PROTEIN-LIPID FILMS: APPLICATIONS IN FOOD SYSTEMS

By

***[1]Enujiugha V. N., [1]Ayodele R. O. and [2]Seidu K. T.**

[1]Department of Food Science and Technology Federal University of Technology, Akure, Nigeria.

[2]Department of Food Science and Technology Federal Polytechnic, Ado-Ekiti, Nigeria.

*Correspondence author: venujiugha@yahoo.com

Abstract

Protein-lipid films are films developed by overlaying of protein and lipids when proteineous foods are heated. Protein-lipid film is formed as a result of endothermic polymerization of heat denatured proteins or lipoprotein monomers at the liquid surface promoted by surface dehydration. Covalent bonding of lipids to proteins through lipolization offers unique opportunities for film formation with improved properties. Proteins and lipids are capable of interacting in many different ways to form effective edible films and coatings. Protein-lipid films have received considerable attention in recent years because of some known advantages over synthetic films, including use as edible packaging materials. This could contribute to the reduction of environmental pollution caused by packaging wastages. This paper reviews recent research on film formulation and modification of these films. Also, application of protein-lipid films to food system and interaction between protein and lipids during the production of protein-lipid films are discussed. Protein-lipid films can be prepared from various protein foods. A remarkable example of protein-lipid film is a traditional soybean food which is a cream-yellow bland flavoured surface film of high nutritional value (soy protein-lipid film, designated as Yuba or soymilk skin), which is formed during the heating of soymilk. The understanding of component interaction at the interfacial level is essential if advances are to be made in the control and manipulation of multiphase foods during production and storage.

Key words: Protein-Lipid Films, Properties, Applications

IJAFS 4, 2013, 18: 553- 570
Accepted for publication, September, 2013.
Cite as IJAFS 4 (1&2), Pp 553-570

Introduction

Currently there has been a renewed interest in edible films made from renewable and natural polymers such as proteins, polysaccharides and lipids. Edible films can be used for versatile food products to reduce loss of moisture, restrict absorption of oxygen, lessen migration of lipids, improve mechanical handling properties, provide physical protection, and/or offer an alternative to the commercial packaging materials. The films can enhance the organoleptic properties of packaged foods provided that various components (such as flavourings, colourings and sweeteners) are used (Bourtoom, 2009). The films can function as carriers for antimicrobial and antioxidant agents (Bourtoom, 2008). The films can also be used for individual packaging of small portions of food, particularly products that are currently not individually packaged for practical reasons. These include pears, beans, nuts and strawberries. In a similar application they also can be used at the surface of food to control the diffusion rate of preservative substances from the surface to the interior of the food

When soymilk is heated in flat, shallow, open pans at about 90°C, a cream-yellow, bland flavored surface film gradually forms. The films (yuba) are successively removed from the surface, hung to air dry and marketed or stored as dried sheets, sticks and chips, or further fabricated into texturized food products. The films can be consumed directly as an ingredient of soups or used as a sheet for wrapping and shaping ground meats or vegetables into various forms.

The protein digestion rate of protein-lipid film is almost 100%. Protein-lipid film is a very popular food material in China; the yield per year was over 200,000 tons at the end of 20th century. Protein-lipid film from soyabeans is called yuba and is one of the popular traditional soybean foods in Japan as well. The formation mechanism of protein-lipid film is entirely different from that of tofu which is another traditional soybean food.

Tofu is a kind of gel formed by the addition of solidification reagents such as $CaCO_3$ and is mainly composed of protein, lipid and water. On the other hand, protein-lipid film is formed as a result of endothermic polymerization of heat denatured proteins or lipoprotein monomers at the liquid surface promoted by surface dehydration. Heating of soymilk leads to a change in the three-dimensional structure of proteins and results in exposing sulphydryl groups and hydrophobic side chains. In tofu processing, proteins create a framework, while lipid and water are buried in networks. Therefore, high protein concentration is beneficial for tofu gel formation. During the film-formation of yuba, lipid acts as a surfactant which moves to the air/water interface and interacts with proteins by hydrophobic interactions.

Furthermore, some of the lipids can be buried in a protein network structure during the protein-lipid film formation. It has also been widely suggested that protein creates a framework in the protein-lipid film structure, while lipids are dispersed in it as droplets. Since protein and lipid account for about 80% of the film based on a dry basis, it is assumed that the effects of soybean cultivars on the productivity of protein-lipid film was mainly due to the concentrations of protein and lipid in the soymilk. Therefore, suitability of soybean cultivars for protein-lipid film production would be different from that for tofu making.

Various reports have shown the effect of protein and lipid contents on the productivity of protein-lipid film. Wu and Bates (1972) observed that poor productivity of protein-lipid film occurred in systems with low protein-lipid ratio under 1.00. The suitability of soybean cultivars for protein-lipid film production is still not clear. Protein-lipid film producers often think that the protein content of soybean is “the higher, the better” from the experience of tofu making. Thereby, they tend to select the soybeans with high protein content. However, since the formation mechanism of protein-lipid film is different from that of tofu, there may be some other factors than protein content which are dominant in protein-lipid film productivity.

Edible Films and Coatings

Characteristics and properties

Edible film is defined as a thin layer of material which can be consumed and provides a barrier to moisture, oxygen and solute movement for the food. The material can be a complete food coating or can be disposed as a continuous layer between food components (Guilbert, 1986). Edible films and coatings have received considerable attention in recent years because of their advantages over synthetic films.

The main advantage of edible films over traditional synthetics is that they can be consumed with the packaged products. There is no package to dispose even if the films are not consumed they could still contribute to the reduction of environmental pollution. The films are produced exclusively from renewable, edible ingredients and therefore are anticipated to degrade more readily than polymeric materials. The films can be applied inside heterogeneous foods at the interfaces between different layers of components. They can be tailored to prevent deteriorative inter-component moisture and solute migration in foods such as pizzas, pies and candies. The films can function as carriers for antimicrobial and antioxidant agents (Bourtoom, 2008). Production of edible films causes less waste and pollution, however, their permeability and mechanical properties are generally poorer than synthetic films (Kester and Fennema, 1986). The development of coatings based on polysaccharides has brought a significant increase in their application and in the amount of products that can be treated, extending the shelf life of fruits and vegetables due to the selective permeability of these polymers to O_2 and CO_2. Table 1 summarizes some of these compounds and their effects.

Table 1. Summary of different components of edible films and edible coatings used as active packages

COMPONENTS	EFFECT
Gellan gum	Increase of phenolics
Alginate and gellan gum	Gas permeability modification
Sorbic acid, benzoic acid, sodium benzoate, citric acid	Antimicrobial
Potassium sorbate	Antimicrobial
Nicines, pediocin	Antimicrobial
Natamycin in a chitosan matrix	Antimicrobial
Tea tree essential oil in HPMC matrix	Antimicrobial
Chitosan-oleic acid	Antimicrobial, Shelf life extension, Tissue firmness conservation, Respiration rate reduction and Fungistatic
Essential oils	Antimicrobial and antioxidant

Source: Falguera *et al.* (2011)

Some researchers have proved the effectiveness of edible films and coatings on the control of browning processes and polyphenol oxidase activity. In the application of chitosan coatings on "Daw" longan (Dimocarpus longan Lour.) fruits, findings revealed that these treatments reduced increasing activities of polyphenol oxidase during the 20 days of storage at 4^{o}C, slightly reducing pericarp browning. Chitosan coatings were also used by Eissa (2008), who found that they delayed discolouration associated with reduced enzyme activity of polyphenol oxidase and other enzymes, and had a good effect on the evolution of colour characteristics and parameters of fresh-cut mushroom during storage at 4^{o}C.

