Scrivener First

Valorization of Agricultural Wastes for the Production of Protein-Based Biopolymers

Nuria Burgos, Arantzazu Valdés and Alfonso Jiménez*

Analytical Chemistry, Nutrition and Food Sciences Department, University of Alicante, Campus San Vicente, 03690, San Vicente del Raspeig, Alicante, Spain

Received March 07, 2016; Accepted April 11, 2016

ABSTRACT: In this study we provide an overview of the latest developments on the extraction, production, modification and applications of fruit residues and by-products in the formation of protein-based biopolymers, in particular for the formulation of edible films. Our aim was mainly to demonstrate the highly transdisciplinary character of these topics by giving an overview of the main developments and research topics in the chemistry and engineering aspects of protein-based biopolymers. These innovative raw materials have been evaluated for the production of biomaterials to be used in some key sectors, such as food packaging.

KEYWORDS: Biopolymers, by-products, proteins, valorization

1 INTRODUCTION

In building a biobased economy, the efficient use of biomass is crucial. The production of a huge amount of agricultural residues, also called agro-wastes, which are currently underutilized with no economical revenue, is an issue in many countries all around the world, particularly in those where the environmental concerns have been raised in the last few years. This lack of utilization results in missing important opportunities to valorize and further reduce the environmental impact of biobased industries. The current practical trends and potential solutions are essentially aimed at extracting valuable components and using the remaining residues in a sustainable manner to close agricultural cycles.

In fact, biomaterials should combine the reduction of waste streams, particularly those obtained from biobased resources, with the development of innovative chemicals and compounds. These bioadditives and biopolymers could help to improve the properties and competitiveness of alternative materials to the traditional fuel-based and non-biodegradable polymers and additives [1, 2], leading to a new and promising research avenue: the use of food supply chain waste (FSCW) as a renewable biorefinery feedstock. FSCW could be defined as "the organic material produced for human consumption discarded, lost or degraded primarily at the manufacturing and retail stages." In the case of many agricultural practices and food processing industries, the yearly production of large amounts of waste requires the urgent proposal of solutions, since the current treatments can be recognized as inefficient, producing large and accumulative quantities of useless and valueless waste. In general terms, the European Union produces just around 90 million tons of food waste (FW) each year, 38% of which is directly produced by the food manufacturing sector, and extensive research is necessary to help overcome this environmental and economic deficit. In addition, residues produced from many vegetable harvesting and processing procedures have been proven to contain valuable functionalized molecules, such as polyphenols, flavonoids, tannins, polysaccharides, proteins and fatty acids, among many others [3–5].

It is known that the main current uses of the food supply chain, agricultural and forestry residues have low added value, mainly meeting needs that concern farming activities (bed and feed for livestocks), soil fertilization and compensation (composting) or energetic requirements (pellets for combustion). This is the current situation in agricultural commercial production, since companies and farmers are not offered opportunities for waste treatment other than accumulating, burning or landfilling. These uses do not cover the real potential of this feedstock from the technological and profitability points of view. Considering this

*Corresponding author: alfjimenez@ua.es

fact, agricultural residues should be considered for the sustainable and renewable source of products due to their high availability and potential.

The extraction, isolation and further incorporation of natural additives and biopolymers in a variety of commercialized products depict the current trend for the limitation of the use of synthetic substances in massive applications. Many industrial sectors (pharmaceutical, nutraceutical, food packaging, cosmetics, biotechnology, and fine chemicals) are focusing on the utilization of functional biomolecules to offer advanced eco-friendly products [6]. In fact, biomass, which represents nature's largest renewable reservoir of potentially fermentable carbohydrates and other valuable compounds, is mostly wasted in the form of pre-harvest and post-harvest agricultural losses and wastes of food processing industries. Due to their abundance and renewability, there has been a great deal of interest in utilizing agro-wastes for the production and recovery of many high added-value products.

In this context, the use of selected agro-wastes to form some interesting building blocks in the synthesis of biopolymers and bioadditives is a growing area of interest where researchers all over the world are focusing their efforts and research resources. In particular, the synthesis and production of protein-based biopolymers is gaining some ground in the research of current trends [7, 8]. The aim of this work is to review in a comprehensive manner the use of some agro-wastes for the production of protein-based biopolymers to permit the valorization of residues and the production of a strong alternative to some plastic commodities.

2 CROP PLANT BY-PRODUCTS FROM INDUSTRIAL PROCESSING

The increasing interest in the production of bioethanol and biopolymers obtained from natural sources, such as sucrose and starch fractions, as a way to replace petroleum-based fuels and materials has represented an important step towards the use of biobased and renewable agro-wastes in massive applications [9, 10]. Figure 1 shows the synthesis process of bioethanol from starch and sugar fractions of grain. The increasing demand for ethanol as a fuel additive and the decreasing dependency on fossil fuels have resulted in a dramatic increase in the amount of grains used for ethanol production, mainly maize, wheat and corn grains [11]. This fact could be considered as a social and economic problem in some countries and controversies have arisen from the difficult ethical debate regarding the depletion of human food to obtain biomaterials [12].

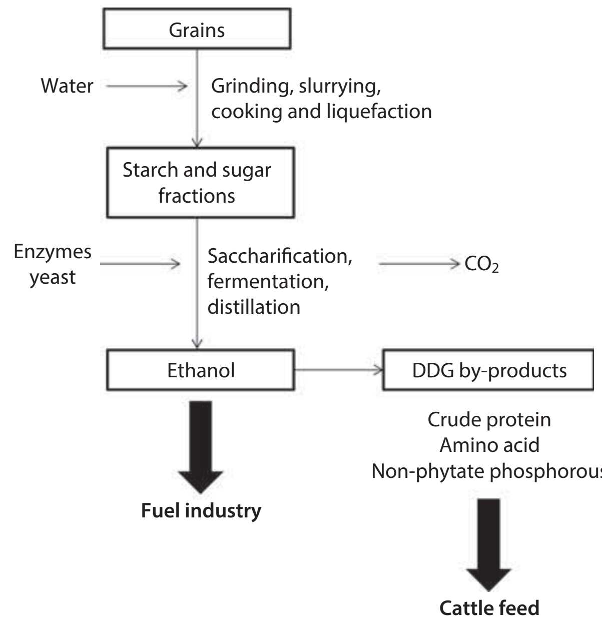

Figure 1 Industrial processing of bioethanol obtained from grain. Note: DDG = Distiller's Dried Grains. (Adapted from [11])

The main process to obtain ethanol from grains and legumes is the dry grinding method. Basic steps of this method include grinding (dry milling), slurrying, cooking, liquefaction, saccharification, fermentation and distillation resulting in bioethanol and some byproducts, which are known as distiller's dried grains (DDG) [13]. They are mainly crude proteins, amino acids and non-phytate phosphorous compounds. The market value of DDGs is quite low ($170 per ton) and it should be improved to obtain valuable chemicals and building blocks [14]. Currently DDG are used as cattle feed but when oversupplied with an increase in the colonies of *E.coli* O157 the possibilities of alternative uses are underlined [14].

Up to now, studies on the extraction, isolation and valorization of isolated amino acids in the manufacture of protein-based biomaterials have been widely reported in the literature [15–18]. In this context, glutamic acid is a five-carbon amino acid present in wheat DDG and has shown high potential as a building block for polymerization. As a result, glutamic acid can be converted into diols, diacids and aminodiols, which are monomers used in the formation of polyesters and polyamides [18]. For this purpose, a selective dehydrogenation (reduction) in the presence of amines allows the conversion of the acid moieties to alcohols under non-extreme conditions of atmospheric pressure and low temperature. On the other hand, proline can be chemically transformed into pyrrolidone, a N-vinylpyrrolidone precursor, with a production of more than 15,000 €/ton for this polymer, common in the cosmetic and medical sectors [17]. The production is separated in two different steps. First, the conversion of proline to pyrrolidone by oxidative decarboxylation, via the imine, in the presence of iodobenzene. Then, pyrrolidone is manufactured conventionally by the reaction of butyrolactone (derived from 1,4-butanediol) with ammonia to be used as a precursor for the manufacture of N-vinylpyrrolidone. However, the current procedures for the production of this amino acid show some drawbacks since a neutralization step is required, followed by purification and conversion steps from the sodium salt, with high costs and environmental impact.

