CHAPTER 2
LITERATURE REVIEW

2.1 High pressure processing of food

Commercial high pressure food products have been manufactured worldwide. For example, fruit jams in Japan; fruit juices in France, Japan, the USA and Mexico; acidified avocado puree in the USA; fish, sake and rice products in Japan; oysters in the USA; sliced ham and other meat products in Spain (de Lamballerie-Anton *et al.*, 2002; Tewari *et al.*, 1999). Applications of these technologies are now found extensively in food industry, and are attracting the attention of more and more researchers worldwide every year (Balny, 2006).

2.1.1 Principles of high pressure

The application of high pressure technology has become the subject of much renewed interest in the food industry (Cheah and Ledward, 1996). High pressure technology originally derived from material sciences, such as ceramics, super alloys and artificial diamond processing, is defined for food use as being a pressure treatment between 100 and 1,000 MPa (Cheftel and Culioli, 1997; de Lamballerie-Anton *et al.*, 2002). Reactions of food components under pressure are determined by Le Chatelier's principle (Hugas, *et al.*, 2002) according to a process associated with a decrease in volume which is favored by an increase in pressure and vice versa. Pressure transmission occurs in a uniform ('isostatic') and quasi-instantaneous manners throughout the biological sample or solution but it may not hold to these assumptions when a large volume of gas is present. The time necessary for pressure processing is therefore essentially independent from sample size. This is in contrast to the situation prevailing for most thermal processing. Food products are most frequently pressurised in a liquid pressure-transmitting medium packaging. A pump is use to generate pressure in the liquid and maintained in a steel cylinder of adequate thickness and resistance. The sample can be maintained under pressure for an

extended period of time without any additional energy requirement (Cheftel and Culioli, 1997).

2.1.2 Advantages of high pressure processing

High pressure processing is gaining its popularity in many countries. It results in the almost complete retention of nutritional and sensory characteristics of fresh food without reducing shelf-life. Moreover, high pressure treatments can induce special effects on the products' texture as novel texture foods (Ledward, 1995). Pressure will modify the structure and function of many proteins. For example, myosin from both meat and fish will be denatured by pressure and subsequently form a gel-like texture (Cheftel and Culioli, 1997). The gels are reported to have different characteristics from heat-induced gels, being glossy and soft by comparison and retaining their original colour and flavour (Ledward, 1995). In addition, high pressure processing over advantage than traditional thermal processing include a reduction in processing times; minimal heat damage to the product; retention of freshness, flavor, texture, and color; little or no vitamin C loss, no undesirable changes in food during pressure-shift freezing due to reduced crystal size and multiple ice-phase form; and minimal undesirable functionality alterations. High pressure processing is also independent of size and geometry of the sample since it is essentially an isostatic process, thus pressure is applied uniformly through out the product. This is in contrast to conventional heating, which generates thermal gradients and may thus over-process parts of the food. These advantages may improve functional properties of food constituents resulting in value-added products (Tewari *et al.*, 1999). Such as, Rodrigo *et al.* (2007) found that no colour degradation of tomato puree and strawberry juice appeared under combined thermal and high pressure treatment compared to heat treatment. Sancho *et al.* (1999) studied the effect of a high pressure treatment on the retention of the hydrosoluble vitamins B_1. B_6, and C after pressure treatment in a multivitamin model system is egg yolk and in strawberry "coulis" and compared it to the retention achieved after a high temperature pasteurization (20 s at 76°C) and sterilization (20 min at 120°C). They found that high pressure trearment does not have a significant effect on the retention of either B_1 or B_6 vitamins when compared with thermal treatments, and that retention was always greater than 99%. On the other

hand, the retention of vitamin C was found to be significantly different depending on the process. In strawberry coulis, retention of vitamin C was not significantly different between pasteurization and high pressure processing (91.52 and 88.68%, respectively), but was significantly different when samples were sterilized (67.11% retention). Deliza *et al.* (2005) pointed that pressurised pineapple juice had sensory properties similar to fresh juice, which is a major advantage in juice processing as it matches consumer demand for healthy, nutritious and "natural" products. Rubio *et al.* (2007) stated that high pressure treatment was an efficient method for preserving the safety of dry cured beef (Cecina de León) with out decreasing their sensory properties. Martino *et al.* (1998) stated that pressure-assisted freezing in pork frozen , both at the surface and at the central zones, showed small uniform and small-sized ice crystals whereas air-blast and cryogenic fluid freezing, having thermal gradients, showed non-uniform ice crystal distributions. Moreover, high pressure processing generates no environmental pollution and has lower energy requirements and reduced running costs (Heremans, 1995).