Oil consumption reduction in deep-fat fried products

Deep-fat frying is a widely used method in the preparation of tasty food with an attractive appearance. The tenderness and humidity of the inner part of these products combined with a porous crunchy crust provides an increase in palatability that is responsible for their great acceptance. The development of more acceptable products

for consumers, who are increasingly more conscious and concerned about their health, has led to the need to reduce oil incorporation during the frying process.

Albert and Mittal (2002) carried out an extensive piece of work comparing eleven hydrocolloid materials including gelatine, gellan gum, k-carrageenan-konjac-blend, locust beans gum, methyl cellulose (MC), microcrystalline cellulose, three types of pectin, sodium caseinate, soy protein isolate (SPI), vital wheat gluten and whey protein isolate (WPI), as well as some composite films made of different combinations of these compounds. Two of them, SPI/MC and SPI/WPI composite coatings, provided the highest index reduction in fat uptake/decrease of water loss value, and reduced the fat uptake up to 99.8%.

Transport of bioactive compounds

Consumers require fresh and minimally processed foods that are exempt from chemically synthesized substances and look for those enriched with natural substances that bring health benefits and maintain nutritional and sensory characteristics (Falguera *et al.*, 2011). Rojas-Grau *et al.* (2007) proved the ability of edible coatings based on sodium alginate and gellan gumto transport N-acetylcysteine and glutathione as anti-browning agents, besides the positive effect of the addition of vegetable oils in these edible coatings to increase resistance to water vapor transport in minimally processed fruits of Fuji apple. The transport and release of various active compounds (antioxidants, flavourings, anti-browning and antimicrobial compounds, vitamins or enzymes) is one of the most important aspects within the features of edible films and coatings.

Shelf life extension of highly perishable products

One of the most important uses of edible films and coatings is focused on the shelf life extension of horticultural products. Carrot is one of the most popular vegetables, but its marketing is limited by its rapid deterioration during storage, mainly due to physiological changes that reduce its shelf life. The product suffers a loss of firmness, with the production and release of a characteristic odour generated by anaerobic catabolism, due to high respiration rate and microbial spoilage (Barry-Ryan *et al.*, 2000). Durango *et al.* (2006) developed coatings based on yam (Dioscorea sp.) starch and chitosan. The maximum antimicrobial activity was obtained in the edible coating containing 1.5% of chitosan, which was completely effective on the growth of molds and yeast reducing the count by 2.5 log unit in the carrot stick that were stored for 15 days. Coating with a chitosan concentration of 0.5% controlled the growth of mold and yeast for the first 5 days of storage. After this time, tested samples generated a count similar to the one of the control sample. Thus the use of antimicrobial coatings based on chitosan and yam starch significantly inhibited the growth of lactic acid bacteria, total coliforms, psychrotrophic microorganism, mesophilic aerobes, molds and yeast.

Composite films

Edible films and coatings may be heterogeneous in nature, consisting of a blend of polysaccharides, protein, and/or lipids. This approach enables one to utilize the distinct functional characteristics of each class of film former (Kester and Fennema, 1986). The combination between polymers to form films could be from proteins and carbohydrates, proteins and lipids, carbohydrates and lipids or synthetic polymers and natural polymers.

The main objective of producing composite films is to improve the permeability or mechanical properties as dictated by the need of a specific application. These heterogeneous films are applied either in the form of an emulsion, suspension, or

dispersion of the non-miscible constituents, or in successive layers (multilayer coating or films), or in the form of a solution in a common solvent.

The method of application affects the barrier properties of the films obtained (Guilbert, 1986). Kamper and Fennema (1984) introduced the emulsion films from methyl cellulose and fatty acids to improve water vapor barrier of cellulose films. Recently, many researchers have extensively explored the development of composite films based on the work of Kamper and Fennema (1984). Examples of these studies include using lipid and hydroxypropyl methyl cellulose (Hagenmaier and Shaw, 1990), methyl cellulose (MC) and lipid (Greener and Fennema, 1989b), MC and fatty acid (Sapru and Labuza, 1994), corn zein, MC and fatty acid (Park *et al*., 1996), whey isolate and lipids (McHugh and Krochta, 1994), casein and lipids (Avena Bistillos, 1993), gelatin and soluble starch (Arvanitoyannis *et al.,* 1997), hydroxypropyl starch and gelatin, corn zein and corn starch (Ryu *et al*., 2002), gelatin and fatty acid (Bertana *et al*., 2005), soy protein isolate and gelatin (Cao *et al*., 2002), soy protein isolate and polylactic acid (Rhim et al., 2007).

Modifications of edible protein films

Modification of edible protein films by chemical method

Chemical treatments with acid, alkali or crosslinking agents have been extensively used to improve the properties of films. Hydrolyzed protein results in greater solubility at high pH and high temperature. Guilbert (1986) reported that denatured protein forms less flexible and transparent but more moisture-resistant films. Theoretically, the more protein interaction from chemical treatment such as alkaline or acid modification would occur with extended chain structures; less permeability and greater tensile strength should be obtained. However, Brandenburg *et al*. (1993) found that alkaline treatment on soy protein isolate did not affect water vapor permeability, oxygen permeability and tensile strength. However, alkaline treatment improved a film's appearance (making it clearer, more uniform, with less air bubbles) and elongation at breaking points. The presence of reactive functional groups in the amino acid side chain of protein makes this crosslinking process possible through chemical, enzymatic or physical treatments.

Chemical agents used for covalent cross-linking of protein have included formaldehyde, Glutaraldehyde, glyoxal and others (Orliac, *et al*., 2002; Hernandez-Munoz *et al.,* 2004). Formaldehyde is the simplest of cross-linking agents and has the broadest reaction specificity. Although formaldehyde contains a single functional group, it can react bi-functionally and can therefore crosslink. Protein cross-linking by glyoxal involves lysine and arginine side chain groups (Marquie, 2001) at alkaline pH. The expected reaction scheme (Gueguen *et al.,* 1998) is as shown on. The reaction between formaldehyde and protein is a two step process: the first step corresponds to the formation of the methylol compound and the second one corresponds to the formation of methylene bridges that is cross-links between protein chains.

Hernandez-Munoz *et al*. (2004) reported that addition of cross-linking agents to the film-forming solution of glutenin-rich films with glutaraldehyde (GTA), glyoxal (GLY) and formaldehyde (FA) enhances the water barrier properties of the films, an increase in the resistance to breakage, and decreased film deformability (Table 2). The formation of more resistant films suggests the occurrence of new covalent bonds between glutenin proteins via chemical reaction through FA, GTA and GLY and amino acid side chain reactive groups.

The FA is the most effective cross-linker in terms of these properties. Formaldehyde is a low molecular weight molecule and could easily migrate between the protein chains and establish new covalent bonds with the Lys, Cys and His groups of the proteins (Gallieta *et al.,* 1998). In addition, the higher TS values for films treated with formaldehyde can be due to the lack of specificity of this chemical with respect to the different amino acid side chain groups.