Therefore, in order to avoid this important drawback, alternative strategies to valorize wheat DDGS proteins are required. Different grain proteins have been proposed for the development of new biomaterials, such as zein, wheat and soy, all of them biodegradable, abundant in nature and with the ability to increase the soil fertility [19]. In this section, the main sources, properties and applications of grain proteins used to obtain protein-based biomaterials are described.

2.1 Zein Protein from Corn Grain

According to FAOSTAT (Food and Agriculture Organization corporate statistical database), the worldwide annual production of corn or maize grain was over 1000 million tons in 2013 [20]. The endosperm and the germ are the major parts of the corn kernel and different industrial processing is carried out depending on the structure of the corn plant and kernel (Figure 2): a) wet milling to obtain starch and oil for human consumption, and b) dry grinding to obtain ethanol. As a result, corn gluten meal and corn gluten feed are the main by-products obtained from wet milling, whereas in the dry-grinding ethanol process, by-products are mostly DDG [21]. During the dry milling procedure, corn grains are steeped with a small amount of water for 30 to 40 hours to break the starch and protein bonds. Then, the germ is separated from the rest of the kernel by milling. The slurry obtained in such a way,

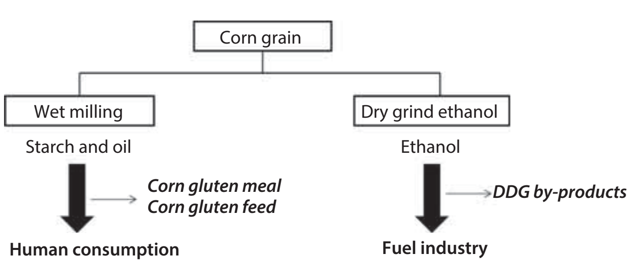

Figure 2 By-products obtained from corn grain. (Adapted from [21])

rich in fiber, starch, and proteins, is finely ground and screened to separate the fiber. Finally, starch is separated from proteins in hydrocyclones.

Zein is the major storage protein obtained from corn (45–50% of the total protein weight) and it can be isolated as a by-product of the wet milling process into the corn gluten meal as well as from the dry grinding ethanol process, in this case as DDG. As a result of its poor nutritional value for human consumption, recent studies in the literature have highlighted its value as an industrial biopolymer with no implications of using food as raw material. In fact, zein is able to form hydrophobic, tough, resistant and flexible edible coatings with a high barrier to oils and antimicrobial activity [21, 22]. As a consequence, zein-based coatings have been used in the packaging industry in medical pills [23], biodegradable plastics [22] and edible films for food packaging [24]. Table 1 summarizes the main applications of zein in the formulation of biopolymers with different applications.

Zein films obtained from corn- and zein-based coatings have been used as oxygen and moisture barriers in packaging materials for nuts and candies [25]. Zein protein coated with paper has been proven as an effective packaging material with good oil barrier for packaging of quick-service restaurant sandwiches, being considered a good alternative in fully compostable paper-based wraps and boxes for food delivery [26]. The potential of these edible zein films with antimicrobial and antioxidant properties to increase the quality of intermediate moisture apricots has also been reported [27].

Different thermoplastic protein biomaterials have been obtained by compression molding. For example, injection molding of corn starch formulations blended with zein protein resulted in materials with good elongation at break and tensile properties when glycerol was added as plasticizer [29]. Differences in the molecular weight of soy protein and corn zein obtained by compression molding were also used in the formulation of single- and double-coated laminates [30]. Laminates of zein sheets and oleic acid were obtained by solvent casting of the individual components followed by hot pressing at 120 °C to limit the presence of voids and defects, resulting in the improvement of oxygen barrier and mechanical properties [31]. Zein has been proven to form odorless, tasteless, clear, hard and almost invisible edible films. The development of zein-based edible films and coatings through conventional techniques (casting, extrusion and co-extrusion) allows obtaining uniform and homogeneous materials with appropriate thickness. Electrospraying has also been applied in the production of food nanoparticles [37], nanofibers [38], nanocapsules [39] and films [28] obtained from zein protein.

Table 1 Main applications of the zein protein biomaterials.

Application	Improved properties	Ref.
Biodegradable sheet materials	High tensile strength. Low water absorption.	22
Medical pills coating	Insoluble in water. Low water vapor permeability (WVP).	23
Packaging for nuts and candies	Oxygen, moisture, and grease barrier. Desirable film appearance: strong and glossy.	25
Zein coated with paper for quick-service restaurant sandwiches	Uniformity of the coating. Similar effectiveness of zein-coated papers than polyethylene laminates regarding grease barriers.	26
Active films for apricots	Reduction of fruit color changes. Inhibition of microbial growth in two orders of magnitude.	27
Food packaging films	Electrospraying produces better films than casting in terms of thickness, transparency, smoother and homogeneous surface and lower WVP.	28
Thermoplastic protein biomaterials blended with corn starch	Strength retention after 1 week without significant changes: 26 MPa tensile strength and3.7% elongation.	29
Double coating laminates with soy protein	Brittle layered structure for the corn-zein component but low WVP.	30
Zein sheets and oleic acid laminate materials	Clearer, tougher, smoother and flexible laminates. Lower O_2 and CO_2 permeability. Lower WVP. Coating increased tensile strength and elongation.	31
Chewing gum	Biodegradation. Safe for human ingestion. Suitable as drug delivery system. Good fracturability.	32
Controlled and targeted delivery of bioactive tissue engineering materials	Hydrophobic, biodegradable, biocompatible, economic and excellent carrier for poorly water-soluble drugs to be delivered by oral route.	33–36
Food nanoparticles	Compact nanostructures for 2.5% and 5% zein solutions. Particles size from 175 to 900 nm. Curcumin encapsulation efficiency: 85–90%. Dispersion of curcumin in semi-skimmed milk.	37
Food nanofibers	Better flexibility, water and oxygen permeability than poly(hydroxyalcanoates).	38
Food nanocapsules	300–450 μm droplets	39

Zein is approved as a generally recognized as safe (GRAS) excipient by the US Food and Drug Administration (FDA) for use in film coatings for pharmaceuticals, medical devices, nanoparticles, nanofibers and films as delivery carrier [40]. Novel applications in biomedical materials, such as those used for the controlled and targeted delivery of bioactive chemicals and tissue engineering, are the main applications of these zein-based films [33]. In this context, zein films, with particle diameters 100–500 and 500–2500 nm, have been reported as suitable materials for culturing human liver cells (HL-7702) and mice fibroblast cells (NIH3T3). Zein was used as scaffold since over 60% of both cells were attached to zein films only after 3 hours from seeding [35]. In a different study, zein films were used as coating materials for human umbilical vein endothelial cells and platelets, with some reduction in platelet adhesion [36]. A fluorescent nanocomposite based on cadmium sulphate and zein was also developed as support for the attachment and the proliferation of the mesenchymal stem cells and fibroblasts [34]. Zein films are also completely safe for human ingestion and they have been recently used to produce chewing gum [32].

