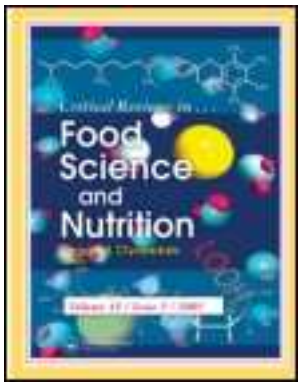

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Edible Films and Coatings from Whey Proteins: A Review on Formulation, and on Mechanical and Bioactive Properties

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Edible Films and Coatings from Whey Proteins: A Review on Formulation, and on Mechanical and Bioactive Properties

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The latest decade has witnessed joint efforts by the packaging and the food industries to reduce the amount of residues and wastes associated with food consumption. The recent increase in environmental awareness has also contributed toward development of edible packaging materials. Viable edible films and coatings have been successfully produced from whey proteins; their ability to serve other functions, viz. carrier of antimicrobials, antioxidants, or other nutraceuticals, without significantly compromising the desirable primary barrier and mechanical properties as packaging films, will add value for eventual commercial applications. These points are tackled in this review, in a critical manner. The supply of whey protein-based films and coatings, formulated to specifically address end-user needs, is also considered.

Keywords Packaging, dairy products, milk proteins, physicochemical properties, rheological properties, permeability

INTRODUCTION

Edible films and coatings have traditionally been used to improve food appearance and preservation, while attempting assure the barrier and mechanical properties of their synthetic counterparts (Khwaldia et al., 2004). They have the intrinsic ability to control mass transfer between the food and its environment, and even between food components, which will thus contribute favorably to extend shelf-life and improve quality thereof (Siew et al., 1999; Khwaldia et al., 2004).

Edible films and coatings are generally manufactured from proteins, polysaccharides, and lipids, used solely, or in combination with each other. Whey protein-based ones have demonstrated mechanical and barrier properties better than competitive protein-based (e.g., corn zein, wheat gluten, and soy protein isolate) or polysaccharide-based (e.g., starch, cellulose, carrageenan, and pectin) films, and they are somewhat comparable to the best synthetic polymer films in the market (Khwaldia et al., 2004). However, their absolute moisture barrier properties and mechanical features suffer from a few limitations, so plasticizers (e.g., sorbitol or glycerol) are to be added to improve resistance to moisture transfer, as well as to avoid brittleness while enhancing flexibility and extensibility.

There are no basic differences in material composition between films and coatings, other than their thickness: films can be used to produce pouches, wraps, capsules, bags, or casings, depending on the extent of the fabrication processes; coatings are a particular form of films, which are applied directly onto the surface of materials. Although removal of coatings may be possible, they are not typically designed to be disposed off separately from the coated material itself; hence, coatings are normally regarded as part of the final product.

The edibility, as well as the biodegradability of edible films and coatings are additional features not usually offered by conventional packaging materials (Cuq et al., 1995; Han, 2002). The use of edible films and coatings as carriers of active compounds has thus been suggested to be a promising application in the field of active food packaging (Cuq et al., 1995; Han, 2000; 2001).

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This review will focus on the composition, and its relation to mechanical, barrier, and active properties, of whey protein-based films and coatings intended for food applications.

FEATURES OF EDIBLE FILMS AND COATINGS

Edible coatings are thin layers of edible material, which are applied directly onto the surface of a food product via dipping, spraying, or brushing, in order to: create a modified overhead atmosphere (Krochta and de Mulder-Johnston, 1997; McHugh and Senesi, 2000; Sonti, 2000); avoid migration of moisture, oxygen, or carbon dioxide, or of any other solutes; and serve as carrier of such food additives as antioxidants, antimicrobials, or specific nutrients while increasing the shelf-life of the product, or even improving its quality by the time of consumption (Guilbert et al., 1996; Sonti, 2000). This is made explicit in Fig. 1.

Edible films should accomplish a number of specific requirements, for example, function as a moisture barrier or as a solute/gas barrier, avoid water/lipid solubilization, keep color and appearance, assure mechanical and rheological characteristics, and be non-toxic; these properties depend obviously on the type of raw materials used, the manufacture process, and the final intended application (Guilbert et al., 1996; Sonti, 2000).

Polysaccharides that have typically been used in film and coating formulations encompass starch, pectin, cellulose, chitosan, and alginate (Sonti, 2000; Baldwin, 2005). These films and coatings perform well in terms of oxygen, aroma, and oil barriers, while providing good strength and structural integrity; however, they are not effective moisture barriers, due to their hydrophilic nature (Krochta, 2001). These coatings may, in addition, retard ripening and thus increase the shelf-life of the coated food product, without generating severe anaerobic conditions (Baldwin et al., 1995; Sonti, 2000).

Proteins that have typically been used in film and coating formulations encompass whey protein, soy protein, casein, cornzein, egg albumin, collagen, and wheat. All these proteins can be obtained from renewable resources, and are degraded more readily then other polymeric materials. Their oxygen barrier properties are associated to a tightly packed, ordered hydrogen-bonded network structure (Baldwin et al., 1995). In particular, milk protein-based coatings possess the extra advantages of constraining enzymatic browning of fresh-cut products; furthermore, the presence of several amino acid residues (mainly cysteine) in the coating can inhibit polyphenoloxidase adventitious in the food (Tien et al., 2001).

Recall that milk possesses a protein system formed by two major families: caseins (which are insoluble) and whey proteins (which are soluble). The former account for ca. 80%(w/w) of the whole protein inventory, and can easily be recovered from skim milk via isoelectric precipitation, through addition, or in situ production of acid, or via rennet-driven coagulation, both of which release whey as a by-product. Whey proteins may in turn be recovered from whey via ultrafiltration, or

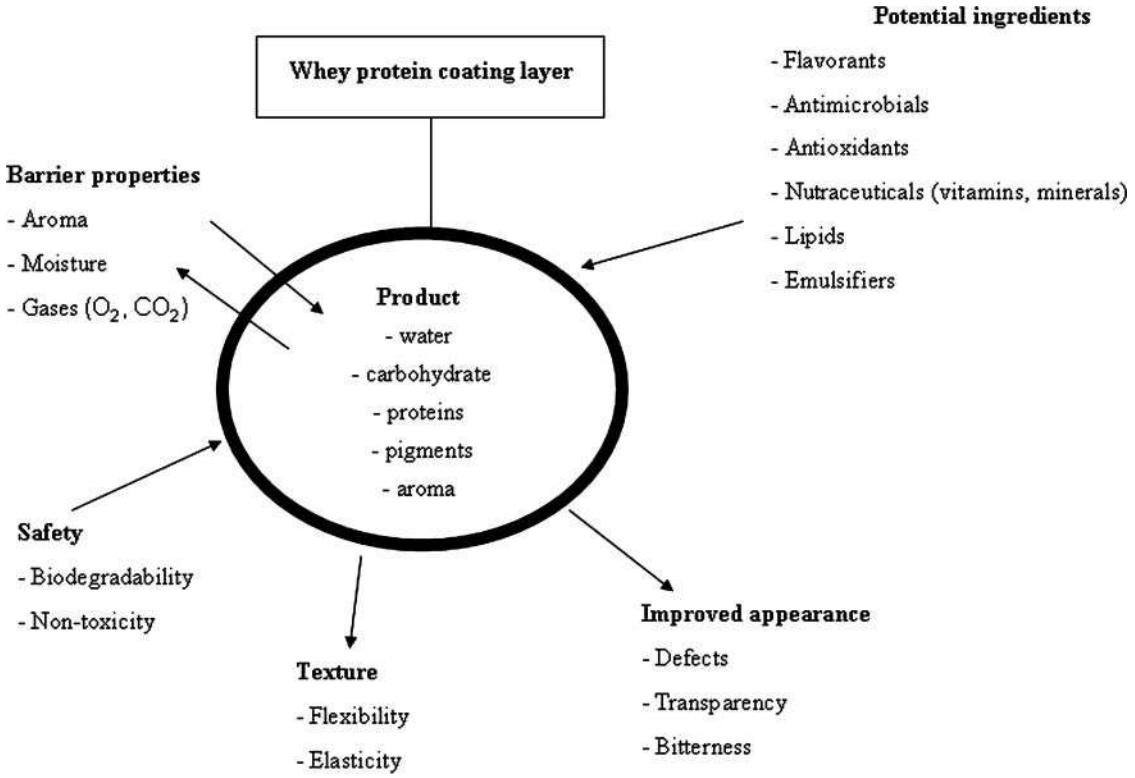

Figure 1 Quality attributes provided specifically by whey protein films and coatings to food products.

else via centrifugation or regular filtration following thermal precipitation. Besides their intrinsically nutritious properties, whey proteins exhibit several functional properties that are essential for the formation of edible films, as will be seen below (McHugh and Krochta, 1994a). On the other hand, the presence of triglycerides in the milk protein network significantly improves water vapor barrier properties, due to their low polarity; however, when present, they also lead to more opaque and relatively inflexible films and coatings (Guilbert et al., 1996).

