

Chapter 9

Protein-Based Bioproducts



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Chapter Highlights

- Plant proteins can be used for the production of a variety of bioproducts, including films and coatings, adhesives, fibres and pharmaceuticals.
- Proteins derived from plant production systems have many advantages: they are safe, low-cost and rapidly deployable, allow for simple product storage and result in proteins that are properly folded, assembled and post-translationally modified.
- While plant-derived protein-based products are natural, renewable, biodegradable and environmentally friendly, they tend to be lower in strength and elasticity than their corresponding synthetic products.
- Current research in this area is focused on overcoming challenges in plant production platforms related to yield, purification, regulatory approval and customer acceptance.

9.1 Introduction

The production of protein-based textile fibres, foams for fire extinguishers and plastics started 60–70 years ago (Wormell 1954). Ulrich and Ursula Kölsch, in Essen Germany, assembled a collection of thousands of plastic articles, including items produced from bio-based plastics and composites. The collection includes items that date back to the 1840s, evidence that the manufacture of bio-based materials is not a new phenomenon. In 1855, Francois Charles Lepage patented, in France and England, an extruded plastic composite material manufactured from ebony or rosewood (*Dalbergia latifolia*) sawdust and diluted egg albumin (Lepage, UK patent

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No. 2232). This material was compressed in steel moulds under pressure with steam heat to produce a composite material known as **bois durci** or '**hardened wood**'. Different products were made from bois durci, including portrait plaques, plaques for attaching to furniture and pianos, picture and mirror frames, inkstands, pen trays, blotters and letter racks, barometers, belt buckles and brooches, album and book covers, boxes, clocks, dishes, paper weights, statuettes, purses, caskets and other articles.

The first protein bioproduct patent was based on a mammalian source. It was granted to a German chemist, Adolf Spitteler, and his business partner, Ernst Wilhelm Krische, in 1899, for making plastic from milk casein (protein) and formaldehyde. The process for producing the casein semisynthetic plastic was accidentally discovered when Spitteler's cat knocked over a small bottle of formaldehyde one night from the chemist's counter into the cat's milk on the floor. The next morning, the chemist found that the cat's milk had turned into a hard, celluloid-like substance. Spitteler experimented with casein and formaldehyde mixtures and found that casein could be transformed into water-insoluble plastic by letting it sit in formaldehyde for extended periods of time.

His businessman partner, Krische, was the owner of a small book binding and school supplies manufacturing company. He was trying to manufacture washable white writing boards for export to Turkey and was experimenting by coating cardboard with milk curdle, since casein was commonly used as a binding material. In fact, casein has had a long history of nonfood applications. The industrial use of casein goes back to at least 2 centuries BCE in Egypt, where casein was used as an adhesive material for colour pigments in paint manufacturing. Spitteler and Krische found each other and worked together on developing the milk protein plastic. They named it Galalith, a Greek word from gala (milk) and lithos (stone) or milk stone. The other trade names that were used for casein plastic were Aladdinite (in the USA) and Lactoid. In Britain, the trade name Erinoid, derived from the Gaelic word for Ireland, which was the source of most British cheese curds, was used for the milk protein plastics.

When lactic acid is added to skimmed milk, it separates into curds and whey. The curds, after being dried and powdered, can be formed into dough by soaking in water and extruded into rods. When these rods are treated with formaldehyde, they harden into a thermoset plastic. This is a lengthy process, sometimes taking months. One advantage of the casein thermoplastic material is that it is easy to colour.

Casein-based plastics were not utilized in the USA until 1919, and the material had some problems, including moisture absorption, shrinkage after drying, a lengthy and costly manufacturing process and difficulties in disposal of manufacturing waste. In 1929, P.C. Christensen added aluminium stearate to hornlike casein plastic and converted it to a soft plastic for the automotive industry. Worldwide casein production increased from 10,000 tons in 1930 to 30,000 in 1932. In 1937, William S. Murray patented a method for converting the milk sugar in skimmed milk to an aldehyde, thus eliminating the use of formaldehyde in the plastic hardening process

and reducing manufacturing waste (Murray and Utica 1937). The main products made from casein in this era were imitation pearl, tortoiseshell and ivory for buttons, belt buckles, knitting needles and jewellery. World War II resulted in a large reduction in the production and use of casein. Today, casein is still used to manufacture buttons and knitting needles.

Soybean was early plant source for protein-based plastics. This crop was domesticated in China between 1500 and 1027 BCE (Hymowitz and Singh 1987). With the development of sea and land routes, the cultivation of soybean spread to the rest of Asia but remained a minor crop between the first and eleventh century AD (Hymowitz 1990). Samuel Brown, an East India Company employee, introduced soybean from India-Pakistan to North America in 1765, where Henry Young planted it in Savannah, Georgia (Hymowitz and Harlan 1983). However, soybean crops were not developed in North America until World War I, when a shortage of vegetable oil made it an alternative source for this purpose (Ralston and Osswald 2008). Today, soybean is one of the most important sources of oil and protein in the modern world. The dry soybean seed contains approximately 40% protein by weight (Liu et al. 2007). The first patent on soybean protein plastic was granted in Europe (France and UK) in 1913 and in the USA in 1916 to a Japanese researcher named Sadakichi Satow. Unfortunately, soy protein plastics had similar drawbacks to casein, including shrinkage, porosity and moisture absorption after drying in formaldehyde.

Manufacturing products from agricultural production was a major interest of Henry Ford, who was the owner of the Ford Company, and the inventor Thomas Edison also became involved in the development of these products. Ford prepared moulded plastics from soybean meal and hardened them with formaldehyde. By 1936, one million Ford vehicles were on the road containing 15 pounds of soymeal plastic parts. However, the auto parts made from this extruded soymeal were moisture-sensitive. Prior to World War II, some progress was made to produce slightly hydrophobic soy-based materials, but the war impaired the opportunity. In any case, these developments in the production of bio-based materials in the 1800s and early 1900s played significant roles in shaping the modern materials industry.

The idea of utilizing renewable, biodegradable and/or edible materials to manufacture industrial goods received significant attention in the 1980s, when the cost of fossil fuel-derived raw materials rose dramatically and people became newly interested in preserving the global environment. New interest evolved in the scientific community to use bio-based technologies in the context of the knowledge and resources available today. Customer willingness, health and environmental concerns and the efficient utilization of agricultural production have been the key driving factors for the re-emergence of protein-based products. Plant proteins from soybean, bean, wheat and corn are now being widely tested for their utility in producing bioproducts. In addition, plant systems, especially tobacco, are being used as platforms for producing proteins from a wide range of species for novel applications, including pharmaceuticals.

9.2 Protein-Based Products

In addition to their importance in human nutrition, proteins are increasingly utilized to produce bioproducts of various sorts, including fibres, films and coatings, adhesives and glues and pharmaceuticals. These protein-based products have a large number of applications in food packaging, pharmaceutical encapsulation, agricultural mulching, novel textile production, medical suturing, protective coating, bonding materials and medicine. In the following sections, the major protein-based bioproducts will be discussed. The major focus, however, will be on the use of proteins from plants.

9.2.1 Films and Coatings

The production of films and coatings is the most studied use of proteins for bioproduct manufacturing. A **protein film** is an independently produced sheet or membrane formed from a protein isolate and a plasticizer by solvent casting or extrusion methods, which have known physicochemical properties that are suitable for a particular use. **Coatings** are films formed from proteins directly on the surfaces of objects and provide some separation from the environment. In some cases, these coatings may be edible, especially if they are deposited on food products. For example, edible coatings are applied commercially to citrus fruit, apples and pears to improve gloss and control weight loss.

Protein-based films and coatings are generally manufactured from native proteins dissolved in different solvents, depending on their solubilities (Table 9.1). For example, corn zein, wheat gluten and sorghum (*Sorghum bicolor*) kafirin are soluble only in aqueous ethanol. Soy, peanut, common bean, cottonseed and rice bran proteins are soluble in water and alkaline water. Like all other physical and chemical properties, the solubilities of native proteins also depend on their constituent amino acid residues.

