CHAPTER 2
LITERATURE REVIEW

2.1 Nutritional value of ostrich meat and its products

Ostrich (*Struthio camelus Linn.*) breeding, a long-developed industry in South Africa, has expanded into Australia, North and South America, Israel, Italy, China, and more recently, into South East Asian countries including Malasia, Indonesia and Thailand (Paleari *et al.*, 1998; Shanawany, 1999). The most popular variety of ostrich farmed in Thailand is “Black Necks” because of their tame character and ease of domestication (Trecharee, 2001).

Fat content of ostrich, chicken, turkey and beef are 1.60, 3.08, 3.80 and 4.50% (w/w), respectively, this is amongst the lowest fat content when compare with other meats (Paleari *et al.*, 1998; Sales and Hayes, 1996). In addition, ostrich meat has a “good” profile of fatty acids when compared with those of beef and chicken owning to the higher polyunsaturated fatty acid content of ostrich meat (reported at 35.1, 19.0 and 5.0%, respectively) (Cooper and Horbañczuk, 2002; Sales, 1998).

The relatively high total ω-3 fatty acid content of ostrich meat would be advantageous for promoting product, since the intake of ω-3 fatty acids is reported to lower the incidence of coronary disease, is essential for growth and development in man throughout the life cycle, and have more effective antithrombotic and antiatherogenic properties than the corresponding ω-6 polyunsaturated fatty acids (Cooper and Horbañczuk, 2002). The total fatty acids composition (saturated and unsaturated) of ostrich is similar to turkey and bovine; however the ostrich meat had quantities of highly unsaturated fatty acids, that further confirmed the nutritional characteristic of this meat (Paleari *et al.*, 1998).

The cholesterol content of ostrich meat (33.8 mg/100 g) is lower than that of corresponding turkey meat (36.6 mg/100 g), and markedly lower than those of both bovine and chicken meat (50.1 and 57.0 mg/100 g, respectively) (Sales and Hayes, 1996; Sale *et al.*, 1996). For mineral composition, phosphorus, manganese and iron

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are higher while sodium is lower in ostrich meat (43 mg/100 g) than either that of beef (63 mg/100 g) or chicken (77 mg/100 g) (Cooper and Horbañczuk, 2002).

Potassium, calcium, magnesium copper and zinc classify ostrich meat as intermediate values between beef and chicken as seen in Table 2.1 (Sales and Hayes, 1996).

Table 2.1 Mineral composition of ostrich meat in comparison to beef and chicken.

Minerals (mg/100g)	Ostrich	Beef	Chicken
Sodium	43	61	77
Potassium	269	350	229
Calcium	8	7	12
Magnesium	22	20	25
Phosphorus	213	180	173
Iron	2.3	2.1	0.9
Copper	0.10	0.14	0.05
Zinc	2.0	4.3	1.5
Manganese	0.06	0.04	0.02

Source: Sales and Hayes (1996)

Of particular interest is the high levels of some of the amino acids in ostrich meat. As in beef and chicken, ostrich meat is characterised by high content of lysine, leucine, aspartic acid and glutamic acid. Ostrich meat is higher in phenylalanine and lower in histidine than either beef or chicken and intermediate with regard to valine, methionine, isoleucine and leucine. Except for arginine and aspartic acid it is lower in the content of the non-essential amino acids serine, glutamic acid, glycine, tyrosine and alanine than that of either beef or chicken. It is similar in overall protein content to chicken, turkey and bovine meat (22.2, 21.39, 20.4 and 20.1% (w/w), respectively) (Paleari *et al.*, 1998; Sales and Hayes, 1996).

Hence, ostrich meat may be considered avaluable food source and has a similar protein content, amino acid composition, and some mineral composition as other foodgroups. The overall lower levels of fat as well as its relatively high

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polyunsaturated fatty acid content when compared with beef and chicken as well as the low cholesterol content of ostrich meat may be useful for people trying to control their weight and those at high risk from coronary heart disease and arteriosclerosis.

The relatively low sodium content of ostrich meat may be advantageous for those on a low sodium diet, for example in cases of hypertension. For this reason, ostrich meat can be categorized as a "healthy" food and it is suitable for processing into commercial meat products. In particular, tenderness plays a central role in orienting consumer preference (Girolami *et al.*, 2003).

Ostrich meat is usually served cooked, grilled, or dried (biltong). The numerous "value added" ostrich products within South Africa such as Viennas (a thin diameter emulsified and scalded product) and chopped ham (Hoffman and Mellett, 2003). Böhme *et al.* (1996) proved that ostrich meat can be used successfully in Italian style fermented sausage with the use of specific starter cultures (Fisher *et al.*, 2000). In addition, a conventional ground ostrich stir-fry and stew were prepared by Walter *et al.*, 2000 to assess the sensory attributes of ground ostrich compared with those of the ground beef dish. The results revealed that the cooked ground ostrich was an acceptable alternative to cooked ground beef, however the sample was found to be so dry upon cooking that it needed additional ingredients that supplied extra moisture in order for it to be pleasing.

Likewise, Hoffman and Mellett (2003) investigated the suitability of replacing pork fat with a modified starch in the production of ostrich patties to improve cooking yield, juiciness and tenderness because texture was determined to be the most important aspect of eating quality that controlled overall acceptability (Girolami *et al.*, 2003). The relatively high pH value of ostrich meat makes it an ideal processing meat, since the natural water-holding capacity is high, a good characteristic in the formulation of cooked meat products (Fisher *et al.*, 2000). The final products, although having a dark appearance, were acceptable in chemical composition and other sensory characteristics. The low fat and high protein content of ostrich bolognas will help to ensure that, if marketed in sufficient quantities, ostrich meat can successfully compete with other healthy meat products (Fernández-López *et al.*, 2003).