2.2 Gluten Protein from Wheat Grain

Among all the by-products obtained from DDG proteins, wheat is of particular importance due to its high production and nutritional value. According to FAOSTAT, the worldwide annual production of wheat grain was over 713 million tons in 2013 [20]. Wheat protein is mainly composed of gluten (80–85% of the total protein weight), while the remaining fraction comprises a heterogeneous group of globulins and albumins. Up to now, and as a representation of wheat DDG valorization, only initial studies in the production of glutamic acid and/or proline from wheat proteins have been reported [17–19]. Wheat proteins have been used to produce biopolymers with elastic behavior, good barrier properties to carbon dioxide, oxygen and aromatic compounds, but poor ductile properties and high fragility. These major drawbacks could be overcome by adding plasticizers, such as glycerol, to gluten matrices during processing to improve ductility, resulting in flexible biofilms [41]. These authors reported that casting films formed by gluten/methyl-cellulose blends containing (25 wt%) glycerol resulted in improved tensile properties, in particular tensile strength (13.6 ± 1.4 MPa) and modulus (261.0 ± 42.7 MPa). These values were clearly higher than those obtained in biofilms with the same composition obtained by melt blending and further hot-press forming (1.4 ± 0.4 and 27.7 ± 5.2 MPa, respectively). Other authors proposed the use of thermoforming under chemically reductive conditions to form translucent sheets with improved tensile properties [42]. The linear deformation resistance of wheat gluten sheets at different moisture conditions (30–70%) (elastic modulus $4.5 \pm 7.8 \times 10^7$ Pa) was comparable to results obtained for polypropylene ($5.3 \pm 4.5 \times 10^7$ Pa).

Paper and paperboard are commonly used in packaging applications due to their biodegradation abilities, high availability, low cost, good mechanical properties and printability. But their hydrophilic character provides low barrier properties. They are usually coated with other materials, such as wheat gluten and montmorillonites, to decrease the effect of water and moisture in multilayer coatings, with a significant reduction in oxygen permeability of these biofilms [43]. Other authors proposed two different treatments to obtain paper-based composites (a surface-treated paper, coated on two sides with calcium carbonate and starch, and an untreated paper) with wheat gluten coatings and they evaluated the effect of the addition of this biopolymer to surface-treated and non-treated papers [44]. They reported that coating with wheat gluten clearly improved the main properties for packaging applications in non-treated papers, since they displayed a higher level of protein penetration (61% against 43% in the treated paper), resulting in better penetration of the gluten coating into the bulk of the paper structure.

By consideration of their adhesive, cohesive and film-forming properties, wheat proteins have also been evaluated for the development of edible coatings in combination with different biobased matrices such as lipids [45]. These combinations have been recently reported to extend the shelf life of different food products, such as dry-roasted peanuts and fried chicken pieces, packaged in gluten edible coatings [46]. Rainbow trout and Atlantic mackerel fish have also been coated with wheat gluten, getting an extension of two and three weeks in their shelf life [47]. Sea bass fish and other seafood products were also coated with wheat gluten, increasing their shelf life by 28 days [48]. The antimicrobial activity of gluten edible coatings with thymol added to hot smoked trout was successfully reported [49], where some decrease in the microorganisms growth with acceptable sensory quality during a five and six week period was observed.

2.3 Soy Protein from Soya

According to the FAOSTAT database, the worldwide annual production of soybean was over 276 million tons in 2013 [20]. Soy protein resins, which are obtained from soybean harvesting and processing as a by-product, have been recognized as adequate candidates for biopolymer formulations for decades [50]. They are abundant, cheap and biodegradable, with high nutritional value. They are also able to form edible films due to the bonds rearrangement in their structures during processing and blending with additives. However, as a consequence of their high hydrophilic character, soy protein films show low barrier properties to moisture. Two main strategies are considered to overcome this drawback: a) mixture with hydrophobic compounds such as lipids [51], and b) modification of the protein network arrangement through crosslinking of the protein chains by using physical, enzymatic or chemical treatments [52]. For example, agar-based films with soy proteins and glycerol as plasticizer were successfully processed by increasing their processing time. This strategy permits the higher exposition to hydrogen bonding interactions among soy proteins and agar, resulting in a clear increase of the tensile strength, from 4.1 MPa in control films to 24.6 MPa in blends with 65% (w/w) of soy protein content [53].

Commercial soy protein isolates or concentrates are mainly used to obtain innovative biocomposites after the removal of carbohydrates and oil components from the main protein structures [54]. These commercial formulations are composed by 70 wt% of protein and their prices range between $3.25/kg and $4.64/kg of protein [14]. Table 2 reviews the most recent uses of soy proteins to obtain different environmentally friendly biocomposites.

Composites formed by soy proteins reinforced with jute fibers were developed in water without any plasticizer, showing good flexural and tensile properties. Composites with 60 wt% soy proteins exhibited the highest flexural properties with an offset yield load of 55 N, stiffness of 11.5 N mm^{-1} and flexural strength of 24 MPa. The increase in the proportion of soy proteins from 40 to 50 wt% also increased their flexural strength by 15% and modulus by 9%. Thermoplastic soy proteins can act as binders to provide higher flexural and tensile properties when compared to commodities such as polypropylene. The use of water as plasticizer also decreased the final cost and possibility of chemical contamination of the matrix [55]. Reinforced biocomposites were developed at different pH values with the addition of hemp yarn to soy protein concentrate resins [56]. Improved interactions between resins and fibers were obtained at pH 10. Biocomposites formed by soy protein concentrates with jute fibers modified with glycerol or halloysite nanotubes showed lower heat release capacity than petrochemical-based resins, such as epoxies and vinyl esters, representing a clear green alternative to the most common resins used in structural applications [57]. In another study, two different processing methods (compression molding and extrusion-injection molding) were tested to obtain new soy protein-based biocomposites [58]. In particular, natural by-products obtained during the formation of agar/agar formulations were used as fibers to reinforce soy protein resins, resulting in sustainable biocomposites with good barrier and mechanical properties. Biocomposites obtained from soy protein isolates reinforced with starch nanocrystals were obtained by casting, allowing the formation of films with improved physical-chemical properties by the decrease in water solubility, high swelling capacity and low water vapor permeability, while mechanical properties were also modified [59]. As the amount of nanocrystals increased in the film up to 40% (w/w), the initial tensile strength (1.10 ± 0.20 MPa) and the initial elastic modulus (26.9 ± 11.2 MPa) increased to 5.08 ± 0.48 MPa and 310 ± 22 MPa, respectively. However, a reduction in the elongation at break was reported from 65.9 ± 17.8 to 21.3 ± 10.5%, showing that the material became more rigid as a consequence of major interactions between the protein matrix and the reinforcement.

Table 2 Main applications of soy protein isolates and concentrates to obtain biomaterials.

Application	Biomaterial matrix	Reference
Reinforcement	Soy protein-jute fiber composites	55
	Soy protein concentrate resin-hemp yarn composites	24, 28, 56
	Soy protein concentrate-jute fiber modified with glycerol and/or halloysite nanotubes	57
	Soy protein isolate with algae waste filler	58
	Soy protein isolate with starch nanocrystals	59
Paper coating	Soy protein isolate with organically modified montmorillonite paperboards	60
Active packaging	alpha-tocopherol encapsulation with soy protein	61
	Ferulic, caffeic, gallic acids and their oxidized products into soy protein films	62
	Soy protein isolate with carboxymethylcellulose and catechin	63
	Chitosan chloride and soy protein alternately assembled on cellulose acetate	64
	Soy protein isolate, virgin coconut oil, and soy lecithin	65
	Soy protein isolate and poly(lactic acid) with natamycin and thymol	66
Coatings	Soy protein isolate and 15 wt% gelatin (on SPI dry basis) with 30 wt% glycerol	59
	Soy protein isolate and gum acacia	67
	Soy protein isolate and beeswax	68
	Soy protein isolate, olive oil, hydroxy propyl methyl cellulose and potassium sorbate	69
	Soy protein isolate and TiO_2	70
	Soy protein and glycerol	71
	Soy protein isolate addition to the wheat flour-based batter	72

Films with excellent barrier to oxygen (at low to intermediate relative humidity) and good mechanical properties were obtained when soy protein was used as a coating for paperback matrices. However, the hydrophilic nature of soy protein results in poor barrier to water vapor, making it necessary to combine soy protein isolates or concentrates with different chemicals to improve these properties. In fact, crosslinking of soy protein isolate with formaldehyde and formation of composites with organically modified montmorillonite were the most effective treatments in decreasing the water vapor permeability of coated paperboards [60]. However, the use of films treated with such crosslinking agents in food packaging applications is questionable for toxicological reasons and further studies are necessary to analyze their migration into food to ensure the high safety requirements in these applications.