Table 9.1 Native protein solubility in protein solvents

Protein	Protein solvents			
	Water	Acidic water	Alkaline water	Aqueous ethanol
Corn zein				X
Sorghum kafirin				X
Wheat gluten		X	X	X
Rice bran protein		X	X	
Soy protein	X		X	
Peanut protein			X	
Cottonseed protein			X	
Common bean protein			X	

Adapted from Krochta (2002)

Table 9.2 Plasticizers used in protein-based films

Plasticizer	Physical property	Solubility	Chemical formula
Stearic acid	Viscous solid	Alkaline water	$\text{CH}_3(\text{CH}_2)_{16}\text{CO}_2\text{H}$
Glycerol	Viscous liquid	Water	$\text{C}_3\text{H}_8\text{O}_3$
Sorbitol	Solid	Water	$\text{C}_6\text{H}_{14}\text{O}_6$
Polyethylene glycol	Viscous liquid	Water	$(\text{CH}_2\text{-O-CH}_2)_n$
Propylene glycol	Liquid	Water	$\text{C}_3\text{H}_8\text{O}_2$
Triethylene glycol	Liquid	Water	$\text{C}_6\text{H}_{14}\text{O}_6$
Sucrose	Solid	Water	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$

Plant proteins, such as soybean protein and corn zein, need a small quantity of plasticizer to weaken the intra- and inter-peptide cross-linking and attractive forces and to reduce the brittleness and stiffness in the films and coatings. **Plasticizers** are low molecular weight and low volatility substances that work as spacers to reduce the strength of intermolecular attractive forces and lower the glass transition temperature of amorphous or partially crystalline protein films. Generally, plasticizers increase the molecular flexibility and extensibility but decrease elasticity, mechanical resistance and barrier properties of protein films and coatings (Gounga et al. 2007). Water is the most effective plasticizer in biopolymer materials, enabling them to undergo glass transition at a lower temperature as well as facilitating deformation and processability of the biopolymer matrix (Hernandez-Izquierdo and Krochta 2008). Besides water, common plasticizers for films include monosaccharides, oligosaccharides (sucrose), polyols (glycerol, sorbitol, propylene glycol and polyethylene glycol or polyethylene oxide), lipids (stearic acid) and their derivatives (Table 9.2) (Sothornvit and Krochta 2005). Plasticizer composition, size, shape and ability to attract water are important for the mechanical and barrier properties of protein films (Sothornvit and Krochta 2000).

Glycerol is the most widely used plasticizer in protein films (Cuq et al. 1997; Sothornvit and Krochta 2001; Cho and Rhee 2002). Its high plasticizing effects are attributed to the ease with which the glycerol molecule inserts and positions itself within the three-dimensional protein network (di Gioia and Guilbert 1999). The critical factors for a good protein plasticizer are that it has a low melting point, low volatility and compatibility (Pommet et al. 2005). In addition to these characteristics, the retention of the plasticizer by the film and amount needed should be taken into account when choosing a plasticizer (di Gioia and Guilbert 1999; Sothornvit and Krochta 2001). The relative effects on the mechanical and barrier properties of films can vary a great deal among different plasticizers, tested in different testing conditions (temperature and relative humidity).

9.2.1.1 Film Preparation Methods

The main ingredients of protein-based films and coatings are proteins, solvents, plasticizers and additives. Native proteins exist as folded structures that need to be unfolded for film and coating formation. Generally, higher temperatures,

high pH and water are used to unfold native protein structures. Other ingredients such as plasticizers and additives such as antioxidants, antimicrobials, nutraceuticals, flavours and colourants are also added to film formulations (Han 2003; Suppakul et al. 2003).

9.2.1.2 Physicochemical Properties of Protein-Based Films and Coatings

Protein films have great potential to be used for producing environmentally friendly food and drug packaging (Janjarasskul and Krochta 2010). Films and coatings made from renewable resources, such as plant proteins, could create new uses for agricultural products and byproducts that could protect, extend the shelf life and add value to food and drug products. To provide physical protection, the films require strength and elasticity, and to extend shelf life, they must act as barriers to water, oxygen, oil, aromas and microbes. If they are used as food coatings, they could add value by incorporating antioxidants, antimicrobial agents, nutrients, colours and flavours. In general, protein films have acceptable strength and elasticity and they are good barriers to oxygen, oil and aromas, but they are poor barriers to moisture at high humidity. However, at low to medium humidity, protein films are acceptable water vapour barriers. Generally, the mechanical and barrier properties are evaluated in a laboratory before industrial production.

Mechanical Properties of the Protein Films

The **mechanical properties** that are commonly measured for films include their strength, elasticity and plasticity. These measurements are made by clamping the film between the jaws of a **tensometer** and applying a strain at a fixed linear rate to the sample and measuring the stress. The resulting stress-strain curves can be used to calculate the **tensile strength** (TS), **elastic modulus** (EM), **yield point** and **break point** of the film (Fig. 9.1). Tensile strength and EM are usually expressed in pascals (Pa); one pascal is equal to one newton (1 N) of force applied over one metre squared (1 m²). **Strain**, which is the geometrical measure of deformation, is expressed in percent elongation (E) and represents the relative displacement between particles in the material body (Jacob 2008). The EM measures the stiffness of the film (Banker 1966). It is calculated by drawing a tangent to the initial linear portion of the stress-strain curve (Fig. 9.1), selecting any point on the tangent curve and dividing the tensile stress by the corresponding strain. Yield strength is the amount of stress at which the film starts to plastically deform. Prior to the yield point, the film deforms elastically and will return to its original shape and size when the stress is released (Dieter et al. 2003). Tensile strength at break point is the amount of force per unit of the original cross-sectional area to pull the film to point where it breaks (Banker 1966). The distance between the yield point and the tensile strength point along the stress-strain curve indicates the degree of plasticity of the film.

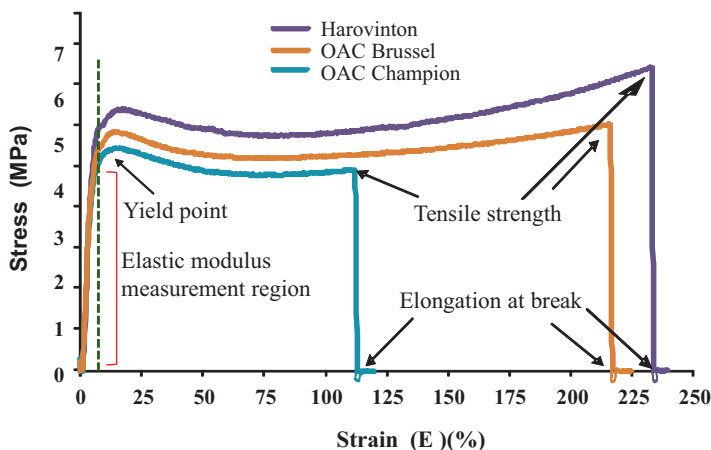


Fig. 9.1 Stress-strain curve of six soybean variety (Ontario, Canada) protein film show tensile strength, elastic modulus and elongation at break point (ASTM 2012)

Table 9.3 Protein-based films plasticized with glycerol

Protein film ^a (protein: plasticizer)	TS (MPa)	E (%)	Reference
Corn zein (2:0.6)	7	2.6	Parris and Coffin (1997)
Wheat gluten (2.7:1)	4.4	142	Park et al. (1994)
Wheat gluten (3:1.1)	1.9–4.4	170–208	Gennadios et al. (1993)
Soy protein isolate (2:1.2)	3.1–5.2	66–86	Brandenburg et al. (1993)
Peanut protein (1:0.67)	4.35	105	Jangchud and Chinnan (1999)
LDPE ^b	8.6–17	500	Salame (1986)
HDPE ^c	17–35	300	Smith (1986)
Pp ^d	38	400	Loo and Sudesh (2007)

E, elongation at break point; TS, tensile strength

^aTest condition: temperature ~25 °C, relative humidity ~50%

^bLow-density polyethylene; ^chigh-density polyethylene. ^dPolypropylene

To produce films, proteins are initially denatured (Gennadios et al. 1994), which exposes their functional groups and allows them to interact with each other to form three-dimensional intermolecular networks when the temperature returns to ambient (Wang and Damodaran 1991; Subirade et al. 1998). The tensile properties of films are affected by protein composition (Table 9.3), protein concentration, amount of plasticizer, pH, ionic strength and heating temperature (Sze et al. 2007). Attractive forces between proteins in the protein film matrix, including hydrogen bonds between backbone amino and carbonyl groups to stabilize α -helix and β -sheet secondary structures within the proteins and to form links between protein molecules, or with the plasticizer (Choi et al. 2003), **van der Waals forces** or electrostatic forces among polymer chains (Takashi et al. 2007), ionic interactions or salt linkages or bridges between oppositely charged functional groups of amino acids in protein side chains and disulphide (S – S) bonds within and between different protein chains (Subirade

Table 9.4 Soy protein secondary structure

Protein secondary structures	7S subunit (%)	11S subunit (%)
α -helices	~ 12	~ 10
β -sheets	~ 37	~ 39
Random coils	~ 22	~ 20
Unordered	~ 28	~ 31

Adapted from Sze et al. (2007)

et al. 1998; Sang et al. 2000), allow the development of a film matrix from denatured protein. During the film drying period, water is progressively eliminated, and protein conformations change, including the degree of protein unfolding, which determines the types and numbers of bonds that establish interactions between proteins (Denavi et al. 2009; Mauri and Anon 2006). The cohesion of the film network is a function of all these interactive forces, which determine the properties of the film.