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2.2 Processing of Meat Yor

Traditional sausages, known in Thailand as "Yor", are the most popular meat product in the country. It is made by grinding and blending the meat with ice cubes, fat, and various curing and flavoring agents (sodium chloride, phosphate, garlic, pepper, sugar, starch and monosodium glutamate). The finished product has a paste-like texture in its raw state but, gradually changes into a more rigid structure during cooking (Barbut *et al.*, 1996; Thai Industrial Standards Institute, 1996).

In meat yor production, the meat is comminuted in the presence of salt to provide adequate ionic strength to induce swelling, water binding and partial extraction of myofibrillar protein components. Another main ingredient, phosphate has the ability to enhance water holding in muscle tissues and to modify textural properties of comminuted meat products. When used in conjunction with salt (NaCl), phosphates can markedly improve cooking yield and palatability (juiciness) of meat and poultry products (DeFreitas *et al.*, 1997; Xiong *et al.*, 2000).

He and Sebranek (1996) showed that frankfurters typically contain 2.0-2.5% (w/w) salt. Studies have shown that levels of 1.5-2.5% (w/w) NaCl are needed to produce acceptable emulsion-type products, whereas lower salt levels of 1.0-1.5% (w/w) may result in unstable emulsions or batters. Many phosphates are suitable for use in meat products however sodium tripolyphosphate (STPP) has been the most commonly used. The 0.25% (w/w) STPP added to high collagen frankfurters improved overall emulsion stability and cooking yield when compared to zero phosphate controls, but there were no reported effects of STPP on the texture of high-collagen frankfurters (Ladwig *et al.*, 1989). Puolanne and Matikkala (1980) reported that phosphate decreased "firmness" in cooked sausage and showed that the water-holding capacity (WHC) of the sausage increased while an increased firmness in the sausages formulated with addition of alkaline phosphates was found by Keeton *et al.* (1984).

Other components in meat yor, non-meat protein such as soy protein, wheat gluten, whey protein and hydrocolloids can be added to alter the appearance, flavour, emulsification and texture of food-products (Pietrasik, 2003; Thai Industrial Standard Institute, 1996).

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2.3 Gum or hydrocolloid in meat products

Macromolecular hydrocolloids or gums, are mainly responsible for the functional properties of processed food systems and the quality of many foods. They are commonly used in comminuted meat products because of their functional properties including emulsification, water- and fat-binding capacity, improvement of texture, rheological properties, stability and appearance. In addition, polysaccharide gums have been reported to affect the thermal transition temperatures of meat proteins (DeFreitas *et al.*, 1997; Fonkwe *et al.*, 2003; Pietrasik, 2003). Hydrocolloid are ordinarily added to myosystems in the form of an unhydrated, dry power, since water is a limiting factor affecting the texture of the final product. Addition of prehydrated or thermally activated hydrocolloids may structurally interfere with the cross-linking required for the protein gel network formation, giving rise to gel weakening. Another difference with respect to aqueous systems is the interaction with protein, lipid, and other components in myosystems (Pérez-Mateos *et al.*, 2001).

Carboxymethylcellulose is a water-soluble, anionic, linear polymer that comes from cellulose and is universally known as CMC. Functional properties, include thickening, mouthfeel modification, moisture retention, stabilisation, of suspensions, water binding, and texturisation properties. Some or all of these can be achieved by the inclusion of cellulose derivatives in foods at levels of 0.1-0.5% (w/v), generally less than 1% (w/v) (Nussinovitch, 1997; Zecher and Gerrish, 1997).

Locust bean gum (LBG) is a commercially available galactomannan. The structure of galactomannan is linear chain polysaccharide, with an average galactose to mannose ratio is 1:4. The content used in food formulations is usually about 0.5-1% (w/v). The galactomannans produce a light texture which can recover after shearing and act as thickening/gelling agent (Nussinovitch, 1997; Ramírez *et al.*, 2002). Specific applications of LBG are as an ingredient in sausage formulations (to prevent weeping), in pumped meat blends (as a suspending agent) and in fish fillets (as a binder and/or phosphate replacer). In processed cured meat products such as sausages, salami and bologna, LBG is added to improve comminution of ingredients, to improve yield through the binding of free water, to facilitate the easy

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extrusion of the material and to prevent phase separation of the ingredients during cooking, smoking, and storage (Fox, 1997).

Another gum, that is of interest with regards to yor processing, is xanthan gum. Xanthan is a non-linear anionic microbial polysaccharide produced by aerobic fermentation by *Xanthomonas campestris*. The levels used in meat products is usually in the range of 0.5-1.0% (w/v) (Nussinovitch, 1997). Xanthan is able to form highly viscous solutions at low concentrations. Therefore, xanthan has many uses in the food industry. Xanthan shows quite spectacular synergistic interactions with other non-gelling polysaccharides of the galactomannan family leading to an increase in viscosity and eventual gel formation. The use of xanthan/locust bean gum combinations has been proposed as non-meat ingredients for the formulation of low-fat ground meat patties. Locust bean gum has been reported to be successful in the formulation of low fat meatballs with good sensory properties. However, several workers reported an detrimental effects on the product structure when using xanthan gum alone. Xanthan gum effected texture properties of low-fat sausage (Solheim and Ellekjaer, 1994), structured beef rolls (Shand *et al.*, 1993) and reduced fat turkey products (Hachmeister and Herald, 1998), these products were less firm and less elastic than ordinary samples.