Nevertheless, one of the main applications of soy protein isolates and concentrates is in active packaging systems. The addition of active agents to the packaging materials and their further release into food allowed enhancing their shelf life, quality and safety against microbiological and oxidative degradation. In general terms, biopolymers obtained from biomass, such as proteins, show three main advantages for this purpose. They are ideal carriers for many active agents, increasing the possibilities for their controlled release, while they could be combined to obtain biocomposites with improved properties. In this context, the encapsulation of antioxidants extracted from plants and essential oils, such as alpha-tocopherol, in hydrocolloid matrices (whey protein isolate, zein and soy protein isolate) have been recently reported in the literature [61, 73]. These innovative formulations could be directly processed by electrospraying in coating layers onto one side of a thermoplastic wheat gluten film [61]. The addition of ferulic, caffeic and gallic acids, as well as their oxidized products to soy protein films, has been recently studied for their antioxidant performance [62]. Gallic acid films exhibited the highest tensile strength and elongation at break, followed by those with caffeic and ferulic acids. Oxidized phenolic acids produced higher tensile strength and elongation at break in films based on soy proteins in contrast to their non-oxidized counterparts. Regarding barrier properties, lower water vapor permeability and water solubility were observed after the addition of phenolic acids [62].

In a different study, active films were obtained by the combination of soy protein isolate with carboxymethylcellulose and using catechin as active agent [63]. The addition of cellulose and catechin to the soy protein isolate decreased transparency, while some improvement on water solubility, decrease in water vapor permeability, higher tensile strength and lower elongation at break were observed. Emulsion-based edible films based on soy protein isolate, virgin coconut oil and soy lecithin plasticized with glycerol were formulated by casting [65]. The applicability of soy protein isolate emulsion-based films was evaluated in their use with olive oil individual containers. The authors concluded that the incorporation of coconut oil and soy lecithin to these films decreased their moisture affinity and increased their ductile properties. In this sense, the increase in the virgin coconut oil concentration increased the elongation at break of films from 104 ± 17% of control to 212 ± 10% in active films. This difference in ductile properties could be attributed to the plasticizing effect of the oil in the matrix. However, the tensile strength values decreased from 12.0 ± 0.6 to 8.1 ± 0.8 MPa and this could be explained by the addition of lipids to the matrix to induce the development of discontinuities in the polymer network. It was also reported that the peroxides index in the oil increased slightly up to 28 days of storage.

Soy protein isolates and concentrates have also been tested to produce antimicrobial active packaging systems. In fact, the antimicrobial activity of chitosan chloride and soy protein isolate assembled on cellulose acetate matrices was successfully reported against *Escherichia coli* and *Staphylococcus aureus* by using the disk diffusion method [64]. In a different study, the antifungal and antibacterial activities of bilayer films prepared with isolated soy protein and poly(lactic acid) (PLA) were successfully tested when natamycin and thymol were added as active agents [66]. However, the effect of soy protein to significantly extend the food shelf life is still unknown, but several natural soy protein coatings have been recently reported to extend the shelf life of different products, such as tomatoes [67], fresh-cut artichoke [68], Phalsa fruit [69], strawberry [70], mozzarella cheese [71], fried breaded shrimp [72] and beef patties [74].

3 AGRO SEEDS AS PROTEIN SOURCES

It is known that fruit manufacturing industries discharge a considerable amount of peels and seeds as industrial waste. Depending on the raw materials and the different cultivars used in the fruit processing, seeds and peels represent different percentages of the total weight and can be used as sources for new valuable products. In general terms, the main components of food seeds are hydrocarbons and relatively small amounts of proteins and fats, with moisture contents around 80–90% in weight [75]. Up to now, only a few agro seeds have been reported as protein sources since there is a minimum amount of proteins in the

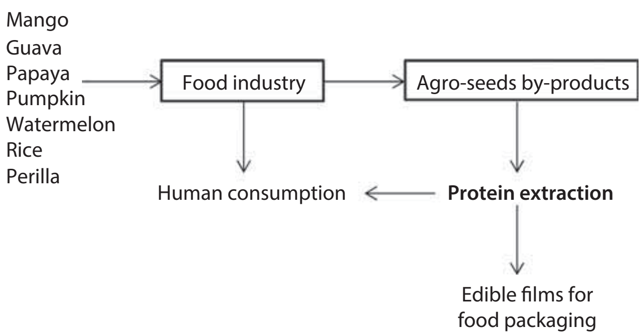

Figure 3 Main sources of agro seeds by-products for their valorization in protein-based films.

fruit composition to obtain good production yields, which is only possible in those seeds with high protein contents. Figure 3 shows the main uses of agro seeds coming from fruits such as mango, guava, papaya, pumpkin, watermelon, rice and perilla, obtaining proteins which could be potentially used in the production of edible films.

Among them, papaya is one of the most popular tropical fruits, with 15.4% of the total production. A large amount of by-products, especially seeds and peels representing about 20 to 25% of the total fruit weight, are obtained during processing. These residues are currently discarded as organic wastes into the environment, resulting in large amounts of residues which are usually burned with production of atmospheric carbon dioxide [76]. Recently, some authors reported that the application of pulsed electric field (PEF) assisted extraction and supplementary aqueous extraction (SAE) at 50 °C, pH 7 during 3 hours, enhanced yield (about 200%) and antioxidant capacities (about 20%) of the compounds extracted from papaya seeds. This method has shown high potential for industrial applications to recover the valuable components from papaya seeds, which can be used as food additives and nutraceutical supplements [77].

The global demand for new sources of proteins has focused on oilseeds and their agro-industrial wastes, defatted oil cakes, by their high potential in the extraction of proteins with high yields [78]. For example, perilla (*Perilla frutescens*) seeds are widely used as an oil source in several Asian countries, such as Korea, China or Japan, due to their antioxidant, antihypertensive and immunostimulant beneficial effects on human health. Perilla seeds contain abundant oil (~43 wt%) and protein (~18 wt%) and, after the oil extraction, their protein content increases up to approximately 40 wt%, being primarily composed of globulin (84 wt%). Although being an abundant source of proteins with relative low cost, this agricultural by-product is currently just used as animal feed or in the production of fertilizers [79]. Therefore, there is a growing interest in finding new applications with higher added-value for the perilla seeds such as food ingredients and edible films based on proteins. In this context, the experimental conditions of ultrasound-assisted alkaline extraction of proteins from dried perilla seeds were optimized [80]. The authors concluded that by using ultrasonic power of 61 W at 40 °C, a liquid to solid ratio of 40 mL g^{-1} and 12 minutes of total extraction time, the extracted content of proteins from perilla seeds was around 10.8%. The analysis of the amino acid profile of perilla proteins denoted balanced amounts of the main essential amino acids, except for lysine [79]. Recently, it has been reported that perilla proteins obtained from the seed oil residues can be combined with 3 wt% of red algae (*Gelidium corneum)* to improve the poor mechanical properties and water vapor permeability of the protein-based films caused by their hydrophilic characteristics, resulting in suitable films for food packaging applications. Furthermore, the incorporation of clove oil (1.2 wt%) into the protein-red algae composite films showed antimicrobial and antioxidant performance in active packaging systems for pork sausages stored at 4 °C [81].

