CHAPTER 1
INTRODUCTION

1.1 LYCHEE

Lychee is one of the most important members of the Sapindaceac family that has over 2,000 species and 150 genera (Menzel, 2002). It is botanically designated *Litchi chinensis* Sonn. [*Nephelium litchi* (ambers)] and widely known as litchi and regionally as lichi, lichee, laichi, lecchee or lychee (Morton, 1987). It is native to the area between southern China, northern Vietnam, and Myanmar, but is now cultivated in many countries with subtropical climates such as India, Thailand, Bangladesh, and Nepal (Menzel, 2002). The major lychee production areas in the world are China, Taiwan, Vietnam, Thailand, India, South Africa, and the Malagasy Republic (Menzel *et al.*, 1988). Lychee is a nonclimacteric fruit. The harvest season in the Northern Hemisphere is between late May and early July. In the Southern Hemisphere, lychees are harvested between November and February. The ripe fruit is spherical, about 2.5-4.0 cm in diameter, and composed of a reddish prickly leather-like pericarp (shell or peel), a white juicy aril (flesh), and a shiny brown seed in the center. The flesh has a pleasant floral flavour and delicious sweet taste (Wu and Sheu, 1996).

The first record about lychee in Thailand was dated back to the year 1854 (King Rama IV). It is very likely that the lychee fruit first came along with Chinese traders and immigrants during the Ratanakosin Era (since 1782) (Sethpakdee, 2004). Lychee ranks eleventh in the list of economic fruit crops in Thailand and can be divided into two groups based on their cool temperature requirements.

(1) Moderate low temperature: Cultivars in this group are grown mainly in the lowland and the central area (including eastern and western regions). More than 10 cultivars has been recorded but the most well known is the “ Kom”(dwarf, 11 % of plantings). Other cultivars of less importance are Kra-lek Bai-Yaw, Sampao Kaew, Kiew Waan, Dang Payom, Kratone Tong Pra-rong and Kra-lok Bai Dum. Recently “Pantip” lychee variety emerged and is grown mainly in Kanchanaburi province.

(2) Low temperature cultivars : There are a few cultivars in this group which

are grown mainly in the north. The lowest temperature requirement belongs to “Hong Huey” (“Tai So” or “Mauritius”, 65 % of production) which contributes more than two-thirds of the whole group. This is followed by Chakrapad (Chacapat), Kim Cheng (Wai Chee), O-Hia (Haak Yip) and a few other less important cultivars. Chakrapad usually fetches the highest price due to its larger fruit size (Sethpakdee, 2004).

Hong Huey is a common cultivar in Thailand, China and Australia and is called as “Tai So” or “Mauritius”. Trees often flower poorly or have insufficient numbers of female flowers to provide good fruitset. Trees are vigorous and spreading with an open crown, and have branched weak crotch angles that can split. Even large trees may suffer damage. Leaves are large, glossy dark almost canoe-shaped. The new flush of growth is bronze, which changes to dull mid-green to pale green with advancing maturity.

Fruits are large (22-26 g) and somewhat egg-shaped, with flat shoulders and a round tip. The thin skin is bright red changing to dull red at maturity. Protuberances are hair-like and sharp-pointed when the fruit are ready to harvest. Fruit are not of good quality until fully mature. The flavour is sweet-acid when immature, sweet when fully ripe, and bland when overripe. The flesh is slightly chewy becoming moderately crisp when fully mature. Seeds are medium, giving a fair flesh recovery of 60 to 70 %. Up to 50 % of fruit have chicken tongue seeds, depending on the season.

As lychee fruits mature, the concentration of sugars, principally sucrose, glucose and fructose increase, while the concentrations of organic acids, predominantly malic acid, decrease. The most reliable guide to maturity is titratable acidity (TA) or the ratio of total soluble solids (TSS, degree Brix) to titratable acidity. Recommendations vary, but a TSS : TA ratio of 40 or greater is recommended for commercial fruit. In practice, most orchards in the region are harvested on the basis of taste and general appearance (Morton, 1987).

The chemical composition of lychee has been reviewed by Cavaletto (1980), Menzel (2002) and Morton (1987). It varies among cultivars. Moisture content ranges from 77 % to 84 % wt. Protein content is 0.7-1.1 % and fat less than 1 %. The physical composition and soluble solid contents of twenty-three cultivars grown in Taiwan were evaluated by Yen (1988 ; cited by Wu and Sheu, 1996). Soluble solid contents of these cultivars ranged from 14.0° to 20.3°Brix.

Sugar composition of "Brewster" lychee was investigated by Chan *et al.* (1975). They reported a total sugar content of 16.8 g/100 g fruit. Sucrose was found to be the predominant sugar (51.1 % of the total sugar content), followed by glucose (30.1 %) and fructose (18.8 %).

Chan and Kwok (1974) analysed the organic acids in "Brewster" lychee. The total organic acid content was 5.18 meq as malic acid/100 g flesh. The predominant organic acid was found to be malic acid which made up 80 % of the total organic acid content. Other nonvolatile organic acids identified were citric acid (10 %), ascorbic acid (5 %) and trace amounts of succinic, malonic, phosphoric, lactic, glutaric and levulinic acids.