Foegeding and Ramsey (1986) investigated the effects of adding locust bean gum, xanthan gum, methycellulose, iota-carrageenan (ι-carrageenan), kappa-carrageenan (κ-carrageenan), guar gum, and a locust bean gum/κ-carrageenan mixture to low-fat, high moisture meat batters. All gums were added in meat batters at 0.2% (v/w) of the raw batter weight. The results showed that the methycellulose treatment caused an increase in weight loss between 60 °C and 70 °C, while with other treatments the weight remained similar throughout the heating process. Xanthan gum and guar gum at 0.2% (v/w) altered the textural parameters as determined by texture profile analysis (TPA). Increasing the concentration of xanthan gum decreased batter hardness without affecting batter stability. Sensory evaluation indicated that low-fat frankfurters (11-12% fat) were as acceptable as the higher fat control frankfurters (27% fat).

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Thirteen edible gum-hydrate formulations were made by re-hydrating 1% of the edible gum ingredient in 20% of water and adding it to the Kung-wan to replace some of its pork fat. Results indicated that eight gum hydrates, which included the κ-carrageenan group (κ-carrageenan, κ-carrageenan with KCl and κ-carrageenan with dietary salt), the konjoc group (konjac and konjac premix with $Ca(OH)_2$, LBG, guar gum and xanthan gum, produced Kung-wans with higher cooking yields than the positive control (Hsu and Chung, 1999). Xanthan gum showed significantly lower texture profile analyses indices except for its brittleness, which although it was lower it was not statistically significant. LBG, guar gum and xanthan showed colour modification of the product with more "yellow" colours occuring. Six gum-hydrates, which included κ-carrageenan, sodium alginate, sodium alginate with $CaCO_3$, curdlangum, gellan gum and LBG, produced products with similar sensory characteristics as the control samples. Overall, κ-carragenan, sodium alginate, curdlan gum and locust bean gum (LBG), appeared to be good fat substitutes for making low fat emulsified meatballs. Within these, κ-carrageenan and LBG were considered to give the better products (Hsu and Chung, 1999).

Pérez-Mateos *et al.* (2001) examined the interactions of κ-carragenan plus other hydrocolloids in fish myosystem gels. The hydrocolloids used in this study were locust bean gum (LBG), guar gum, xanthan gum, iota-carrageenan, kappa-carrageenan, sodium carboxymethylcellolose (CMC) and sodium alginate. They were added in powder blend to the washed minced tissue usually at a level of 0.5% (w/w) (1% (w/w) in the case of locust bean gum). Mixtures of κ-carrageenan plus other hydrocolloids were examined for their effects on the mechanical and water holding properties of heat-induced gels made from washed blue whiting mince. The overall gel structure and thermal behaviour were also studied. No synergistic effects were noted in the functional properties of the samples except for the formulation containing the mixture of κ-carrageenan with locust bean gum. In these samples light microscopy revealed that that the hydrocolloids expanded as inclusions, forming cavities of varying morphology and size. The anionic hydrocolloids were mixed throughout the protein matrix, probably through interaction with the myofibrillar protein. The non-ionic hydrocolloids were dispersed throughout the matrix but did not interact with it and were located simply by inclusion. The thickening hydrocolloids (locust bean gum,

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guar gum, xanthan gum, carboxymethylcellulose and alginate) formed a mesh of filaments inside the cavities; while the gelling hydrocolloids (carrageenans) that were mainly inside the cavity interiors formed a continuous structure. The blend of κ-carrageenan with sodium alginate exhibited thermally strong synergistic interactions but no particular effects were induced on corresponding functional properties.

2.4 Effects of high pressure on hydrocolloids

Hydrocolloids are the most widely used ingredients added to meat products such as bologna, frankfurter and Vienna. They play a significant role in the structure, stability and textural characteristics of many food colloids through their aggregation and gelling behaviour (Galazka *et al.*, 1999).

Polysaccharides have been used extensively in the manufacture of food products to impart new functional properties or to manipulate the texture of restructured food products. Aqueous solutions of nonstarch polysaccharides can undergo a coil-to-helix, sol-gel transition that is sensitive to temperature, pressure, pH, and ionic strength. Carrageenans and alginates are the most studied nonstarch hydrocolloids. The effects of high pressure processing on hydrocolloids have been investigated. Gekko (1992; 1994) gathered previous experimental data to examine the influence of pressure on the sol-gel transition of several food macromolecules (carrageenan, agarose, gelatin, milk casein, ovalbumin, and soy protein). The results showed the evolution of the typical sol-gel transition temperature as a function of pressure, using concentration as a parameter for κ-carrageenan. The several-phase diagrams showed a linear decrease of the melting temperature as the pressure increased and ι-carrageenan behaved very similarly. An upward shifting of the temperatures of transition with increasing concentration is also apparent in the diagrams.

On the other hand, Tsen and King (1994) studied the gelation of carrageenan under high pressure using a three-variable, three-level designed response surface methodology, finding that the best overall acceptability could be obtained by using the optimum combination of pressure, temperature, and concentration of 334 MPa, 45 °C, and 1% (w/v), respectively. In addition, the effects of high hydrostatic pressure

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(200, 400 MPa) and temperature (20, 40 and 60 °C) on the ι-carrageenan gelation were studied by Steyer *et al.* (1999). The high hydrostatic pressure carrageenan gels were compared with those obtained by heat treatment at the same temperature and atmospheric pressure. It was found that gels obtained under high pressure showed a maximum hardness at 200 MPa for 120 min at 60 °C and a minimum at 400 MPa for 60 min at 40 °C. Results indicated that the textural properties of ι-carrageenan gels could be adapted by high hydrostatic pressure treatment (pressure, pressurisation time and temperature).