Rice is another massive source of proteins obtained from the harvesting residues and processing by-products. The large annual worldwide production of milled-rice (about 460 million tons) results in huge amounts of by-products such as rice bran, oils, flour, wax and hulls [82]. Among them, rice bran and flour are products with a high content of proteins, with about 14–20 wt% and 7–9 wt% respectively. However, these by-products are mainly used as animal feed and they are currently underutilized as protein sources for the production of biopolymers. Proteins can be extracted from defatted rice bran by using enzymatic methods, which are highly specific, require mild conditions, and are readily accepted by food regulatory agencies, getting up to 95% of protein recovery under optimal conditions. These products show essential amino acid profiles comparable to those of casein. Recent studies showed that the treatment of rice bran with subcritical water at temperatures of 200–220 °C for 30 minutes allows the recovery of >90% of the protein in soluble form with limited hydrolysis parallel processes. In this environmentally friendly extraction process, water is put under high pressure to maintain its liquid state at an increased range of temperatures, permitting versatile extractions where proteins can be obtained with high purity and recoveries [83]. The formation of edible films based on rice bran is not only expected to extend their applications, but also for the development of new functional food additives [84]. In this sense, Shin *et al.* [85] used rice bran oil residues as an adequate protein source for the formation of edible films by their high content of isolated proteins (12–20 wt%) obtained after fat extraction, providing a solution to the accumulation or burning of these currently underutilized food processing by-products. These authors prepared edible films based on rice bran with fructose as plasticizer, but poor mechanical properties and water vapor permeability were obtained, making these films useless for food packaging applications. Red algae or gelatin were incorporated into the film-forming solution to improve these properties and the formulation with 4 wt% rice bran/4 wt% gelatin showed a significant increase on tensile strength (from 0.94 to 23.9 MPa) and on elongation at break (from 25.5 to 141.0%) [85]. The conditions that could effectively affect the formation of rice bran proteins-based films and their physical-chemical and functional properties were evaluated. The film-forming variables, such as the protein purity and concentration, plasticizer type and concentration, pH of the film-forming solution and temperature, significantly affected the physical-chemical properties of these protein-based films. Alkaline conditions (pH 8.0), and low glycerol concentrations (2 w/v%) resulted in better films in terms of puncture strength and water solubility. The authors concluded that rice bran proteins-based films showed mechanical and functional properties comparable to those of the soy protein-based counterparts [84].

The interest in the use of pumpkin (*Cucurbita* sp.) seeds as a source of proteins is based on their high content, ranging from 24.5 to 36 wt%. The major protein fraction in pumpkin seeds is represented by the 12S globulin, called cucurbitin, followed by the 18S globulin. These two protein fractions and other smaller amounts of albumins represent 59% of the total protein content in pumpkin seeds [86]. The increased production of pumpkin seed oils results in large amounts of by-products, where protein contents can reach up to 60–65 wt%. But this residue is only currently used as livestock feed [87]. Some authors have reported the functional properties of pumpkin seed protein isolates (PSPI), such as solubility, interfacial and emulsifying properties, as function of pH, ionic strength and PSPI concentration [86].

The watermelon seed meal, which is generated from the watermelon processing industry to obtain juices, nectars and fruit cocktails is another agro-seed residue produced in high amounts, especially in tropical regions. The watermelon oil cake is the residue obtained after oil pressing and it is currently used for animal feeding. Watermelon *(Citrullus lanatus*) seed meal contains high levels of extractable proteins, which can be used as raw material for the production of high quality protein products, such as nutritional and functional ingredients, to be directly introduced in food formulations. Protein isolates and concentrates can be extracted from the defatted watermelon seed meals by using alkali and NaCl, respectively, with high yields in the case of protein isolates (around 38 wt%) besides the good functional properties shown by the protein concentrates. Globulin was the main protein obtained in the extraction of watermelon seed meal, followed by albumin and glutelin, while the amino acids profile was dominated by arginine, aspartic acid and glutamic acid [88]. It has been observed that some relationship between the proteins quality and the extraction conditions can be established to select the functional properties of the protein-based products. The excellent nutritional and functional properties of these proteins obtained from watermelon seed meal makes this residue highly attractive for its potential use in food formulations [78]. The effect of the matrix on the mineral bioavailability of defatted flour and proteins isolated from watermelon seed meal has also been reported [89]. The authors found that the protein isolates were a good source of iron and zinc and that the bioaccessibility of all the minerals present in these formulations is correlated with the phytate, tannin and oxalate concentrations. The chemical composition and functional properties of these proteins suggest their suitability for mineral fortification in food formulations.

Mango (*Mangifera indica* L.) fruit is mainly produced in Asian and Pacific countries (~77% of the worldwide production), while approximately 14% and 9% are produced in American and African tropical countries respectively [20]. During the mango processing, only the edible parts of the fruit are used and a considerable amount of residues and by-products, especially peels and seeds, are produced and discarded as industrial waste or used as animal feed. Mango puree, nectar, juice, leather, pickles, slices in syrup, canned slices and chutney are the main industrial products obtained from mango fruits. Depending on the final product and the fruit origin, peels and seeds can represent between 35% and 60% of the total weight of the fruit [90]. However, these by-products are considered a good and cheap source of valuable food and nutraceutical compounds due to their high content of health-enhancing substances, such as phenolic compounds, carotenoids, vitamin C and dietary fibers [91]. The mango seeds are basically composed of the tegument and the kernel. Depending on the varieties, the kernel represents from 45 wt% to 75 wt% of the seed and about 20 wt% of the whole fruit, and contains starch, cellulose, hemicelluloses, lignin and fatty acids, such as oleic, stearic, palmitic and linoleic. The tegument is the external shell that covers the kernel and mainly contains cellulose, hemicelluloses and lignin [92]. Mango seed kernels contain a considerable amount of the total phenolic compounds and lipids with unsaponifiable matter of the whole fruit, also including a small amount of crude proteins (6.7 wt% on dry basis). However, the quality of these proteins is high because they contain all the essential amino acids, with the highest values of leucine, valine and lysine, depending on the mango variety [90]. It has also been reported that the essential amino acids in mango seed kernels, except methionine, threonine and tyrosine, were at higher levels than in the FAO/WHO reference proteins [93]. Mango seed kernel could be considered as a potential source of proteins for biopolymer formulations, but a current lack of information about the extraction methods for the recovery of these compounds from mango by-products has delayed the possible uses of these proteins in high added-value applications.

Guava seeds could be considered as an excellent source of oil and protein pastes due to their composition of around 7.6 wt% protein, 16.0 wt% fat, 61.4 wt% raw fiber and 4.1 wt% of water [94]. Approximately 6% of the total guava weight corresponds to seeds that are currently discharged as waste, causing important environmental problems in their production sites. However, this by-product has shown high potential as fiber and protein sources. Some authors [95] studied the extraction process of the guava seed protein isolate as well as their functional and nutritional qualities. These authors obtained the protein isolated from the guava seed meal by using isoelectric precipitation at pH 5, with a previous solubilization process at pH 11.5 and 40 °C for 30 min, reaching an extraction yield of 78.2% and a very high protein content of 96.7 wt%. The oil absorption capacity of guava seed extracts, as well as the foaming capacity and stability, were lower than those obtained from other seed proteins. The authors also concluded that the essential amino acid profile of the guava seed protein isolates satisfied the FAO/WHO pattern requirements for adults, except for lysine. In another work, glutelins (86–90 g/100 g) and globulins (10 g/100 g) were identified as the main components of the guava seed proteins extract, while albumins and prolamins were minor components (~2 g/100 g) [96]. The high content of tannin, saponin and phytic acid may exert an antinutritional effect if consumed excessively. However, thermal treatments or germination can be applied to guava seeds to modify their chemical composition, reducing the content of those chemicals and enabling their use in the food and feed industries [97]. Therefore, the guava seed could be an interesting source of proteins not only for animal and human consumption but also for the preparation of edible films, reducing the pollution problems associated with the waste discarded by the processing industry.