Table 2. Tensile strength (TS) and percentage of elongation at break (%E) for glutenin-rich films plasticized with 33% glycerol (w/w) at different concentrations of cross-linker

Treatment	TS (MPa)	%E
Control	5.9 +0.8	260 + 17
Formaldehyde(%)		
2	13.8 + 1.8	100 + 27
4	13.4 + 0.9	96 + 22
8	13.1 + 1.5	96 + 18
Glutaraldehyde (%)		
2	8.6 + 1.1	131 + 32
4	9.6 + 2.3	165 + 22
8	9.1 + 0.8	164 + 24
Glyoxal(%)		
2	7.2 + 0.9	206 + 39
4	7.7 + 1.3	209 + 33
8	7.9 + 0.9	199 + 37

Source: Hernandez-Munoz *et al.* (2004)

Modification of edible protein films by enzymatic treatment

Many studies have been carried out in an attempt to improve the performance of protein films. The alternative to improving protein film functionality is to modify the polymer network through the cross-linking of the polymer chains. An enzyme that has received extensive recent attention for its capacity to cross-link protein is transglutaminase. Transglutaminase (Tgse, protein-glutamine γ-glutamyl transferase, E.C.2.3.2.13) catalyzes acyltransfer reactions between λ-carboxyamide groups of glutamine residues (acyl donor) and ε-amino groups of lysine residues (acyl acceptor), resulting in the formation of ε-(λ-glutaminyl) lysine intra and intermolecular cross-linked proteins (De Jong and Koppelman, 2002). The reaction catalyzed by glutamyltransferases is shown in Figure 1 (Yee *et al*., 1994). The formation of the cross-linking does not reduce the nutritional quality of the food as the lysine residue remains available for digestion. In the past, the limited availability and high cost of transglutaminase limited its applications.

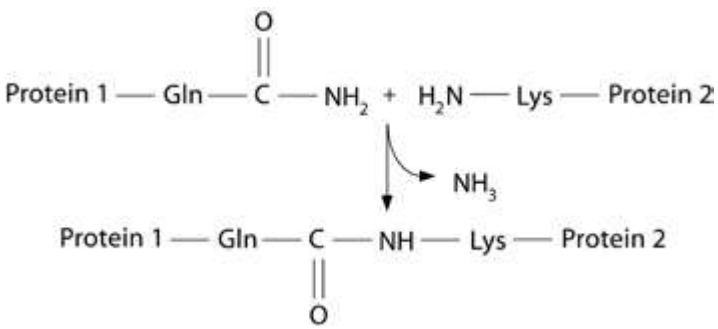

Figure 1. Cross-linking reactions catalyzed by glutamyltransferases (Source: Yee *et al.*, 1994)

Polymerization using transglutaminase has been investigated with various protein sources including a-casein, soy proteins and gelatin, where different responses in gel strength were dependant on the reaction conditions and on the different protein sources (Sakamoto *et al*., 1994). The increase in gel strength of proteins submitted to the action of transglutaminase depended on the order and intensity by which the enzyme produced cross-links, and the extent to which these new covalent linkages could impede the 'physical' cross-linkages occurring during denaturation and formation of the triple helix during gel formation (Babin and Dickinson, 2001). Mahmoud and Savello (1993) investigated the production of whey protein films using transglutaminase as the catalytic cross-linking enzyme. Transglutaminase could catalyze the covalent polymerization of whey protein. However, the effect of using transglutaminase on a film's permeability was not available. Stuchell and Krochta (1994) studied enzymatic treatments on edible soy protein films.

The results showed that treatment with horseradish peroxidase provided no further improvement in water vapor permeability, but increased tensile strength and protein solubility and decreased elongation. Yildirim *et al*. (1996) prepared biopolymer from crosslinking whey protein isolate and soybean by transglutaminase. The biopolymer showed excellent stability, thus using polymers should result in the formation of better water moisture barrier films. Larre *et al*. (2000) showed that transglutaminase was effective in introducing covalent bonds into films obtained from slightly deamidated gluten. The establishment of these covalent bonds induced the formation of polymers of high molecular weight that were responsible for the greater insolubility of the treated films but a reduced surface hydrophobicity. Mechanical properties showed that the addition of covalent bonds by the use of transglutaminase increased the film's integrity and heavy-duty capacity as well as its capacity to stretch (Table 1).

Modification of edible protein films by combination with hydrophobic materials

The barrier properties of bio-polymeric films are important parameters when considering a suitable barrier for use in foods and food packaging. Protein films are generally good barriers against oxygen at low and intermediate relative humidity (RH) and have good mechanical properties, but they are poor barriers against water vapor. Being a poor barrier is due to their hydrophilic character. In many applications, a better barrier against water vapor is preferable since low levels of water activity must be maintained in low-moisture foods to prevent texture degradation and to minimize deteriorative chemical and enzymatic reactions (Kester and Fennema, 1986). Therefore, the hydrophobic properties of lipids are exploited for their great water barrier properties, and especially high melting point lipids, such as beeswax or carnauba wax (Shellhammer and Krochta, 1997).

A composite film made of a protein and a lipid can be divided into laminates (in which the lipid is a distinct layer within or atop the biopoly-meric films) and emulsions (in which the lipid is uniformly dispersed throughout the biopolymeric film). Both the laminate and emulsion films offer advantages. The laminate films are easier to apply with regard to the temperature, due to the distinct natures of the support matrix and lipid (Koelsch, 1994). During the casting of the lipid onto the protein film, the temperatures of the film and lipid can easily be controlled separately. When producing the emulsion films, the temperature of the emulsion must be above the lipid-melt

temperature but below the temperature for solvent volatilization of the structural network.

The main disadvantage of the laminated films, however, is that the preparation technique requires four stages; two casting and two drying stages. This is why the laminated films are less popular in the food industry despite their being good barriers against water vapor. The preparation of the emulsion films requires only one casting and one drying stage, but the finished films are still rather poor barriers against water vapor, since the water molecules still permeate through the non-lipid phase. The reason for this is the nonhomogeneous distribution of lipids. However, they have the advantages of exhibiting good mechanical resistance, and to require a single step during the manufacture and application process, against one step per layer for multilayer films. It has been shown that for emulsion-based films the smaller the lipid globule size is, and the more homogeneously distributed they are, the lower the water vapor permeability (McHugh and Krochta, 1994).

Many researchers have examined the water vapor permeability and mechanical properties of composite films made from proteins with added lipids. For example, composite protein-lipid films had lower water vapor permeability values than control protein films from caseinates (Avena-Bustillos and Krochta, 1993), whey protein (McHugh and Krochta, 1994; Banerjee and Chen, 1995), zein (Weller *et al*., 1998), and wheat gluten (Gennadios *et al*, 1993; Gontard *et al*., 1994). McHugh and Krochta (1994a) developed whey protein-lipid emulsion films and found that the water vapor permeability of films was reduced through lipid incorporation. Fatty acid and beeswax emulsion films exhibited very low water vapor permeability. Gontard *et al*. (1994) reported that beeswax was the most effective lipid to improve moisture barrier of films prepared from wheat gluten.