Lychee is an excellent source of vitamin C. Depending on the cultivar, lychee contains 40-90 mg ascorbic acid/100 g flesh. It also contains thiamin, riboflavin, calcium, phosphorus and iron (Cavaletto, 1980 ; Menzel, 2002 ; Morton, 1987).

Johnston *et al.* (1980) studied the volatile profile of lychee. Of forty-two compounds identified β-phenethylalcohol, its derivatives and terpenoids comprised the major and characteristic portion of the volatiles.

Thailand is a country in Asia with a significant export industry. Export of lychee to Malaysia and Singapore is by road but export over longer distances such as Hong Kong and European countries, is by air cargo. Below grade fruit is sold as raw material for processing. The export statistics for fresh and canned lychee from 1995-1999 are shown in table 1.1 (Sethpakdee, 2004).

Table 1.1 Export of lychee products in Thailand from 1995-1999 (Unit =Million tons)

Type of lychee	1995	1996	1997	1998	1999
Fresh	3,257	11,603	11,158	1,511	12,496
Canned	8,930	14,084	15,525	5,404	12,886

In 1999, Hong Kong was the largest importer of fresh Thai lychee (8,644 MT) while Malaysia and USA were the major importers of canned lychee (3,767 and 2,049 MT respectively).

1.2 HIGH PRESSURE PROCESSING OF FOODS

1.2.1 Evolution of high pressure processing

High pressure processing has advanced further than the other alternative physical technologies applied to food with the exception of irradiation. To some extent, this is because of the efficacy of high pressure processing. If the applied pressure is high enough, all vegetative and spore forms of microorganisms can be inactivated. In addition, the engineering aspects of high pressure processing have advanced to such an extent that commercially economic processes have become viable within the last decade or so, at least for high-value niche market products. As with so many new technologies, the original observations on which high pressure processing was built were made a long time ago. The first reports that microorganisms could be inactivated by high pressure were made more than 100 years ago.

1.2.2 The history of high pressure

The initial experiments on the use of high pressure for the preservation of food materials were performed at the turn of the century in America by Hite (1899 ; cited by Gomes, 1997). In his experiments, meat samples subjected to a pressure of forty tons at 52°C for 1 hour showed no evidence of microbial growth after 3 months of storage. In addition, milk samples treated at a pressure of ninety tons for an hour was not altered for about four days. He also concluded that some fruits and vegetables such as peaches, pears, bananas, plums, elderberries, beans, peas and tomatoes could be satisfactorily preserved by pressure treatment (Hite *et al.*,1914 ; cited by Gomes, 1997).

Modification in protein structure and reactivity due to high pressure was also shown to happen in 1914 by Bridgman who observed the coagulation of egg white under a pressure of 6,000 kg/cm^2 for 1 hour. Likewise, Basset *et al.*(1933 ; cited by Gould, 2001) reported that pressure caused the gelation of horse serum proteins.

However, equipment and the packaging of such materials of that period were not suitable for general application of the technique, and so its use was very little until the 1980s. During this period, the little research of note included : investigation of microorganism death by high pressure treatment (Timson and Short, 1965; Sale *et al.*, 1970), tenderisation of meat by pressure-heat treatments (Bouton *et al.*,1977) and investigations of protein denaturation by pressure treatment (Suzuki, 1960 ; Hawley,

1971 ; cited by Gomes, 1997). However more recently, many studies have been reported involving the use of high pressure to preserve food. Japanese scientists have made a major contribution to this field and high pressure processed foods such as jams, fruit jellies, fruit sauces, fruit yoghurts and salad dressings have been commercially available in Japan since April 1990 (Gomes, 1997).

1.2.3 General principles, advantages and units of high pressure

Two principles underlie the effect of high pressure. Firstly, the principle of Le Chatelier, according to which any phenomenon (phase transition, chemical reactivity, change in molecular configuration, chemical reaction) accompanied by a decrease in volume will be enhanced by pressure. One would expect that temperature would have an antagonistic effect because increasing temperature results in a volume increase. On the other hand, the reaction rate increases with increasing temperature according to Arrhenius's law. Secondly, pressure is instantaneously and uniformly transmitted independent of the size and the geometry of the food. This is known as isostatic pressure.

In recent years, high pressure processing of foods has received attention due to its potential benefits compared to thermal treatments. The advantages of high pressure include : i) retention of flavour, colour, vitamin content and taste because covalent bonds (rupture of peptide bonds and disulphide bonds) are not affected by the pressures while they may be broken by temperature. ii) the effect of pressure is independent of the size and geometry of the sample since it is an isostatic process, thus pressure is applied uniformly throughout the product, while heat generates gradients and may thus over-process part of the food. iii) improvement of food texture. iv) additive-free foods are produced. v) there is no environmental pollution and vi) low energy and running costs are involved (Heremans, 1995; Galazka and Ledward, 1995).