4 CONCLUSIONS

The use of agricultural biowastes obtained from harvesting and processing by-products has been shown to be a very interesting field of research in which valuable chemicals and building blocks are extracted for the production of a new generation of biopolymers with enhanced mechanical and barrier properties, which are highly bioavailable and compatible with all uses for human consumption. Particularly, proteins extracted from fruit and vegetable residues (i.e, non-edible components, such as peels and seeds) showed potential in the formation of biopolymers and other fully renewable materials.

As with any emerging technology, a significant effort with extensive and interdisciplinary collaborations and partnerships is required to move concepts from research and development to fruitful commercialization and real applications. But the use of agro-wastes and residues in the industrial production of biopolymers ensures their fully renewable character and important developments in production will be effective in the near future.

REFERENCES

1. J. Vartiainen, M. Vaha-Nissi, and A. Harlin, Biopolymer films and coatings in packaging applications – A review of recent developments. *Mater. Sci. Appl.* **5**, 708–718 (2014).
2. M.G. Rao, P. Bharathi, and R.M. Akila, A comprehensive review on biopolymers. *Sci. Revs. Chem. Commun.* **4,** 61–68 (2014).
3. A. Valdés, A.C. Mellinas, M. Ramos, M.C. Garrigós, and A. Jiménez, Natural additives and agricultural wastes in biopolymer formulations for food packaging. *Front. Chem.* **2**, 6 (2014).
4. A. Valdés, L. Vidal, A. Beltrán, A. Canals, and M.C. Garrigós, Microwave-assisted extraction of phenolic compounds from almond skin byproducts (*Prunus amygdalus*): A multivariate analysis approach. *J. Agric. Food. Chem.* **63**, 5395–5402 (2015).
5. L.A. Pfaltzgraff, M. De Bruyn, E.C. Cooper, V. Budarin, and J.H. Clark, Food waste biomass: A resource for high-value chemicals. *Green Chem.* **15**, 307–314 (2013).
6. M.G.A. Vieira, M.A. da Silva, L.O. dos Santos, and M.M. Beppu, Natural-based plasticizers and biopolymer films: A review. *Eur. Polym. J.* **47**, 254–263 (2011).
7. C. Mellinas, A. Valdés, M. Ramos, N. Burgos, M. Del Carmen Garrigós, and A. Jiménez, Active edible films: Current state and future trends. *J. Appl. Polym. Sci.* **133**, (2016).
8. P. Gupta and K.K. Nayak, Characteristics of protein-based biopolymer and its application. *Polym. Eng. Sci.* **55**, 485–498 (2015).
9. A. Valdés, M. Ramos, E. García-Serna, M.C. Garrigós, and A. Jiménez, Polymers extracted from biomass, in *Reference Module in Food Science*, Elsevier (2016).

10. A. Valdés and M.C. Garrigós, Carbohydrate-based advanced biomaterials for food sustainability: A review. *Mater. Sci. Forum* **842,** 182–195 (2016).
11. J. Han and K. Liu, Changes in composition and amino acid profile during dry grind ethanol processing from corn and estimation of yeast contribution toward DDGS Proteins. *J. Agric. Food. Chem.* **58**, 3430–3437 (2010).
12. A.-S. Voisin, J. Guéguen, C. Huyghe, M.-H. Jeuffroy, M.-B. Magrini, J.-M. Meynard, C. Mougel, S. Pellerin, and E. Pelzer, Legumes for feed, food, biomaterials and bioenergy in Europe: A review. *Agron. Sustain. Dev.* **34**, 361–380 (2013).
13. O.A. Olukosi and A.O. Adebiyi, Chemical composition and prediction of amino acid content of maize- and wheat-distillers' dried grains with soluble. *Anim. Feed Sci. Tech.* **185**, 182–189 (2013).
14. D.J. Cookman and C.E. Glatz, Extraction of protein from distiller's grain. *Bioresour. Technol.* **100**, 2012–2017 (2009).
15. C.Y. Lin and J.C. Liu, Modular protein domains: An engineering approach toward functional biomaterials. *Curr. Opin. Biotechnol.* **40**, 56–63 (2016).
16. B. Aghaei-Ghareh-Bolagh, S.M. Mithieux, and A.S. Weiss, Elastic proteins and elastomeric protein alloys. *Curr. Opin. Biotechnol.* **39**, 56–60 (2016).
17. E. Scott, F. Peter, and J. Sanders, Biomass in the manufacture of industrial products – the use of proteins and amino acids. *Appl. Microbiol. Biotechnol.* **75**, 751–762 (2007).
18. T. Werpy and G. Petersen. *Top Value Added Chemicals from Biomass: Vol. I — Results of Screening for Potential Candidates from Sugars and Synthesis Gas*. Pacific Northwest National Laboratory, National Renewable Energy Laboratory, Oak Ridge (2004).
19. M.F. Villegas-Torres, J.M. Ward, and G.J. Lye, The protein fraction from wheat-based dried distiller's grain with solubles (DDGS): Extraction and valorization. *New Biotechnol.* **32**, 606–611 (2015).
20. Food and Agriculture Organization of the United Nations, FAOSTAT database, http://www.faostat.fao.org (2015).
21. R. Shukla and M. Cheryan, Zein: The industrial protein from corn. *Ind. Crop. Prod.* **13**, 171–192 (2001).
22. H.-M. Lai, G.W. Padua, and L.S. Wei, Properties and microstructure of zein sheets plasticized with palmitic and stearic acids. *Cereal Chem. J.* **74**, 83–90 (1997).
23. R.A. Reiners, J.S. Wall, and G.E. Inglett. *Corn proteins: Potential for their Industrial Use*, pp. 285–302, American Association of Cereal Chemists, St. Paul, Minn. (1973).
24. A. Gennadios, *Protein-Based Films and Coatings*, CRC Press, Boca Raton, FL (2002).
25. K. Khwaldia, E. Arab-Tehrany, and S. Desobry, Biopolymer coatings on paper packaging materials. *Compr. Rev. Food Sci. F.* **9**, 82–91 (2010).
26. T.A. Trezza and P.J. Vergano, Grease resistance of corn zein-coated paper. *J. Food Sci.* **59**, 912–915 (1994).
27. T. Baysal, S. Ersus, and E. Apaydın, The effect of corn zein edible film coating on intermediate moisture apricot (*Prunus Armenic* L.) quality. *Gida* **35**(4), 245–249 (2010).
28. V.A. Gaona-Sánchez, G. Calderón-Domínguez, E. Morales-Sánchez, J.J. Chanona-Pérez, G. Velázquez-de la Cruz, J.V. Méndez-Méndez, E. Terrés-Rojas, and R.R. Farrera-Rebollo, Preparation and characterisation of zein films obtained by electrospraying. *Food Hydrocolloid* **49**, 1–10 (2015).
29. S. Lim and J. Jane, Storage stability of injection-molded starch-zein plastics under dry and humid conditions. *J. Environ. Polym. Degrad.* **2**, 111–120 (1994).
30. H. Pol, P. Dawson, J. Acton, and A. Ogale, Soy Protein isolate/corn-zein laminated films: Transport and mechanical properties. *J. Food Sci.* **67**, 212–217 (2002).
31. A.M. Rakotonirainy and G.W. Padue, Effects of lamination and coating with drying oils on tensile and barrier properties of zein films. *J. Agric. Food Chem.* **49**, 2860–2863 (2001).
32. F. Mehta, R. Rajagopalan, and P. Trivedi, Formulation, optimization and texture characterization of environment friendly gum. *Der Pharmacia Sinica* **3**, 664 (2012).
33. R. Paliwal and S. Palakurthi, Zein in controlled drug delivery and tissue engineering. *J. Control. Release* **189**, 108–122 (2014).
34. B. Dhandayuthapani, A.C. Poulose, Y. Nagaoka, T. Hasumura, Y. Yoshida, T. Maekawa, and D.S. Kumar, Biomimetic smart nanocomposite: In vitro biological evaluation of zein electrospun fluorescent nanofiber encapsulated CdS quantum dots. *Biofabrication* **4**, 025008 (2012).
35. J. Dong, Q. Sun, and J.Y. Wang, Basic study of corn protein, zein, as a biomaterial in tissue engineering, surface morphology and biocompatibility. *Biomaterials* **25**, 4691–4697 (2004).
36. H.J. Wang, Z.X. Lin, X.M. Liu, S.Y. Sheng, and J.Y. Wang, Heparin-loaded zein microsphere film and hemocompatibility. *J. Controlled Release* **105**, 120–131 (2005).
37. J. Gomez-Estaca, M.P. Balaguer, R. Gavara, and P. Hernandez-Munoz, Formation of zein nanoparticles by electrohydrodynamic atomization: Effect of the main processing variables and suitability for encapsulating the food coloring and active ingredient curcumin. *Food Hydrocolloid* **28**, 82–91 (2012).
38. M.J. Fabra, A. Lopez-Rubio, and J.M. Lagaron, Nanostructured interlayers of zein to improve the barrier properties of high barrier polyhydroxyalkanoates and other polyesters. *J. Food Eng.* **127**, 1–9 (2014).
39. A. Jaworek, Micro- and nanoparticle production by electrospraying. *Powder Technol.* **176**, 18–35 (2007).
40. Y. Zhang, L. Cui, X. Che, H. Zhang, N. Shi, C. Li, Y. Chen, and W. Kong, Zein-based films and their usage for controlled delivery: Origin, classes and current landscape. *J. Control. Release* **206**, 206–219 (2015).
41. M. Zuo, Y. Song, and Q. Zheng, Preparation and properties of wheat gluten/methylcellulose binary blend film casting from aqueous ammonia: A comparison with compression molded composites. *J. Food Eng.* **91**, 415–422 (2009).
42. F.M. Pallos, G.H. Robertson, A.E. Pavlath, and W.J. Orts, Thermoformed wheat gluten biopolymers. *J. Agric. Food Chem.* **54**, 349–352 (2006).
43. H. Türe, M. Gällstedt, E. Johansson, and M.S. Hedenqvist, Wheat-gluten/montmorillonite clay multilayer-coated paperboards with high barrier properties. *Ind. Crop. Prod.* **51**, 1–6 (2013).