According to Bian-Sheng *et al.* (2001) investigated the rheological properties of several food gum solutions including carrageenan, agar, high-methoxyl (HM) pectin, sodium alginate, xanthan, and guar gums after high-pressure treatment. They found that the viscosity of carrageenan and agar solutions increased; the HM pectin, sodium alginate, and guar gum solutions did not change much; and xanthan gum solutions decreased in viscosity. Experimental results also showed that the storage modulus (G') of carrageenan and agar solutions were reduced in magnitude, and their G' values were lower than their G'' after treatment. The loss tangents ($\tan \delta = G''/G'$) of HM pectin, sodium alginate, and guar gum solutions after the treatment were almost the same as those before the treatment. Results implied that the gelation of carrageenan and agar occurred within a pressure range of 200-600 MPa at various temperatures (30-60 °C) and over differing times (10-120 min), while HM pectin, sodium alginate, and guar gum solutions were not change in rheological properties.

In case of alginates (a family of ionic linear copolymers of β-D-mannuronic and α-L-guluronic acids units linked [1-4]), different pressure conditions have been reported from 100 to 900 MPa. The results showed that sodium alginate mixed with various calcium salts formed gels when a hydrostatic pressure of 900 MPa was applied (Shioya *et al.*, 1994). The texture of resultant gels varied with the calcium salt used and their concentration level. Both soluble and insoluble calcium salts could be used to make gels. Calcium chloride provided opaque, homogeneous, and firm gels. Calcium citrate provided transparent, homogeneous, and soft gels. These results suggested that insoluble calcium salts dissociate, and the hydration of sodium alginate was accelerated, under high hydrostatic pressure.

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The gelation of pectin (ionic hydrocolloids formed by a family of polymers with linear chains of [1-4]-linked α-D-galacturonic acid units) under high pressure has been examined. It was found that pressurised gels of low methoxyl (LM; -$COOCH_3$ content<50%) pectins (200 and 400 MPa for 30 min) combined with sugar were more elastic and less "sticky" than classical thermally treated gels (130 °C for 10 min). LM pectins went from a disordered solution to a well ordered gel structure, apparently due to a redistribution of calcium throughout the system. For HM pectin, these solutions only gelled in a very narrow range of sugar concentration, beginning at 55% and ending at 62% (w/w), with more pectin becoming necessary as the sugar concentration increased. The gels were also less brittle and sticky as well as more elastic than corresponding thermally processed gels. Water availability, strictly regulated by the sugar and a specific polysaccharide concentration seemed to be fundamental requirements for the gelling of HM pectins (Gustin *et al.*, 1997a; 1997b).

Onwulata and Elchediak (2000) have used dynamic pulsed pressure treatment to study the functional properties induced in MCC (microcrystalline cellulose) and a modified waxy maize starch. The slurry (12% (w/v), pH = 5) was heated (25 °C and 70 °C for 15 min), transferred by gravity into the high-pressure chamber, and processed (one or five times) by pulsed pressure (414 and 620 MPa) at 120 cycles/min. Flow rate through the pressure chamber ranged from 57 to 100 L/h, and residence time was 1.0 sec. The pressurised samples were freezedried and then evaluated for water-holding capacity, viscosity (5% v/v suspensions), thermal characteristics, and enthalpy of melting (DSC on samples equilibrated at 5% humidity). After pressure treatment of MCC fiber at 25 °C, shifts in melting energy were significantly affected as evidenced by the DSC results. At 70 °C, peak melting energy did not significantly differ from unpressurised cellulose; the lower pressure increased density and the higher pressure dramatically reduced water-holding capacity (the potential increase of fiber amounts in food formulations). It has already been proposed that the preheating at 70 °C may be a main factor for the results obtained, particularly in the case of the modified waxy maize starch, for which molecular degradation after processing was reported via NMR analysis.

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2.5 Influences of high pressure on meat texture

Tenderisation of meat by high pressure was first shown by Macfarlane (1973) using pre-rigor meat. When various ovine and bovine muscles excised soon after slaughter were subjected to high pressure (103 MPa, 30-35 °C, 1-4 min), a very firm and contracted raw meat was obtained. However, after cooking, the meat was tender and with a higher moisture content and lower Warner-Bratzler (W-B) shear value than non-pressurised meat removed from the carcass either pre- or post-rigor (Cheftel and Culioli, 1997).

Improvement in the tenderness of meat by pre-rigor pressure treatment can be achieved using a wide range of treatment conditions these include, the pressure applied, temperature attained during pressure treatment, duration of the pressure treatment, and the rate of pressure increase and decrease. Pre-rigor pressurisation of meat has been reported to improve the tenderness over pressure ranges 90 to 150 MPa, using temperature ranges from 25 °C to 40 °C, and at treatment times of only a few minutes. The specific combinations, of course, depend on the species and muscle type concerned (Macfarlane, 1973; Montero and Gómez-Guillén, 2005).

When high pressure is applied post-rigor, no contractions are induced, but there are extensive modifications to the sarcomere structure (Cheftel and Culioli, 1997). Despite these changes, when pressure conditions are applied to retain the raw appearance of meat, around 150 MPa at ambient temperature (25 °C, 1 h) for the biceps femoris muscle (Bouton *et al.*, 1977), or chilled (0 °C, 3 h) in the case of the longissimus and semimembranosus muscles (Macfarlane *et al.*, 1981), there is no improvement in the beef tenderness. Again, the tenderness, shear strength, or juiciness of cooked bovine semimembranosus muscle (after cooking for 1 h at 80 °C) is not improved with the pressurisation at moderate temperature (30 °C), even when the treatment is prolonged to as much as 24 h (Cheftel and Culioli, 1997).