MANUFACTURE OF WHEY PROTEIN EDIBLE FILMS AND COATINGS

An edible whey film is basically a dried, extensively interacting polymer network that possesses a three-dimensional gel-type structure. Despite the specific film-forming process, the final films should form a spatially rearranged gel structure including any added film-forming agents.

The most distinctive characteristics of whey proteins, when compared to other film-forming biopolymers, are conformational denaturation, electrostatic charges, and amphiphilic nature. Several factors affect the conformation of whey proteins, for example, charge density and hydrophilic-hydrophobic balance; those factors can ultimately determine the physical and mechanical properties of films and coatings prepared therefrom.

In order for a film or coating to be generated, gelation is a prerequisite; this phenomenon is the result of both physical (electrostatic and hydrophobic) and chemical (disulphide) interactions, established among whey protein molecules. Destabilization of the (otherwise stable) soluble proteins in whey can be induced via addition of chemicals, change in net charge, increase in hydrostatic pressure, heating, cooling, or partial enzymatic hydrolysis. Each of these processes induces partial (or total) unfolding of the initial proteins, thus resulting in protein aggregation and eventual gel formation.

Emulsions are heterogeneous systems containing at least one immiscible liquid dispersed in another, which is stabilized in droplet particles. Emulsion stability is affected by film morphology and by the characteristics of the continuous phase (pH, viscosity, and ionic strength) and by the dispersed phase (size and density of lipid droplets) (Pérez-Gago and Krochta, 1999). In protein-stabilized emulsions, the net charge of the adsorbed protein layer is highly dependent on pH; when the pH is close to the isoelectric point (pI) of the major whey protein (which is ca. 5.0), its net charge approaches zero, so electrostatic repulsions become weak and aggregation will likely occur. At pH above and below pI, the emulsion droplets exhibit a net charge; hence, electrostatic repulsions develop between droplets (see Fig. 2). In protein-lipid systems, protein acts as an emulsifier agent, thus lowering the interfacial tension between protein and lipid phases (Baldwin et al., 1997). Changes in pH may also affect the emulsion stability and the film permeability, but scarce data on the effect of pH have been made available up to now; adjustment of pH towards pI may increase the lipid phase connectivity when the net protein charge approaches zero (Perez-Gago and Krochta, 1999).

Formation of whey protein films normally involves heat denaturation of said proteins in aqueous solutions (Perez-Gago and Krochta, 2002); in the absence of thermal processing, such films would readily crack into small pieces upon drying, owing to food intermolecular interactions (McHugh et al., 1994b). Heating indeed modifies the three dimensional structure of proteins, via exposing internal–SH and hydrophobic groups—which promote intermolecular S-S bonding and hydrophobic interactions during drying (Perez-Gago and Krochta, 2002), as schematically depicted in Fig. 3.

Protein films are finally obtained from whey protein gels via dehydration after heat- or cold-set gel formation (Vliet et al., 2004). A common practice in film formation is to dry at room conditions, typically 21–23°C and 50% relative humidity (see Fig. 3). However, control of this drying process is crucial during application of edible coatings onto foods: faster drying results in stiffer, less flexible films, with a less extensive apparent effect upon film tensile strength and elongation. This is attributed to changes in film morphology, with subsequent thinner film

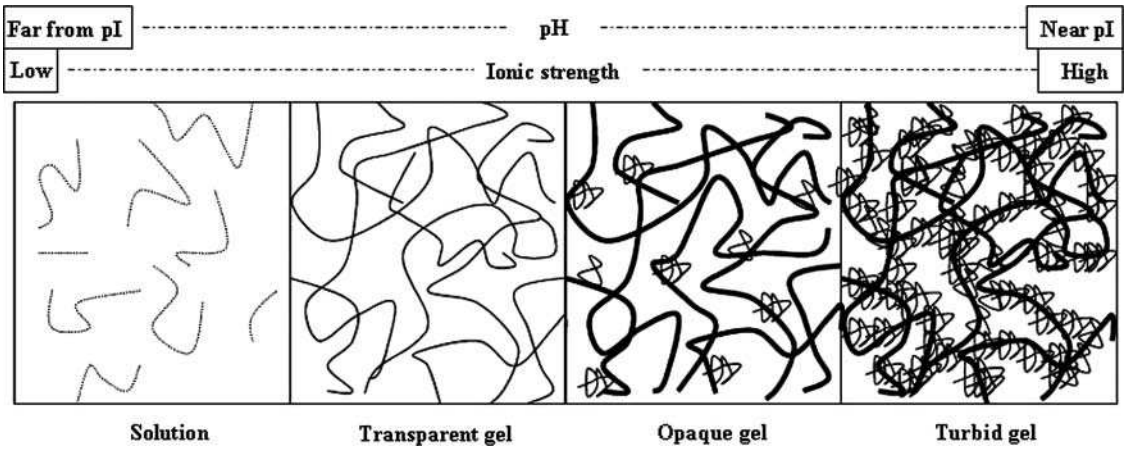

Figure 2 Whey protein gel macroscopic appearance, as influenced by pH and ionic strength, during gelation (adapted from Doi and Kitabatake, 1997).

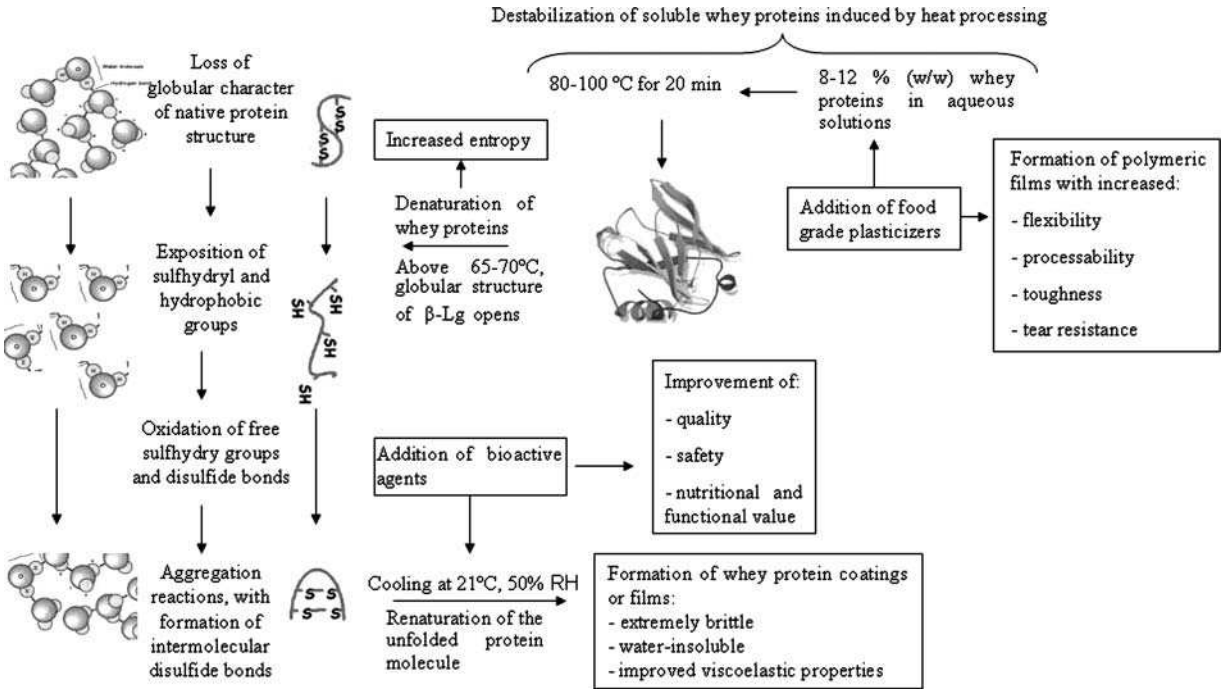

Figure 3 Whey protein gel molecular appearance, as influenced by additives, during heat-induced gelation.

drying achieved at a higher rate. Many procedures have been used to produce whey protein edible films and coatings, viz., dipping, spraying, foaming, fluidization, enrobing, casting, and extrusion. The control of processing conditions is very important, because changes in treatment conditions can alter the kinetics, and even the reaction mechanisms involved in film formation (Guilbert et al., 1996; 1997).

Transparent, flexible whey protein edible films usually go though a thermal-compression molding step; this is an important step prior to extrusion, as it will consequently become a less time-consuming and expensive method. Extruded whey protein films may consequently be formatted into pouches for filling with milk powders (or other dry foods and ingredients).

In order to be considered edible, the whey protein film-forming process should be appropriate for food handling in terms of pH change, salt addition, heating, enzymatic modification, drying, use of organic solvents, and application of other chemicals. Additionally, plasticizers and any other additives should be compatible with the biopolymer (Han, 2002; Nussinovitch, 2003).

FORMULATION OF WHEY PROTEIN EDIBLE FILMS AND COATINGS

Feedstock Materials

The volume of whey protein (WP) production worldwide has increased due to improvements in membrane and ion exchange technologies, which have made it easier to recover whey proteins with desired, useful functional features, viz. acid stability properties, gelation, film formation ability, aeration, and emulsification properties (Huffman, 1996; Perez-Gago and Krochta, 2002).