Pressure treatment is still costly, mainly because of the initial capital expenditure, and this limits its application to high-value products. However, it can be expected that those costs will go down as a consequence of further progress in technology. An illustration is given by the presence of pressure-pasteurised milk on the British market (UK high pressure club for food processing, 2002).

The relationship between the units used in biochemical studies are listed

below.

Unit	MPa	kbar	kgf/mm^2	atm	$psi(lbf/in^2)$	$tonf/in^2$
100 MPa	100	1	10.20	986.9	14504	6.475
10 kgf/mm^2	98.07	0.9807	10	967.8	14224	6.350
10^4psi	68.95	0.6895	7.031	680.5	10000	4.464
10 $tonf/in^2$	194.44	0.6895	15.75	1524	22400	10

1.2.4 Effect of high pressure on proteins

Proteins are macromolecules with different levels of structural organisation. The native conformation of a protein is maintained by a delicate balance of ionic, hydrophobic and van der Waals interactions and hydrogen bonds. Loss of stability of proteins with consequent denaturation can be brought about by pH changes, heat, salts, surface effects and high pressure.

The volume of a protein is the sum of three effects : (a) the compositional or constitutive volume of the atoms. Calculation of constitutive volume is done by summing bond lengths and van der Waals radii of the atoms in the protein ; (b) the conformational volume. Imperfect packing of the atoms in the secondary and tertiary structure of the molecule, due to void and compressed regions is known as conformational volume and (c) the salvation volume ie. the change in volume of the molecule caused by the salvation of peptide bonds and amino acid side chains.

Application of high hydrostatic pressure leads to volume changes in the system by modifying the intramolecular interactions and creating holes in the protein, which may be filled by solvent molecules, thus changing the spatial arrangement of the solvent around the protein, the energy of the system is not affected by pressure. Increase of temperature causes changes in both the energy and volume of the system.

1.2.4.1 Pressure effects in intermolecular interaction

The stability of proteins is associated primarily with three types of interactions : hydrogen bonds, electrostatic interactions and hydrophobic interactions.

The effect of pressure is small in the formation and breakage of hydrogen bonds if anything hydrogen bonds became stronger since the formation of hydrogen bonds is due to shortening of interaction distances which are accompanied by volume decreases (Balny and Masson, 1993).

The formation of ions in aqueous solution is favoured by pressure because it involves a volume decrease due to the electrostrictive effect i.e. the coulombic field of the charged groups produce a compact alignment of water around themselves. Thus electrostatic interactions easily break under pressure.

Hydrophobic effects is considered to be the sum of two contributions : a) hydrophobic salvation : the structure of water is disturbed when hydrophobic groups are exposed in aqueous surroundings and thus compact layer between the water molecules and the nonpolar groups is formed. b) hydrophobic interactions : interactions between solvent and apolar species in the system (Heremans, 1982).

Formation of hydrophobic interactions between aliphatic groups results in a volume increase and thus is not favoured by pressure. On the other hand, grouping of aromatic rings and charge-transfer interactions results in a volume decrease and may be favoured by pressure (Mozhaev *et al.*, 1994). However, in general it is believed that hydrophobic interactions in proteins readily break under pressure.

1.2.4.2 Pressure effects on protein structure

The four levels of protein structure are characterised as primary (amino acids in a polypeptide chain joined by covalent bonding), secondary (coiling of peptide chains joined with hydrogen bonding), tertiary (arrangement of chains into globular shape by non-covalent bonding), and quaternary (various compact structure or sub-units joined by non-covalent bonding) (Leadley and Williams, 1997 ; Heremans, 1995).The primary structure of proteins is not affected by pressure, while the secondary, tertiary and quaternary structures can be significantly affected by pressure ; therefore pressure can result in novel functional properties because tertiary structure is important in determining protein functionality (Tewari *et al.*, 1999).Application of high pressure promotes destabilisation of hydrophobic interactions, which are to some extent responsible for the maintenance of tertiary and quaternary structures. The secondary structure, which is more likely to be stabilised by hydrogen bonds is less affected by pressure, usually changes in the secondary structure requires very high pressures compared the pressures necessary to bring about changes in the tertiary and quaternary structures. Denaturation, dissociation or precipitation of proteins induced by pressure can be reversible or irreversible (Gomes, 1997).

Techniques such as ultraviolet and visible spectroscopy, fluorescence, electrophoresis, circular dichroism and differential scanning calorimetry are widely used to detect conformational changes in proteins. Analyses of the amide band by Raman spectroscopy has also been used to determine changes in the secondary structure induced by pressure (Heremans and Heremans, 1989).

Hayakawa *et al.* (1992) reported changes in the secondary structure of ovalbumin after pressure treatment at 600 MPa for 9 min. The α-helix content of native ovalbumin was 33% while pressure treated ovalbumin had a 25% α-helix content. In addition, a decrease of 39% in the endothermal enthalpy of denaturation of pressurised ovalbumin was observed compared to the native protein. The authors also found that the secondary structure of bovine serum albumin was not affected by pressure under the same conditions.