Protein and lipid interactions in the production of protein lipid film

The nature of protein-lipid interactions

Native proteins are able to bind lipid in two main ways, either in a cavity or binding, or through less well defined hydrophobic patches which lie close to the surface of the protein. Both types of proteins have been found to be interfacially active. One example from milk is the major whey protein β-lactoglobulin, which binds a wide variety of aliphatic components in its binding site, including lipids (Perez *et al*., 1992), whilst from cereals the nonspecific lipid transfer proteins (ns LTP) seem to have an important functional role at interfaces, especially with regards to beer foam stability (Sorensen *et al*., 1993) and is known to bind lipids in a central pocket. One of the remarkable features of many of the lipid binding sites found in these proteins is their ability to accommodate a wide range of aliphatic molecules, and some mammalian LTPS have been found to bind simple lipids, but also expand in size to accommodate triglycerides (Bruce *et al*., 1998).

An example of a protein group with an accessible hydrophobic lipid binding site is the wheat puroindolines (PINs). Their tryptophan rich regions are able to bind a variety of lipids (Wilde *et al*., 1993; Kooijman *et al*., 1997). Despite belonging to the same supergene family as the ns LTPs and sharing a great deal of structural homology, PINs appear to bind lipid by a different mechanism, although this will only be defined once their three dimensional structures have been determined. They also have a functional effect on the stability of air-water interfaces in foods. Thus, the addition of PIN has been found to aid the recovery of beer foam that has been adversely affected by lipid

(Clark *et al*., 1994) possibly by binding residual free lipids in such a way that they no longer cause collapse of the bubble network.

The importance of the interfacial properties of PIN in food systems has also been indicated by its effect on the crumb structure of bread (Dubreil *et al*., 1998). As well as naturally occurring lipid-binding sites, new binding sites can be induced by processing using heat or pressure, or as a consequence of the pH and ionic strength of a food system. Thus, the proteins may unfold to reveal the more hydrophobic sites normally only present in the centre of the protein, or the conditions may cause dimeric proteins to dissociate to reveal the hydrophobic faces normally buried in the dimmer interface. Such alterations form the basis of the treatments frequently used to potentially raise the functional properties of food protein ingredients.

Protein stabilised foams and emulsions

The texture and organoleptic properties of many foods arise as a consequence of their multiphase nature. Thus they may comprise a liquid and an air phase to form a foam structure, such as is found in bread, cakes, mousses and beers, or a liquid and an oil phase to form an emulsion, such as is found in sauces, gravies, and spreads. Foams and emulsions share a common feature; they are a dispersion of one phase (dispersed phase) in another (continuous phase). The two phases are immiscible and the successful stabilisation of the dispersed phase within the continuum results in very different structural and rheological properties to those of the individual phases (Fillery-Travis et al., 2000).

For example, whipping a solution of egg albumin results in a very viscous foam, the textural properties of which are completely different to those of the parent solution or the entrapped air. Molecules that are able to stabilise foams and emulsions must be surface active and form an adsorbed layer at the boundary or interface between the different phases. As a consequence they must possess both hydrophobic and hydrophilic regions within their structure and are, by definition, amphiphilic. The two main classes of amphiphilic molecules used within food dispersions are proteins and low molecular weight surfactants or emulsifiers. This review will focus on the role of proteins in stabilising air-water and oil-water interfaces, and their interactions with lipid at the interface.

Protein stabilized interfaces

For a soluble protein to significantly lower the interfacial tension between two phases, it must first undergo a rearrangement of its structure to expose hydrophobic amino acids to the hydrophobic phase. Thus, the rate of lowering of the interfacial tension can be very slow compared to emulsifiers or surfactants (Wilde, 1996). In addition, proteins are larger than surfactants, so their diffusion to the interface is slower. This rate of change in interfacial tension equates directly to the amount of interfacial area created over the short time periods during homogenisation and foam generation.

Therefore, proteins are generally less efficient at creating dispersions and the foams and emulsions created by surfactants and emulsifiers tend to have smaller droplet and bubble size distributions for a given energy input. The mechanism of interfacial stabilisation for proteins and small molecule surfactants also differs considerably. Surfactants or emulsifiers form a very dense, fluid interfacial layer and can reduce the interfacial tension between the two phases to very low values, which corresponds to large increases in surface area. They prevent the dispersion breaking or coalescing by

interfacial movement known as the Gibbs-Marangoni mechanism (Figure 3b), which restabilises any interfacial tension gradients which occur.

Similarly, proteins adsorb at the interface and lower the interfacial tension enabling dispersion, but to prevent coalescence they unfold at the interface and interact with neighbouring proteins through electrostatic, hydrophobic and covalent interactions to form a viscoelastic adsorbed film. The mechanical strength of the viscoelastic adsorbed layer created by proteins is extremely efficient at preventing coalescence in both foams and emulsions, it is also capable of retarding drainage from foams (Wilde 1996).

Protein-lipid interactions at the interface

Whilst both proteins and surfactants can both stabilise foams successfully, problems arise when both are present at the surface. The mobility of the surfactants is compromised by the protein and the visco-elasticity of the protein adsorbed layer is reduced by the presence of the surfactant. This often results in coalescence (Wilde, 1996), a major problem in many food foam systems, but which can also be exploited in the case of antifoaming agents where foam presents a problem during food production.

The shear dependant elasticity of the protein alone shows a long linear region, before at high shear, the surface elasticity is broken down and the interface yields. In the presence of surfactant, the elasticity is reduced, and also the stress at which the surface yields is much lower, demonstrating how the stability of foams and emulsions stabilised by these mixtures can be compromised. This effect can be visualised by imaging the interface using atomic force microscopy (AFM). This methodology involves adsorbing a protein and/or surfactant to an air/water interface, then transferring a section of the surface onto a mica sheet for measurement by the AFM (Mackie, *et al.,* 1999). The AFM .feels' the surface of a sample using a piezo crystal driven device, to give a three dimensional map of a surface with very high resolution. Figure 2 shows an AFM image of a mixed protein: surfactant surface, the dark areas represent the surfactant and the light regions denote thicker, protein areas. It is possible to visualise how the presence of the surfactant domains has reduced the elasticity of the protein network. It is also possible to imagine how the mobility of the surfactant is restricted or caged, which severely limits the ability of the surfactant to stabilise foam lamellae by the Gibbs-Marangoni mechanism, resulting in poor foam stability due to increased rates of coalescence (Wilde, 1996). This also indicates how important the critical stress is for foam drainage. Low stress circumstances such as model foam lamellae drainage will display rigid, elastic properties, but higher stress events such as foam drainage and deformation may cause the breakdown of the surface, and lead to a fluid interface, and different drainage behaviour.