Proteins with different physical properties result in films with different properties (Table 9.4; Wang and Damodaran 1991). For example, higher β -sheet content in the film matrix increases the tensile properties of protein films, and strong protein cross-linking increases film stiffness and strength but decreases the ability of the film to elongate.

Barrier Properties

Films and coatings can be used to protect the objects they surround from various organic and inorganic materials including moisture, oil and microbes. Sometimes the barrier properties of the protein films and coatings have to meet certain standards in order to be used for particular applications, such as packaging foods or drugs. Generally, protein films are permeable to polar substances, such as water, but less permeable to nonpolar substances such as oxygen, oil, aroma and microorganisms compared to low-density polyethylene films (Lim et al. 1999; Krochta 2002; Table 9.5). The high permeability to polar substances reflects the fact that its two major ingredients, namely, proteins and plasticizers, are generally polar in nature. Nevertheless, protein films manufactured from different proteins have different moisture and oxygen permeabilities (Table 9.5).

Similarly, plasticizer type and concentration also affect film properties. High levels of plasticizer weaken the attractive forces in film networks and dramatically reduce film stiffness but elevate elongation properties (Tables 9.6 and 9.7). Different types and amounts of plasticizers interact differently even within a single polypeptide chain. For example, studies of soy protein films plasticized with glycerol revealed that there were two glass transition temperatures, indicating that the films contained two microdomains that interacted differently with glycerol (Chen and Zhang 2005). The presence of these domains suggests that protein and glycerol are not uniformly compatible across the polymer chains, but there is a preferential linking between protein polymer regions and glycerol molecules. Usually, higher

Table 9.5 Water vapour permeability and oxygen permeability of selected protein films plasticized with glycerol

Protein film (Protein: plasticizer)	WVP ^a (g.mm/m ² .d.kPa)	OP ^b (cc ³ .µm/m ² .d.kPa)	Reference
Corn zein (4.9:1)	7.69–11.49 (21 °C, 85% RH) ^c	13.0–44.9 (30 °C, 0%RH)	Park and Chinnan (1995)
Corn zein (2.3:1)	32.52 (25 °C, 100% RH)	–	Parris and Coffin(1997)
SPI(1.7:1)	154 (25 °C, 50/100% RH)	4.75 (25 °C, 0% RH)	Brandenburg et al. (1993)
Wheat gluten (2.5:1)	–	3.82 (23 °C, 0% RH)	Gennadios et al. (1993)
Wheat gluten (2.5:1)	108.4 (26 °C, 50%/100% RH)	6.7 (38 °C, 0% RH)	Aydt et al. (1991)
Peanut protein (1:0.67)	9.03 (37.8 °C, 50%RH)	0.46 (30 °C, 0% RH)	Jangchud and Chinnan (1999)
LDPE	–	1870	Salame (1986)
HDPE	0.02	427	Smith(1986)

^aWVR, water vapour permeability; ^bOP, oxygen permeability

^cTest condition: temperature ~25 °C, relative humidity ~50%

Table 9.6 Selected protein films as affected by plasticizer types and amounts

Protein film (protein: plasticizer)	WVP (g.mm/m ² .d.kPa)	OP (cc ³ .µm/m ² .d.kPa)	TS (MPa)	E (%)	Reference
WG:EG (2:1)	–	–	2.7	393	Sánchez et al. (1998)
WG:DEG (2.7:1)	–	–	2.5	479	
WG:TEG (3.2:1)	–	–	3	423	
WG:G (3.8:1)	–	–	1.8	562	
WPI:G (5.7:1)	–	18.5	29.1	4.1	McHugh and Krochta (1994)
WPI:G (2.3:1)	–	76.1	13.9	30.8	
WPI:S (2.3:1)	–	4.3	14	1.6	
WPI:S (1:1)	–	8.3	14.7	8.7	
PPI:G(1:0.67)(g/g)	9.03	0.46	4.4	105	Jangchud and Chinnan (1999)
PPI:G (1:1.67)(g/g)	8.97	1.20	4.1	164	
PPI:G(1:1.71) (g/g)	10.64	0.11	5.1	125	
Polypropylene			38	400	Loo and Sudesh (2007)
LDPE	0.02	1870	10	620	

DEG, Diethylene glycol; E, elongation at break point; EG, ethylene glycol; G, glycerol; OP, oxygen permeability; PEG, polyethylene glycol; PPI, peanut protein isolates; S, sorbitol; TEG, tetra ethylene glycol; TS, tensile strength; WG, wheat gliadin; WVR, water vapour permeability
Test condition: temperature ~25 °C, relative humidity ~50%

quantities of plasticizers reduce mechanical and barrier properties (Cuq et al. 1997). Extensive research efforts have been focused on modifying the properties of protein-based films to improve their mechanical and barrier properties for industrial applications (Rhim 2004; Rhim and Weller 2000; Rhim et al. 1999, 1998, 2000; Micard et al. 2000; Gennadios et al. 1993, 1998; Ghorpade et al. 1995; Park et al. 1993).

Table 9.7 Effect of plasticizer types and quantity on mechanical and water barrier properties of egg white protein films

Plasticizer	WVP(g.mm/m ² .d.kPa)	TS (MPa)	E (%)
30% G	210.48	4.12	12.4
40% G	246.48	2.23	18.7
50% G	256.32	1.26	32.2
50% PEG	149.28	3.84	59.7
60% PEG	149.04	3.37	88.1
50% S	117.6	3.71	15.0
60% S	136.56	2.22	18.6

E, elongation at break point; G, glycerol; PEG, polyethylene glycol; S, sorbitol; TS, tensile strength; WVP, water vapour permeability

Test condition: temperature ~25 °C, relative humidity ~50%

Adapted from Gennadios et al. (1996)

Film age also affects its properties. Over a period of time, protein films can change chemically and/or physically. Chemical changes such as oxidation degrade the protein chains, while glycerol plasticizer has the tendency to migrate to the film surface (Anker et al. 2001) with the passage of time and water in the film also evaporates. These changes reduce the intermolecular spaces between proteins, which facilitate attractive forces to increase cross-linking and make the film harder and also more brittle (Kim et al. 2002).

For commercial applications, it is desirable that protein films meet industry standards set for petroleum-based plastics, particularly polypropylene and low-density polyethylene films. However, generally, protein-based films have lower mechanical and water barrier properties than synthetic plastics. For example, protein-based films have tensile strengths of 2–24 MPa, elongation at break points of 3–210% and water vapour permeabilities of 6–300 g.mm/m².d.kPa, compared to tensile strengths of 8–38 MPa, elongation at break points of 300–500% and water vapour permeabilities of 0–0.02 g.mm/m².d.kPa measured for polypropylene and low-density polyethylene (Tables 9.6, 9.8, and 9.9). Protein films, however, have better oxygen barrier properties than synthetic films. For example, protein-based films have oxygen permeabilities of 2–45 cc³.µm/m².d.kPa, compared to 427–1870 cc³.µm/m².d.kPa for polyethylene (Tables 9.5 and 9.6).

Although the mechanical properties of protein films are sufficient for a number of industrial applications including food wraps, pouches, medical capsules and bandages (Krochta 2002), research efforts have been focused on modifying protein properties to enable the manufacture of films that have properties that are closer to standard industry mechanical and barrier properties (Rhim 2004; Rhim and Weller 2000; Rhim et al. 1998, 1999, 2000; Micard et al. 2000; Gennadios et al. 1993, 1998; Ghorpade et al. 1995; Park et al. 1993). In order to improve emulsification, gelation, water-holding capacity, foaming and solubility properties of films produced from proteins, various treatments have been used, including acylation, alkylation, phosphorylation, enzymatic modifications and conjugation with polysaccharides (starch) and lipids (Achouri et al. 2005). In addition, various film

Table 9.8 Physical properties of protein-based fibres

Fibre	Denier ^a	Breaking tenacity (MPa)	Elongation at break (%)	Tensile modulus (GPa)	Moisture regain (%)	Reference
Soy protein	–	37–104	0.4–5.9	–	–	Reddy and Yang (2007)
Zein	–	36–60	1.8–5.0	–	–	Yang et al. (1996)
Gluten	34	115	23	5	18	Reddy and Yang (2007)
Wool	8–15	174–260	30–40	4.3–6.5	16	Huang et al. (1995)

^aA den is a unit of measure for the linear mass density of fibres. Mass in gram per 9000 metres.