Denaturation of chymotrypsinogen was reported to start at 370 MPa and irreversible denaturation was achieved at 760 MPa (Wong and Heremans, 1988)

A phase change from liquid to solid was observed when soy protein was subjected to a pressure of 500 MPa for 30 min (Kajiyama *et al.*, 1995). Aggregation of pressurised β-lactoglobulin (450 MPa, 25°C, 15 min) has been reported (Funtenberger *et al.*, 1995) and Galazka *et al.* (1996) have shown that pressure treatment modifies the functional (emulsifying) properties of this protein.

Electrophoretic and chromatographic analysis conducted by Defaye and Ledward (1995) indicated that a pressure of 750 MPa for 20 min at pH 7 induces dimerisation of metmyoglobin but had little effect on the structure at pH 5.

1.2.5 Effect of high pressure on enzyme activity

Enzymes are a special group of protein molecules characterised by two striking properties, their enormous catalytic power and their specificity. Quite often the rate of an enzyme-catalysed reaction is 10^6 to 10^{20} times that of an uncatalysed one. Enzymes are highly specific, both in the type of reaction catalysed and the choice of substrate. Generally, the specificity is determined by the rate at which the enzyme catalyses similar reactions or by its ability to distinguish between closely related substrates. A "good" substrate may be cleaved 10^4 times faster than a "poor" substrate. Some amino acid side chains are important in determining enzyme specificity or in accelerating the reaction rate. Enzymes thus contain two important

regions or sites one that recognises the substrate and one that catalyses the reaction once the substrate has been bound. These two regions are called collectively the active site, which takes up only a relatively small part of the total protein volume. The active site is a three-dimensional entity formed by amino acid groups from different parts of the linear polypeptide chain, brought into proximity in the folded enzyme structure (Ludikhuyze *et al.*, 2001a).

The delicate balance of stabilising and destabilising interactions emanating from various intramolecular forces, as well as interactions with the surrounding solvent, makes proteins and hence enzymes exhibit only small intrinsic stability. Changing temperature, pressure, or microenvironmental conditions may disturb this subtle balance and bring about changes in the overall enzyme conformation or local changes at or near the active site, which may result in a loss of enzyme activity. Thermal inactivation generally takes place in the temperature range 40°C to 80°C. Pressure inactivation of enzymes can occur at pressures exceeding about 200 to 300 MPa (Ludikhuyze *et al.*, 2001a).

In view of the specificity of enzymatic reactions, enzymes may be affected by pressure in several ways (Cheftel, 1992) : (1) Pressurisation at room temperature may bring about reversible or irreversible, partial or complete enzyme inactivation resulting from conformational changes in the protein structure. These changes depend on the type of enzyme, microenvironmental conditions, pressure, temperature, and processing time. (2) Enzymatic reactions may be enhanced or inhibited by pressure, depending on the volume change (positive or negative) associated with the reaction. Pressure-induced changes in the catalytic rate may be due to change in the enzyme-substrate interaction, changes in the reaction mechanisms, or the effect of a particular rate-limiting step on the overall catalytic rate. (3) A macromolecular substrate (protein, starch) may become more sensitive to enzymatic action once it has been unfolded or gelatinised by pressurisation. (4) Provided that the cell membrane or the membrane of intracellular organelles is altered, intracellular enzymes may be released from extracellular fluids or cell cytoplasm, hereby facilitating enzyme-substrate interactions

1.2.6 Effects of high pressure on chemical related to food quality

In response to the growing consumer demand for manufactured food

products, the food industry is interested in high pressure technology as a demand for high-quality, nutritious potential food processing/preservation method that minimally affected sensory and nutritional quality attributes. High pressure allows inactivation of pathogenic/ spoilage microorganisms and food-spoiling enzymes while leaving most attributes of food quality intact. This advantage is attributed to the fact that high pressure keeps covalent bonds intact and affects only non-covalent bonds. Important characteristics of high-quality foods are texture, flavour, colour, and nutritive value. The first three properties are quality attributes that determine consumer acceptance of the product. Nutritive value (i.e., vitamins, minerals, and other nutrients) is a hidden quality. Chemical or biochemical reactions occurring in food products can bring about undesirable changes in or deterioration of these attributes of food quality during preservation / processing treatments and subsequent storage (Ludikhuyze and Hendrickx, 2001).

Reactions leading to such undesirable changes include nonenzymatic and enzymatic browning, lipid hydrolysis or oxidation, protein denaturation, hydrolysis or cross-linking, polysaccharide hydrolysis or synthesis, and degradation of natural pigments. In the context of conventional preservation/processing methods, a great deal of research has been carried out to investigate the effects of various processing variables (e.g., temperature, time, heating rate, pH, water activity) on reactions leading to deterioration of food products in order to design processes so that they induce the required microbial and enzymatic inactivation while retarding or inhibiting specific deteriorative reactions. Knowledge about the effects of pressure is indispensable for implementation of high pressure technology as an alternative preservation/processing method in food industry. The most likely route that the food industry will take is to combine high pressure processing with other processing methods, especially moderately elevated temperature, because bacterial spores and some enzymes are very pressure stable (Sale *et al.*,1970 ; Hendrickx *et al.*,1998 ; Smelt, 1998).