The profiles of WP products depend on several factors, such as the type of feedstock whey (acid or sweet), the source of milk (bovine, caprine, or ovine), the positioning within the season, the type of feed, and the stage of lactation. However, the effect of each of these factors upon variability of the final commercial products can be circumvented via control of the processing parameters by the manufacturers, in order to fully meet market specifications.

Depending on their concentration, there are standard whey protein concentrates (WPC) containing typically 35, 50, 65, and 80%(w/w) protein. When the threshold of 90%(w/w) protein is reached, usually via the preparative chromatography technique, a whey protein isolate (WPI) is accordingly obtained.

Structuring Agents

The formulation of protein-based films generally requires incorporation of a minimal content of some structuring agents, for example, plasticizers to avoid brittleness (Kokoszka, Debeaufort, Lenart, and Voilley, 2010), polysaccharides to improve barrier features (Krochta, 2001), emulsifiers to stabilize the base emulsion (Krochta, 2002), and lipids to improve water vapor barrier properties (Guilbert et al., 1996).

Plasticizers

Plasticizers are usually required for the manufacture of edible films and coatings, particularly when polysaccharides or

proteins are used as starting material; such film structures are often brittle and stiff, because of extensive interactions between their polymer molecules (Krochta, 2002). Plasticizers are low molecular weight agents incorporated into the polymeric film-forming materials that decrease the glass transition temperature of the polymers, owing to a decrease in the ratio of the crystalline to the amorphous region, which is associated to some extent with lowering thereof (Guilbert et al., 1997; Krochta, 2002).

There are two main types of plasticizers (Sothornvit and Krochta, 2000; 2001): (i) agents capable of forming many hydrogen bonds which thus interact with polymers by interrupting polymer-polymer bonding and maintaining appropriate distances between polymer chains (e.g., glycerol, polyethylene glycol, sorbitol, and water); and (ii) agents capable of interacting with large amounts of water, which thus retain more water molecules, and lead to a higher moisture content and a larger hydrodynamic radius (e.g., sucrose). However, owing to the hydrophilic nature of water, and to the abundant hydrogen bonds associated with their molecular structures, it is very difficult to separate the aforementioned two mechanisms. Water is actually a very good plasticizer, but it can easily be lost by dehydration at low relative humidities (Guilbert and Gontard, 1999); therefore, addition of hydrophilic chemical plasticizers can reduce the said water loss, while increasing the amount of bound water and maintaining a high water activity.

The molecules of a plasticizer position themselves between polymer molecules, and interfere with polymer-polymer interactions in such a way that flexibility, processability, toughness, and tear resistance of the film are improved (Guilbert and Gontard, 1996; Krochta, 2002); in a sense, those agents increase the free volume of the polymer structures, and thus the molecular mobility of the polymer molecules (Sothornvit and Krochta, 2000). The most common food grade plasticizers are glycerol, polyethylene glycol, sorbitol, propylene glycol, sucrose, and water; in terms of physicochemical properties, they affect not only the elasticity, but also the permeability to vapors and gases (Sothornvit and Krochta, 2000; 2001).

The mechanical strength of WPI-based films decreases with the increase of the ratio of plasticizer to WPI; concomitantly, the water sorption increases, so the tensile strength decreases and the elongation increases (Lourdin et al., 1997; Mathews and Dufresne, 2002), as depicted in Table 1. The effects of plasticizers upon elongation of WPI-based films are more apparent for glycerol than sorbitol, as observed in this table (Chick and Ustunol, 1998; Khwaldia et al., 2004). Due to their intrinsically hydrophilic nature, WPI-based films added with hydrophilic plasticizers tend to increase the permeability to gases and absorb larger quantities of water, especially under high relative humidity; this can easily be concluded from inspection of Table 2 (Perez-Gago and Krochta, 2002). Most plasticizers are indeed very hydrophilic and hygroscopic in nature, so they can attract water molecules to form large hydrodynamic plasticizer-water complexes (Krochta, 2002).

Table 1 Effects of plasticizer type (glycerol, G, or sorbitol, S), and ratio of whey protein isolate (WPI) to plasticizer (or whey protein concentrate, WPC) on physical properties of whey protein based-films (adapted from Perez-Gago and Krochta, 2002; Khwaldia et al., 2004)

Film[a]	Tensile strength (MPa)	Elongation (%)	Water vapor permeability (g.mm/m^2.h.kPa)	Oxygen vapor permeability (g.mm/m^2.h.kPa)
WPI:G (1:1)	–	–	6.4	–
WPI:G (1.6:1)	–	–	1.6	–
WPI:G (2:1)	5.76	22.7	–	–
WPI:G (2.3:l)	13.9	30.8	–	76.1
WPI:G (5.7:l)	29.1	4.1	–	18.5
WPC:G (2:1)	3.49	20.8	–	–
WPI:S (1:l)	14.7	8.7	0.9	8.3
WPI:S (2.3:1)	14.0	1.6	–	4.3
WPI:G (2.3:1)	–	–	–	76.1

[a]Film formation conditions: denaturing 10%(w/w) solutions, at 90°C for 30 min, followed by drying at 23°C under 50% RH.
(-) Without known result.

The plasticizing efficiency and the water-binding capacity of plasticizers depend on the size and shape of their molecules, as well as the number of oxygen atoms and the distance to each other within their molecular structure (Sothornvit and Krochta, 2001). Besides the effect of hydrogen bonding, repulsion forces between molecules of similar charge or lack of attraction forces between polar and non-polar polymers (e.g., acetylated starch) can increase the distance between the polymer molecules; hence, the function of plasticization is readily achieved in the case of charged polymeric film structures. When compared to their neutral polymer counterparts (e.g., starch), the flexibility of charged polymer films (e.g., soy protein, carboxymethyl cellulose, or alginate) is thus more significantly affected by changes in pH and salt levels, for a given water activity. On the other hand, WPI-based films plasticized with sorbitol were found more effective, in terms of moisture and oxygen barriers,

Table 2 Effects of plasticizer type (glycerol, G, or sorbitol, S), ratio of whey protein isolate (WPI) to plasticizer, and relative humidity (RH) on physical properties of whey protein-based films (adapted from Perez-Gago and Krochta, 2002)

Film[a]	Test conditions[b]	Water vapor permeability (g.mm/m^2.h.kPa)	Oxygen vapor permeability (g.mm/m^2.h.kPa)
WPI:G (1:1)	25°C, 0/50% RH	6.4	–
WPI:G (1.6:1)	25°C, 0/11% RH	0.2	–
WPI:G (1.6:1)	25°C, 0/50% RH	1.6	–
WPI:G (1.6:1)	25°C, 0/65% RH	5.0	–
WPI:S (1:1)	25°C, 0/10% RH	0.2	–
WPI:S (1:1)	25°C, 0/50% RH	0.9	8.3
WPI:S (1:1)	25°C, 0/65% RH	2.3	–
WPI:S (1:1)	25°C, 0/75% RH	3.5	–
WPI:S (3.5:1)	23°C, 40% RH	–	0.7
WPI:S (3.5:1)	23°C, 70% RH	–	43.3

[a]Film formation conditions: denaturing 10%(w/w) solutions, at 90°C for 30 min, followed by drying at room temperature.
[b]RHs are those applied on the top and bottom sides of film (top/bottom).
(-) Without known result.

than those plasticized with glycerol (Chick and Ustunol, 1998; Perez-Gago and Krochta, 2002) as apparent in Table 2.

Polysaccharides

Polysaccharides were the earliest, and hence the most extensively studied materials for biopackaging. A variety of such compounds (and derivatives thereof) have been tested for potential use as biodegradable/edible films including alginate, pectin, carrageenan, konjac, chitosan, pullulan, cellulose, and starch.

Recall that whey protein films exhibit, in general, poor barrier properties. Several efforts have meanwhile demonstrated that conjugation of proteins with other polysaccharide materials may be useful in attempts to strengthen such barrier properties. Addition of polysaccharides has indeed a significant effect upon the physical properties of protein-based edible films (Ciesla et al., 2006). However, compatibility issues should be taken into account when dealing with mixtures of biopolymers, as mixing drastically alters the performance of these materials relative to their plain counterparts (Diab et al., 2001).

The three-dimensional structure of polysaccharides is rather complex, as their molecular weights are much higher than those of proteins. Several carbohydrates are neutral, whereas some gums are negatively charged. The dominant electrostatic neutrality of carbohydrates is not expected to significantly affect the properties of protein-based films and coatings. However, the occurrence of relatively large numbers of hydroxyl groups (or other hydrophilic moieties) in their structure suggests that hydrogen bonds may play significant roles during film formation and upon its final characteristics. Some of the negatively charged gums (e.g., alginate, pectin, and carboxymethyl cellulose) do indeed exhibit significantly different behavior under acidic than neutral or alkaline conditions (Gennadios et al., 1997; Perez-Gago and Krochta, 2002).