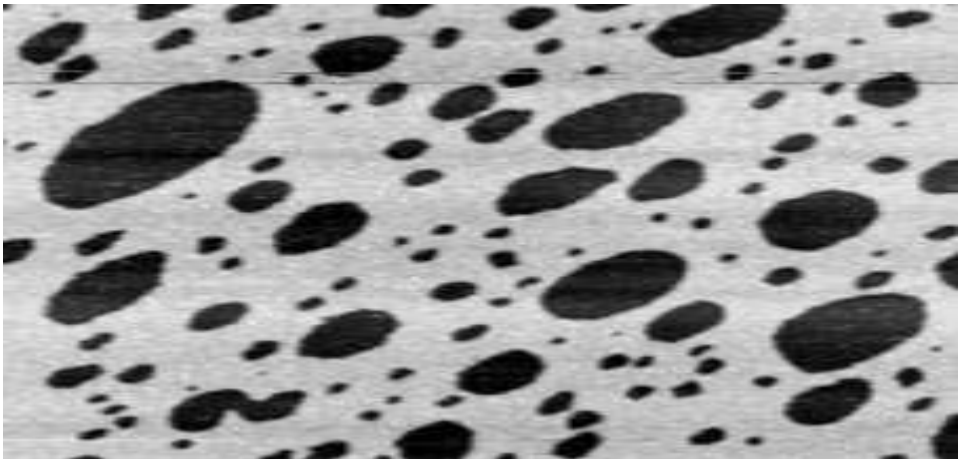

Figure 2: An AFM image of a mixed protein (α-casein):surfactant (Tween 20) surface. The darker regions represent thin, fluid surfactant domains. The light areas represent the thicker, viscoelastic, protein network.

The influence of protein structure and stability on interfacial behaviour

The main physicochemical properties which define the ability of a protein to form and stabilize dispersions are size, solubility, hydrophobicity, charge and flexibility. In addition to the effect of size on the rate of adsorption to an interface, a protein must be soluble to be available to adsorb. Insoluble proteins tend to form large aggregates which are incapable of rapid diffusion and adsorption, and result in poor quality dispersions.

The hydrophobicity of a protein will also determine the interfacial tension value which changes as the number and density of hydrophobic residues at the interface increases (Toledano and Magdassi, 1998). Increased surface hydrophobicity has also been associated with increased rates of adsorption. Protein flexibility is another important factor in protein adsorption (Mitchell, 1986; Townsend and Nakai, 1983) and affects the final number of hydrophobic residues exposed to the surface and the rate. Finally charge can affect a proteins interfacial behaviour in two ways, through interfacial protein-protein interactions, and electrostatic repulsion. The former are noncovalent in nature (comprising of van der Waals forces and H-bonds) and contribute to the overall interfacial visco-elasticity by strengthening the gel layer of protein at the interface.

The contribution of charge is highlighted by the observation that surface visco-elasticity reaches a maximum when the pH is close to a proteins' isoelectric point, i.e., its net charge is zero. The structure of the adsorbed protein can also influence stability of the dispersion through longer range colloidal forces which prevent aggregation/flocculation. Pair-wise interaction potentials for emulsion or suspension particles consider three major contributions to the interaction; van der Waals attraction, electrostatic repulsion and steric hindrance. By increasing the overall charge on a dispersed phase droplet through the charge of the adsorbed protein the dispersion can be stabilised by the electrostatic repulsion of the droplets hindering their close approach. Reduction of the charge can have drastic effects on dispersion stability (Husband *et al*., 1997). Similarly, the presence of large hydrophilic loops or tails can provide a steric hindrance to particle encounter.

Conclusion

For many years studies of food systems at the molecular level have, partly out of necessity arising from the limitations of the analytical methodologies, been largely limited to investigating single components within a food system. However, much is lost with such an approach and it is only as the interactions between food components become understood that the functional properties of food systems, so clearly and well characterised at the macro level, can be explained. The present review shows how we are applying such an approach to studying the lipid-protein interactions in multiphase food systems and hence defining the role they play in determining the bulk properties of such foods. Only through such endeavours will we be able to develop knowledge-based strategies for improving the utilisation of raw materials and increasing processing efficiency.

The concept of edible films and coatings represents a stimulating route for creating new packaging materials. This is because edible films and coatings are available with a wide range of properties that can help to alleviate many problems encountered with foods. Edible films can be produced from materials with film forming ability. Components used for the preparation of edible films can be classified into three categories:

hydrocolloids, lipids and composites. Hydrocolloid films possess good barrier properties to oxygen, carbon dioxide, and lipids but not to water vapor. Most hydrocolloid films also possess superb mechanical properties, which are quite useful for fragile food products. However, potential functions and applications of the films and coatings warrant increased considerations.

Extensive research is still needed on the methods of films formation and methods to improve film properties and the potential applications.

References

Ahmad, M. and Khan, I.. Effect of waxing and cellophane lining on chemical quality indices of citrus fruit. *Plant Foods Human Nutrition* Vol. 37 (1992). pp 47- 57.

Alikonis, J. J. Candy Technology. Westport CT: AVI Publishing Company, Inc. 1979.

Andrady, A. L. and Xu, P.. Elastic behaviour of chitosan films. *Journal of Polymer Science* Vol.5 (1997), pp. 307- 521.

Anand,G., Juilee, T. and Murali, S.. Protein diffusion into thermally evaporated lipid films: role of protein charge/mass ratio. Colloids and surface B: *Biointerfaces* Vol. 28 (2003), pp. 209-214

Arvanitoyannis, I., Nakayama, A. and Aiba, S.. Edible films made from hydroxypropyl starch and gelatin and plasticized by polyols and water. *Carbohydrate Polymers* Vol. 36 (1998), pp. 105-119.

Arvanitoyannis, I., Psomiadou, E., Nakayama, A., Aiba, S. and Yamamoto, N. Edible films made from gelatin, soluble starch and polyols, Part 3. *Food Chemistry* Vol. 60 (1997), pp. 593-604.

Avena-Bustillos, R. J. and Krochta, J. M.. Water vapor permeability of caseinate-based edible films as affected by pH, calcium crossing and lipid content. *Journal of Food Science Vol.* 58 (1993), pp. 904-907.

Babin, H. and Dickinson, E.. Influence of transglutaminase treatment on the thermoreversible gelation of gelatin. *Food Hydocolloids Vol.* 15 (2001) pp. 271–276. .

Baker, R. A., Baldwin, E. A., and Nisperos-Carriedo, M. O.. Edible coatings and films for processed foods. In Krochta, J.M., Balwin, E. A. and Niperos-Carriedo M.O. (Eds.). Edible Coatings and Films to Improve Food Quality, 1994 p. 89-104. Lancaster, Basel: Technomic Publishing.

Banerjee, R. and Chen, H.. Functional properties of edible films using whey protein concentrate. *Journal of Dairy Science* Vol.78 (1995), pp. 1673-1683.

Bertana, L. C., Tanada-Palmua, P. S., Sianib A. C. and Grosso C. R. F.. Effect of fatty acids and 'Brazilian elemi' on composite films based on gelatin. *Food Hydrocolloids* Vol.19 (2005), pp. 73–82.

Bourtoom,. Edible films and coatings: Characteristics and properties. *International food research journal* Vol.15 (2008), pp 33-41

Bourtoom,. Edible protein films; Properties enhanced. *International food Research Journal* Vol.16 (2009), Vol. 1-9

Bradenburg, A. H., Weller, C. L. and Testin, R. F.. Edible films and coatins from soy protein. *Journal of Food Science* Vol.58 (1993), pp. 1086-1089.

Brault, D., DAprano, G. and Lacroix, M.. Formation off re- standing sterilised edible films from irradiated caseinates. *Journal of Agricultural and Food Chemistry* Vol. 45 (1997), pp. 2964–2969.