Table 9.9 Properties of soy protein fibres affected by plasticizers (salts were used as plasticizers), post-spinning chemical reagent treatments

Treatment	Fibre process	Tenacity (g/tex)	Elongation at break %	Flexibility (mm)	Moisture uptake %
<i>Common plasticizers</i>					
0% glycerol	Extruded	1.49	0.5	45	1.59
15% glycerol	Extruded	1.57	1.6	21	1.61
15% sorbitol	Extruded	0.38	0.7	45	1.20
7.5% glycerol, 7.5% sorbitol	Extruded	1.23	1.3	21	1.24
<i>Salts used as plasticizers</i>					
Control (15% glycerol)	Extruded	1.57	1.6	21	1.61
ZnCl ₂ 4%	Extruded	1.12	2.1	5	1.37
CaCl ₂ 4%	Extruded	0.81	1.3	11	1.36
ZnCl ₂ , CaCl ₂ 2% each	Extruded	0.74	1.2	11	1.20
Na ₂ HPO ₄ 4%	Extruded	0.75	1.8	11	1.53
NaCl ₂ 10%	Wet-spun	0.68	0.5	45	1.06
ZnCl ₂ 10%	Wet-spun	0.26	0.7	16	1.47
CaCl ₂ 10%	Wet-spun	1.06	0.6	45	2.58
ZnCl ₂ -CaCl ₂ -NaCl ₃ . 3% each	Wet-spun	1.84	0.5	45	1.61
<i>Post-spinning chemical reagents treatments</i>					
Acetaldehyde 25%	Extruded	2.19	0.9	11	0.76
Acetic anhydride/Acetic acid (9:1)	Extruded	2.31	4.7	2.0	0.77

Adapted from Huang et al. (1995)

additives and production modifications have been tested to improve the mechanical and barrier properties of the protein films. These include (1) various plasticizer types and protein/plasticizer concentrations and ratios; (2) various additives such as cysteine, propylene glycol alginate, methylcellulose, bee wax, gossypol, fatty acids, mineral oil and different casting solvents; (3) adjustments in pH and drying conditions; and (4) post-casting film treatments with mild acid and alkali or exposure to UV radiation (Krochta 2002).

9.2.1.3 Protein Film and Coating Applications

Protein films have a wide range of potential applications, including incorporation into food covers, wraps, separation layers, casings, pouches, bags, capsules, microcapsules, labels, trash bags, water-soluble fertilizer and pesticide bags and agricultural mulches. They can be used as coatings for drugs, paper and paper products (such as disposable plates) and disposable laboratory items (such as gloves, gowns and disposable diapers) (Krochta 2002). Soy proteins are very useful for papermaking and paper coatings. Their film-forming characteristics improve the strength and heat resistance of paper, allowing it to be used at higher production speeds in print applications. Their amphoteric nature (possessing both positive and negative charges) and high water-holding capabilities also improve ink receptivity and printability (Brentin 2014).

For some applications, edibility and biodegradability are two important properties. For example, most of the food and drug coatings manufactured from biomaterials are edible, and this property is determined by its formulation, method of manufacture and modification treatments that were used (Krochta 2002). The biodegradability of films by microorganisms in composting environments at the end of their life cycle, by naturally occurring microorganisms into water, carbon dioxide, methane, biomass and mineral residues, is an important attribute of many protein-based bioproducts, as it helps to reduce environmental pollution due to packaging. ASTM International (American Section of the International Association for Testing Materials) standard methods for aerobic composting (D6400–12) and aerobic biodegradation (D5338) have been developed to measure the biodegradability of materials. In order for a plastic product to be labelled compostable, it must meet the US Standard ASTM D6400 and/or the European Norm EN 13432. Both specifications require that materials be completely biodegraded during composting at a rate similar to other known compostable materials (90% of organic carbon to CO₂ within 180 days) and should not leave visual or toxic residue.

Opportunities for utilizing protein films are increasing because of the application of new technologies to improve their mechanical and barrier properties while retaining their biodegradability and edibility. In addition, the food, pharmaceutical and biofuel industries are producing large amounts of protein meals, concentrates and isolates as byproducts, which are available for industrial-scale protein-based product manufacturing. Therefore, this bioindustry is moving from a pilot project stage to a commercial stage. For example, industrial-scale corn zein protein-coated

confectionaries, nuts and drug tablets are already on the market (<http://www.zein-products.com/zeinapplications.html>), and these films provide a relatively effective water vapour barrier compared to other edible films. Other potential uses of plant proteins include coatings for fresh vegetables, fruits and fried foods (Trezza and Krochta 2002; Shukla and Cheryan 2001). The use of protein coatings decreases water loss, reduces pigment degradation, prevents undesirable pigment development, delays ripening, improves gloss, intensifies peel colouration and reduces oxygen, aroma and oil transfer.

In addition, an interesting property of edible coatings is their ability to incorporate active ingredients that can enhance their functionality, including antimicrobials (such as organic acids, fatty acid esters, polypeptides and plant essential oils, nitrites and sulphites), texture enhancers (such as calcium salts) and nutraceuticals (such as certain fatty acids and vitamins). All of these effects can improve quality, shelf life and safety and reduce postharvest losses and additional packaging costs. Some of the problems and limitations that are associated with these coatings include anaerobic respiration in fruits and vegetables when the coating is too thick and restricts exchange of CO₂ and O₂, undesirable tastes associated with particular compounds like essential oils and food safety issues such as allergic reactions (Dhall 2013).

9.2.2 Protein Adhesives

Adhesives are nonmetallic liquids or gels that bind the surfaces of materials together and resist separation (<https://www.britannica.com/technology/adhesive>). Adhesives (or glues) hold objects together through adhesive forces between adhesive materials and the surfaces of materials (called **adherents**) and cohesive forces within the glue (Fig. 9.2). The physical and chemical properties of the adhesive, the type of adherents and the nature of the surface pretreatments are important factors in glue performance, in the short and long terms. To make initial molecular contact, adhesives have the ability to wet and spread evenly on the surfaces of the materials one wishes to joint. Once that is achieved, intrinsic attractive forces are generated across interfaces through a number of mechanisms including **adsorption** (occurs when adhesive molecules are attracted to a specific site on a solid surface through weak van der Waals forces or chemisorption through covalent bonding), **mechanical interlocking** (occurs when the adhesive flows into pores or solidifies around the projections), **interdiffusion** (occurs when the adhesive dissolves and diffuses into the substrate material) and **electrostatic attractions** (occur when electrons are transferred across the interface, thus creating positive and negative charges that attract one another). Generally, several mechanisms contribute to the performance of adhesives with various types of adherents.

In the formation of an adhesive joint, a transitional region arises in the interface between the joint surface and adhesive. In this transitional region, the chemical and physical properties of the adhesive may be considerably different from those in the noncontact portions or cohesive region (Fig. 9.2). It is generally believed that the

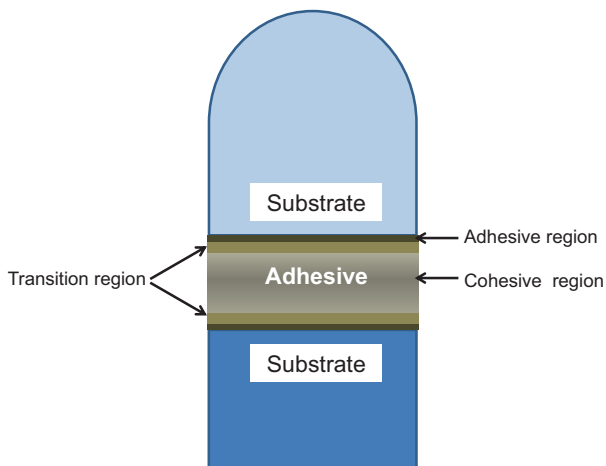


Fig. 9.2 Schematic presentation of adhesives and substrate interaction

interphase composition controls the durability and strength of an adhesive joint and is primarily responsible for the transference of stress from one surface to the other. This transitional region is frequently the site of environmental attack, leading to joint failure (Encyclopaedia Britannica).