2.1.3 General effect of high pressure on protein conformation

As a thermodynamic parameter, pressure, has far-reaching effects on the conformation and structure of macromolecules. Such phenomena are accompanied by a decrease in volume, resulting in molecular conformation changes, intramolecular rearrangements and chemical reactions (Cheftel and Culioli, 1997). It has been suggested that the weaker interactions such as hydrogen, hydrophobic and ionic associations are affected by high pressure treatment, whereas covalent bonds are believed to be largely unchanged (Apichartsrangkoon *et al.*, 1998; de Lamballerie-Anton *et al.*, 2002).

Electrostatic interactions occur between ionized groups from amino acid side chains and from the C- and N-terminal amino acids in the polypeptide backbone. Protein molecules behave as zwitterions carrying a net positive or negative charge depending on their isoelectric point and on the pH of the environment. When charged groups are present in an ionic medium, a diffuse layer of ions is formed around the charged particle to form an electrical double layer. Volume is increased upon the formation of this layer at about 10-20 ml/mol per interacting group. However, high

pressure is able to disrupt these electrostatic interactions, leading to protein denaturation, aggregation, or gelation (Messens *et al.*, 1997).

Hydrophobic effects arise from the repulsion between water molecules and the non-polar protein residues. Water molecules are also involved in the volume changes associated with hydrophobic bond formation. The aliphatic or aromatic side-chain regions of protein molecules have low affinity for an aqueous environment. As a result the layer of water molecules immediately surrounding the hydrophobic region is oriented to provide a tightly packed structure. The change of the released water molecules from a tightly ordered to a random state also causes a slight volume increase. As a result, hydrophobic interactions are disrupted by high pressure treatment at pressures up to about 100 MPa. Above this value high pressure tends to stabilize hydrophobic interaction (Ledward, 1995).

Formation of protein molecules involves both strong covalent bonds, such as peptide bonds, and weaker bonds, such as disulfide bonds. The disulfide bonds play an important role in the high-pressure-induced aggregation and gelation of whey proteins, especially at neutral and alkaline pH values. An increased reactivity of SH groups at such pH values may cause the increased aggregation (Messens *et al.*, 1997). Pressure has an effect on the covalent bonds that are not broken but the weak energy bonds like hydrogen bonds and the hydrophobic bonds can be irreversibly modified (Cheftel, 1995).

High pressure effects on proteins and biopolymers are primarily related to the rupture of non-covalent interactions within protein molecules and to the subsequent reformation of intra-and intermolecular bonds within or between protein molecules (Messens *et al.*, 1997). Molina *et al.* (2001) stated that proteins or complex biopolymers subjected to high pressure may have their structures modified by disruption of hydrophobic and electrostatic interactions. Moreover, Apichartsrangkoon *et al.* (1998) and Messens *et al.* (1997) pointed out that pressure induced unfolding of protein, and subsequent aggregation leads to the formation of gels, which may ultimately affect the textural quality of a food. Pressure-induced gels of water-soluble proteins differ from those induced by heat, by being glossier, smoother and softer, and having greater elasticity (Ledward, 1995). Oligomeric proteins can be dissociated into their subunits using moderate pressure (<200 MPa)

and unfolding of single-chain proteins occurs beyond 300 MPa (Apichartsrangkoon *et al.*, 1998). In addition, pressure modifies the quaternary structure by destroying hydrophobic interaction. However, many quaternary structures are insensitive to pressure or show more complex behaviors, such as partial dissociation followed by the aggregation of subunits or precipitation. Generally, the primary structure is not modified by pressure, whereas secondary and tertiary structures of proteins can be significantly modified at pressures > 200 MPa, For example the secondary structure undergoes irreversible unfolding, but the tertiary structure shows reversible unfolding. The pressure-induced denaturation of globular proteins can lead to aggregation and, ultimately, under appropriate conditions, (high concentrations) to gelation or precipitation. Additionally, an important factor in high pressure-induced aggregation of proteins is the increased reactivity of any SH groups present in the system (Galazka *et al.*, 2000).