Emulsifiers

An emulsifier (also known as an emulgent) is a compound aimed at stabilizing an emulsion, and it is often a surfactant; such type of agents of amphiphilic nature are able to reduce the surface tension of water-lipid or water-air interfaces. Emulsifiers are essential toward formation of protein-based films and coatings when lipid particles are present; they can also modify the surface energy, thus allowing control of adhesion and wettability of the film surface itself (Krochta, 2002). Although several biopolymers (i.e., casein, egg yolk, and whey proteins) possess emulsifying capacity to a certain extent, it is usually necessary to incorporate extra emulsifiers into film-forming solutions so as to produce stable lipid emulsion films.

Examples of commonly employed food emulsifiers encompass egg yolk (accounted for mainly by lecithin), honey, and mustard, in which a variety of chemicals, present in the mucilage surrounding the seed hull, act as emulsifiers; however, WPC and low-molecular weight emulsifiers are also common (Han, 2002; 2003).

Plain lecithin is often used in the industrial formulation of foods, and is probably the most frequently incorporated emulsifier in protein-based films and coatings. Addition of lecithin to whey protein dispersions increased the strength of heat-induced gels (Dickinson and Yamamoto, 1996) and their heat-stability (Dickinson and Yamamoto, 1996; Euston et al., 2001; Suender et al., 2001; Jiménez-Flores et al., 2005), as well as when incorporated in dairy-based products (Hardy et al., 1985; McCrae and Muir, 1992; Singh et al., 1992; van der Meeren et al., 2005). It is known that phosphatidylcholine, the most abundant component of lecithin, can form lamellar mesophases and vesicles in aqueous media, and thus interact with whey proteins (Brown, 1984; Nakamura et al., 1988; Cornell and Patterson, 1989); as filler particles, they interact with the gel network, and consequently tend to reinforce it with resulting increases in the gel strength. Improvement in heat stability is achieved via a combination of interfacial protein displacement (Courthaudon et al., 1991; Dickinson et al., 1993) and formation of protein/surfactant complexes (McCrae and Muir, 1992; Singh et al., 1992; Jiménez-Flores et al., 2005). Lefévre and Subirade (2001) demonstrated that a phospholipid/β-lactoglobulin interaction stabilized this whey protein against thermally induced unfolding. However, the exact heat stabilizing mechanism is not fully understood to date, and a number of factors are induced believed be involved (el-Bakry et al., 2005).

Lipids

Whey proteins can generate films and coatings bearing good oxygen, aroma compound, and oil barrier properties at low RH, but they will still show poor moisture barrier properties; topical incorporation of lipids has proven positive, in attempts to improve those properties (Perez-Gago and Krochta, 2002). Used as such, however, lipids require solvent or high temperature casting, and furthermore exhibit poor mechanical properties (Krochta, 2002); furthermore, these compounds exhibit certain disadvantages with respect to application, and mechanical and chemical stabilities, as well as organoleptic quality.

The effect of lipid type (viz. anhydrous milkfat fractions, as well as bee, carnouba, and candelilla waxes) and its concentration upon the mechanical properties of whey protein films have been studied (Shellhammer and Krochta, 1997); the film strength and its elasticity decreased with increasing lipid concentration. In addition, carnouba wax emulsion films appeared the strongest at every lipid level tested, whereas candelilla counterparts were the weakest (Khwaldia et al., 2004).

Crystalline lipids provide better barrier characteristics to moisture transport than liquid lipids. Consequently, high melting fractions of fatty acids and monoglycerides, hydrogenated fats, and waxes have proven useful in composite edible films to reduce moisture permeability, owing to their intrinsic nonpolarities and hydrophobicities (Kester and Fennema, 1986; Hagenmeier and Shaw, 1992). More specifically, orthorhombic lipid crystals found, for example, in bee and paraffin waxes, perform better as barriers than their hexagonal counterparts found

in tristearin or acetylated monoglycerides (Martin-Polo et al., 1992). This is explained by a denser and more compact structure of the former crystals, which contain less void volume available for water molecule migration.

Bioactive Agents

Edible films and coatings can carry various agents with physical function, such as antimicrobials, antioxidants, and nutraceuticals, or even flavors and colorants, for that matter. All of these can contribute to enhance food quality and safety, but only up to the level at which such additives start interfering with the physical (including mechanical) properties of the films themselves (Kester and Fennema, 1986; Baldwin et al., 1995; 1997; Guilbert et al., 1997; Howard and Gonzalez, 2001; Han, 2002; 2003) (see Fig. 1). Each of these families of compounds will be discussed below at some length.

Antimicrobials

Growth of both deteriorating and pathogenic microorganisms in food may be prevented via incorporation of antimicrobial agents into the films or coatings (Debeaufort et al., 1998). Antimicrobial films and coatings were indeed a rather innovative concept under the global scope of active packaging which has in general been developed to delay, reduce, or even inhibit growth of microorganisms on the surface of packaged food (Appendini and Hotchkiss, 2002). Hence, antimicrobial agents have traditionally been added to the food during matrix formulation, but their activity may be inhibited by many compounds in the matrix itself, thus constraining their efficiency. In such cases, use of antimicrobial compounds in the films or coatings may turn out to be more efficient, as these compounds will selectively and gradually migrate from the package material onto the surface of the food, and diffuse thereafter into the bulk of the food, so relatively high concentrations will be maintained on the surface of the food for extended periods of time (Ouattara et al., 2000).

The antimicrobial agents most commonly utilized in edible coatings include organic acids, bacteriocins (e.g., nisin), enzymes (e.g., lysozyme), inorganic gases (e.g., carbon dioxide), polysaccharides (e.g., chitosan), fatty acids, fungicides (e.g., natamycin), and such natural antimicrobial crude mixtures as spices (Weng and Hotchkiss, 1992; Ouattara et al., 1997; Han, 2000; Tharanathan, 2003). A few compounds (viz. nisin and lysozyme) have been found to be efficient food preservatives when previously added to the edible films, and are essentially safe for human consumption (Padget et al., 2000; Hoffman et al., 2001; Dawson et al., 2002; Cagri et al., 2004; Min et al., 2005). Fruit and plant extracts (e.g., oregano and rosemary) have recently been introduced as antimicrobial agents, owing to their ability to control various food-borne bacteria, for example, *Salmonella* spp. (Paster et al., 1990; Helander et al., 1998) and *Escherichia coli* O157:H7 (Burt and Reinders, 2003).

A few selected examples of antimicrobial agents (and corresponding E numbers), approved for use in contact with foods and concentrations used to control various target food-borne microorganisms, are depicted in Table 3. However, detailed information about their incorporation into whey protein edible films and coatings is almost nonexistent to date. Recently, a few studies involving incorporation into whey protein films and coatings of such antimicrobial agents as potassium sorbate and natamycin (Krochta, 2003), lactoferrin, lactoperoxidase, and lysozyme (Krochta, 2004), *p*-aminobenzoic, and sorbic acids (Cadri et al., 2006), citric, lactic, malic, and tartaric acids and nisin (Eswaranandam et al., 2006), oregano, rosemary, and garlic essential oils (Seydim and Sarikus, 2007), and even TiO_2 nanoparticles (Zhou et al., 2006) have been carried out. The beneficial effects attained in terms of physical, mechanical, and biochemical features of the resulting films and coatings have been duly discussed elsewhere (Krochta and De Mulder-Johnston, 1997; Han, 2001).

Antioxidants

Antioxidants are compounds that can help overcome the detrimental effects of free radicals formed via oxygen during normal metabolism, coupled with aggressive external factors (e.g., chemical pollution and radiation), mainly before vital molecules become damaged (Pelli and Lyly, 2003).

Several pieces of experimental evidence have indicated that addition of antioxidants to edible whey films and coatings prevents off-flavor development, discoloration, and even textural decay, thus contributing to extend the shelf life of foods; however, exhibition of functional features towards specific health issues (e.g., cancer prevention, and cognitive or immune function improvement) have also been claimed (Valko et al., 2007). Recall that incorporation of synthetic antioxidant compounds, for example, butylated hydroxytoluene or hydroxyanisole, in high-density polyethylene has been shown to protect food products from oxidation (Miltz et al., 1988; Wessling et al., 2000). However, a growing concern by consumers at large regarding food safety has urged the food industry to resort to natural compounds, as is the case of α-tocopherol in active whey protein packaging materials, given that it is a natural lipophilic, chain-breaking antioxidant able to protect the cell membrane from free radical-mediated decay.

Most natural antioxidants are obtained from plant extracts, for example, oils, fruits, spices, seeds, leaves, and husks (Okonogi et al., 2007); their antioxidant capacity has been found comparable to, and sometimes even higher than that of synthetic antioxidants (Cuvelier et al., 1990; Pokorny, 1991). In particular, rosemary and sage (from the *Lamiaceae* family) have been widely used, and most of their antioxidant compounds, namely the phenolic ones, have been duly identified (Das and Pereira, 1990; Pokorny, 1991; Schwarz and Ternes, 1992).