Adhesives are used in every sphere of human life, including aerospace, automotive, electronics, construction, furniture construction, carpet manufacturing, musical instrument building, packaging, plywood manufacturing and agriculture. In 1998, the share of natural adhesives was 0.03% of the total North American wood industry (Seller 2001). In Canada, about 80% of adhesives and sealants are used in industrial applications including packaging, automotive, construction and the furniture industry and the remainder by individual consumers for home maintenance and renovation.

Adhesives are formulated by mixing base materials such as proteins, starch and lignin with fillers, pigments, stabilizers, plasticizers and other additives to yield products with desirable characteristics. Adhesives may be synthetic or natural, depending on their base materials. Many synthetic adhesives are formaldehyde-based resins derived from petrochemicals including phenol formaldehyde and urea formaldehyde resins. Although synthetic adhesives have high-performing characteristics, including excellent bond strength, environmental resistance and durability, their base chemical, formaldehyde, is a human carcinogen (International Agency for Research on Cancer, <https://www.iarc.fr>), and they are derived from nonrenewable resources. Alternatively, natural adhesives are thus being developed to replace these formaldehyde-based adhesives currently on the market because of these concerns.

Natural adhesives and sealants are derived from natural biopolymers obtained from plants, animals and microbes (Lambuth 2003; Imam et al. 2013). Proteins have been used to formulate commercial adhesives, and sealants, for many years, but initially, animal proteins were used for glues. Protein adhesives are used in antique

furniture and old religious texts. These adhesives have excellent flexibility and non-warp characteristics, as well as permanent and tenacious adhesion. They are water-soluble, easy to clean up, nontoxic, eco-friendly, biodegradable, recyclable and repulpable. Protein adhesives have some limitations, however, such as a lack of specific adhesion on coatings and nonporous surfaces and sensitivity to temperature and humidity changes. The use of plant proteins as adhesives is more recent with soy protein-based adhesives widely used between 1930 and 1960. However, they were completely replaced thereafter by cheaper and stronger synthetic adhesives.

Today, soy protein alone, or in combination with animal proteins such as casein, gelatin and blood proteins, is used to produce adhesives that are widely used as glues in paper, book binding, packaging, furniture and wood industries (Frihart 2009; Lambuth 2003). Generally, protein adhesives have sufficient strength in dry conditions but are susceptible to moisture and mould (Lambuth 2003). The nature of the protein determines the formulation, mixing and application of the adhesive. The manufacturing steps, however, are common and include grinding dry protein extracts to glue particle sizes (typically with surface areas between 3000 and 6000 cm² per gram), sufficiently dispersing the ground proteins in alkaline water for maximum binding efficiency and addition of fungicide (such as sodium orthophenylphenate, sodium pentachlorophenate, copper-8-quinolinate or copper naphthenate) to prevent mould.

Adhesive durability has been a problem for protein glues. Several protein modifications including physical, chemical and enzymatic treatment are used to enhance the functional properties of protein adhesives such as bonding strengths and environmental and moisture resistance. Treatment of proteins with organic or inorganic alkali, such as sodium hydroxide or trisodium phosphate, breaks the internal hydrogen bonds of protein molecules, unfolds the protein structure and exposes the polar functional groups of the amino acid residues for adhesion to binding surfaces such as wood (Brother et al. 1940; Lambuth 2003). The combination of alkali treatment and mild heating of the protein in deionized water breaks the inter- and intramolecular protein hydrogen and disulphide bonds and unfolds the protein structure to improve the adhesive and viscosity and hydrophobic properties of the glues (Graham and Krinski 1983). For example, soy protein heated at 50 °C and a pH of 10.0 improved adhesive strength by 118% and the hydrophobic properties of soy protein glues by 92% (Hettiarachchy et al. 1995). The amount of alkali in the protein adhesives depends on the usage in the final product. For example, high-alkali (Table 9.10) soy protein adhesives prevent glue swelling, maintain glue viscosity and improve moisture resistance by forming insoluble proteinates (Laucks and Davidson 1928). However, it also burns wood cellulose and causes reddish-brown stains on wood surfaces (Truax 1929). On the other hand, low-alkali soy protein adhesives are less dispersive and have lower bonding strengths. This makes them good for paper and softboard lamination, but not for structural usage such as sheathing plywood (Sheeran 1957; Lambuth 2003). Furthermore, salt treatment of disulphide bond-containing proteins, such as soy protein, results in cleavage of the disulphide bonds and unfolded protein structures, which improves the viscosity of glues without

Table 9.10 Soybean protein adhesives: ingredients and mixing procedure

Ingredients and mixing procedure	High-alkali amount (kg)	Low-alkali amount (kg)
Water at 16–21 °C	87.5	112.5
Adhesive-grade soybean flour ^a	48.5 ^b	48.5 ^b
Pine oil or diesel oil defoamer: Mix 3 min or until smooth	1.5 ^b	1.5 ^b
Water at 16–20 °C: Mix 2 min or until smooth	72.5	75.0
Fresh hydrated lime: (as a slurry in)	6.0	15.0
Water at 16–21 °C: Mix 2 min or until smooth	12.0	25.0
50% sodium hydroxide solution: Mix 1 min	7.0	–
Sodium silicate solution: Mix 1 min	12.5 ^c	–
Orthophenyl phenol or other preservative: Mix 10 min	2.5	2.5

Adapted from Lambuth (2003)

^a44% protein, specific surface 3000–6000 cm²/g

^bNormally dry-blended for easier handling and dust control

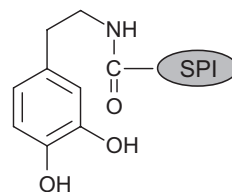
^c8.90% Na₂O, 28.70% SiO₂, 41 ° Baume´

reducing adhesive strengths and water resistance at certain concentrations. High concentrations of salts, however, reduce viscosity, adhesive strengths and water resistance (Kalapathy et al. 1996).

The addition of chemicals such as urea, guanidine hydrochloride, sodium dodecyl sulphate, maleic anhydride, polyethylenimine and polyamidoamine-epichlorohydrin, which react with the carboxylic acid and amino groups in proteins, results in cross-linking of protein molecules and the formation of three-dimensional networks. These networks improve the adhesive and moisture resistance properties of protein-based glues. For example, soy protein modified with urea and guanidine hydrochloride increases the average shear strengths in walnut (*Juglans* spp.), cherry (*Prunus* spp.) and pine plywoods by 34 and 37%, respectively. In addition, both urea and guanidine hydrochloride-modified soy protein exhibited 100% moisture resistance. These chemicals are known to increase the production of secondary structures in globular proteins, which may be responsible for enhancing adhesive strength and also expose hydrophobic amino acids, which might enhance water resistance (Huang and Sun 2000). Furthermore, the addition of polyamidoamine-epichlorohydrin to maleic anhydride-grafted soy protein isolates improves the adhesive properties of glues to such an extent that they exceed those of commercial phenol formaldehyde glues (Liu and Li 2007).

Enzymatic hydrolysis of soy protein with proteases such as trypsin is another method for improving its adhesive properties. Glue strength increases of 58–119% have been observed with these treatments (Kalapathy et al. 1995; Hettiarachchy et al. 1995). Other soy protein modifying enzymes, including urease, pepsin and transglutaminase, also improve the adhesive and water resistance properties of soy protein glues (Thames et al. 2010; Imam et al. 2013).

Fig. 9.3 Dopamine-grafted soy protein.
(Adapted from Liu and Li (2002))



Marine mussel adhesive proteins allow the adhesion of objects in seawater (Waite 1987) and contain a substantial quantity of 3,4-dihydroxyphenylalanine (DOPA). DOPA incorporated into synthetic polypeptides mimics marine adhesives and plays an important role in moisture-resistant adhesion (Liu and Li 2002; Yu and Deming 1998). Above and beyond its effectiveness on wet surfaces, this adhesive protein has several other advantages, such as strong adhesive strengths and resistance to biological degradation. However, marine adhesive proteins are difficult to produce at reasonable costs. For example, DOPA content in soy proteins can be increased through genetic engineering, and dopamine-grafted (Fig. 9.3) soy protein showed significant increases in adhesive strengths and water resistance in wood glues (Liu and Li 2002).