2.1.4 High pressure effects on meat products

High pressure processing is a major emergent technology with ever greater possibilities for its application in the meat industry (Cheftel and Culioli, 1997; Hugas *et al.*, 2002). Pressure-assisted gelation depends on the protein system such as source species, type of protein, its structural condition and the presence of other compounds. Pressure and temperature are necessarily associated, given that the effects of high pressure on meat system constituents depend on the actual temperature at which pressurisation occurs and any indirect heating which follows. The mechanism of protein denaturation differs according to the pressure/temperature combination (Messens *et al.*, 1997), so that not only the levels of pressure and temperature are important, but also the sequence in which they are applied (Colmenero, 2002). Combining pressure with heat does tenderise meat, but the final products have a cooked appearance, and therefore cannot be sold as fresh meat (Cheftel and Culioli, 1997).

Pressure treatment at up to 150 MPa has been shown to increase the binding achieved between meat particles in patties when they are subsequently cooked (Macfarlane *et al.*, 1984). Additionally, pressurization above 150 MPa causes colour changes different to those observed in cooked meats (Hugas *et al.*, 2002). In a more

detailed study, minced beef meat was pressurised at 10°C for 10 min, L^* colour values increased significantly in the range 250-350 MPa, the meat becoming pink, while a^* values decreased at 400-500 MPa, the meat becoming grey-brown (with a "cooked aspect"), (Cheftel and Culioli, 1997).

Pressure, 100-300 MPa at 6°C for 5-30 min, has been found to cause a significant increase in the penetration force required for a raw meat batter (Carballo *et al.*, 1996). Cooked sausages were treated at 500 MPa for 5 min or 15 min at 65°C and compared to those of sausages treated with a conventional heat pasteurisation (80-85°C for 40 min). In this study it was found that pressure treatment induced higher yield than heat treatment. Colour attributes did not change, however pressurised sausages were more "cohesive" and "less firm" than the heat-treated sausage. Pressurised samples were preferred because of their better appearance, taste and most especially texture (Mor-Mur and Yuste, 2003).

2.2 Ostrich

2.2.1 Introduction

Ostrich has a scientific name of *Struthio camelus linn.* (Horbañczuk *et al.*, 1998; Paleari *et al.*, 1998). The first ostrich was bred in South Africa, then extensively farmed in several countries such as Egypt, Australia, New Zealand, the United State and Asia, e.g. China, Malaysia and Indonesia (Paleari *et al.*, 1998; Shanawany, 1999; Sukwanmanee, 2002). Sukwanmanee (2002) reported a number of ostriches farmed in several countries such as 750,000 in South Africa, 500,000 in United State, 100,000 in Europe, 40,000 in Australia, 30,000 in China and 750 in Malaysia. In 1995, the Animal Husbandry station at Prachinburi province of Thailand imported 21 ostriches from America for the first attempt in breeding (Sukwanmanee, 2002). In 1999, there were 2,815 ostriches which subsequently increased to 18,262 in 2000 (Department of Livestock Development, 2002). The most popular variety of ostrich farmed in Thailand is Black Necks because they are quite tame (Trecharee, 2001). Subsequently ostrich farming is still increasing extensively all over the country and has become a popular source of red meat for human consumption.

2.2.2 Nutritional aspect of ostrich meat

Ostriches produce red meat that is similar in taste and texture to veal and beef (depended on age at slaughter) (Shanawany, 1999; Sukwanmanee, 2002). It is high in protein, but low in intramuscular fat. The protein content of ostrich meat is 21.74%, as compared to 21.4, 20.4 and 20.35% for chicken, turkey and bovine meat, respectively (Paleari *et al.*, 1998; Sales and Hayes, 1996; Shanawany, 1999). As in beef and chicken, ostrich meat is characterized by high content of lysine, leucine, aspartic and glutamic acids. Ostrich meat has higher phenylalanine and lower histidine than beef and chicken, and intermediate value content of valine, methionine, isoleucine and leucine. Except for arginine and aspartic acid, it is lower in its content of non-essential amino acids such as serine, glutamic acid, glycine, tyrosine and alanine than both beef and chicken (Sales and Hayes, 1996). The fat content of ostrich is 1.8% (w/w) as compared to 3.6, 3.8 and 4.5% (w/w) for chicken, turkey and beef, respectively (Paleari *et al.*, 1998; Shanawany, 1999). Moreover, the fatty acid profile of ostrich meat is higher in polyunsaturated fatty acid than either beef or turkey, but the total fatty acid content is similar in all the types of meats (Paleari *et al.*, 1998). Like beef, pork, mutton and poultry, ostrich is considered rich in oleic acid (Hoffman and Fisher, 2001). In addition the saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acid contents in ostrich meat are 48.0, 41.8 and 9.0% (w/w), respectively (Paleari *et al.*, 1998).