A few selected examples of antioxidant agents approved for use in contact with foods, including crude aqueous extracts from plants and corresponding activities [EC_{50}], are tabulated in Table 4. Note, however, that information about their specific incorporation into whey protein edible films and coatings is

Table 3 Selected examples of antimicrobial agents approved for use in contact with foods, and concentration ranges used to control food-borne microorganisms

Anti-microbial agent	Active compound (E-number)	Concentration (range) (assay pH)	Microrganism	Reference
Bacteriocin	Nisin (E 234)	1.56–100 μg/mL	*Listeria monocytogenes*; *Listeria innocua*; *Brochothrix thermosphacta*; *Lactobacillus helveticus*; *Micrococcus flavus*; *Pediococcus pentosaceus*	Daeschel et al., 1992; Siragusa et al., 1999; Coma et al., 2001; Ko et al., 2001; Janes et al., 2002
		4.8–19 μg/mL	*L. monocytogenes*	Neetoo et al., 2008
		10 mg/mL		Limjaroen, 2003
Enzyme	Glucose oxidase (E 1102)	10 mg/mL ($pH_{optimum} = 5.50$)	*Talaromyces flavus*	Kim et al., 1990; Murray et al., 1997; Massa et al., 2001
			Pleurotus ostreatus	Shin et al., 1993
			Penicillium amagasakiense	Wohlfahrt et al., 1999
			Penicillium canescens	Simpson, 2006
	Lactoperoxidase	10 mg/mL ($pH_{optimum} = 6.00$)	Gram-positive and Gram-negative microorganisms	Zapico et al., 1991; Bjorck et al., 1975; Santos et al., 2008; Zapico et al., 1995
		10 mg/mL	*Salmonella enteritidis*;	Min et al., 2005
		27.5 mg/mL	*Escherichia coli* O157:H7	
	Lysozyme (E 1105)	5 mg/mL ($pH_{optimum} = 6.24$)	Gram-positive bacteria	Appendini and Hotchkiss, 2002
	Lactoferrin	32 mg/mL	*E. coli* O157:H7	al-Nabulsi and Holley, 2005
Organic acid	Lactic acid (E 270)	26 mg/mL	*Salmonella* spp.	
		45 mg/mL (pH 7)	Fungi	Lin and Krochta, 2005
		45 mg/mL (pH 5)		
		14.4 mg/mL (pH 3)		
	Salt			
	Sodium lactate (E 325)	25 mg/mL	*L. monocytogenes*; *S. enteritidis*	Shelef et al., 1997
		10 mg/mL	*Clostridium perfringens*	Thippareddi and Juneja, 2004
		46–56 mg/mL	*L. monocytogenes*	Neetoo et al., 2008
	Citric acid (E 330)	9 mg/mL	*L. monocytogenes*	Hettiarachchy and Eswaranandam, 2007
		26 mg/mL	*Salmonella* spp.	
	Tartaric acid (E 334)	9 mg/mL	*L. monocytogenes*	
		26 mg/mL	*Salmonella* spp.	
	Malic acid (E 296)	9 mg/mL	*L. monocytogenes*	
		26 mg/mL	*E. coli* 0157:H7; *Salmonella* spp.	
	Acetic acid (E 260)	25–50 mg/mL	*L. monocytogenes*	Samelis et al., 2001
		30 mg/mL (pH 7)	Fungi	Lin and Krochta, 2005
		1.2–7.2 mg/mL (pH 5)		
		0.6 mg/mL (pH 3)		
	Lauric acid	80 mg/mL	*L. plantarum*	Padgett et al., 2000
		0.5 mg/mL	*Aspergillus niger*	Řiháková et al., 2001
	Propionic acid (E 280)	37 mg/mL (pH 7)	Fungi	Lin and Krochta, 2005
		1.5–8.9 mg/mL (pH 5)		
		0.74 mg/mL (pH 3)		
	Salt			
	Sodium propionate (E 281)	0.125–12.5 mg/mL ($pH_{optimum} = 5.5$)	Yeasts, fungi	Keeney and Broyles, 1943
		16–60 mg/mL ($pH_{optimum} = 5.5$)	Bacteria	
	Calcium propionate (E 282)	0.125–12.5 mg/mL	Molds, fungi	Droby et al., 2003
		50 mg/mL	*Botrytis cinerea*	
		37 mg/mL		Mills et al., 2004
	Salt			
	Sodium benzoate (E 211)	2.5–5.0 mg/mL	*L. monocytogenes*	Neetoo et al., 2008
		10 mg/kg	*Alternaria alternata*	Combina et al., 1999
	Potassium benzoate (E 212)	50 mg/mL	*L. monocytogenes*	SSamelis et al., 2001
	Sorbic acid (E 200)	15 mg/mL	*L. monocytogenes*	Limjaroen, 2003

(Continued on next page)

Table 3 Selected examples of antimicrobial agents approved for use in contact with foods, and concentration ranges used to control food-borne microorganisms *(Continued)*

Anti-microbial agent	Active compound (E-number)	Concentration (range) (assay pH)	Microrganism	Reference
	Salt			
	Sodium sorbate (E 201)	0.25–1.0 mg/mL	*A. alternata*	Combina et al., 1999
	Potassium sorbate (E202)	3.8–7.5 mg/mL	*L. monocytogenes*	Neetoo et al., 2008; Chen et al., 1996; Han and Floros, 1997; 1999
		20 mg/mL		Limjaroen, 2003
		10 mg/kg	*A. alternata*	Combina et al., 1999
	Chitosan	0.4–1 mg/mL	*Staphylococcus aureus*	Uchida et al., 1989
		0.5–2 mg/mL	*E. coli*	
	Chitosan			
	MW = 470 kDa	0.5–0.8 mg/mL	Gram–negative	No et al., 2002
	MW = 1106 kDa	0.5–0.8 mg/mL	Gram-positive bacteria; *L. plantarum*; *Lactobacillus brevis*; *Lactobacillus bulgaricus*;	
	Chitosan oligomer			
	MW = 11 kDa	2–3 mg/mL	*Bacillus cereus*	Sekiguchi et al., 1994
	MW = 1 kDa	5 mg/mL	Gram-negative bacteria	Uchida et al., 1989
	MW = 2–4 kDa	5 mg/mL	Gram-positive bacteria	
Oil extract	Oregano	20 mg/mL	*S. enteritidis*, *E. coli* O157:H7; *L. monocytogenes*; *S. aureus*; *L. plantarum*	Dadalioglu and Evrendilek, 2004; Burt and Reinders, 2003
	Garlic	30 mg/mL		Pranoto et al., 2005
	Rosemary	>40 mg/mL		Smith-Palmer et al., 1998; Hammer et al., 1999; Pintore et al., 2002
	Cynara scolymus	≥ 2.5 mg/mL	*Bacillus subtilis*; *S.aureus; Agrobacterium tumefaciens*; *Micrococcus luteus*; *E. coli*; *Salmonella typhimurium*; *Pseudomonas aeruginosa*	Zhu et al., 2005
		≥ 2.5 mg/mL	*Candida albicans*; *Candida lusitaniae*; *Saccharomyces cerevisiae*; *Saccharomyces carlsbergensis*; *A. niger, Penicillium oxalicum*; *Mucor mucedo*; *Cladosporium cucumerinum*	
Polyene	Natamycin (E 235)	10 μg/mL	*Penicillium discolour*	Brik, 1981

rather limited; only ascorbyl palmitate and α-tocopherol have been thoroughly tested, following addition to whey protein isolates (Han and Krochta, 2007).

When antioxidants are deliberately added to the film to act preferentially as nutraceutical ingredients rather than as preservatives, it is extremely important to assess their bioavailability not only upon ingestion, but also through the digestive tract, and after absorption through the intestinal walls into the blood circulation system (Ross and Kasum, 2002).

Nutraceuticals

This category of compounds has received increasing attention in recent years, by both the scientific community and the market at large. Besides antioxidants, the list of nutraceutical compounds includes vitamins, probiotic cultures and bioactive peptides, and scientific evidence to support health claims is steadily growing (Wildman, 2001). The pathway of the activities of nutraceutical entities when performing physiological functions in the human body has not yet been fully elucidated; however, it is well recognized that their addition to whey protein films and coatings aids in preventing the risk of disease, so they hold a strong promise in terms of public health (Elliott and Ong, 2002).