Blending together different proteins is another way to enhance the functional properties of glues. For example, soybean proteins have good adhesive properties but weak water resistance, while casein proteins have good water resistance but poor adhesion. A blend of these proteins results in an adhesive with better properties than those derived from the either single protein. However, for this approach to be successful, the proteins in the mixture must be compatible and have similar processing requirements to convert them into glues. Soy-blood and soy-casein blends have successfully been used in interior plywood and softwood manufacturing during the 1930s to 1960s and again during the oil embargo in 1973. Generally, a soy-casein mixture provides an excellent adhesive for softwood and millwork assembly (Lambuth 2003).

9.2.3 Protein Fibre

Fibres have extensive uses in textile and clothing manufacture; they are used for protection and have medicinal and aesthetic applications. A fibre is a continuous filament or discrete, elongated, piece of material. The word 'fibre' comes from the Latin word *fibra* or *fillum*, meaning thread. Fibres can have micro (10^{-6} m) or 10^{-9} nano- (10^{-9} m) diameters and almost limitless lengths (Castano et al. 2012).

Synthetic fibres are made from petroleum-derived plastics, such as polyester, nylon and rayon. Plants produce natural fibres such as cotton and bast fibres (e.g. derived from flax, hemp, jute [*Corchorus* spp.], ramie [*Boehmeria nivea*], kenaf [*Hibiscus cannabinus*] and abaca [*Musa textilis*]), and fibres can also be synthesized from natural sources such as **alginate** (a natural polymer that exists widely in many

species of brown seaweed.), cellulose (to produce lyocell fibres from cellulose), polylactic acid (from sugars extracted from crops like corn and sugar beet), polyhydroxyalkanoate (from bacterial sources) and protein. Natural fibres are generally biodegradable; they are mostly hydrophilic and made up of short, flexible chains with low levels of crystallization. They often have chain backbones with oxygen or nitrogen links and/or pendant groups containing oxygen or nitrogen atoms. Biodegradable fibres are suitable for all applications, including knitwear, intimate apparel, shirts, trousers, dress material, bath linen, floor coverings, bed linens, furnishing and industrial yarns. Biodegradable fibres impart colour brilliance to fabrics and garments, which remain bright and true even after repeated washes. The fibres often give fabrics a soft and bouncy feel.

Wool and silk are two natural protein fibres that have been used for centuries in textile manufacturing. These natural protein fibres are made from filamentous animal proteins. Generally, they have good physical properties, but they also have some limitations, such as variable fibre diameters, propensity to shrink and high cost. Fibres produced from plant globular proteins, especially seed storage proteins, are alternatives to wool and silk (Wormell 1954). Kajita and Inoue in Japan and Boyer in the USA first patented fibre development from soybean protein in 1940. Fibres from soybean protein and corn zein called **prolons** were investigated extensively in the 1930s and 1940s, but they were not commercialized because of their high cost and poor/inconsistent physical properties. The cheaper and excellent physical properties of synthetic fibres called **synthons**, manufactured from petroleum, at that time led to a rapid commercialization of synthetic textiles (Huang et al. 1995). Renewed interest in eco-friendly and renewable protein fibre materials has led to the commercialization of soy protein fibres and garments manufactured from these textiles by Chinese companies around the globe (Zhang et al. 2003). Plant protein fibres have excellent properties, including natural lustre and smooth surfaces, as well as good physical and dyeing properties. Garments manufactured from soybean fibre textiles have good breathability and comfort, and they also have a fine appearance with excellent drape (Fig. 9.4).



Fig. 9.4 Men's and ladies' soybean cotton spandex jersey shirts [52% cotton, 43% azlon (protein from soybean), 5% spandex] (<http://www.nyfifth.com/ash-city-e-c-o-knits-88622-mens-soybean-cotton-spandex-jersey-polo-p-34520.html>, used with permission, searched on Feb. 27. 2018)

9.2.3.1 Properties of Protein Fibres

The morphological and mechanical properties of protein-based synthetic fibres are important determinants of their commercial utility. The morphological properties of importance include surface texture, fibre diameter, length and circularity. Scanning electron microscopy (SEM) is a useful tool to examine and measure the morphological properties of fibres, including surface properties, cross-sectional area and circularity. SEM and atomic force microscopy can also be used to measure some mechanical properties such as the strength of the electrospun nanofibre. Mechanical properties of fibres, including intra- and intermolecular alignments and crystallinity, can also be determined by X-ray diffraction and differential scanning calorimetry, respectively. Tensile tests determine the strength, elongation at break point and flexibility of the fibre (Table 9.8).

Several prior- and post-spin factors can affect the physical properties of protein-based fibres (Table 9.9). The prior-spinning factors include source, type and concentration of protein, additives and solvents, blends with other polymers, pH, temperature and viscosity of the protein solution and extrusion/spinning instrument set-up. Post-spinning factors include washing, drying, drawing, chemical treatments, thermal and conditioning treatments, as well as annealing and testing conditions. The variations among proteins from different sources described above necessitate that specific formulations, with different additives, blends and electrospinning conditions need to be investigated and optimized to produce fibres with specific properties.

9.2.3.2 Protein Fibre Applications

Plant protein fibres have been on the market for decades with different trade names, including Vicara, Zycon and Wavcrape for corn zein fibres (Lawton 2002), Prolon and Alysol for soy protein fibres and Ardil for peanut protein fibres. Today, **Azlon** is the common generic name for all fibres regenerated from plant proteins (<http://info.fabrics.net/meet-the-azlons-from-a-to-z-regenerated-rejuvenated/>). Azlon blended textile fabrics are commercially available (Fig. 9.4), and a considerable amount of research is currently being carried out to improve the technology and properties of the plant protein-based fibres in public and private sectors. Some proteins such as soybean protein, corn zein, wheat gluten and peanut protein have greater potential for use in producing fibres than others because they are readily available for industrial availability (Xu et al. 2012). Some of the ongoing limitations of protein fibres involve performance characteristics, such as moisture sensitivity and mechanical properties. However, like any field of material research, improvements in protein-based fibres are being explored. In particular, blends with other polymers, including polyethylene oxide, polylactic acid, polyvinyl alcohol, polycaprolactone, polyacrylonitrile, hydroxyapatite and polysaccharides, are the wave of the future and considered as the next generation of materials.

In addition to the general usage of protein-based fibres for textiles and clothing, they have other speciality uses in (1) medicine as medical sutures and for drug delivery, bandaging and enzyme mobilization; (2) cosmetics and skin care; (3) tissue engineering of blood vessels, bone tissues, heart tissues and cartilage tissues; (4) electronics including nano-sensors; and (5) military protective clothing and body armour (<http://news.bbc.co.uk/2/hi/science/nature/379338.stm>). Historically, silk and animal gut were widely used as surgical sutures because they are eventually degraded by human proteolytic enzymes, but recently they have been replaced by synthetic sutures (made of polyglycolic, polylactic acid, polydioxanone and caprolactone) because of concerns about possible contamination of gut sutures with prions. Plant protein fibres are an ideal alternative to synthetic sutures because they are renewable and absorbable.

The ideal wound dressing is one that is sterile, breathable and supports a moist healing environment. Such a dressing will reduce the risk of infection, help the wound heal more quickly and reduce scarring. Conventional dressings do not efficiently induce haemostasis (the mechanism that stops bleeding) or adhere in moist environments around wounds. With the advances in nanotechnology seen in the last two decades, it is now possible to design and produce **nanofibre-based wound dressings** that contain an electrospun nanofibrous layer applied to a basic support fabric material. These wound dressings have very high surface area to volume ratios. They are able to control the release of drugs such as antibiotics and analgesics copun with protein nanofibre; prevent haemostasis, high filtration and liquid absorption efficiencies; and stimulate the growth of live cells. Thus, the combination of nanotechnology with electrospinning and the development of new wound dressing materials from plant proteins with highly desirable properties may lead to bioproducts that can could enhance the healing of wounds significantly compared to the conventional fibrous dressing materials.

Nanofibres have also been used for drug delivery because their very small sizes and extraordinarily large surface areas make them highly efficient delivery and carrier systems. Some nanofibres can also control the release of active ingredients and protect the chemical integrity of drugs. For example, protein (gelatin)-polyvinyl alcohol nanofibres containing a model drug have been produced, and their encapsulation and delivery efficiencies have been demonstrated (Yang et al. 2007). The development of nanofibres into efficient drug delivery systems is attracting much attention, and in particular, the use of electrospun nanofibers manufactured from biodegradable polymers, such as proteins, for drug delivery systems is being actively studied. Variables that affect their efficiencies and drug release rates include the physical properties of the drug and the protein microfibre.