Other important chemical constituents of ostrich meat are its cholesterol and mineral components. The cholesterol content at about 33.8 milligrams/100 grams is consistently lower than those of 36.6 milligrams/100 grams typically found for turkey and 50.1 milligrams/100 grams for beef, and markedly lower than that for chicken (85 milligrams/100 grams) (Paleari *et al.*, 1998; Shanawany, 1999). Studies on the mineral content by Sales and Hayes (1996) reported that while the phosphorus, manganese and iron contents were higher in ostrich meat than those in beef and chicken the sodium levels were lower. Potassium, calcium, magnesium, copper and zinc contents of ostrich were found to be intermediate between those for beef and those of chicken meat.

Therefore, in summary, ostrich meat has a similar in protein content and amino acid composition compared to other conventional animal meats. The low

intramuscular fat, sodium contents and cholesterol levels of ostrich meat could be considered to give it a considerable marketing advantage in the "healthy product" market, especially in countries where coronary heart disease is a major health factor. One of the most important aspects of eating quality that determines overall acceptability of ostrich meat is its texture (Girolami *et al.*, 2003). This makes ostrich flesh suitable for further processing into various meat products, either on its own or combined with other types of protein (Sales and Hayes, 1996).

2.2.3 Ostrich meat products

Ostrich meat is perceived and marketed as a healthy alternative to other red meats (Fisher *et al.*, 2000). A special feature of ostrich meat is its high protein content, and low fat and cholesterol contents (Shanawany, 1999). Because tenderness is the most important quality characteristic sought by the average meat consumer (Fernández-López *et al.*, 2003), ostrich meat can be made into many suitable products. This is because ostrich meat has relatively high pH (>6.2), which is an ideal for meat processing to obtain high natural water holding capacity as a useful characteristic in the production of cooked/processed meat products (Fernández-López *et al.*, 2003; Hoffman and Mellett, 2003).

Ostrich meat is usually served as cooked, grilled, or dried (biltong), fillet streaks, in addition to fresh meat cubes and stir-fry material (Fernández-López *et al.*, 2003; Shanawany, 1999). Within South Africa value added ostrich products such as viennas and ham are made (Fisher *et al.*, 2000). Böhme *et al.* (1996) proved that ostrich meat can be used successfully in Italian-style fermented sausages. For example, it has been extensively used in bologna sausage (Fernández-López *et al.*, 2003) as well as the previously mentioned viennas and chopped hams. The products were found to be darker in colour, but acceptable with regards to both their composition and other sensory characteristics. The low fat and high protein content of ostrich products can be highly competitive in healthy product market, if supplied in sufficient quantities (Fisher *et al.*, 2000).

2.3 Yor

Yor is a popular traditional Thai meat sausage, made from pork or fish by mixing the minced meat with fat, water, salt and ground spices to improve both the flavor and taste. Finely comminuted yor is chopped into a fairly homogeneous mass. This mixture has a paste-like texture in the raw state but is gradually changed into a rigid structure usually by steaming (Thai Industrial Standards Institute, 1996).

2.4 Non-meat ingredients

Various ingredients are added to processed meat products, this may be for several reasons. Some of these are to assist in protein extraction, enhance flavor, provide new flavor notes, bind moisture, increase juiciness, improve texture, modify texture and potentially reduce cost of the overall meat formulation (Amako and Xiong, 2001; Barbut, 2002; Hongsprabhas and Barbut, 1999a). The non-meat additives can be divided into several sub-groups. They include sodium chloride, phosphate salts, various other curing salts, water, spices, binders (e.g., high protein items such as whey, soy) and fillers (e.g., low protein items such as starch). Each has its own unique role in the final formulation of the product. The most common non-meat additives used by the poultry and meat processing industry, and found in yor are described below.