Eshtiaghi and Knorr (1993) compared the effect of high pressure processing (HPP) with water blanching on quality (softness), leaching of potassium and loss of ascorbic acid of potato cubes. Water-blanched and high-pressure-treated samples had similar softness but potassium leaching was reduced by 20%. In addition, ascorbic

acid was better retained (90% at 5°C to 35% at 50°C) in high-pressure-treated vacuum-packaged samples. Rovere *et al.* (1997 ; cited by Tewari *et al.*, 1999) studied the effects of HPP on chopped tomatoes. They pre-treated tomatoes at 25, 50, or 85°C, followed by cooling and vacuum packaging. After this, the tomatoes were subjected to pressures. They reported that colour, sugar content, and pH were affected by pressure, and that viscosity decreased with increasing blanching temperatures and increased with increasing pressures. They also found that pressure has a significant inactivating effect on polygalacturonase type pectic enzymes, but had only little effect on pectin esterases (PE). They concluded that HPP may control various quality parameters of chopped tomatoes.

Kimura *et al.* (1994) compared the quality (volatile flavour components, anthocyanins, browning index, furfural, sucrose, and vitamin C content) of pressure-treated and heat-treated jams during storage at 5 and 25°C for 1-3 months. Immediately after processing, the pressure-treated jams had better fresh quality than heat-treated jams, and the quality was maintained in both at low temperature storage, but not at room temperature for pressure-treated jams. The presence of dissolved oxygen and enzymes was believed to have resulted in deterioration of pressure-treated jam held at ambient temperature. However, pressure-treated jam could be stored at refrigeration temperature with minimal loss in sensory and nutritional characteristics for up to 3 months. Gow and Hsin (1996) compared the quality and shelf life of pressure-treated with thermally pasteurised (88-90°C for 24 s) “guava puree”. A substantial inactivation of microbes (< 10 cfu mL^{-1}) was observed at 600 MPa, and the pressure-treated samples showed no colour-change, no degradation of pectin, no cloud formation, and had the same ascorbic acid content as fresh samples. However, enzyme inactivation was more pronounced in thermally-treated samples. The pressure-treated guava puree (600 MPa) maintained good quality (similar to freshly extracted guava puree) for 40 days when stored at 4°C. It is to be noted that guava puree is particularly sensitive to enzymatic browning reactions which are inhibited during HPP to some extent.

Dong *et al.*(1996) studied the effect of HPP on the shelf life and sensory characteristics of *Angelica keiskei* juice by subjecting it to a pressure of 558 MPa for

7 min and storing it at 4°C. Pseudomonas spp., *E. coli*, and coliform bacteria were totally inhibited by HPP. During storage, microbiological quality and sensory characteristics were monitored and it was found that HPP did not significantly influence freshness, sweetness, and bitterness but after 8 days (at 4°C) pressure-treated juices had better freshness rating than controls (non-pressurised juice). Kloczko and Radomski (1996 ; cited by Tewari *et al.*, 1999) studied preservation of fresh fruits , vegetables, fruit- and vegetable-juices by subjecting them to pressures and subsequently storing them at 6°C. They reported that HPP had no beneficial effects on keeping quality of fruits and vegetables, whereas immediately after pressurisation, and after 55 days of refrigerated storage, pressure-treated juices had better aroma, flavour, and microbiological quality than untreated controls, and vitamin C content remained the same or declined slightly. Pehrsson (1996) described experiments on HPP of microbially stable citrus juice, where they processed juices for 60-90 s at refrigeration or freezing temperature. The product was stored and distributed under refrigeration. The pressure-treated juice was stable for 6 months at 4°C without losing any freshness (as compared to juices thermally treated at 98°C for 10 s). Donsi *et al.*(1996 ; cited by Tewari *et al.*, 1999) studied the high pressure stabilisation of orange juice, by evaluating microbial activity and the chemical composition of orange juice treated at different pressure levels for various operating times. They obtained a 2 months shelf life for pressure-treated orange juice (at 350 MPa for 1 min at 30°C) stored under refrigeration. Butz *et al.* (1997) studied the effects of HPP on anti-mutagenic activities of fruits and vegetables juice. The anti-mutagenic activity was compared with raw and heated samples (100 or 50°C for 10 min). They reported that anti-mutagenicity of strawberry and grapefruit juices was not affected by heat and pressure. Also, vegetable juices exhibited moderate to strong anti-mutagenicity, whereas, the anti-mutagenic activity of carrot, leek, spinach, kohlrabi, and cauliflower juices was sensitive to heat treatment but remained unaffected by pressure treatment.

Severini *et al.* (1997 ; cited by Ludikhuyze and Hendrickx, 2001) investigated the effects of high pressure on the lipid oxidation of extra virgin olive and seed oils. Peroxidase value, p-anisidine value, rancimat test, and volatile

hydrocarbons were the analytical parameters measured to study the oxidative stability of the pressure-treated oils. They reported pressure treatment changed p-anisidine values, but not others (peroxidase values and volatile hydrocarbons). Other parameters affecting high pressure treatment were origin, composition, initial quality, and age of the oils, and it was found that olive oils were more resistant to oxidation which suggests need for replacement of seed oil with extra virgin olive oil during HPP to extent shelf life of foods. They conducted their study using only one pressure, temperature and time period. Further studies should be conducted using different combinations of pressure, temperature and time intervals, which may result in other value-added products and opportunities.