The application of higher pressure (up to 500 MPa) to meat in cold conditions could permit tenderisation; however, using pressure treatments in excess of 300 MPa, causes the meat not to appear as "raw" and therefore, would not be commercialy acceptable. Myofibril fragmentation has been shown to be considerably increased after pressurisation (100-300 MPa, 5 min and 20 °C) and the ultrastructure

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is also extensively modified (Suzuki *et al.*, 1990). Decreases of 60%, 20%, and 10% in the hardness (determined from the texture profile) of pressurised beef muscle have been reported with treatments at 10 °C for 5 min at 100, 150, and 300 MPa, respectively; however, no changes were found in elasticity (Suzuki *et al.*, 1992). When meat was treated at higher pressures (100-300 MPa), considerable modifications were induced in sarcomere structure, even at low temperature (20 °C) and using relatively short treatment times of about 5 min (Suzuki *et al.*, 1990; Suzuki and Ikeuchi, 1991).

The application of high pressure even at the lower temperatures of 5-10 °C induced drastic changes in the colour of both red pork and beef muscle samples (Montero and Gómez-Guillén, 2005). On a more detailed level, Carlez *et al.* (1995) studied the effect of different packing/pressure conditions on the colour of meat and the subsequent levels of extractable myoglobin. In minced beef packed under vacuum (without air or oxygen) and pressurised at 10 °C for 10 min, the L* values increased significantly in the range 250-350 MPa, turning the meat pink, whereas a* values decreased at 400-500 MPa, giving the meat grey/brown colour with it acquiring a "cooked" appearance. The total extractable myoglobin decreased with pressure in the range 250-500 MPa, whereas the proportion of the metmyoglobin increased at the expense of the oxymyoglobin using pressures of 400-500 MPa. Packaging of meat under vacuum with an oxygen scavenger partly protected the meat colour (samples processed at 400 MPa turned pink with no change in the a* value or metmyoglobin content). There was a similar protective effect when chilled minced meat was blended with $NaNO_2$ and NaCl 18 hours before processing at 350-500 MPa. However, cystein, ascorbic acid, nicotinamide, and nicotinic acid had no protective effect under similar conditions (Carlez *et al.*, 1995). These authors also observed that the meat discolouration from pressure processing appeared to be as a result of a "whitening" effect that occurred in the range 200-350 MPa, possibly due to globin denaturation and/or heme displacement or release. It was also suggested that the oxidation of ferrous myoglobin to ferric metmyoglobin at or above 400 MPa may also play some part in this phenomenon. Because both degradation phenomena took place only 2-5 min after pressure was reached, they appear to be more dependent on critical pressure thresholds than on exposure times. Only the second phenomenon was

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prevented by total oxygen removal or prior formation of nitrosylmyoglobin. Cheftel and Culioli (1997) suggesting that the pressure resistance of the nitrosylmyoglobin and nitrosylmyochromogen (Fe^{2+}) pigments were probably directly related to their resistance to oxidation.

2.6 Effects of high pressure on meat products

The use of high pressure to induce gelation of proteins in foods first aroused interest in the late 1980s. This technology can be used to create new products from meat or fish muscle (new textures and/or flavors), or to create analogs of existing products, in which colour, flavor, and nutritional value are only minimally affected (Cheftel and Culioli, 1997; Messens *et al.*, 1997). There is an additional advantage in that pressure allows gels to form faster than in normal thermal gelation and also enhances the gel-forming capacity of poor functional quality fish mince (Pérez-Mateos *et al.*, 1997).

Although the basic mechanisms of pressure-induced gelation of myofibrillar proteins differ to some extent from those of heat-induced gelation, both processes require initial protein conditioning to facilitate refolding at a later stage, to form a gel network. Briefly, thermal gelation involves the first stage of protein denaturation and solubilization during which a certain concentration of saline ions is introduced; this disrupts the internal forces maintaining the native conformation of the proteins, thus favoring interaction with water (solubilisation). Subsequent heating further facilitates denaturation through the unfolding of proteins and allows formation of new chemical bonds and interactions between protein molecules (aggregation) (Ferry, 1984). The outcome is a three-dimensional mesh (gel) in which the degree of order will depend on the processing conditions, such as time/temperature, ionic strength, pH, and protein concentration (Montero and Gómez-Guillén, 2005).

Pressure-induced protein gelation was initially used as an alternative to thermal treatments, the latter being, in principle, more aggressive on proteins as they can cause more severe denaturation (Cheftel and Culioli, 1997). Pressurising above 100-150 MPa at low temperature induces protein denaturation, thus favoring solubilization and unfolding, which are necessary to the first stage of gelation.

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The extent of the protein denaturation will essentially depend on the intensity of the pressure applied, the temperature, and the ionic strength introduced (Montero and Gómez-Guillén, 2005). Carlez *et al.* (1995), by measuring the total enthalpy of denaturation, found that high-pressure processing in bream surimi (300 MPa, 5 °C, and 15 min) caused much less protein unfolding (23%) than thermal treatment at 90 °C for 30 min (67%). The pH is also a factor; given a pH proximate to the isoelectric point of the proteins, high pressure is not an effective tool for inducing denaturation (Denda and Hayashi, 1992). Also, the pH tends to increase with pressure, a fact attributed to the denaturation of certain protein fractions (Angsupanich and Ledward, 1998).

When pressure is released, the refolding process of proteins begins, establishing new interactions to produce the protein aggregation necessary for the formation of a gel network. This aggregation may even begin during the partial unfolding of myofibrillar proteins (Carlez *et al.*, 1995). As shown by studies conducted largely on fish muscle proteins, the entire process can take place at less than 10 °C in several minutes (Carlez *et al.*, 1995; Ohshima *et al.*, 1993; Pérez-Mateos and Montero, 1997; Shoji *et al.*, 1992;).