2.4.1 Salt or sodium chloride (NaCl)

Salt or sodium chloride is the most widely used ingredient added to meat products. It carries out several major functions including, enhanced protein solubilisation, both enhancing and providing flavor and altering microbial growth. Salt-soluble protein extraction, mainly myosin and actin, is essential for meat processing. Addition of 1.5-2.0% (w/w) salt during extraction increases the binding, yield and juiciness of the product (Tseng *et al.*, 2000). In raw meat batters, these proteins can bind moisture and increase water holding capacity (WHC), as well as assisting in the emulsification of fat particles in comminuted the products by coating the fat globules. Upon heating, the extracted proteins coagulate and provide binding of the meat particles, binding of moisture (minimize cooking losses) and help form a coherent matrix to hold the melted fat (Barbut, 2002). Also Hsu and Chung (1998);

Hsu and Yu (1999), reported that higher salt addition in meatball production caused more salt-soluble protein to be extracted from the muscle cells which in turn formed a more stable meat emulsion. This made the final product harder with a minimum cooking weight loss at 2.5% (w/w) NaCl. Addition of salt at 1% (w/w) and 3% (w/w) significantly increased cooking yield, diameter and cohesiveness, but decreased brittleness, gumminess and Hunter-Lab values of meatball, when compared to those made with no added salt (Hsu and Chung, 2001).

Whereas, increasing salt from 0 to 1% (w/w) reduced cooking shrinkage of chicken muscle from 35 to 18%(w/w) and addition of 2 or 5% (w/w) salt resulted in 16% (w/w) of shrinkage (Hongsprabhas and Barbut, 1999b). Increasing NaCl from 1.5 to 2.5% (w/w) resulted in different protein extraction profiles in chicken breast meat batters. Therefore, relatively low levels of NaCl are used in batter/emulsion-type meat products where solubilization of myofibrillar proteins is necessary to enhance water-binding/holding (Rhee, 1999).

2.4.2 Phosphates

Phosphates which are salts of phosphoric acid carry out four main functions namely; increasing water holding capacity due to protein extraction, assisting stabilization meat emulsions due to the hydrophilic or hydrophobic structure of the molecule and retarding oxidation due to the chelating activity such as binding iron to prevent it from acting as a pro-oxidant and finally enhancing flavor.

Difference types of phosphates are available in the market, but the most popular form is alkaline polyphosphate such as tripolyphosphate which represents more than 50% of the phosphates used in general meat processing (Barbut, 2002). Sodium tripolyphosphate is the most widely used in meat products (Muguruma *et al.*, 2003) and a maximum level of 0.5% is allowed in poultry products according to Thai and US food regulations (Hsu and Yu, 1999; Thai Industrial Standards Institute, 1996).

Polyphosphate, consisting of sodium polyphosphate and sodium pyrophosphate in a 50/50-ratio w/w used at less than 0.4% significantly increased cooking yields (Hsu and Chung, 2001). In addition, phosphate can work together with sodium chloride to enhance muscle protein extraction, improve water-holding

capacity, improve cooking yield, palatability (juiciness) and reduce cooking shrinkage of meat and poultry products (Barbut, 2002; Hsu and Yu, 1999; Rhee, 1999; Xiong *et al.*, 2000).

2.4.3 Non-meat protein

Non-meat proteins are derived from a variety of plant and animal sources, for example, egg white, soy protein, sodium caseinate, wheat gluten, whey protein and various hydrocolloids, are routinely used in the manufacture of comminuted meat products because of their functional properties including emulsification, water binding capacity, improvement of texture and appearance (Jarmoluk and Pietrasik, 2003; Pietrasik, 2003; Pietrasik and Jarmoluk, 2003).

For many years, different protein isolates have been used as functional ingredients in a number of grounds and emulsified meat products, to enhance the binding properties of the meat. This has, in recent years, resulted in the production of more stable meat products with better textural properties. Gelling ability of proteins and physical properties of protein gels are important for use as texture modifiers in foods (Pietrasik and Li-Chan, 2002).

2.4.3.1 Soy Protein Isolate

The soy protein isolate (SPI) contains more than 90% (w/w) protein (N x 6.25 on a dry basis). SPI is approved for use in ground meat and surimi type products (McMindes, 1991; Park, 2000).

Chin *et al.* (1999); Lin and Mei (2000) reported that SPI is commonly used in processed meat products as a binder to reduce processing costs, control water loss, increase yield and viscosity, and to stabilize the emulsion in emulsion-type meat products.

Major proteins in soy protein are globulin, which consist of several subunits, such as 2S, 7S, 11S and 15S. Among them, 7S (conglycinin) is 180 kDa and 11S (glycinin) is 360 kDa which play a major role in the gel formation of soy proteins (Apichartsrangkoon, 2003). Such gelation is primarily as a result of aggregated caused by hydrophobic interactions, since the 11S and 7S globulins contain about 39 and 41% w/w hydrophobic amino acid residues.