The effectiveness of nutraceuticals in providing physiological benefits depends on their eventual bioavailability: delivery of the active molecules will therefore require food manufacturers to provide protection mechanisms that (i) preserve the active molecular form until the time of consumption, and (ii) deliver this form to the cellular target within the human organism upon ingestion. Whey protein-based systems for delivery of compounds of interest within molecular, edible networks have been developed to some extent by biomedical and pharmaceutical companies (Peppas et al., 2000; Langer and Peppas, 2003). Gels can indeed be formed by controlling the assembly of protein molecular chains, thus offering the possibility of developing

Table 4 Selected examples of antioxidant agents approved for use in contact with foods, and average (± standard deviation) of activities via DPPH radical method

Antioxidant compound/extract (E-number)	$[EC_{50}]^1$	Reference
Ascorbic acid, vitamin C (E 300)	5.5 ± 0.1 μg/mL	Kim et al., 2006
	19.9 ± 2.3 μg/mL	Desai et al., 2008
	6.49 ± 1.07 μg/mL	Saleem et al., 2004
α-Tocopherol, vitamin E (E 307)	40.60 ± 0.29 μM	Kweon et al., 2001
	12.64 ± 0.42 μg/mL	Saleem et al., 2004
	15.6 μM	Sang-Myung et al., 2003
Gallic acid	11.2 ± 0.9 μM	Baratto et al., 2003
Propyl gallate (E 310)	6.4000 ± 0.0003 μg/mL	Sultanova et al., 2001
Butylated hydroxyanisole, BHA (E 320)	20 μg/mL	Wang et al., 2007
	10.8 μM	Sang-Myung et al., 2003
Butylated hydroxytoluene, BHT (E 321)	10 μg/mL	Pourmorad et al., 2006
Caffeic acid	3.2 ± 0.1 μg/mL	Kim et al., 2006
	45 μg/mL	Sai Mokbel and Suganuma, 2006
Ferulic acid	36.5 ± 0.23 μM	Kweon et al., 2001
Chlorogenic acid	12.3 ± 0.12 μM	
Oxalic acid	1.1 mM	Lee et al., 2000
Quercetin	5.9 ± 0.7 μg/mL	Kim et al., 2006
Rosmarinic acid	2.90 ± 0.30 μg/mL	Tepe et al., 2006
Chitosan		
MW = 950 kDa	1 mg/mL	Pasanphan, 2008
MW = 2.8 kDa	0.87 mg/mL	
Green tea	0.40 ± 0.05 μM	Aynur and Nehir, 2008
Black tea		
	0.62 ± 0.01 mg/mg	
with lemon	0.54 ± 0.30 μM	
with bergamot	0.48 ± 0.20 μM	
with clove	0.52 ± 0.18 μM	
with grounded cinnamon	0.53 ± 0.57 μM	
with stick cinnamon	0.52 ± 0.04 μM	
Sage	4.95 ± 0.05 μM	
Peppermint		
	0.58 ± 0.01 Mm	
with lemon	0.37 ± 0.12 μM	
Thyme	0.99 ± 0.09 μM	
Absinthium	2.98 ± 0.09 μM	
Roselle	3.93 ± 0.01 μM	
Olive leaves	4.86 ± 0.01 μM	
Shrubby blackberry	6.22 ± 0.01 μM	

[1]Efficient concentration [EC_{50}]: amount of test sample needed (measured as concentration of stock solution added to the reaction mixture) to decrease the initial DPPH concentration by 50%.

GRAS status-holding biocompatible carriers for oral administration of such sensitive drugs as retinol (Beaulieu et al., 2002), or living microorganisms as bifidobacteria (Guérin et al., 2003).

In spite of their success elsewhere, many of the synthetic polymers employed as vectors cannot be used in food applications, either because of cost or compatibility with the food matrix (including unacceptable organoleptic effects).

However, a number of researchers have succeeded in incorporating nutraceutical compounds into biodegradable edible films and coatings to enhance the nutritional value of some food products (i.e., fruits and vegetables), in which these micronutrients are present in low levels. Tapia et al. (2008) reported that addition of 1%(w/v) ascorbic acid to alginate and gellan-based edible coatings helped preserve the natural ascorbic acid content in fresh-cut papaya, thus assuring its nutritional quality throughout storage. Han et al. (2004) reported that chitosan-based coatings could hold relatively high concentrations of calcium cations or vitamin E, which would significantly increase their content in fresh and frozen strawberries and red raspberries.

Tapia et al. (2007) developed the first edible films containing probiotic bacteria, aimed at coating fresh-cut apple and papaya; viable numbers above 10^6 cfu/g of *Bifidobacterium lactis* Bb-12 were maintained for 10 days during refrigerated storage, of both papaya and apple pieces wrapped in alginate or gellan film, thus demonstrating the feasibility of these polysaccharide coatings to carry viable probiotics on fresh-cut fruit. Not until recently have whey proteins been considered as an alternative to alginate, owing to their better nutritional value, as well as their ability to form gels (Lefèvre and Subirade, 2000; Remondetto and Subirade, 2003) and emulsions (Lefèvre and Subirade, 2003). However, the concentration of nutraceutical added to the coatings should be carefully assessed, in particular regarding the effects on their basic functionality arising from the barrier and mechanical properties of the film. For instance, when evaluating the feasibility of milk protein-based edible films to carry high concentrations of calcium, 5 to 10%(w/v), or vitamin E, 0.1% to 0.2%(w/v), Mei and Zhao (2003) concluded that protein-based edible films can carry active compounds, but the film functionality will be compromised. Conversely, Park and Zhao (2004) reported that the water barrier properties of chitosan-based films would be improved if the concentration of zinc lactate (in the range 5–20%,w/v) or vitamin E were increased in the film matrix.

Flavors and Colorants

Consumers have for long used flavors and colors to judge a food, so manufacturers have taken advantage of the said realization to develop additives to improve it. As part of the secondary function of foods, color provides visual information about quality that also psychologically conditions perception of its flavor. Furthermore, colorants or flavor enhancers in such foods as meats and vegetables may also contain ingredients of nutritional and health value, as recently demonstrated for cactus fruits from *Aloe vera* (Stintzing et al., 2001; 2003; Butera et al., 2002; Galati et al., 2003).

Flavorants, more commonly known as taste or flavor enhancers, are largely based on amino acids and nucleotides, and are traded as their sodium or calcium salts. These agents are usually included in whey protein-edible films and coatings to enhance/mask original flavors of the packaged food, but they may also play a role in preservation: this is the case of citric,

Table 5 Selected examples of coloring agents approved for use in contact with foods

Naturally occurring colorants			
Coloring agent (E-number)	Color	Source	Reference
Anthocyanins (163)	orange-red to red to blue	berries, grapes, apples, roses, hibiscus, red cabbage, sweet potato	Schwarz and Winterhalter, 2003
Betacyanins (163)	red	red beets, red chard, cactus fruit, bougainvillea	Cai and Corke, 1990; Cai et al., 1998
Caramel (150)	beige to brown	heated sugars	Butera et al., 2002; Galati et al., 2003; Stintzing et al., 2001;, 2003
Carmine (120)	red	cochineal insects	Greenfield, 2005
Carotenoids (150)	yellow to orange to red	saffron, tomatoes, paprika, corn, butter, palm oil, red salmon	Cai et al., 2001
Chlorophylls (141)	green to olive green	green plant leaves	Duhard et al., 1997; Stintzing and Carle, 2004
Riboflavin (101)	yellow	vegetable leaves, milk, eggs, organ meats, malt	Butera et al., 2002; Galati et al., 2003; Stintzing et al., 2001; 2003
Turmeric (100)	yellow	*Curcuma longa* rhizomes	Duhard et al., 1997; Stintzing and Carle, 2004

malic, and tartaric acids (Eswaranandam et al., 2006; 2006), as well as lactic and acetic acids (Lin and Krochta, 2005).

The sensory attributes of WPI/candelilla wax emulsion films were assessed (Kim and Ustunol, 1991) in terms of transparency, odor, sweetness, and adhesiveness, using a (trained) sensory panel. The films manufactured had no distinctive milk odor, but were perceived as slightly sweet and adhesive: WPI films without candelilla wax looked glass clear and transparent, whereas the corresponding candelilla wax-containing films were opaque. Even though reports regarding the details of inclusion of several substances in whey protein edible films and coatings designed for contact with foods (and consequences thereof) are not yet fully available, a number of natural substances (and corresponding color and E number) are listed in Table 5. The safety of use of food colorants and flavorants, both natural and synthetic, remains however a controversial topic, and will thus likely elicit debate, motivate scientific studies, and entertain legislative actions in the near future.

PHYSICOCHEMICAL PROPERTIES OF WHEY PROTEIN EDIBLE FILMS AND COATINGS

The potential use of whey protein edible packaging depends heavily on their intrinsic physicochemical properties; the criteria that apply to conventional food packaging materials are obviously to be met by those edible materials as well. These criteria relate to barrier properties (e.g., water vapor, gases, light, and aroma), optical properties (e.g., transparency) and mechanical properties (Haugaard et al., 2001; Weber et al., 2002). Therefore, the importance of accurate methodologies to determine film performance is obvious (Debeaufort et al., 1998). These methods are derived from classical ones applied to synthetic materials, yet they have been adapted to whey film specifications, mainly due to the major influence of RH and temperature upon the final film properties (Krochta and Mulder-Johnston, 1997).

Barrier Properties

The barrier properties that are commonly considered in attempts to characterize the ability of edible films to protect packaged foods from environmental aggression and adjacent ingredients are water vapor and gas permeabilities; aroma compound and oil permeability are at least as important for many foods, yet they have received far less attention.

Permeability is defined as the rate of vapor (or gas) transmission through a unit area of flat material of unit thickness, induced by a unit pressure difference between the two sides of the material, under specified temperature and humidity conditions (Yang and Paulson, 2000). The primary mechanism for gas (or vapor) transmission through a film can be rationalized as active diffusion: the penetrant dissolves (in liquid form, in the case of vapor) in the matrix on the high concentration side, diffuses through the film driven by a concentration gradient within the liquid phase, and eventually evaporates (in the case of vapor) or is released (in the case of gas) on the other surface (Kester and Fennema, 1986). Permeation can be mathematically described by Fick's first law (Landrock and Proctor, 1952; Jost, 1960; Crank, 1975; Chang, 1981).