Bruce C., Beamer, L.J., Tall, A.R.. The implications of the structure of the bacteriacidal/permeability increasing protein on the lipid-transfer function of the cholesteryl ester transfer protein. Current Opinion in *Structural Biology* Vol.8, (1998), pp 426-434.

Butler, B. L., Vergaro, P. J., Testin, R. F., Bunn, J. M., and Wiles, J. L.. Mechanical and barrier properties of edible chitosan films as affected by composition and storage. *Journal of Food Science* Vol. 61 (1996), pp. 953-961.

Cao, N., Fua, Y. and He, Y.. Preparation and physical properties of soy protein isolate and gelatin composite films. LWT-*Food Science and Technology* Vol. 35 (2002), pp. 680–686.

Cheftel, J. C., Cuq, J. L. and Lorient, D.. Amino acids, peptides, and proteins. In Fennema, O.R. (Ed.), *Food Chemistry,* Vol. 6 (1985) pp. 279-343. New York: Marcel Dekker.

Clark, D.C., P.J. Wilde, Marion, D.. The protection of beer foam against lipid-induced destabilisation. *Journal of the Institute of Brewing* Vol.100, (1994), pp. 23-25.

Cohen, E., Shalom, Y. and Rosenberger, I.. Posthar vest ethanol buildup and off-flavour in 'Murcott' tangerine fruits. *Journal of American Society of Horticultural and Science* Vol. 115 (5) (1990), pp. 775–778.

Davies, D. H., Elson, C. M. and Hayes, E. R.. N, O-carboxymethyl chitosan, a new water soluble chitin derivative. In Skjak-Braek, G., Anthosen, T. and Sandford, P. (Eds.). Chitin and Chitosan: Source, Chemistry, Biochemistry, Physical Properties, and Application, 1989 p. 467-472. New York: Elsevier Applied Science

Debeaufort, F. and Voilley, A. Z.. Effect of surfactants and drying rate on barrier properties of emulsified films. *International Journal of Food Science and Technology* Vol. 30 (1995), pp. 183-190.

Debeaufort, F., Martin-Polo, M. and Voilley, A.. Polarity homogeneity and structure affect water vapor permeability of model edible films. *Journal of Food Science* Vol. 58 (1993), pp 426-434.

De Jong, G. A. H. and Koppelman, S. J.. Transglutaminase catalyzed reactions: impact on food applications. Journal of Food Science 67: 2002 2798– 2806.

Dickey, L. C. and Parris, N.. Ethanolic extraction of zein from maize. *Industrial Crops and Products an International Journal* Vol.13 (2001), pp 67–76.

Dickey, L. C. and Parris, N.. Serial batch extraction of zein milled maize. *Industrial Crops and Products an International Journal* Vol.15 (2002), pp 33–42.

Dubreil, L., Méliande, S., Chiron, H., Compoint, J.-P., Quillien, L., Branlard, G., Marion, D.. Effect of purindolines on the breadmaking properties of wheat flour. *Cereal Chemistry* Vol. 75 (1998), pp 222-229.

El Ghaouth, A., Arul, J., Ponnampalam, R. and Boulet, M. Chitosan coating effect on stability of fresh strawberries. *Journal of Food Science* Vol. 57 (1991a) pp 1618-1620.

El Ghaouth, A., Arul, J., Ponnampalam, R. and Boulet, M.. Use of chitosan coating to reduce water loss and maintain quality of cucumber and bell pepper fruits. *Journal of Food Processing and Preservation* Vol.15 (1991b), pp. 359-368.

Elson, C. M. and Hayes, E. R.. Development of the differentially permeable fruit coating Nutri- Save® for the modified atmosphere storage of fruit. Proceedings of the 4th National Controlled Atmosphere Research Conference: Controlled Atmosphere for Storage and Transport of Perishable Agricultural Commodities, 1985 p. 248-262. Raleigh, m North Carolina.

Falguera, V., Juan, P.Q., Alberto, J., Jose, A.M., Albert, I.. Edible films and coatings: structures, active functions and trends in their use. *Trends in food science and technology* Vol. 22 (2011), pp 292-308

Filali-Mouhim, A., Audette, M., St-Louis, M. *et al*, Thauvette, L., Denoroy, L., Penin, F., Chen, X., Rouleau, N., Le, C. J., Rossier, J., Potier, M. and Le, M. M.. Lysozyme fragmentation induced by g-radiolysis. *International Journal of Radiation Biology* Vol.72 (1997), pp 63– 70.

Fillery-Travis A., Mills, E., Wilde, P.. Protein-lipid interaction at interfaces. Grasas Aceites 51: 2000 50-55.

Galietta, G., di Gioia, L., Guilbert, S. and Cuq, B.. Mechanical and thermomechanical properties of films based on whey proteins as affected by plasticizer and cross-linking agents. *Journal of Dairy Science* Vol. 81(1998), pp. 3123–3130.

Gennadios, A., Brandenburg, A. H., Weller, C. L. and Testin, R. F.. Effect of pH on properties of wheat gluten and soy protein isolate films. *Journal of Agricultural and Food Chemistry* Vol.41 (1993), pp. 1835-1839.

Gennadios, A., McHugh, T. H., Weller, C. L. and Krochta, J. M.. Edible coating and films based on protein. In Krochta, J. M., Balwin, E. A. and Niperos-Carriedo, M. O. (Eds.). Edible Coatings and Films to Improve Food Quality, 1994 p. 201-277. Lancaster. Basel: Technomic Publishing.

Gontard, N., Duchez, C., Cuq, J.-L. and Guilbert, S.. Edible composite films of wheat and lipids: Water vapour permeability and other physical properties. *International Journal of Food Science and Technology* Vol. 29 (1994) pp. 39–50

Gontard, N., Guilbert, S. and Cuq, J. L.. Edible wheat gluten film: Influence of the main process variable on film properties using response surface methodology. *Journal of Food Science* Vol.57 (1992), pp 190-195.

Gueguen, J., Viroben, G,, Noireaux, P. and Subirade, M.. Influence of plasticizers and treatments on the properties of films from pea protein. *Industrial Crops and Products an International Journal* Vol.7 (1998), pp 149–57.

Guilbert, S.. Technology and application of edible protective films. In Mathlouthi, M. (Ed.), Food packaging and preservation, 1986 p. 371–394. London, UK: Elsevier Applied Science.

Greener, I. K. and Fennema, O.. Barrier properties and surface characteristics of edible bilayer films. *Journal of Food Science* Vol.54 (1989a), pp 1393-1399.

Greener, I. K. and Fennema, O.. Evaluation of edible, bilayer films for use as moisture barrier for food. *Journal of Food Science* Vol.54 (1989b), pp 1400-1406.

Hagenmaier, R. D. and Baker, R. A.. Reduction in gas exchange of citrus fruit by wax coatings. *Journal of Agricultural and Food Chemistry* Vol.41 (2) (1993) pp. 283-287.

Hagenmaier, R. D. and Shaw, P. E.. Moisture permeability of edible films made with fatty acid and (hydrocypropyl) methylcellulose. *Journal of Agricultural and Food Chemistry* Vol.38(9) (1990), pp 1799-1803.