Disulphide bond formation is a major factor in stabilizing the gel structure. With increasing soy concentration both the 7S and 11S globulins provide two or more active free-SH groups. Hydrophobic groups may also become exposed during heat treatment of the protein; thus both types of linkage may contribute to the final gel structure. In addition, the extent of any gelation depends on heating temperature and ionic strength of the system. Glycinin and conglycinin form a gel at 95°C whereas glycinin forms only loose aggregates under similar conditions, with conglycinin gelling at 85°C when 0.2 M salt is added (Damodaran, 1996; Fukushima, 1980; Park, 2000, Sheard *et al.*, 1986; Zayas, 1997).

When gelation was induced using high pressure (range of 300-700 MPa for 15 min), SPI and its major globulins; conglycinin and glycinin, produced self-supporting gels. Such pressure-induced gels showed significant lower values of adhesiveness and hardness when compared to the equivalent heat-treat gels. The water holding capacity of conglycinin, and SPI gels is also enhanced by high pressure treatment at 300 and 400 MPa (Molina *et al.*, 2002).

Chin *et al.* (1999) reported that increasing the levels of SPI between 30-50% as a meat replacement in comminuted meat systems increased yield stress and decreased pseudoplastic behavior of the emulsion system as the degree of soy protein denaturation increased with heating. Furthermore, Chin *et al.* (1999) also cited that frankfurters formulated to a 15% (w/w) fat endpoint had sensory characteristics similar to the 30% (w/w) fat control sample when 20-30% (w/w) of meat was replaced with hydrated SPI. However, an increased soy flavor and decreased juiciness were observed when more than 30% (w/w) of lean meat was replaced with hydrate SPI. Thus, the replacement of lean meat with pre-hydrated SPI up to 30% (w/w) may not be detrimental to product quality and could be used to increase the yield of emulsified products. It was also found that a replacement of the meat protein with 2.2% (w/w) SPI in bologna formulations was not detrimental to textural properties; however, replacement with 4.4% (w/w) SPI resulted in a softer texture. Dumoulin, *et al.* (1997) were found that a soy protein solution (17% w/w) pressurised at 400 and 500 MPa, at -5-50°C for 30 min produced gels whose hardness increased with increasing pressure and temperature. The equivalent pressure-induced gels were significantly softer and more deformable than heat-induced gels formed at

atmospheric pressure. Apichartsrangkoon (2003) also studied effects of high pressure on rheological properties of soy protein gels. The results showed that the rheological properties of soy protein gels were more affected by temperature than pressure. The shape of the storage (G') and loss (G'') moduli, as functions of frequency, minimally changed with temperature and/or pressure treatment. Limited disulphide bond formation occurs in temperature- and pressure- induced gel systems.

2.4.3.2 Whey proteins

Whey is obtained from cheese manufacture, and contains approximately 20% (w/w) of original milk protein. It is a heterogeneous mixture of non-casein milk proteins which provides an edible source of protein and is relatively cheap when compared to other binders and extenders used in the food industry (Hughes *et al.*, 1998; McIntosh *et al.*, 1998). Whey protein consists principally of β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulins. These are globular proteins which, in their native state, have a tertiary structure stabilized by intramolecular disulfide bonds between cysteine residues.

β-lactoglobulin and bovine serum albumin (BSA) each contain free sulfhydryl groups. However, β-lactoglobulin is located in the interior of the globular protein structure and is therefore unavailable for interaction with free sufhydryl groups or disulfide groups on other protein molecules. If temperature, pH or ionic strength is changed or other chemicals are added, then the protein may unfold and the free sulfhydryl group could become available for interaction (McClements *et al.*, 1993). In addition, α-lactalbumin gels at 62-68°C, whereas β-lactoglobulin gels at 78-83°C. Elastic gels are formed at pH 6.0-7.5 (Morr and Ha, 1993; Park, 2000).

Whey proteins are used in numerous food products for their functional characteristics that include gelation, emulsification and foam formation as well as stabilization and nutritional properties. The gel-forming ability of whey protein contributes to its usefulness as a stabilizer and texture modifier (Lowe *et al.*, 2003). Whey protein isolate (WPI) and whey protein concentrate (WPC) have been used extensively in a variety of meat products including meatballs, beef patties and various types of sausage including knockwurst, non-smoked and non-spiced sausage, also smoked sausages such as frankfurter and wieners (Hughes *et al.*, 1998).