9.3 Plant Crops as Platforms for Speciality Protein Products

Proteins play crucial roles in living organisms, including humans, to enable a large number of fundamental processes, such as cell signalling, immune responses, cell adhesion, cell division and cell growth and differentiation. The continuous progress

in biotechnology, including genetic and protein engineering, in the last few decades has made it possible to manipulate different platforms for the commercial-scale production of proteins in transgenic bacteria, yeast, filamentous fungi, insects, mammalian and plant cell cultures and transgenic animals and plants. These biotechnological advances have significantly affected many industries, including food, pharmaceutical, nutraceutical, enzyme, hormone, textile, leather, paper, pulp, polymer, plastics and agriculture industries. For example, there are more than 200 approved peptide and protein pharmaceuticals in the US Food and Drug Administration list, including human insulin, serum albumin, human growth hormone, various antibodies, edible vaccines, collagen, human epidermal growth factor and blood coagulating protein (Factor VIII), among many others.

Of the different recombinant proteins that are produced on a commercial scale, 39% are made in *Escherichia coli*, 35% in Chinese hamster ovary (CHO) cells, 15% in yeasts, 10% by other mammalian systems and 1% by other bacteria and systems (Rader 2008). Microorganisms and cell cultures are robust recombinant protein synthesis production systems. They possess certain challenges, however, such as high culture development costs, high cell culture maintenance costs, cell culture variability and limitations concerning the production of large molecular weight proteins.

In principle, DNA from any source can be manipulated in any living system. Genetically engineered animals have been created that produce recombinant proteins in their tissues, milk, blood or urine (<http://www.youtube.com/watch?v=q0WCjX8jUE4>). By the late 1980s, it was shown that transgenic plants could be used as alternative, commercial-scale, recombinant protein production platforms, after immunoglobulins and the assembly of functional antibodies were successfully achieved at 1.3% of the total leaf protein in tobacco leaves (Hiatt et al. 1989). This opened many new windows of opportunity to use genetically engineered plants for the production of recombinant proteins in whole plants or in their tissues, seeds and cell culture. Some transgenic plants carrying human protein genes are given in Table 9.11. Further progress made in biotechnological fields in the 1990s and early 2000s prompted interest in the production of pharmaceuticals in plants, known colloquially as ‘pharming’ (Hunter 2011).

Plant crop protein production platforms have certain advantages over animal and microbial systems. Mammalian cell culture systems are complicated and expensive processes; they require large bioreactors and high energy inputs for commercial-scale production. In contrast, plant systems are cost-effective, quicker to scale up, easy to propagate and simple to distribute. In addition, there is no risk of contamination by human pathogens (such as viruses and prions), and relatively cheap systems exist for purification and concentration of the therapeutic proteins. Plant platforms can synthesize and accumulate valuable proteins to high levels. These proteins are properly assembled and folded and can be post-transcriptionally modified to yield complex protein molecules. In addition, if the plants are engineered to accumulate the proteins in storage tissues and cellular compartments, they may be stably stored without refrigeration.

Table 9.11 Plant platforms for the production of human and animal recombinant proteins

Product	Plant platform	Level	Application	Reference
<i>Human protein</i>				
Protein C	Tobacco	<0.01% TSP ^a	Anticoagulant (human)	Cramer et al. (1999)
	Canola	0.30% seed protein	Thrombin inhibitor	
Epidermal growth	Tobacco	<0.01% TSP	Wound repair and control of cell proliferations	
Interferon- α	Rice; turnip	–	Hepatitis C and B treatment	
Haemoglobin α,β	Tobacco	0.05% seed protein	Blood substitute	
Somatotropin	Tobacco	<0.01–7.00% TSP	Growth hormone	Staub et al. (2000)
Erythropoietin	Tobacco	<0.01% TSP	Anaemia	Kusnadi et al. (1997)
Enkephalins	<i>Arabidopsis</i>	0.10% seed protein	Anti-hyper analgesic	
Interferon- β	Tobacco	0.01% FW ^b	Hepatitis C and B treatment	
Lactoferrin	Potato	0.10%tsp	Antimicrobial	Chong and Langridge (2000)
Homotrimeric collagen	Tobacco	<0.01% FW	Collagen	Ruggiero et al. (2000)
<i>Non-human proteins</i>				
α -Trichosanthin from TMV-U1 subgenomic coat protein	Tobacco	2.00% TSP	HIV therapies	Giddings et al. (2000)
Glucocerebrosidase	Tobacco	1.00–10.00% TSP	Gaucher disease	Cramer et al. (1999)

^aTotal soluble protein^bFresh weight

Plants are capable of assembling two or more subunits of proteins into complex three-dimensional structures. For example, spider dragline silk genes were successfully expressed in tobacco (*Nicotiana* spp.) and potato (*Solanum tuberosum*) plants, and spider silk proteins accumulated in transgenic tobacco leaves and potato tubers up to at least 2% of total soluble proteins with >90% homology to *Nephila clavipes* native proteins (Scheller et al. 2001; Menassa et al. 2004). Even more dramatic was the production of spider silk protein in transgenic *Arabidopsis thaliana*, which accumulated to 18% of total soluble proteins (Yang et al. 2005).

Plant platforms, including major crops such as alfalfa (*Medicago sativa*), potato, wheat, rice, tobacco, soybean, carrot (*Daucus carota* subsp. *sativus*) and turnip (*Brassica rapa* subsp. *rapa*), have been extensively tested for their ability to produce human and animal antibodies and vaccines (Table 9.12). For example, the hepatitis B surface antigen has been produced in transgenic tobacco plants, and

Table 9.12 Plant platforms for the production of antibodies and vaccines

Product	Plant platform	Level	Application	Reference
<i>Antibodies</i>				
ZMapp	Tobacco	–	Ebola virus	Qiu et al.(2014)
FVIII	Tobacco	370 µg/g ^a	Haemophilia A	Sherman et al. (2014)
Influenza HA	Tobacco	400–1300 mg/kg leaves	Influenza (humans)	Shoji et al. (2011)
ScFvT84.66 (ScFv)	Wheat	900.0 ng/g leaves; 1.5 µg/g seed	Cancer treatment; carcinoembryonic antigen	Stoger et al. (2000)
	Rice	29.0 µg/g leaves 32.0 µg/g; seed 3.8 µg/g callus 27.0 µg/g leaves		Stoger et al. (2000); Torres et al. (1999)
T84.66 (IgG)	Tobacco	1.0 µg/g leaves	Diagnostic; antihuman IgG	Vaquero et al. (1999)
Guy's 13 (SIgA)	Tobacco	500 µg/g FW ^a leaves	Dental caries; streptococcal antigen I or II	Ma et al. (1998); (1995)
Anti-HSV-2 (IgG)	Soybean	–	Herpes simplex virus 2	Zeitlin et al. (1998)
<i>Vaccine</i>				
Heat-labile toxin B-subunit	Maize	–	Enterotoxigenic <i>E. coli</i> (humans)	Streatfield et al. (2000)
	Tobacco	<0.01% TSP	Enterotoxigenic <i>E. coli</i> (humans)	Haq et al. (1995)
	Potato	0.19% TSP	Enterotoxigenic <i>E. coli</i> (humans)	Haq et al. (1995); Mason et al. (1998); Tacket et al. (1998)
Cholera toxin B-subunit	Potato	0.30%tsp	<i>Vibrio cholerae</i> (human)	Puchta (2000); Arakawa et al. (1998)
Envelope surface protein	Potato	<0.01% FW	Hepatitis B virus (humans)	Richter et al. (2000)
	Lettuce; lupin	<0.01% FW	Hepatitis B virus (humans)	Kapusta et al. (1999)
Capsid protein	Tobacco	0.23% TSP	Norwalk virus (humans)	Mason et al. (1996)
Capsid protein	Potato	0.37% TSP	Norwalk virus (humans)	Mason et al. (1996); Tacket et al. (2000)
Rabies virus glycoprotein	Tomato	1.00% TSP	Rabies virus	McGarvey et al. (1995)

(continued)

Table 9.12 (continued)

Product	Plant platform	Level	Application	Reference
Glycoprotein S	<i>Arabidopsis</i>	0.06% TSP	Transmissible gastroenteritis corona virus (pigs)	Gómez et al. (1998)
	Tobacco	0.20% TSP	Transmissible gastroenteritis corona virus (pigs)	Tuboly et al. (2000)
	Maize	<0.01% FW	Transmissible gastroenteritis corona virus (pigs)	Streatfield et al. (2000)

^aFresh weight

^bTotal soluble protein

human insulin has been produced in transgenic *A. thaliana* seeds at levels of 0.13% of the total soluble seed protein (Nykiforuk et al. 2006). The quality of these antibodies and vaccines was equivalent to the present commercially produced proteins in microorganism-based systems. For example, a 44 kDa fragment of human collagen I α 1 (CI α 1) expressed in corn grains was molecularly equivalent to that produced in recombinant yeast (*Pichia pastoris*).

