev. Food Sci. Food Saf.* **2007**, *6*, 60–75. [CrossRef]
289. Parris, N.; Sykes, M.; Dickey, L.C.; Wiles, J.L.; Urbanik, T.J.; Cooke, P.H. Recyclable zein-coated kraft paper and linerboard. *Progr. Pap. Recycl.* **2002**, *11*, 24–29.
290. Gällstedt, M. Films and Composites Based on Chitosan, Wheat Gluten or Whey Proteins—Their Packaging Related Mechanical and Barrier Properties. Available online: http://kth.diva-portal.org/smash/get/diva2:9580/FULLTEXT01.pdf (accessed on 4 December 2015).
291. Kim, J.-Y.; Jung, C.-K.; Kim, D.-H.; Kim, S.-B. Properties of edible biofilm manufactured from yellowfin tuna (Thunnus albacares) skin gelatin. *Korean J. Chem. Eng.* **2012**, *29*, 786–791. [CrossRef]
292. Chiou, B.-S.; Avena-Bustillos, R.J.; Bechtel, P.J.; Jafri, H.; Narayan, R.; Imam, S.H.; Orts, W.J. Cold water fish gelatin films: Effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties. *Eur. Polym. J.* **2008**, *44*, 3748–3753. [CrossRef]
293. Gómez-Guillén, M.C.; Pérez-Mateos, M.; Gómez-Estaca, J.; López-Caballero, E.; Giménez, B.; Montero, P. Fish gelatin: A renewable material for developing active biodegradable films. *Trends Food Sci. Technol.* **2009**, *20*, 3–16. [CrossRef]
294. Hosseini, S.F.; Rezaei, M.; Zandi, M.; Ghavi, F.F. Preparation and functional properties of fish gelatin-chitosan blend edible films. *Food Chem.* **2013**, *136*, 1490–1495. [CrossRef] [PubMed]
295. Al-Hassan, A.A.; Norziah, M.H. Starch–gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocoll.* **2012**, *26*, 108–117. [CrossRef]
296. Rivero, S.; García, M.A.; Pinotti, A. Composite and bi-layer films based on gelatin and chitosan. *J. Food Eng.* **2009**, *90*, 531–539. [CrossRef]
297. Ozcalik, O.; Tihminlioglu, F. Barrier properties of corn zein nanocomposite coated polypropylene films for food packaging applications. *J. Food Eng.* **2013**, *114*, 505–513. [CrossRef]
298. Martelli, S.M.; Moore, G.; Paes, S.S.; Gandolfo, C.; Laurindo, J.B. Influence of plasticizers on the water sorption isotherms and water vapor permeability of chicken feather keratin films. *LWT-Food Sci. Technol.* **2006**, *39*, 292–301. [CrossRef]
299. Guazzotti, V.; Marti, A.; Piergiovanni, L.; Limbo, S. Bio-based coatings as potential barriers to chemical contaminants from recycled paper and board for food packaging. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* **2014**, *31*, 402–413. [CrossRef] [PubMed]
300. Coltelli, M.B.; Coiai, S.; Bronco, S.; Passaglia, E. Nanocomposites Based on Phyllosilicates: From Petrochemicals to Renewable Thermoplastic Matrices. In *Advanced Nanomaterials*; Geckeler, K.E., Nishide, H., Eds.; Wiley: Weinheim, Germany, 2010; pp. 403–458.
301. Castiello, S.; Coltelli, M.B.; Conzatti, L.; Bronco, S. Comparative study about preparation of poly(lactide)/organophilic montmorillonites nanocomposites through melt blending or ring opening polymerization methods. *J. Appl. Polym. Sci.* **2011**, *125*, E413–E428. [CrossRef]
302. Sothornvit, R.; Rhim, J.W.; Hong, S.I. Effect of nano-clay type on the physical and antimicrobial properties of whey protein isolate/clay composite films. *J. Food Eng.* **2009**, *91*, 468–473. [CrossRef]
303. Kumar, P.; Sandeep, K.P.; Alawi, S.; Truong, V.D.; Gorga, R.E. Preparation and characterization of bio-nanocomposite films based on soy protein isolate and montmorillonite using melt extrusion. *J. Food Eng.* **2010**, *100*, 480–489. [CrossRef]
304. Salam, A.; Lucia, L.A.; Jameel, H. A preliminary assay of the potential of soy protein isolate and its hydrolysates to provide interfiber bonding enhancements in lignocellulosic furnishes. *React. Funct. Polym.* **2014**, *85*, 228–234. [CrossRef]
305. Flory, A.R.; Requesens, D.V.; Devaiah, S.P.; Teoh, K.T.; Mansfield, S.D.; Hood, E.E. Development of a green binder system for paper products. *BMC Biotechnol.* **2013**, *13*. [CrossRef] [PubMed]
306. Mayer, F. Adhesive Based on Potato Pulp and Preparation and Use of this Adhesive. Patent EP0732386, 18 September 1996.
307. Pervaiz, M.; Sain, M. Protein extraction from secondary sludge of paper mill wastewater and its utilization as a wood adhesive. *BioResources* **2011**, *6*, 961–970.
308. Cutter, C.N. Opportunities for bio-based packaging technologies to improve the quality and safety of fresh and further processed muscle foods. *Meat Sci.* **2006**, *74*, 131–142. [CrossRef] [PubMed]

309. Johansson, C.; Bras, J.; Mondragon, I.; Nechita, P.; Plackett, D.; Simon, P.; Aucejo, S. Renewable fibers and bio-based materials for packaging applications—A review of recent developments. *Bioresources* **2012**, *7*, 2506–2552. [CrossRef]
310. Jane, L.; Lim, S.T.; Paetau, I. Degradable Plastics Made of from Starch and Proteins. In *Biodegradable Polymers and Packaging*; Ching, C., Kaplan, D.L., Thomas, E.L., Eds.; Technomic: Lancaster, PA, USA, 1993; pp. 63–73.
311. Schnabel, W. *Polymer Degradation: Principles and Practical Applications*; Macmillan Publishing Co. Inc.: New York, NY, USA, 1981.
312. Fishman, M. Edible and biodegradable polymer films: Challenges and opportunities. *Food Technol.* **1997**, *51*, 16.