Hernandez, E.. Edible coating from lipids and resins. In Krochta, J. M., Balwin, E. A. and Niperos- Carriedo, M. O. (Eds.). Edible Coatings and Films to Improve Food Quality, 1994 p. 279-303. Lancaster. Basel: Technomic Publishing.

Hernandez-Munoz, P., Villalobos, R. and Chiralt, A.. Effect of cross-linking using aldehydes on properties of glutenin-rich films. Food Hydrocolloids 18: 2004 403– 411.

Husband, F.A., Wilde, P.J., Mackie, A.R., Garrood, M.J.. A comparison of the functional and interfacial properties of □-casein and dephosphorylated □-casein. *Journal of Colloid and Interface Science*, Vol.195, (1997), pp 77-85.

Kamper, S. L. and Fennema, O. N.. Water vapor permeability of an edible fatty acid, bilayer films. *Journal of Food Science* Vol.49 (1984), pp 1482-1485.

Kaplan, D. L., Mayer, J. M., Ball, D., McCassie, J., Allen, A. L. and Stenhouse, P.. Fundamental of biodegradable polymer. In Ching, C., Kaplan D., and Thomas E. (eds.). Biodegradable Polymers and Packaging, 1993 p. 1-42. Lancaster: Technomic Publishing.

Ke, D. and Kader, A. A.. Tolerance of 'Valencia' oranges to controlled atmospheres as determined by physiological responses and quality attributes. *Journal of American Society of Horticultural and Science*, Vol.115(5) (1990), pp770–783.

Kester, J. J. and Fennema, O. R.. Edible films and coatings: *A review*. *Food Technology* Vol. 40 (1986), pp 47-59.

Kinsella, J. E. and Phillips, L. G.. Film properties of modified proteins. In Kinsella, J. E. and Soucie, W. G. (Eds.). Food Protein, 1979 p.78-99. Champaign, IL: The American Oil Chemists's Society.

Kinsella, J. E., Damodaran, S. and German, B. 1985. Physiochemical and functional properties of isolated proteins with emphasis on soy proteins. In Altschul, A.M. and Wilcke, H. L. (Eds.) New Protein Foods, p. 107-179. Orlando, FL: Academic Press.

Koelsch, C. M. and Labuza, I. P.. Functional, physical and morphological properties of methyl cellulose and fatty acid-based edible film barriers. LWT- *Food Science and Technology* Vol. 25 (1992), pp 404-411.

Koelsch, C. Z.. Edible water vapour barriers: properties and promise. *Trends in Food Science and Technology* Vol.5 (1994), pp 76-81.

Kooijman, M., Orsel, R., Hessing, M., Hamer, R.J. and Bekkers, A.C.A.P.A.. Spectroscopic characterisation of the lipid binding properties of wheat puroindolines. *Journal of Cereal Science* Vol.26, (1997), pp145-159.

Krochta, J. M. and De Mulder-Johnston, C. D. Edible and biodegradable polymer films: challenges and opportunities. *Food Technology* Vol.51 (1997), pp 61-74

Kunte, L. A., Gennadios, A., Cuppett, S. L., Hanna, M. A. and Weller, C. L. Cast films from soy protein isolates and fractions. *Cereal Chemistry* Vol. 74 (1997) pp.115-118.

Lacroixa, M., Le, T. C., Ouattara, B. *et al*, Yu, H., Letndre, M., Sabato, S. F., Mateescu, M. A. and Patterson, G. Use of γ-irradiation to produce films from whey, casein and soya proteins: structure and functional characteristics. *Radiation Physics and Chemistry* Vol.63 (2002), pp 827-832.

Landry, J.. Comparison of extraction methods for evaluating zein content of maize grain. *Cereal Chemistry* Vol.74(2) (1997), pp 188–189

Larre, C., Desserme, J., Barbot, J. and Gueguen, J. Properties of deamidated gluten films enzymatically cross-linked. *Journal of Agricultural and Food Chemistry* Vol.48 (2000), pp 5444–5449.

Mackie, A. R., Gunning, A. P., Wilde, P. J. and Morris, V. J.. The orogenic displacement of protein from the air/water interface by surfactant. *Journal of Colloid and Interface Science* Vol.210, (1999) pp. 157-166.

Mahmoud, R. and Savello, P. A.. Solubility and hydrolyzability of films produced by transglutaminase catalytic crosslinking of whey protein. *Journal of Dairy Science* Vol.76 (1993), pp 29–35.

Mark, A. M., Roth, W. B., Mehltretter, C. L. and Rist, C. E. Oxygen permeability of amylomaize starch films. *Food Technology Vol.*20 (1966), pp 75-80.

Marquie, C.. Chemical reactions in cottonseed protein cross-linking by formaldehyde, glutaraldehyde, and glyoxal for the formation of protein films with enhanced mechanical properties. *Journal of Agricultural and Food Chemistry* Vol.49 (2001), pp 4676–4681.

McHugh, T. H., Aujard, J. F. and Krochta, J. M.. Plasticized whey protein edible films: water vapour permeability properties. *Journal of Food Science* Vol.59 (1994), pp 416-419.

McHugh, T. H. and Krochta, J. M.. Water vapour permeability properties of edible whey protein-lipid emulsion films. JAOCS 71: 1994a 307-312.

McHugh, T. H. and Krochta, J. M.. Dispersed phase particle size effects on water vapour permeability of whey protein-beeswax edible emulsion films. Journal of Food Processing and Preservation 18: 1994b 173-188.

Mitchell, J.R.. Foaming and emulsifying properties of proteins. In Developments in Food Proteins - 4 (1986) p. 291-338, B.J.F. Hudson (Ed.), Elsevier, London.

Murray, D. G., Luft, L. and Low, D. E.. Corn starch hydrolysates. *Food Technology* Vol.27 (1973), pp 32-39.

Murray, J. F.C., Limited, H. and Reigate.. Cellulose. In Phillips, G.O. and Williams, P.A. (Eds.). Handbook of hydrocolloids, 2000 p 219-245. Cambridge, England: Woodhead Publishing Limited.

Muzzarelli, R. A. A.. Chitosan-based dietary foods. *Carbohydrate Polymer* Vol. 29: (1996), pp 309-316

Nelson, K. L. and Fennema, O. R.. Methylcellulose films to prevent lipid migration in confectionery products. *Journal of Food Science* Vol. 56 (1991), pp 504-509.

Orliac, O., Rouilly, A., Silvestre, F. and Rigal, L.. Effect of additives on the mechanical properties, hydrophobicity and water uptake of thermo-moulded films produced from sunflower protein isolate. *Polymer* Vol. 43 (2002), pp. 5417–5425.

Park, H. J.. Edible coatings for fruit and vegetables: Determination of gas diffusivities, prediction of internal gas composition and effects of coating on shelf life. Georgia, USA: University of Georgia, Ph.D. Thesis. 1991.

Park, H. J., Bunn, J. M., Weller, C. L., Vergano, P. J. and Testin, R. F.. Water vapor permeability and mechanical properties of grain protein-based films as affected by mixtures of polyethylene glycol and glycerin plasticizers. *Transaction of the American Society of Agricultural Engineers* Vol. 37: (1994a) pp.1281-1285

Park, H. J., Chinnan, M. S. and Shewfelt, R. L.. Edible corn-zein coatings storage life tomatoes. *Journal of Food Processing and Preservation* Vol.18 (1994b) pp. 317-333.