WPI and WPC are different in their protein contents. WPI contains ≥ 90% (w/w) protein, whereas WPC contains about 50 to 70% (w/w) protein (Morr and Ha, 1993). Hongsprabhas and Barbut (1997) reported that pre-heated WPI could form a gel at a 6% protein concentration (1°C with salt was added). Substitution of 2% of a meat batter protein with pre-heated WPI improved textural parameters and reduced cook loss compared to no substitution or 2% (w/w) regular WPI addition (Hongsprabhas and Barbut, 1999b). Pre-heated WPI did not cause any detrimental effects on the meat system. It was also found that pre-heated WPI substitution was beneficial in increasing WHC, reducing cook loss and increasing gel strength of the raw and cooked products, particularly when used at low salt level (Hongsprabhas and Barbut, 1999a).

McClements *et al.* (1993) reported that, in an oil/water system, the protein molecules of WPI may interact with neighboring molecules adsorbed onto the same droplet, or on different droplets via a combination of non-covalent bonds and/or covalent disulphide bonding, (where the cysteine thiol groups interact). Formation of such bonds increases the viscoelasticity of the interfacial film giving it an enhanced stability, reducing coalescence, and leading to the promotion of flocculation.

2.4.3.3 Wheat gluten

Wheat gluten is obtained from wheat starch manufacture. It is currently used in meat protein systems to supply additional protein and specific amino acids. It is a key ingredient in food formulations because of its water and fat binding as well as texturising properties (Comfort and Howell, 2003).

The basic mechanism for the gel formation of gluten is via disulphide bonding. The uniqueness of gluten is its dough-forming ability promoted by gliadin and glutenin content (Park, 2000). Gliadin is a low molecular weight protein, 30 to 50 kDa, soluble in ethanol and responsible for viscous or flow properties of the material, whereas glutenin is a high molecular weight protein, 69 to 88 kDa, soluble in water or dilute acid or alkali and responsible for the inherent elastic behavior (Tatham *et al.*, 1990).

When gluten is heated, both rheological properties of storage (G') and loss (G'') moduli are increased, indicating an increase in the overall number of

rheologically effective cross-links (Anderson *et al.*, 1988; Comfort and Howell, 2003; Park, 2000; Schofield *et al.*, 1984; Tatham *et al.*, 1990).

Apichartsrangkoon (2002) reported that heat treatment of gluten, soy concentrate, and mixtures of both at 90°C for 0.5 to 6 hours caused large changes in the rheological properties of these systems, with higher cross-link densities being observed in the gluten-rich samples rather than in soy-rich samples. Given that such viscoelastic gel structures were formed this suggests that gluten is modified to a greater extent by heat than the soy.

The formations of covalent disulphide cross-links are important in the heat-treated systems. Apichartsrangkoon *et al.* (1998, 1999) studied physicochemical properties and dynamic viscoelastic behavior of high pressure treated wheat gluten at temperature of 20, 40 and 60°C, pressure of 200, 400 and 600 MPa with holding times of 20 and 50 min. It was found that at 20 and 40°C, pressure could alter gluten structure but disulphide cross-linking only became significant when samples were held at 800 MPa for 50 minutes. Wheat gluten treated at 60°C markedly increased in hardness and the degree of disulphide bonding noted, especially in the pressure range of 400 to 800 MPa. It was also observed that the G' and the G'' were more affected by temperature than by pressure (see earlier). In addition, when wheat gluten, soy protein and mixtures with both gluten and soy were treated at 700 MPa for 50 minutes at 20 and 60°C, the samples formed "solid-like" gel structures. Both the G' and the G'' of high gluten gels tended to increase with increasing pressure and temperature, whereas those for high soy protein gels only slightly increased with increasing treatment. Thus, the combined effect of temperature and pressure was much greater on the large complex gluten molecule than on the smaller "simpler" soy globulins (Apichartsrangkoon and Ledward, 2002).

2.5 Rheological measurements

Rheological measurements are becoming increasingly relevant to the food industry as a tool for the physical characterization of raw material prior to processing, for intermediate products during manufacturing, and for assessing the finished foods. There are several approaches to conduct these rheological characterizations, and the

selected technique depends on the specific product and the functional characteristics in need to be assessed (Tabilo-Munizaga and Barbosa-Cánovas, 2004b).

Rheology is concerned with the flow and deformation of substances and in particular, to their behavior in the intermediate area between the behaviour of "true solids" and "Newtonian fluids". Moreover, rheology attempts to define a relationship between the stress acting on given material and the resulting deformation and/or flow that takes place (Steffe, 1996).