1.2.7 Effect of high pressure on microorganisms

Application of HPP as a method for microbial inactivation has stimulated considerable interest in the food industry. The effectiveness of HPP on microbial inactivation has to be studied in great detail to ensure the safety of food treated in this manner. Currently, research in this area has concentrated mainly on the effect of HPP on spores and vegetative cells of different pathogenic bacterial species. Detectable effects of HPP on microbial cells include an increase in the permeability of cell membranes and possible inhibition of enzymes vital for survival and reproduction of the bacterial cells (Farr, 1990). To design appropriate processing conditions for HPP of food materials, it is essential to know the precise tolerance levels of different microbial species to HPP and the mechanisms by which that tolerance level can be minimised. A knowledge of critical factors that affect the baroresistance of different bacterial species will help in the development of more effective and accurate high pressure processors. Inappropriate use of a variety of parameters like pressure range, processing temperature, initial temperature of sample, holding time, and packaging type may adversely affect the outcome of HPP. Thus a through understanding of the effect of a variation in critical factors on the intracellular changes undergone by pressure-treated microbial species is essential for documentation of a safe HPP. The physicochemical environment can adversely change the resistance of a bacterial species to pressure. Factors such as the water activity and pH also influence the extent to which foods need to be treated to eliminate pathogenic microorganisms. In most cases, the effect of HPP on gram-positive bacteria is less pronounced than on gram-

negative species. Vegetative cells during the early growth phase are normally more barosensitive than cells in the stationary, dormant, or death phase (Isaacs *et al.*, 1995). Pressure has a greater destructive effect on cells in acid media than in neutral media. Cells in distilled water are more susceptible than those in buffer, and cells pressurised at 5°C or 40°C are more sensitive to pressure than those pressurised at room temperature (Arroyo *et al.*, 1997 ; Apichartsrangkoon, 1998). Yeast, lactic acid bacteria and moulds are very sensitive, while bacterial spore are most resistant and can even survive pressures above 1000 MPa. Viruses also appear to have a high resistance to pressure (Cheftel, 1992).

1.2.7.1 Mode of action of HPP on microorganisms

It can be expected that the mode of action of pressure on whole organisms is not necessary the same, but dependent on the pressure level. Elevated pressure between 30 and 50 MPa can influence gene expression and protein synthesis. High pressure can induce tetraploidy in *Saccharomyces cerevisiae*, indicating that high pressure processing can interfere with replication of DNA. At pressure of ~ 100 MPa the nuclear membrane of yeasts was affected, and at more than 400-600 MPa further alteration occurred in the mitochondria and the cytoplasm. In particular, metal ions are released at pressure over 300 MPa. Pressure-inducible proteins have been found in *Methanococcus thermolyticus*, *Rhodothorula rubra* and *E. coli*, a pressure of 53 MPa could induce protein similar to those found at elevated temperature. Although it is not yet known whether pressure can indeed enhance resistance to physical treatment, cells subjected to stress other than temperature (e.g. by sublethal heat) become more resistant to pressure. The mechanism might be stabilisation of the structures of membrane-bound enzymes. A perturbation of the bacterial membrane is almost always involved during pressure treatment (Smelt, 1998).

Cell membranes which are held together by hydrogen bonds, separate the intracellular constituents from their environment, are comprised of a twin layer of phospholipids consisting of hydrophobic moieties (fatty acids) and relatively hydrophilic moieties (glycerol), with a protein layer between the two phospholipids layers. Cell membranes, which play an important role in cell transport, permeability and respiration, are the first point of attack of high pressure, which breaks up phospholipids molecules, denatures proteins and alters permeability (Chong and

Cossius, 1993 ; cited by Apichartsrangkoon, 1998). Fatty acids of barophilic microorganisms become more polyunsaturated with increase in growth pressure (DeLong and Yayanos, 1985). A food spoilage organism, *Lactobacillus plantarum* in the exponential phase was more resistant to pressure when the cells were grown at suboptimal temperature (Smelt *et al.*, 1994). Under these conditions, fatty acids were more unsaturated than in cells grown at optimal temperatures. When cholesterol is included, the fluidity of cell membranes of prokaryotes decreases and the cells become more sensitive to pressure. The protective effect of different carbohydrates on the membrane during freezing is in the order glycerol < fructose < glucose < sucrose < trehalose and the same order was found for the protective effect of these carbohydrates against pressure (Smelt, 1998). Propidium iodide and ethidium bromide bind to nucleic acids, but they can only penetrate into the cell when the membrane is damaged. By contrast with untreated cells, pressurised bacteria can be stained with propidium iodide or ethidium bromide (Mackey *et al.*, 1995 ; Smelt *et al.*, 1994). Pressure-inactivation is also accompanied by an increase of extracellular ATP again showing leakage of the membrane. Integral and peripheral membrane proteins become more detached from the plasma membrane when the membrane bilayer is sufficiently perturbed by pressure. HPP can also induce enzyme denaturation. There is an optimum temperature at which enzymes are most resistant to pressure (Hawley, 1978). As this is similar to that what has been found for microorganisms and bacteriophages, there is some circumstantial evidence that some microbial enzymes constitute the main target of pressure inactivation (Smelt, 1998). Enzymes of extreme thermophiles are not only more heat resistant but also more pressure resistant than mesophilic microorganisms stabilised by pressure (Jaenicke, 1991). High pressure can presumably directly affect enzymes and carriers of transport systems. A decrease intracellular pH after pressure treatment has also been found.