Park, J. W., Testin, R. F., Vergano, P. J., Park, H. J. and Weller, C. L.. Fatty acid distribution and its effect on oxygen permeability in laminated edible films. *Journal of Food Science* Vol. 61 (1996), pp 401-406.

.Pérez, M.D., Sanchez, L., Aranda, P., Ena, J.M., Oria, R., Calvo, M.. Effect of beta-lactoglobulin on the activity of pregastric lipase. A possible role for this protein in ruminant milk. *Biochimica Biophysica Acta* Vol.123, (1992) pp.151-155.

Petracek, P. D., Dou, H. and Malik, I.. A postharvest pitting of Temple oranges stimulated by high temperature storage and wax application. *Florida State Horticultural Society* Vol.110 (1997), pp 211–214.

Petracek, P. D., Dou, H. and Pao, S.. Influence of applied waxes on postharvest physiological behaviour and pitting of white grapefruit. *Post-harvest Biology and Technology* Vol.14 (1998), pp 99–106.

Rhim, J. W., Gennadios, A., Handa, A., Weller, C. L. and Hanna, M.A. Solubility, tensile, and color properties of modified soy protein films. *Journal of Agricultural and Food Chemistry* Vol.48 (2000(, pp 4937-4941.

Rhim, J. W., Gennadios, A.,Weller, C.L. and Hanna, M.A.. Sodium dodecyl sulfate treatment improves properties of cast films from soy protein isolate. *Industrial Crops and Products an International Journal* Vol.15 (2002) pp.199–205.

Rhim, J. W., Lee, J. H. and Ng, P. K. W.. Mechanical and barrier properties of biodegradable -based films coated with polylactic acid. *LWT - Food Science and Technology* Vol.40 (2007), pp 232-238.

Rodriguez, M., Oses, J., Ziani, K. and Mate, J. I.. Combined effect of plasticizers and surfactants on the physical properties of starch based edible films. *Food Research International* Vol. 39 (2006), pp 840-846.

Ross, P. I.. Gelatin. In Mark, H. F., Bikales, N. M., Overberger C. G., Menges, G. and Kroschwitz, J. I. (Eds.). Encyclopedia of polymer science and engineering, volume 7: Fibers, optical to hydrogenation, 1987 p. 488. New York: Wiley- Interscience.

Ryu, S. Y., Rhim, J. W., Roh, H. J. and Kim, S. S.. Preparation and Physical Properties of Zein-Coated High-Amylose Corn Starch Film, *LWT - Food Science and Technology* Vol.35 (2002), pp 680-686

Sakamoto, H., Kumazama, Y., and Motoki, M. Strength of protein gels prepared with microbial transglutaminase as related to reaction conditions. *Journal of Food Science* Vol. 59 (1994), pp 866–871.

Salame, M.. Barrier polymers. In Bakker, M. (Ed.). The Wiley Encyclopedia of Packaging Technology, 1986 p. 48-54. New York: Johh Wiley and Sons.

Sandford, P. A.. Chitosan: commercial uses and potential application. In Skjak-Braek, G., Anthosen, T. and Sandford, P. (Eds.). Chitin and Chitosan: Source, Chemistry, Biochemistry, Physical Properties, and Application, 1989 p. 51-69. New York: Elsevier Applied Science.

Sapru, V. and Labuza, T. P.. Dispersed phase concentration effect on water vapor permeability in composite methyl cellulose-stearic acid edible films. *Journal of Food Processing and Preservation* Vol.18 (1994), pp 359-368

Schuessler, H. and Schilling, K.. Oxygen effect in the radiolysis of proteins. *International Journal of Radiation Biology* Vol.45 (1984), pp 267–281.

Scope, R. K.. Separation by precipitation. In Scope, R.K. (Ed.), Protein purification; Principles and practice, 1994 p. 71-101. New York: Springer-Verlag.

Shellammer, T. H. and Krochta, J. M.. Whey protein emulsion performance as affected by lipid type and amount. *Journal of Food Science* Vol. 62 (1997), pp. 390–394.

Shukla, R. and Cheryan, M.. Zein: The industrial protein from corn. *Industrial Crops and Products an International Journal* Vol.13 (2001), pp. 171–192.

Sorensen, S.B., Bech, L.M., Muldberj, M., Beenfeldt, T., Breddam, K.. Barley lipid transfer protein 1 is involved in beer foam formation. MBAA Tech. Quart. 30, (1993) 136-145.

Stuchell, Y. M. and Krochta, J. M.. Enzymatic treatments and thermal effects on edible soy protein films. *Journal of Food Science* Vol. 59 (1994), pp 1332–1337.

Subirade, M., Kelly, I., Gueguen, J., and Pezolet, M.. Molecular basis of film formation from a soybean protein: Comparison between the conformation of

glycinin in aqueous solution and in films. *International Journal of Biological Macromolecules* Vol. 23 (1998), pp 241-249.

Toledano, O. and Magdassi S.. Emulsification and foaming properties of hydrophobically modified gelatin. *Journal of Colloid and Interface Science*, Vol. 200 (1988), pp. 235-240.

Townsend, A. and Nakai, S.. Relationships between hydrophobicity and foaming characteristics of food proteins. *Journal of Food Science*, Vol. 48, (1983) pp. 588-594.

Weller, C. L., Gennadios, A. and Saraiva, R. A.. Edible bilayer films from zein and grain sorghum wax or carnauba wax. *LWT- Food Science and Technology* Vol. 31 (1998), pp 279-285.

Wilde, P. J.. Foam measurement by the microconductivity technique: an assessment of its sensitivity to interfacial and environmental factors. *Journal of Colloid and Interface Science* Vol.178, (1996) pp. 733-739.

Wilde, P.J., Clark, D.C., Marion, D.. The influence of competitive adsorption of a lysopalmitoyl phosphatidylcholine on the functional properties of puroindoline, a lipid binding protein isolated from wheat flour. *Journal of Agricultural and Food Chemistry* Vol. 41, (1993), pp. 1570-1576.

Whistler, R. L. and Daniel, J. R.. Carbohydrate. In Fennema, O. R. (Ed.). Food Chemistry, 1985 p. 69. New York: Marcel Dekker.

Wu, L.C. and Bates R.P.. Soy protein-lipid films: Studies on the film formation phenomenon. *Journal of food science* Vol. 37 (1972), pp. 25- 36

Xu, X.Y., Kim, K.M., Hanna, M.A. and Nag, D. Chitosan-starch composite film: preparation and characterization. *Industrial Crops and Products an International Journal* Vol.21 (2005), pp. 185-192.

Yee, V. C., Pedersen, L. C., Trong, I. L. *et al* Bishop, P. D., Stenkamp, R. E. and Teller, C. C.. Three-dimensional structure of a transglutaminase: Human blood coagulation factor XIII. Procedure National Academy Science 91: 1994 7296-7300.

Yildirim, M., Hettiarachchy, N. S. and Kalapathy, U.. Properties of biopolymers from cross-linking whey protein isolate and soybean 11s globulin. *Journal of Food Science* Vol.61 (1996), pp.1129–1131.