44. C. Guillaume, J. Pinte, N. Gontard, and E. Gastaldi, Wheat gluten-coated papers for bio-based food packaging: Structure, surface and transfer properties. *Food Res. Int.* **43**, 1395–1401 (2010).
45. N. Gontard, C. Duchez, J.L. Cuq, and S. Guilbert, Edible composite films of wheat gluten and lipids: Water-vapor permeability and other physical-properties. *Int. J. Food Sci. Technol.* **29**, 39–50 (1994).
46. I. Fernández-Pan, J. Ignacio, and M. Caballero. *Biopolymers – New Materials for Sustainable Films and Coatings*, p. 233, John Wiley & Sons, Ltd, Chichester, UK (2011).
47. S. Dursun and N. Erkan, The effect of edible coating on the quality of smoked fish. *Italian J. Food Sci.* **26**, 370–382 (2014).
48. N. Erkan, S. Dursun, Ş. Ulusoy, S. Akçay, and M. Yeşiltaş, Combined effects of protein based edible film coatings and vacuum packaging on the quality of fresh sea bass fillets. *Fleischwirtschaft Int.* **28**, 61–68 (2013).
49. N. Erkan, H. Dogruyol, A. Günlü, and I. Genç, Use of natural preservatives in seafood: Plant extracts, edible films and coating. *J. Food Health Sci.* **1**, 33–49 (2015).
50. D. Jagadeesh, K. Kanny, and K. Prashantha, A review on research and development of green composites from plant protein-based polymers. *Polym. Compos.* n/a (2015).
51. F.M. Monedero, M.J. Fabra, P. Talens, and A. Chiralt, Effect of oleic acid–beeswax mixtures on mechanical, optical and water barrier properties of soy protein isolate based films. *J. Food Eng.* **91**, 509–515 (2009).
52. C. Zhang, K. Guo, Y. Ma, D. Ma, X. Li, and X. Zhao, Original article: Incorporations of blueberry extracts into soybean-protein-isolate film preserve qualities of packaged lard. *Int. J. Food Sci. Techol.* **45**, 1801–1806 (2010).
53. G. Tian, B. Xu, G. Yang, and J. Guo, Microstructure and mechanical properties of soy protein/agar blend films: Effect of composition and processing methods. *J. Food Eng.* **107**, 21–26 (2011).
54. R. Kumar, V. Choudhary, S. Mishra, I.K. Varma, and B. Mattiason, Adhesives and plastics based on soy protein products. *Ind. Crop. Prod.* **16**, 155–172 (2002).
55. N. Reddy and Y. Yang, Completely biodegradable soy-protein–jute biocomposites developed using water without any chemicals as plasticizer. *Ind. Crop. Prod.* **33**, 35–41 (2011).
56. J.T. Kim and A.N. Netravali, Development of aligned-hemp yarn-reinforced green composites with soy protein resin: Effect of pH on mechanical and interfacial properties. *Compos. Sci. Technol.* **71**, 541–547 (2011).
57. R. Nakamura, A.N. Netravali, A.B. Morgan, M.R. Nyden, and J.W. Gilman, Effect of halloysite nanotubes on mechanical properties and flammability of soy protein based green composites. *Fire Mater.* **37**, 75–90 (2013).
58. T. Garrido, M. Peñalba, K. de la Caba, and P. Guerrero, Injection-manufactured biocomposites from extruded soy protein with algae waste as a filler. *Composites Part B: Eng.* **86**, 197–202 (2016).
59. A. González and C.I. Alvarez Igarzabal, Nanocrystal-reinforced soy protein films and their application as active packaging. *Food Hydrocolloid.* **43**, 777–784 (2015).
60. J.W. Rhim, J.H. Lee, and S.I. Hong, Water resistance and mechanical properties of biopolymer (alginate and soy protein) coated paperboards. *Lebensm Wiss Technol.* **39**, 806–813 (2006).
61. M.J. Fabra, A. López-Rubio, and J.M. Lagaron, Use of the electrohydrodynamic process to develop active/bioactive bilayer films for food packaging applications. *Food Hydrocolloid.* **55**, 11–18 (2016).
62. A. Insaward, K. Duangmal, and T. Mahawanich, Mechanical, optical, and barrier properties of soy protein film as affected by phenolic acid addition. *J. Agric. Food Chem.* **63**, 9421–9426 (2015).
63. J. Han, S.-H. Shin, K.-M. Park, and K. Kim, Characterization of physical, mechanical, and antioxidant properties of soy protein-based bioplastic films containing carboxymethylcellulose and catechin. *Food Sci. Biotechnol.* **24**, 939–945 (2015).
64. Y. Pan, X. Huang, X. Shi, Y. Zhan, G. Fan, S. Pan, J. Tian, H. Deng, and Y. Du, Antimicrobial application of nanofibrous mats self-assembled with quaternized chitosan and soy protein isolate. *Carbohyd. Polym.* **133**, 229–235 (2015).
65. D. Carpiné, J. Dagostin, L. Bertan, and M. Mafra, Development and characterization of soy protein isolate emulsion-based edible films with added coconut oil for olive oil packaging: Barrier, mechanical, and thermal properties. *Food Bioproc. Technol.* **8**, 1811–1823 (2015).
66. A. González and C.I. Alvarez Igarzabal, Soy protein – Poly(lactic acid) bilayer films as biodegradable material for active food packaging. *Food Hydrocolloid.* **33**, 289–296 (2013).
67. C. Li, J. Wang, J. Shi, X. Huang, Q. Peng, and F. Xue, Encapsulation of tomato oleoresin using soy protein isolate-gum aracia conjugates as emulsifier and coating materials. *Food Hydrocolloid.* **45**, 301–308 (2015).
68. C. Ghidelli, M. Mateos, C. Rojas-Argudo, and M.B. Pérez-Gago, Novel approaches to control browning of fresh-cut artichoke: Effect of a soy protein-based coating and modified atmosphere packaging. *Postharvest Biol. Technol.* **99**, 105–113 (2015).
69. R.A. Dave, T.V.R.A. Rao, and A.S.B. Nandane, RSM-based optimization of edible-coating formulations for preserving post-harvest quality and enhancing storability of phalsa (*Grewia asiatica* L.). *J. Food Process. Preserv.*, **In press** (2015).
70. Y. Zhang, S. Lin, B. Lin, W. Wang, and S. Wang, Studies on fresh-keeping strawberry using TiCVSPI composite film. *J. Chinese Inst. Food Sci. Technol.* **15**, 120–125 (2015).
71. Y. Zhong, G. Cavender, and Y. Zhao, Investigation of different coating application methods on the performance of edible coatings on Mozzarella cheese. *LWT - Food Sci. Technol.* **56**, 1–8 (2014).
72. H.W. Ji, G.K. Pan, S.C. Liu, W.M. Su, H.Y. Lu, and C. Pan, Effect of soy protein isolate on quality of fried breaded shrimp. *Modern Food Sci. Technol.* **30**, 104 (2014).
73. N. Burgos, A.C. Mellinas, E. García-Serna, and A. Jiménez, Nanoencapsulation of flavor and aromas in food packaging, in *Nanotechnology in Food Industry*, Vol. I-X, A.M. Grumezescu (Ed.), Elsevier, **In press** (2016).