Pressure-assisted gelation depend on pressure/temperature combinations, so that not only the level of pressure and temperature is important, but also the sequence in which they are applied. Heating (>40 °C) under high pressure conditions limits the gelling of meat system. When pork or chicken meat batters was heated at 60-80 °C for 30 min at 200-400 MPa, the resulting structures were weaker and had better water-binding properties than the equivalent gels made by heating. These conditions may help to improve the water-binding properties because the resulting three-dimensional gel is less ordered, the resulting gel matrix being less rigid than those formed when meat is heated under atmospheric conditions (Cofrades *et al.*, 2003).

With regard to the behaviour of mixed hydrocolloid/protein aqueous systems under high pressure, Galazka *et al.* (1999) reported that the complexes of anionic polysaccharides with protein material, such as bovine serum albumin, appears to protect the protein againt pressure-induced aggregation due to disulphide bridge formation during or after high-pressure treatment. The microstructure of pressure-

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induced gels of mixed β-lactoglobulin and sodium alginate in an aqueous system was also studied (Dumay *et al.*, 1999).

The behaviour of hydrocolloids added to a myosystem mince has been observed by Montero *et al.* (1998) who found that starch granule structures were more evident in the pressure-induced gels than in the heat induced gels created at atmospheric pressure. Fernández-Martín *et al.* (2000) found by differential scanning calorimetry that starch granule structure in pork meat batters was preserved by pre-pressurisation from subsequent thermal gelatinization. The main effect of pressure on starch materials is to gradually reduce their gelatinization temperatures as a function of the pressure applied (Hibi *et al.*, 1993). Pressure tends to maintain the granule in its original state as a result of the stabilization of the existing hydrogen bonds (Douzals *et al.*, 1998; Thevelin *et al.*, 1981).

Montero *et al.* (2001) determined the behaviour of non-ionic hydrocolloids (locust bean and guar gum) and ionic hydrocolloids (xanthan gum and carboxymethylcellulose) in the gelation characteristics of blue whiting mince under high pressure with different pressure-time-temperature combinations. Heat treatment at atmospheric pressure generally produced higher “adhesiveness” and “yellowness” (b*). High pressure at cold temperatures (7 °C) induced the greatest cohesiveness and the highest values at breaking deformation (except with xanthan) and the overall lowest elasticity. High pressure with moderate heating generally produced the lowest hardness values. The organization of the gum into larger macromolecular structures generally differed according to the treatment used: filamentous structures forming under pressure and aggregates forming with heat (except xanthan). Water holding capacity increased under pressure in gels containing ionic gums. Overall, the combination of hydrocolloid and gelling treatment appeared to offer increased technological possibilities in that it produced a wider range of rheological characteristics, water holding capacity and colour in the gelled product (Montero *et al.*, 2001).

The effects of carrageenans and alginate on physical properties of combined pressure and temperature treated fish mince gels was reported by Pérez-Mateos *et al.* (2002). The results indicated that the effect of the gelling treatment had on the fish mince was largely dependent on the hydrocolloid added. Gelation at atmospheric

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pressure induced gels that were more adhesive, harder (except in the case of iota-carrageenan) and less cohesive. Lower pressure conditions (200 MPa, 10 °C, 10 min) produced more cohesive gels with higher breaking deformation loads and lower overall elasticity; these gels also had the highest values of work required for penetration (especially those containing iota-carrageenan). Higher pressure conditions (375 MPa, 37 °C, 20 min) induced gels with the lowest hardness. The carrageenans (iota or kappa) appeared to form a reticular (composed of small independent filaments) structure in the heat-induced gels, which was not observed with alginate (Montero *et al.*, 2001). In the pressurised fish mince gels, iota-carrageenan was in a globular form, indicating that it had not gelled; however kappa-carrageenan, on the other hand, under similar conditions formed small fine reticular structures. Alginate formed a fine, dense network under the higher pressure (375 MPa) conditions, but this was not observed at lower pressures (200 MPa).

2.7 Chemical changes induced by high-pressure treatment

Pressure induces changes that disturb the balance of noncovalent interactions, which stabilize the native conformation of proteins, particularly myosin and actin (Okamoto *et al.*, 1990). Early studies on isolated muscle proteins showed that myosin and actin underwent depolymerisation under pressures around 100-300 MPa (Ikkai and Ooi, 1969; Macfarlane and McKenzie, 1976). As a consequence of depolymerisation, pressure induced the increased solubilization of myofibrillar proteins. Macfarlane (1974) found that 150 MPa at low or moderate temperature greatly promoted protein solubilization in homogenates of ovine meat in saline solutions. The pressure-treated homogenates subsequently lead to firmer, more cohesive gels and reduced "drip loss" from the products. The amount of protein solubilized was found to be dependent on temperature (greater at 0 °C than at 30 °C), pH, and nature/concentration of salt used.

The application of high pressure to meat products is associated with a small reduction in volume (less than 1% v/v), due mainly to changes in protein hydration and the "packing efficiency" of the amino acids (Masson, 1992). Protein unfolding is much less intense during moderate pressurisation than during heating (Heremans *et al.*, 1997),

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and such pressure induced denaturation, unlike thermal denaturation, may be reversible to some extent (Montero and Gómez-Guillén, 2005). It is generally agreed that high pressure denaturation involves low energy levels and is therefore largely ineffective in the disruption of strong covalent bonds. In fact, the presence of disulphide bonds in a given protein can negatively influence the effectiveness of protein denaturation by pressurisation. However, the greater is the hydrophobicity, the more pronounced is the materials response to pressure (Denda and Hayashi, 1992).