The observations on membrane damage, protein denaturation, decrease intracellular pH and the observations on yeasts suggest that membrane-bound enzymes associated with efflux of protons may be at least one of the major targets in high-pressure inactivation. Elevated pressure can influence gene and protein expression in both 0.1 MPa adapted and high-pressure adapted microorganisms. A further important effect of pressure on membranes would be on ion movements

mediated by ATPase enzymes. *Streptococcus faecalis* could be adapted to grow at a pressure as high as 20 MPa. This strain had a regulatory defect and it produced large amounts of ammonia. Under pressure, non-adapted *S. faecalis* is hypersensitive to acid and the ammonia acted to neutralise metabolic acids. DNA and RNA are very resistant to pressure. However, an extreme condensation of the nuclear material was observed in *Listeria monocytogenes* and *Salmonella typhimurium* (Smelt, 1998).

The structure of spores is much more complex than of vegetative cells. In addition to peptidoglycan, spores contain, dipicolinic acid as well as calcium ions. This complexity affords spores higher resistance to heat, pressure, drying, radiation, acids and chemical disinfectants. The mode of action on bacterial spores is still a matter of speculation. Bacterial spores are killed directly by pressures higher than 1000 MPa and more rapidly at low pH values. Taki *et al.* (1991 ; cited by Apichartsrangkoon, 1998) used a pressure of 600 MPa at 60°C for 20-60 min to inactivate spores of *Bacillus licheniformis*, while to reduce the number of spores of *B. cereus*, by this degree required pressures of 800 MPa at 50°C for 20 min (Fonberg-Broczek *et al.*, 1995 ; cited by Apichartsrangkoon, 1998). Spores of *Clostridium sporogenes* were completely inactivated by pressures of 800 MPa at 80-90°C or 1500 MPa at 60°C (Tewari *et al.*,1999). However, spores are sensitive to pressures between 50 and 300 MPa (Sale *et al.*, 1970 ; Timson and Short, 1965 ; Wuytack *et al.*,1997). It is generally agreed that at such pressures, spores germinate, followed by death of the germinated spore. Sale *et al.* (1970) suggested a two step process to inactivate spores involving a "low" pressure/temperature regime to initiate germination and then a more severe regime to inactivate the germinated forms. This method could inactivate 4×10^5 endospores/ml of *B. cereus* at room temperature by treatment at 200 MPa for 1 min followed by treatment at 900 MPa for 1 min (Tewari *et al.*,1999). Wuytack *et al.* (1997) and Hölters *et al.* (1997) used pressures of 60-200 MPa to induce germination of dormant spores of *B. subtilis* and *B. stearothermophilus* and pressures greater than 300 MPa to inactivate the spores.

1.2.7.2 The effects of food constituents on pressure resistance

The effects of food constituents on pressure resistance is complicated. Some effects are the result of pressure on the molecules and especially water. For instance, high pressure causes water to become solid by formation of different types of ice.

This ice formation can be altered by the presence of high concentrations of solutes. Little attention has been paid to the behaviour of complex foods in this respect. In addition, the pH shift due to pressure is dependent on the type of buffer. The situation is further complicated by the fact that during pressure treatments temperature changes almost always occur (Smelt *et al.*, 2001).

In real food situations, two effects always determine microbiological safety and stability : the effect of the food during treatment and the effect after treatment during recovery of the microorganism. It should also be taken into account that results of studies in buffers or laboratory media can not be directly extrapolated to real food situations. For instance, milk and cream protect microorganisms against pressure (Patterson *et al.*, 1995). Arroyo *et al.* (1997) subjected lettuces and tomatoes to pressure and found a decrease in the number of CFUs of about 2 log cycles at a pressure of 350 MPa for 10 min. Shigehisha *et al.* (1991) subjected pork slurries to pressure and found that pressures as high as 600 MPa were necessary to inactivate *Micrococcus luteus, Staphylococcus aureus,* and *Streptococcus faecalis*. Proteins in general seem to protect microorganisms against pressure inactivation. Also glucose seems to have a protective effect apart from water activity (Simpson and Gilmour, 1997). Szczawinski *et al.* (1997) found that *Listeria monocytogenes* survived in Edam cheese after pressure treatment for 15 min at 500 MPa.