Water Vapor Permeability

Water activity (a_w), which depends on the moisture content and the interactions of water molecules with the other ingredient molecules, is one of the critical factors that affects the sensory quality and shelf life of a food item. During storage, many chemical and enzymatic decay reactions (e.g., lipid oxidation, Maillard, and enzymatic browning), as well as microbial growth proceed at rates governed by the prevailing water activity; in addition, textural properties of certain foods are also largely dependent on a_w. Hence, water vapor permeability is an important property of whey protein edible films and coatings, owing to its effect upon control of water vapor transport and water balance between a food system and its surroundings.

The method most commonly used to determine water vapor permeabilities is ASTM E96–95 (ASTM, 1996), known as the "cup method"; this gravimetric method involves sealing a test film in a cup, partially filled with either distilled water or desiccant, thus leaving an air gap under the film (McHugh and Krochta, 1994a).

The concept of water vapor permeability is also useful in attempts to understand solute-polymer interactions in edible films, as well as to elucidate mass transfer mechanisms (Yang and Paulson, 2000). In fact, the hydrophilic nature of whey proteins limits their ability to form films with good moisture barrier properties: hence, weight loss of the food product (owing to moisture) is likely to occur, when compared with what happens when polymer synthetic films are used. On the other hand, whey protein films can effectively prevent water vapor condensation inside the package which is a potential source of microbial spoilage, especially in the case of fruit and vegetable packaging (Ben-Yehoshua, 1985). For similar reasons, the RH content and the type of plasticizer affect significantly the moisture permeabilities of protein films (see Table 2) (Perez-Gago and Krochta, 2002; Khwaldia et al., 2004).

The moisture sorption isotherm is the classical means to characterize the water sorption property of an edible film which correlates, in turn, to the amount of water eventually transmitted to the product inside. Knowledge of the underlying sorption isotherm is also important to predict stability and quality changes throughout packaging and storage (Srinivasa et al., 2007), but provides little fundamental insight into the interaction of water with film components. Despite the several mathematical models proposed to describe moisture sorption isotherms, none yields accurate results in the whole range of a_w and for all foods (al-Muhtaseb et al., 2004).

In order to reduce the water vapor permeabilities, it is important to include hydrophobic compounds as part of the film formulation; this can be accomplished via lamination of the protein film with a lipid layer, but such composite films tend to later delaminate because of the high surface energy between the two layers. On the other hand, if the lipids can be homogenized into the protein-plasticizer network while in solution, then the aforementioned problem can be overridden, and good mechanical properties will eventually result (Shellhammer and Krochta, 1997).

The capacity of lipids in films to reduce water loss in packaged fruits and vegetables is obviously affected by the type (including chain length) and amount of lipid used (Ayranci and Tunc, 2003; Olivas and Barbosa-Canovas, 2005); studies encompassing dispersed lipid films prepared with WPI (Shellhammer and Krochta, 1997) indicated that addition of glycerol and sorbitol reduces internal hydrogen bonding, thereby increasing film flexibility and water vapor permeability. Alcantara et al. (1998) were in turn concerned with understanding the effect of drying upon the said permeabilities, in the case of WPI-based films, and once again glycerol appeared to produce the best moisture barriers when a fast drying rate was provided.

Gas Permeability

Oxygen permeability is the next most commonly studied transport property of edible films; that gas is involved in many degradation reactions in foods, for example, fat and oil rancidity, microorganism growth, enzymatic browning, and vitamin loss. Therefore, many packaging strategies attempt to exclude oxygen from close vicinity with the food (Gontard et al., 1996). On the other hand, permeability to oxygen and carbon dioxide is essential for respiration of living tissues, as it happens with fresh fruits and vegetables, so moderate barrier films and coatings are more appropriate in this case. If a film or coating with an appropriate permeability is chosen, a respiratory exchange will be established, which, if carefully controlled, will aid in preserving fresh fruits and vegetables (Ayranci and Tunc, 2003). Oxygen permeability can be measured using the standard method (ASTM, 1988), whereas methods to determine CO_2 permeability have been based on modifications of the method employed to measure water vapor permeability (Ayranci et al., 1999).

The oxygen permeability of whey protein films is lower than those of synthetic films, under similar RH conditions and using the same plasticizer (Perez-Gago and Krochta, 2002). This observation is surely related to the more polar nature and linear structure of the latter, which leads to a higher cohesive energy density and a lower free volume (Miller and Krochta, 1997). Such a relatively low oxygen permeability of whey protein films and coatings can be thoroughly employed to enhance chemical quality, including oxidative damage of lipid ingredients and deterioration brought about by aerobic microflora, as happens in nuts, confectionary, fried products, fresh fruits and vegetables, and colored produce (Baldwin et al., 1997).

Whey protein-based films and coatings appear to have greater oxygen permeability than collagen, wheat gluten, and soy protein-based films (McHugh and Krochta, 1994a). These attributes can be used to induce a shiny, smooth surface on the foods besides protecting them from dehydration, aroma loss, moisture migration, and ageing. However, modification of the polymer structure itself combined with optimized plasticizer selection, may affect the polymer free volume, and thus result in further reduction of oxygen permeability. Oxygen permeability values of WPI films as affected by such factors as plasticizer type (glycerol or sorbitol), ratio of WPI/plasticizer, and RH, are depicted in Table 2.

Gas permeability of edible films is influenced by atmospheric RH, as well as by temperature and thickness, likewise water vapor permeability (Cisneros-Zeballos and Krochta, 2002). At higher RH, oxygen permeability increases substantially (see Table 2) (Guilbert et al., 1997; Mate and Krochta, 1998), so it is important to constrain RH in order to maximize the effectiveness of edible films as gas barriers.

Coatings that exceed a critical thickness can cause detrimental effects in the food, owing to a reduced internal oxygen partial pressure while allowing CO_2 concentration to increase, and hence lead to anaerobic fermentation. Since thick coatings

restrict the respiratory gas exchange, the product may accumulate high levels of ethanol and eventually develop off-flavors (Miller et al., 1984; Kays, 1991; Sonti, 2000).

Aroma Compound Permeability

Among the barrier properties exhibited against several compounds by biopolymer-based packaging, molecules implicated with aroma perception are of particular importance: (i) to control losses and adsorption of such molecules, which would otherwise deteriorate the sensory quality of the food; and (ii) to deliberately deliver such molecules from active packages, in a controlled fashion (Chalier et al., 2006). Unfortunately, aroma barrier properties of edible protein films have received too little attention, except for the realization that WPIs are excellent barriers to δ-limonene (Miller and Krochta, 1997; Miller et al., 1998; Perez-Gago and Krochta, 2002). WPI films containing 25% glycerol were indeed comparable to EVOH films in terms of barrier to δ-limonene transport, under similar temperature and RH.

Mechanical Properties

Edible films should possess adequate mechanical strength and extensibility, so as to maintain integrity and withstand the external stresses that prevail throughout processing, handling and storage (Yang and Paulson, 2000) besides durability, when used to separate layers of homogeneous food (Sonti, 2000). Such mechanical properties of edible films and coatings depend obviously on the type of base material, especially on its structural cohesion. However, mechanical properties are also dependent on film-forming conditions, for example, the type of process and solvent, the rate of cooling or evaporation, and the coating technique, for instance, spraying or spreading (Guilbert et al., 1996). The mechanical properties may vary with film thickness and speed of testing used, so a tight control of the working conditions during testing is required (ASTM, 1997).

Classical methods employed in the evaluation of mechanical properties of synthetic materials can also be applied to whey protein edible films; these include puncture and tensile tests (Cuq et al., 1996), although the latter are more often described in the literature. The features ascertained by tensile tests are tensile strength (TS), elongation (E) and Young modulus (Ym): TS is defined as the maximum tensile stress that a material can sustain, and is taken as the maximum load exerted on the test specimen during the test; E is the maximum change in length of the test specimen before breaking, and is expressed as percent change of the original length of the material between the grip of the test machine (Olivas and Barbosa-Canovas, 2005); and Ym is defined as the ratio of stress to strain, in the initial linear part of the stress/strain curve (McHugh and Krochta, 1994a). The magnitudes of TS and E are considerably affected by RH and temperature: hence, film samples should be properly conditioned prior to assaying because TS and Ym decrease, whereas E increases upon increase in water content of the coating (Guilbert et al., 1997; Miller et al., 1998; Wu et al., 2002; Olivas and Barbosa-Canovas, 2005).

When the plasticizer concentration in the film-forming solution increases, less stiff and rigid, and hence more extendible films result; inclusion of a plasticizer thus causes a reduction in TS and an increase in E (see Table 1). These general trends are probably associated with the reduction in magnitude of the interactions between biopolymer chains, an effect that is well known and broadly discussed in the literature (Parris et al., 1995; Cuq et al., 1997; Lourdin et al., 1997; Arvanitoyannis and Biliaderis, 1998; Sobral et al., 2001; Paschoalick et al., 2003; Laohakunjit and Noomhorm, 2004; Mali et al., 2005).