Cold pressurisation (7 °C) at about 200 MPa is believed to cause major rearrangement and/or destruction of ionic saline bonds, due to electrostriction and hydrophobic interactions (Cheftel and Culioli, 1997). As pressure is increased still further, hydrogen bonds may ultimately rupture (Angsupanich and Ledward, 1998). Cleavage of hydrogen bonds at pressures higher than 600 MPa has been reported as the mechanism which may be responsible for the pressure denaturation of globular protein-in-water solutions (Hayakawa *et al.*, 1996). The decreasing in volume is also one of the reasons why collagen is scarcely affected by high pressure, as the typical triple-helix structure is mainly stabilized by pressure-insensitive hydrogen bonds (Fernández-Martín *et al.*, 1998; Heremans, 1995). Protein denaturation observed using pressures as high as 1,000 MPa has been shown to be more severe than that caused by thermal denaturation in several animal protein systems (Hayakawa *et al.*, 1996). Such protein denaturation have been shown to be irreversible under these conditions as the secondary structure is irreversibly altered and aggregate structures are formed by chemical modification of the polypeptide “skeleton” (Masson, 1992).

When pressure is released, the protein is restructured initially by hydrogen bonding, and later any hydrophobic or electrostatic forces will be superimposed on that structure. Thus, the network will be dominated by the formation of an initial hydrogen-bonded gel (Angsupanich and Ledward, 1998; Angsupanich *et al.*, 1999; Defaye *et al.*, 1995). A significant contribution of hydrogen bonds, together with ionic bonds, has also been reported in low-temperature pressure-induced gels made from blue whiting (Pérez-Mateos *et al.*, 1997). Also, hydrophobic interactions have been reported to play a predominant role in stabilizing pressure-induced gels produced from Alaska pollack (Gilleland *et al.*, 1997). This low-temperature gelling mechanism differs from thermal gelation, in which thermolabile hydrogen bonds are readily

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broken upon heating, the subsequent bonding is dominanted by hydrophobic and to some extent electrostatic interactions.

During the refolding process, there may also be some thiol-disulphide interchange, which will induce more protein aggregation (Cheftel and Culuoli, 1997; Masson, 1992). A number of studies have shown a major contribution of covalent disulphide bonds to the polymerization of myosin heavy chains in pressure-induced gels using a relatively high levels of pressure treatment (≥300 MPa) (Angsupanich *et al.*, 1999; Gilleland *et al.*, 1997; Okazaki *et al.*, 1997; Shoji *et al.*, 1992).

Although under pressure, myosin in its monomer form undergoes "head-to-head" interactions to form oligomers. This polymerization process progressively increases as pressure increases (Messens *et al.*, 1997). Yamamoto *et al.* (1993) proposed a mechanism of pressure-induced aggregation of rabbit myosin in which, at 70 MPa or higher, the two "heads" of the same myosin molecule fuse. At higher pressures of approximately 200 MPa, oligomers are formed by head-to-head interactions, creating large clusters in which the "heads" are positioned centrally with the "tails" outward, but without these interacting directly with one another. In these conditions, a gel will not occur because the pressure has not affected the central helical structure of the "tails". However, with heating, which causes a heat-induced helix-coil transition, the myosin molecules can interact through the entanglement of the denatured tails, leading to efficient protein aggregation and subsequent gelation.

Electrophoretic studies on protein solubilized with chemicals, selected for their capacity to selectively disrupt certain types of bonds or interactions in both fish and meat gel systems, have highlighted the importance of the role of polymerization in myosin heavy chain (MHC) and actin, as opposed to troponins, tropomyosin, and myosin light chains (MLCs) in pressure-induced or pressure/heat-induced gels (Angsupanich *et al.*, 1999; Ashie and Lanier, 1999; Jiménez-Colmenero *et al.*, 1998; Montero *et al.*, 1997). However, MHC fragmentation has also been reported, in the case of squid based materials and was largely attributed to pressure induced increased proteolytic activity in the samples (Nagashima *et al.*, 1993). Jiménez-Colmenero *et al.*, (1998) reported such phenomena in both heated-under-pressure pork and chicken batters (*i.e.*, the formation of low-molecular-weight and high-molecular-weight fragments from MHC breakdown, and polymerization of MHC, preserved

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from thermal denaturation), suggesting that both may occur simultaneously and independently.

2.8 Effects of high pressure on microorganisms

High pressure processing effectively inactivates spoilage microorganisms as well as food borne pathogens. This inactivation is due to widespread damage of the microorganisms through modification of morpholgy and the damaging of several major components such as cell membranes, ribosomes and enzymes, including those involved in the replication and transcription of DNA. The effects of high pressure on bacterial survival are influenced by a number of interacting factors such as the magnitude and duration of any treatment regime, the temperature, the environmental conditions, the bacterial species and phase of development of the microorganism (de Lamballerie-Anton, 2002).

At ambient temperature most vegetative cells are inactivated between 400 and 600 MPa. In general, gram-positive bacteria (e.g. *Listeria monocytogenes* and *Staphylococcs aureus*) are more resistant to pressure than gram-negative bacteria (e.g. *Pseudomonas*, *Salmonella* spp., *Yersinia enterocolitica* and *Vibrio parahaemolyticus*), but large differences in survival characteristics can exist between strains even within the same species. Moreover, cocci are more resistant than rods because fewer morphological changes occur under pressure. In addition, cultures in the exponential growth phase have been shown to be far more sensitive than cultures in the stationary phase (Shigehisa *et al.*, 1991). In contrast, spores at ambient temperature can resist pressure up to 1,000 MPa, temperatures of above 70 °C being necessary to obtain any significant level of inactivation. However, it has also been shown that lower pressures (250 MPa) associated with mild temperatures (40 °C) can inactivate spores in a two stage process. Pressure first is used to induce germination and then a subsequent treatment inactivates the baro-sensitive germinated spores. In addition, pressurisation can inactivate some parasites such as Trichinella spiralis but its efficiency for the inactivation of viruses has been shown to be very limited (de Lamballerie-Anton, 2002).