Commercial enzyme production is another area where transgenic plants can play an important role. The global industrial enzyme market will be as high as \$7 billion by 2015 (http://www.prweb.com/releases/industrial_enzymes/peptases_carbohydrases/prweb8121185.htm), according to Global Industry Analysts (Hood and Requesens 2012). These commercial enzymes, including proteases, amylases, cellulases, xylanases, lipases and reduction/oxidation enzymes are utilized by many industries, such as the manufactures of detergent, pulp and paper, textile, chemical, feed, food, biofuels and bio-based products (Hood and Requesens 2012). Currently, the largest markets for technical enzymes are the pulp and paper, food and beverage and animal feed industries (<https://www.freedoniagroup.com/World-Enzymes.html>). These may be replaced, however, by the lignocellulosic-supported biofuel and bio-based product industry market in the near future since large quantities of enzymes, including cellulases, hemicellulases and ligninases, will be required to deconstruct feedstock materials (Hood and Requesens 2012).

In 2011, the US Department of Agriculture approved the first commercial-scale production of **Enogen**, a transgenic corn plant developed by Syngenta US to express α -amylase. Enogen eliminates the need to use liquid α -amylase in dry-grind ethanol production (Table 9.13). Another industrial enzyme, bovine trypsin (a protease) is widely used for commercial purposes to digest other pharmaceutical proteins. This enzyme can be expressed in the corn grain (Woodard et al. 2003) and has been marketed by Sigma Chemicals, USA, with the trade name TrypZean. **TrypZean** produced in plant-based systems could replace other production systems such as animal cell cultures to eliminate the chances of human pathogen contamination of the enzyme preparation. Several plant crops (Table 9.13) including tobacco, *Arabidopsis*, potato, rice, alfalfa, canola, pea, barley, soybean, wheat and corn are

Table 9.13 Transgenic plant platform expressing commercially significant enzymes

Enzyme	Plant platform	Level	Reference
α-Amylase (archaea)	Corn	0.08–0.16% DW ^a	Urbanchuk et al. (2009)
	Tobacco	0.3% TSP ^b	Pen et al. (1992)
	Tobacco	5.0% TSP	Kumagai et al. (2000)
Xylanase	<i>Arabidopsis</i>	1.4–3.2% TSP	Bae et al. (2008)
	Tobacco	4.1 TSP	Herbers et al. (1995)
	Rapeseed	2kU/kg seed	Liu et al. (1997)
Glucanase	Tobacco	0.22–0.38% TSP	Bae et al. (2010)
	Tobacco	0.3% TSP	Lebel et al. (1998)
	Barley	–	Jensen et al. (1996)
	<i>Arabidopsis</i>	26% TSP	Ziegler et al. (2000)
Phytase	Tobacco	1% TSP (seed)	Pen et al. (1993)
	Tobacco	14.4% seed	Verwoerd et al. (1995)
	Soybean	–	Denbow et al. (1998)
Bovine chymosin (rennin)	Brassica	0.5% total seed protein	van Rooije et al. (2008)
	Flax	0.5% total seed proteins	van Rooije et al. (2008)
Bovine trypsin	Corn	58 mg/kg seed	Woodard et al. (2003)

^aDry weight^bTotal soluble protein

being investigated to produce commercial enzymes such as amylase, glucanase, xylanase, phytase and chymosin (Hood and Requesens 2012; Biesgen et al. 2002). Besides enzyme purity, the commercial viability of these products depends on several factors including the cost and demand for the product.

Like any other system, plant platforms also display certain challenges, such as low accumulation of some proteins, difficult purification, biological equivalence, regulatory status and consumer acceptance. Other possible challenges are the potential for horizontal dissemination of transgene(s) to other plants through pollen grains, especially for open-pollinated crops, such as corn, and the contamination of plant tissues and protein products with pesticides, herbicides and toxic plant metabolites (Fitzgerald 2003).

Plant-based systems require methods of purification that are different from other systems, such as mammalian cells. **Protein fusion technology** is being tested to overcome two significant challenges of plant-based systems, namely, low accumulation of the protein and difficulty in terms of purification. In this approach, the DNA encoding the target protein is fused with DNA sequences encoding targeting peptides, stabilizing sequences, elastin-like proteins, hydrophobins and prolamine seed storage proteins (γ -zein), amino acid affinity tags or oil bodies (Conley et al. 2011). Both SemBioSys (no longer trading) and Plant Farm Corp. have developed technologies that combine the high-capacity, low-cost production of therapeutic proteins in seeds with a novel technology that simplifies downstream purification. In this instance, the genes encoding the target proteins are fused with a sequence encoding a small protein called oleosin. The resulting fusion protein accumulates on oil bodies within the seed. These oil bodies and the oleosin/protein fusion are then

Table 9.14 Plant platform-based pharmaceuticals in clinical development

Product	Product type	Developer	Platform	Developmental stage
ELELYSO (taliglucerase alfa)	Enzyme (type 1 Gaucher disease)	Protalix BioTherapeutics	Transgenic carrot cell suspension	Licensed USFDA (http://www.protalix.com)
ZMapp	Antibodies	Mapp/KBP	Transgenic tobacco	Preclinical
Oral PRX-106	Antibodies (immune-mediated hepatitis)	Protalix BioTherapeutics	Transgenic carrot cell suspension	Phase II
PRX-107	Enzyme (human alpha-1-antitrypsin for emphysema)	Protalix BioTherapeutics	Transgenic carrot cell suspension	Phase II
PRX-110	Therapeutic protein DNase I (cystic fibrosis)	Protalix BioTherapeutics	Transgenic carrot cell suspension	Phase II
Influenza virus VLP	Subunit vaccine (avian influenza H5)	Medicago Inc.	<i>N. benthamiana</i> transient expression by agroinfiltration	Phase II complete
Influenza virus HA	Subunit vaccine (avian and swine influenza)	Fraunhofer CMB	<i>N. benthamiana</i> transient expression by agroinfiltration	Phase I complete
2G12	Antibody (indicated as HIV microbicide)	Pharma-planta	Transgenic tobacco	Phase I completed
Biosimilar trastuzumab or Herceptin®	Antibody (cancer)	PlantForm Corp	Transgenic safflower	Phase II completed
MAPP66	Antibodies (HSV/HIV)	Bayer/ICON	<i>N. benthamiana</i> transient expression with MagnICON virus-based vectors	Phase II completed
CaroRx	Antibody (dental caries bacteria prevention)	Planet biotechnology	Launch vector	Phase II in the USA; product licensed in EU as a medical device
RhinoRx	Antibody (cold-causing rhinovirus)	Planet biotechnology	Transgenic tobacco	Phase I completed
DoxoRx	Antibody (drug-induced alopecia)	Planet biotechnology	Transgenic tobacco	Preclinical stage

simply purified from other components in ground seeds by centrifugation because they float. The protein is released from the oil bodies into an aqueous extraction solution by enzymatic digestion of the oleosin/protein linker, and initial purification is accomplished without expensive chromatography.

Research in the last decade has overcome many of the technical challenges that have delayed the application of molecular pharming. Good manufacturing practices for plant-derived proteins are in place, with an emphasis on the containment of therapeutic protein in a particular tissue/organ of the plant such as seed, sustainability in production and similarity to mammalian cell-derived therapeutic protein. Indeed, some plant-derived therapeutic proteins are already on their way to commercialization as described in the Table 9.14.

9.4 Closing Comments

Environmental concerns and consumer awareness have been driving forces for the reintroduction of protein-based bioproducts to the market. Advances in genetics, protein chemistry and the availability of superior tools and technologies have accelerated the utilization of proteins for manufacturing consumers' goods. Continuous efforts are ongoing to make these new products economical, durable and sustainable, and the commercialization of such products is gaining momentum. A few, such as protein-coated nuts and fruit, soybean cotton spandex shirts, fabricated nano-spun dressings and plant platform-multiplied vaccines and enzymes, are already on the market, and many more are close to commercialization.

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