Surface Properties

Surface characteristics of films and coatings, such as cohesion and adhesion on the surface of coated foods, are properties of considerable relevance. Recall that cohesion of a polymer is a consequence of its ability to form strong and/or numerous molecular bonds between adjacent polymeric chains, thus hindering their separation; such an ability depends on the structure of the polymer, and mainly on its molecular strength, geometry, molecular weight distribution, and type and position of lateral functional groups (Guilbert et al., 1996). Assessment of surface morphologies of whey protein-coated apple skin was done via scanning electron microscopy (Choi et al., 2002), as well as via confocal Raman microspectrometry, surface enhanced Raman scattering, and Fourier transform Raman spectrometry (Hsu et al., 2005).

To fully take advantage of edible films, the coating is supposed to completely adhere onto the food surface. However, adhesion of whey protein films (as happens with most hydrophilic edible coatings) is intrinsically poor, because of the distinct chemical nature of the two contacting surfaces. Furthermore, if the film-forming materials contain heterogeneous ingredients that fail to be compatible with whey proteins, the cohesion of the resulting films decreases and film strength weakens. Therefore, when use of new additives is under scrutiny, compatibility between all ingredients should be assured so as to attain the strongest cohesion.

Plasticizers are known to reduce cohesion of film-forming polymers (Guilbert et al., 1996). Hence, surface adhesion of whey-protein edible coatings can be improved via addition of surfactants, for example, Tween or lecithin, which reduce surface tension, and concomitantly improve wettability (Lin and Krochta, 2005).

Optical Properties

Color and transparency (or opacity) of edible films are important features, especially when the films are intended for use in food packaging, as consumers are attracted by the external

Table 6 Optical properties of selected whey protein-films, as compared with synthetic films (adapted from Shiku et al., 2003)

Films	Light transmission (%)[a]									Transparency[b]
	200 nm	280 nm	300 nm	350 nm	400 nm	500 nm	600 nm	700 nm	800 nm	
WPI	0.6 ± 0.1	1.6	68.9 ± 6.5	81.4 ± 3.6	84.0 ± 4.6	85.0 ± 4.3	86.9 ± 4.0	87.8 ± 3.8	88.0 ± 3.7	3.41
Synthetic films[c]										
LDPE	13.1	67.5	–	79.9	83.4	85.6	86.9	87.8	83.6	3.05
OPP	4.6	80.0	–	86.2	87.9	88.8	89.1	89.3	89.6	1.67
PE	0.3	0.3	–	68.3	73.6	82.1	83.5	84.2	84.9	1.51
PVDC	0.3	79.1	–	83.8	86.6	87.5	90.0	87.9	84.9	4.58

[a]Mean (± standard deviation) of three determinations.
[b]Transparency calculated as: A_{600}/X, where A_{600} is absorbance at 600 nm and X is thickness.
[c]LDPE: low-density polyethylene; OPP: oriented polypropylene; PE: polyester; PVDC: polyvinylidene chloride.
(-) Without known result.

appearance of the food matrix, both at the time of purchase and at the time of ingestion (Kunte et al., 1997). The most commonly used methodologies to measure color are Hunter Lab, CIE L*a*b*, CIE LCH, CIE XYZ, and CIE Yxy (Abbot, 1999). Measurements are conducted in a colorimeter, and the color difference is expressed as a single numeric value, ΔE^*, which indicates the magnitude of the color difference, but not the quality of such a difference (Sakai, 1998).

The transparency of films is often determined according to ASTM D1746 (ASTM, 1997b), and is usually calculated by the equation proposed by Han and Floros (1997). The ultraviolet and visible light barrier properties of a film are measured at selected wavelengths, normally from 200 to 600 nm, using a calibrated spectrophotometer. A comparison of light transmission (and transparency) properties of WPI-based edible films is presented in Table 6, including a few synthetic films for the sake of comparison; the former had a transparency of 3.41%, thus indicating that they are more transparent than several synthetic polymer films (Shiku et al., 2003).

In general, ΔE^* increases with addition of plasticizer, except in the case of colorless compounds (e.g., glycerol), owing to a dilution effect (Sobral et al., 2005).

APPLICATIONS OF WHEY PROTEIN EDIBLE FILMS AND COATINGS

Edible films and coatings convey several benefits in terms of safety, convenience, and environment as schematically depicted in Fig. 1. The major advantages of whey protein-based ones are the improvement in quality to extend shelf life, coupled with the reinforcement in surface strength of fragile matrices to ease handling (Baldwin et al., 1995). One current issue encompassing these specific coatings pertains to quality assurance of fruits and vegetables, even after the package is opened (Krochta at al., 1994; Olivas and Barbosa-Canovas, 2005). Besides the essentially protective function, whey protein edible films and coatings are also utilized by the food industry to develop single-dose, premeasured pouches of food ingredients, as well as to mask putatively undesirable tastes thereof (Gennadios and Weller, 1990). Application of the classical extrusion processes of conventional synthetic films to extrude whey protein films allows easy shaping into pouches, for example, for milk powders and other dry foods.

Nowadays, a number of innovative features are under investigation, relating to whey protein edible films, for example, as effective vectors to carry and deliver antimicrobial, antioxidant, and other nutraceutical compounds, as happens with colored/flavored confectionary, glazed bakery, flavored nuts and vitamin-enriched rice, besides agrochemicals (in the form of hard or soft gel capsules, microcapsules, soluble strips, flexible pouches, and coatings on hard particles) (Kester and Fennema, 1986; Gennadios and Weller, 1990; Baldwin et al., 1996); the goal is to avoid significantly compromising the basic barrier and mechanical properties of the (commercial) films themselves. However, the rates of release of active principles by whey protein edible films and coatings to the surrounding media should be as specific and predictable as possible, so as to maximize their effectiveness on the food product. It is also important to consider the chemical interactions between such active principles and the film-forming materials, including their dependence on the outer environmental conditions. Finally, all ingredients of those films, which include functional additives and processing aids, should be non-toxic and food-grade, in addition to assuring specified migration rates (Guilbert and Gontard, 1995; Han, 2002; 2003; Nussinovitch, 2003).

The most beneficial characteristic of whey protein edible films and coatings is obviously their edibility and inherent biodegradability (Guilbert et al., 1996; Krochta, 2002). The latter feature is particularly attractive for food industry and services, because it can reduce the total amount of synthetic materials disposed off, while effectively responding to environmental awareness of consumers at large (Krochta and De Mulder-Johnston, 1997; Amarante and Banks, 2001). However, the period of biological decay of whey proteins in such edible films and coatings should obviously be longer than the expected shelf-life of the packaged food products (Krochta and De Mulder-Johnston, 1997; Amarante and Banks, 2001).

Despite the aforementioned advantages, there are also many limiting factors for full commercial exploitation of whey protein

edible films and coatings, for example, those arising in treatment of immature, flavorless fruits, and in high temperature storage (Park, 2000; Sonti, 2000). Finally, extra regulatory issues will also play a role, as discussed below.

REGULATORY ISSUES PERTAINING TO WHEY PROTEIN EDIBLE FILMS AND COATINGS

Since the package is an integral part of the food product, it should follow all regulations regarding food products (Guilbert and Gontard, 1995) besides being significantly affected by, and the first to be exposed to consumer acceptance (Petersen et al., 1999).

As a first step, it is recommended that whey protein edible film and coating suppliers obtain "no-objection" notification from the established authorities, regarding use of their film and coating products as food ingredients, including careful consideration of proper labeling, viz. nutritional information and possible allergenicity (Han, 2001, 2002; Krochta, 2002). In the case of nutraceutical applications, other pending regulations are to be considered, especially in what concerns health claims and novel foods.

On the other hand, consumer acceptance is an integrated index of subjective preferences by consumers for a given product that includes organoleptic properties, safety assurance, marketing approach, and cultural background. Organoleptic properties may include appealing flavor, tastelessness, and compatibility with texture and appearance (Han, 2002; Nussinovitch, 2003). Marketing factors include the price of the final product, as well as annoying instructions and requirements to handle, open, and dispose off the package. Finally, cultural background encompasses reluctance to use novel materials, and prejudiced concern about the safety of their contact with items that will eventually be ingested.

CONCLUSIONS

This review has attempted to demonstrate a number of features specific to whey protein edible films and coatings as novel packaging materials. These are very promising systems toward improvement of food quality, extension of shelf life, assurance of safety, carrying of functionality, and decreasing of ecological footprint. Furthermore, they can be used as individual pouches of homogeneous ingredients and active ingredient carriers. Their inherent edibility and biodegradability are thus major advantages over conventional synthetic packaging materials. However, industrial implementation of this new technology still hinges upon further scientific research aimed at identifying the film-forming mechanisms, in attempts to optimize performance of both the product and the process. Extra research efforts are sought in terms of consumer studies and long term toxicity assessment, before a substantial market share may be achieved.

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