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Pressure treatment of pork homogenates at 400 MPa and 25 °C for 10 min reduced by at least 6 log cycles the populations of *Escherichia coli*, *Campylobacter jejuni*, *P. aeruginosa*, *Salmonella typhimurium*, *Y. enterocolitica*, *Saccharomyces cerevisiae* and *Candida utilis* inoculated at a level of 10^6-10^7 CFU/g (Shigehisa *et al.*, 1991). However for *Micrococcus luteus*, *S. aureus* and *Streptococcus faecalis*, a similar reduction ratio required a treatment of 500 or 600 MPa for 10 min (Cheftel and Culioli, 1997). *Clostridium sporogenes* putrefactive anaerobe spores were subjected to pressures from 600 to 800 MPa at 90 to 110 °C and the with a processing time up to 20 min. The result showed that the high pressure/temperature combination was more effective than thermal processing alone (Rovere *et al.*, 1999).

2.9 Viscoelastic properties of food

Many food emulsions have intermediate physical properties, they are not pure liquids or pure solids, but have rheological properties that are partly viscous and partly elastic. Viscoelastic materials exhibit both viscous and elastic behaviour simultaneously. In an ideal or elastic solid, all the mechnical energy applied to the material is stored in the deformed bonds and is returned as mechanical energy once the deforming force is removed. On the other hand, in an ideal liquid, all of the mechnical energy applied to the material is dissipated in causing the material to flow. Thus, in a viscoelastic material, part of the energy is stored as mechnical energy within the material, and part of the energy is dissipated. For this reason, when a force is applied to a viscoelastic material, it does not instantaneously adopt its new dimensions, nor does it instantaneously return to its underformed state when the force is removed (McClements, 1999; Steffe, 1996).

Dynamic viscoelastic methods are commonly used to characterise the rheological properties of viscoelastic materials, such a method is one in which creep and stress relaxation measurements are made. In a creep experiment, a constant stress is applied to a material and the change in its dimensions with time is monitored, which results in a strain versus time curve. The data are usually expressed in terms of a parameter called the compliance (J), which is equal to the ratio of the strain to the

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applied stress. The time dependence of the compliance of a material can also be measured when the stress is removed, which is referred to as a creep recovery experiment (McClements, 1999).

It is however possible that instead of applying a constant force and measuring the change in the strain with time, is to apply a constant strain and measure the change in the stresses acting on the material as a function of time. This type of experiment is referred to as a stress relaxation. The same type of information can be obtained from creep and stress relaxation experiments, and the method used largely depends on the type of rheological instrument available and the geometry of the samples to be tested (McClements, 1999).

In order to describe the viscoelastic behaviour of a material, mechanical models may be applied to any data obtained. These models are composed of theoretical "springs" (considered ideal solids, which account for the elastic behaviour of viscoelastic materials) and viscous "dashpots" (representing ideal fluids, which account for the viscous behaviour) and may be combined in different ways to explain the materials behaviour. Three are the most commonly mechanical analogs used: the Kelvin-Voigt model, the Maxwell model (Bruno and Moresi, 2004; Correia and Mittal, 2002), and the standard linear solid one.

The Kelvin-Voigt model, is composed of a spring and a dashpot in parallel, and represents the start point for the development of mechanical analogs describing the creep behaviour. In fact, the Kelvin model is not sufficient to describe creep in many biological materials and several are better modeled by the Burgers model (a Kelvin and a Maxwell element in series). A model consisting of one Maxwell element in series with two Kelvin-Voight elements is able to describe liquid-like viscoelastic behaviour (Steffe, 1992).

The Maxwell model, consisting of a Hookean spring and a Newtonian dashpot in series (Del Nobile *et al.*, 2007), is suitable for understanding stress relaxation data, however does not consider the equilibrium stress. For this reason, the viscoelastic behaviour of food can be better described by using a generalized Maxwell model consisting of several elements in parallel with a spring component (Steffe, 1992). Stress relaxation data are very importance since they supply information about assessing the viscoelastic behaviour of several meat products such

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as Surimi gel (Ma *et al.*, 1996), pork ham muscle (Lachowicz *et al.*, 2003), bologna (Bruno and Moresi, 2005) and beef (Del Nobile *et al.*, 2007).

In a similar model, if the system is subjected to a constant strain, the total stress is the sum of the stress of each element. Since each element may have a different relaxation time, a relaxation spectrum can be obtained for a viscoelastic material. Models containing more exponential components and a residual term have been used to describe the stress relaxation behaviour of myofibrillar protein-based films (Cuq *et al.*, 1996). Ma *et al.* (1996) were able to evaluate the effects of setting and addition of corn starch on the relaxation response of surimi gels and describe the viscoelastic behaviour of gel structure by means of a generalized Maxwell model consisting of seven elements (a three-element Maxwell model with a free spring). A model including one spring and two Maxwell elements was successfully use to describe the stress relaxation of lipids such as beeswax, candelilla wax, carnauba wax and a high-melting milkfat fraction (Shellhammer *et al.*, 1997). The viscoelastic properties of some pork ham muscles were better fitted to a generalized Maxwell model consisting of a parallel coupling of a Hookes body and two Maxwell's bodies (Lachowicz *et al.*, 2003).

The standard linear solid model, also called Zener model, can consist in two different mechanical analogs: a spring in series with a Kelvin model or a spring in parallel with a Maxwell model. In addition to the used model, the equilibrium modulus, the decay modulus, time of relaxation, and specific viscosity in the relaxation model are affected by the specimen orientation and its location with the product (Wang, 2003).

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