74. P. Guerrero, P. Arana, M.N. O'Grady, J.P. Kerry, and K. de la Caba, Valorization of industrial by-products: Development of active coatings to reduce food losses. *J. Clean. Prod.* **100**, 179–184 (2015).
75. N. Mirabella, V. Castellani, and S. Sala, Current options for the valorization of food manufacturing waste: A review. *J. Clean. Prod.* **65**, 28–41 (2014).
76. B.B. Koubala, S. Christiaens, G. Kansci, A.M. Van Loey, and M.E. Hendrickx, Isolation and structural characterisation of papaya peel pectin. *Food Res. Int.* **55**, 215–221 (2014).
77. O. Parniakov, E. Roselló-Soto, F.J. Barba, N. Grimi, N. Lebovka and E. Vorobiev, New approaches for the effective valorization of papaya seeds: Extraction of proteins, phenolic compounds, carbohydrates, and isothiocyanates assisted by pulsed electric energy. *Food Res. Int.* **77**, Part 4, 711–717 (2015).
78. A.A. Wani, D.S. Sogi, P. Singh, I.A. Wani, and U.S. Shivhare, Characterisation and functional properties of watermelon (*Citrullus lanatus*) seed proteins. *J. Sci. Food Agric.* **91**, 113–121 (2011).
79. Y. Takenaka, Y. Arii, and H. Masui, Subunit structure and functional properties of the predominant globulin of perilla (*Perilla frutescens* var. frutescens) seeds. *Biosci. Biotechnol. Biochem.* **74**, 2475–2479 (2010).
80. J. Zhu and Q. Fu, Optimization of ultrasound-assisted extraction process of perilla seed meal proteins. *Food Sci. Biotechnol.* **21**, 1701–1706 (2012).
81. N.B. Song, J.H. Lee, and K. Song, Preparation of perilla seed meal protein composite films containing various essential oils and their application in sausage packaging. *J. Korean Soc. Appl. Biol. Chem.* **58**, 83–90 (2015).
82. Food and Agriculture Organization of the United Nations, FAO Rice Market Monitor, Volume XVIII - Issue No. 4, http://www.fao.org/fileadmin/templates/est/COMM_MARKETS_MONITORING/Rice/Images/RMM/RMM_DEC15_H.pdf (2015).
83. F. Shih, An update on the use of co-products from the milling of rice in value-added food products. *J. Am. Oil Chem. Soc.* **89**, 1–8 (2012).
84. A.P. Adebiyi, A.O. Adebiyi, D.-H. Jin, T. Ogawa, and K. Muramoto, Rice bran protein-based edible films. *Int. J. Food Sci. Tech.* **43**, 476–483 (2008).
85. Y. Shin, S.-A. Jang, and K. Song, Preparation and mechanical properties of rice bran protein composite films containing gelatin or red algae. *Food Sci. Biotechnol.* **20**, 703–707 (2011).
86. S. Bučko, J. Katona, L. Popović, Ž. Vaštag, L. Petrović, and M. Vučinić–Vasić, Investigation on solubility, interfacial and emulsifying properties of pumpkin (*Cucurbita pepo*) seed protein isolate. *LWT-Food Sci. Technol.* **64**, 609–615 (2015).
87. Ž. Vaštag, L. Popović, S. Popović, V. Krimer, and D. Peričin, Production of enzymatic hydrolysates with antioxidant and angiotensin-I converting enzyme inhibitory activity from pumpkin oil cake protein isolate. *Food Chem.* **124**, 1316–1321 (2011).
88. A. Wani, D. Sogi, P. Singh, and U. Shivhare, Characterization and functional properties of watermelon (*Citrullus lanatus*) seed protein isolates and salt assisted protein concentrates. *Food Sci. Biotechnol.* **20**, 877–887 (2011).
89. A. Jyothi lakshmi and P. Kaul, Nutritional potential, bioaccessibility of minerals and functionality of watermelon (*Citrullus vulgaris*) seeds. *LWT-Food Sci. Technol.* **44**, 1821–1826 (2011).
90. M.H.A. Jahurul, I.S.M. Zaidul, K. Ghafoor, F.Y. Al-Juhaimi, K.-L. Nyam, N.A.N. Norulaini, F. Sahena, and A.K. Mohd Omar, Mango (*Mangifera indica* L.) by-products and their valuable components: A review. *Food Chem.* **183**, 173–180 (2015).
91. D.S. Sogi, M. Siddiq, I. Greiby, and K.D. Dolan, Total phenolics, antioxidant activity, and functional properties of 'Tommy Atkins' mango peel and kernel as affected by drying methods. *Food Chem.* **141**, 2649–2655 (2013).
92. M.A. Henrique, H.A. Silvério, W.P. Flauzino Neto, and D. Pasquini, Valorization of an agro-industrial waste, mango seed, by the extraction and characterization of its cellulose nanocrystals. *J. Environ. Manage.* **121**, 202–209 (2013).
93. A.E.M. Abdalla, S.M. Darwish, E.H.E. Ayad, and R.M. El-Hamahmy, Egyptian mango by-product. 1. Compositional quality of mango seed kernel. *Food Chem.* **103**, 1134–1140 (2007).
94. A. El Anany, Nutritional composition, antinutritional factors, bioactive compounds and antioxidant activity of guava seeds (*Psidium Myrtaceae*) as affected by roasting processes. *J. Food Sci. Tech.* **52**, 2175–2183 (2015).
95. A.B. Nicanor, A.O. Moreno, A.L.M. Ayala, and G.D. Ortíz, Guava seed protein isolate: Functional and nutritional characterization. *J. Food Biochem.* **25**, 77–90 (2001).
96. A. Bernardino-Nicanor, A.A. Scilingo, M.C. Añón, and G. Dávila-Ortíz, Guava seed storage protein: Fractionation and characterization. *LWT-Food Sci. Technol.* **39**, 902–910 (2006).
97. Y. Chang, M. Tan, W. Lok, S. Pakianathan, and Y. Supramaniam, Making use of guava seed (*Psidium guajava* L): The effects of pre-treatments on its chemical composition. *Plant Food Hum. Nutr.* **69**, 43–49 (2014).