Rheological properties are determined by measuring force and deformation as a function of time. Rheology is concerned with how all materials respond to applied forces. Basic concepts of stress (force per area) and strain (deformation per length) are key to all rheological evaluation. Stress is always a measurement of force per unit of surface area and is expressed in units of Pascals (Pa). Normal stress occurs when the force is directly perpendicular to a surface and can be achieved during tension or compression. Shear stress occurs when forces act in parallel to a surface. On the other hand, strain represents a dimensionless quantity of relative deformation. (Ferry, 1980).

2.5.1 Oscillatory testing

Dynamic oscillatory testing has several advantages over more traditional destructive methods of testing as it allows the structure build up or breakdown to follow in real time and under comparable conditions. Moreover the study of viscoelastic properties of polymeric materials such as protein could lead to a better understanding of the nature and rate of conformational rearrangements and the disposition and interaction of the macromolecules in a structure, both in terms of their short-range and long-range interactions (Ferry, 1980). From dynamic rheological tests in the linear viscoelastic range, the storage modulus (G') the loss modulus (G'') and the loss tangent ($\tan \delta = G'' / G'$) can be obtained.

G' the storage modulus value is a measure of the deformation energy stored in the sample during the shear process, representing the elastic behavior of a sample. In contrast, G'' the loss modulus value is a measure of the deformation energy used up in the sample during the shear and lost to the sample as heat, and represents the viscous behavior of a sample. If G' is much greater than G'', the material will behave

more like a solid; that is, the deformations will be essentially elastic or recoverable. However, if G'' is much greater than G', the energy used to deform the material is dissipated and the material's behavior is more liquid-like. On the other hand, the lost factor (or damping factor) reveals the ratio of the viscous to the elastic portion of the deformation behavior. A phase angle $\delta = 0°$ or $\tan \delta = 0$ corresponds to an elastic response and $\delta = 90°$ or $\tan \delta = \infty$ is a viscous response. If the phase angle is within the limits of $0 < \delta < 90°$, the material is called viscoelastic (Ferry, 1980; Steffe, 1996; Tabilo-Munizaga and Barbosa-Cánovas, 2004b).

2.5.2 Transient testing

To assess the viscoelastic behaviour of meat emulsions during or after cooking and discriminate between meat emulsions of different compositions, two basic quasi-static (*e.g.* stress relaxation and creep) methods using compression have been reported (Bruno and Moresi, 2004; McClements, 1999). More specifically, the results of stress relaxation allowed such emulsions to be described as viscoelastic solids by using mechanical models consisting of Maxwell elements in parallel with or without a spring. On the contrary, the results of creep experiments exhibited a liquid-like viscoelastic behaviour as described by a Kelvin-Voigt element in series with a spring or Maxwell element (Bruno and Moresi, 2004).

2.5.2.1 Stress relaxation test

In the stress relaxation test, an instantaneous deformation is applied to a body. This may be done while the sample is under compression, extension or shear. A level of strain is to maximize sensitivity of the measurement and minimize sample damage. Deformation or strain is maintained as a constant throughout the test while the stress is monitored as a function of time. For viscoelastic materials, this stress will decay to an asymptotic value. The equation for stress as a function of time is usually expressed as:

$$\sigma(t) = \sigma_e + (\sigma_0 - \sigma_e) \exp(-t / \lambda)$$

Where σ is the stress at the time t, σ_0 is initial modulus, σ_e is the equilibrium stress and λ is the relaxation time.

With a modified Maxwell model the material can be described by three constant factors: initial modulus (σ_0), which is the first reading at maximum strain; equilibrium modulus (σ_e); and relaxation time (λ). The relaxation time constant is the time it takes for the stress to decay to $1/e$ or 36.8% of its initial value (Steffe, 1996; Tabilo-Munizaga and Barbosa-Cánovas, 2004b).

2.5.2.2 Creep and recovery

Creep studies are determined in terms of compliance, $J(t)$, which is the quotient of the deformation resulting from the constant stress applied to the sample (Jiménez-Avalos *et al.*, 2005). In the linear region, the magnitude of the applied stress is very small and the constant stress/recovery response is independent of this stress (Ferry, 1980). The constant stress/recovery response and its mathematical representation use different "models" that include "springs" and "dashpots", which combined in series or in parallel, characterize the viscoelastic properties of fluids and solids. Mathematical equations also correlate the applied stress with the resulting deformation. There are three main models: the Maxwell, the Kelvin-Voigt and the Burgers model, which combines the first two models placed in series, allowing characterization of the viscoelasticity of fluids and solids (Jiménez-Avalos *et al.*, 2005; Steffe, 1996).