Acidity

In considering the effect of pH, one must consider that both temperature and pressure cause a pH shift and each buffer has its own characteristics in this respect. When the activation volume of the buffer is very large, the pH change due to pressure will be large. For instance, the pH shift of phosphate buffer caused by pressure is large and it is generally used as the reference buffer in inactivation studies of microorganisms. In addition, buffers have their specific effects on the physiology of the cell. Yeasts and moulds are quite resistant to low pH and a pH less than 4.0 hardly sensitises these microorganisms against pressure (Smelt, 1998).

Ye *et al.*(1996 ; cited by Tewari *et al.*,1999) studied the pressure tolerance of *Saccharomyces cerevisiae, Escherichia coli,* and *Staphylococcus epidermis* in various media (agar, broth, apple jam, and juice), by subjecting the inocula to a pressure of 300 MPa at 5-25°C for 1-20 min. They reported that media pH played a very

important role in the destruction of microbes ; *Staphylococcus epidermis* was inhibited > 90 % at 300 MPa in 11.2 min at pH 7.2 and in 4.8 min at pH 4.0. Timson and Short (1965) studied on *B. subtilis* showed that the pressure resistance of the bacterium (when subjected to 483 MPa for 30 min) was decreased as the pH in milk medium was lowered or raised from a pH value of 8. This value is not a constant for all microorganisms and the survival of *B. subtilis* at a specific pH can vary with the pressure and temperature of treatment.

By comparison, vegetative bacteria are quite sensitive to pressure, to heat, and low pH. Bacterial spores are most resistant to the direct effects of pressure treatment at neutral pH. Roberts and Hoover (1996) investigated the effects of change in pH values combined with a variety of other factors on the pressure-resistance of *B. coagulans* ATCC 7050. They reported an increase in the effectiveness of pressurisation as the pH of the buffer was lowered. A decrease of an additional 1.5 log was observed as the pH was decreased from 7.0 to 4.0. Timson and Short (1965) observed that at high pressure the spores were most resistant at neutral pH and at low pressure, most sensitive to neutral pH. When spores are killed indirectly, following germination at pressures between 50 and 300 MPa spores are most sensitive to pressure at neutral pH. Bacteria are more sensitive to suboptimal pH after heat or pressure treatment.

In contrast, Sale *et al.* (1970) reported that the inactivation of bacterial spores by pressurisation was maximum when the buffer was at a pH near neutral and was lowest at extreme values of pH.

Acidity not only enhances inactivation during treatment, but inhibits out growth of cells injured sublethally by heat or pressure. Apart from pH effects, no specific effect of organic acids have been observed. This might be due to the fact that pressure favours ionisation and that organic acids particularly inhibitory in the undissociated form. On the other hand, it is conceivable that the undissociated part might be more active under pressure.

Type of culture medium

The type of culture medium used for growing the microbial species can also have a significant impact on the pressure and heat resistance of any microorganism. In general, the richer the growth medium, the better the baroresistance of the

microorganisms. This is thought to be because of the increased availability of essential nutrients and amino acids to the stressed cell. It must be kept in mind that the parameters governing pressure tolerance are not constant for every bacterial species, they vary from one bacterium to another, and may also be different for a single species grown under different conditions or in different growth media.

Hayakawa *et al.* (1994) compared the pressure resistance of spores of six *Bacillus* strains. The spores were cultivated on nutrient agar and suspended in cold sterile distilled water with the filtrate being heated for 30 min at 80°C to destroy any vegetative cells. Spores of these six strains were then treated under pressures ranging from 196 to 981 MPa at 5-10°C for holding times of 20-120 min. It was found that *B. stearothermophilus* IAM 12043, *B. subtilis* IAM 12118 and *B. licheniformis* IAM 13417 had the most resistance to pressure, but *B. coagulans* was actually activated when treated with high pressures. There was no actual correlation between pressure and heat resistance, although they choose rightly a number of spore-forming bacteria varying widely in heat resistance. This could be due to applications of very low pressure in most cases. However, variables including initial and final spore levels and dilution levels were not specified, which may help understanding the results better. It should also be noted that, in most cases, the reference heat treatment is much more inactivating that the pressure treatment to be compared, therefore, direct comparison between heat resistance and pressure resistance is often not fair. Patterson *et al.* (1995) studied the sensitivity of vegetative pathogen (*Yersinia enterocolitica* 11174, *Salmonella typhimurium* NCTC 74, *Salmonella enteritidis, Staphylococcus aureus* NCTC 10652, *Listeria monocytogenes*, *Escherichia coli* O157:H7) in buffer (pH 7.0), UHT milk, and poultry meat to high pressures up to 700 MPa at 20°C. A 10^5 reduction in numbers was obtained in all cases which pressures in the range of 275-700 MPa for 15 min were applied at 20°C. Different strains of *L. monocytogenes* and *E. coli* O157:H7 showed significant variation in pressure resistance, which were further used to examine the effect of substrates on pressure sensitivity, and indicated that substrate affected the baroresistance of the microorganisms significantly.

Water Activity

Although there is a general osmotic effect of water activity (a_w) on the cell, there are also specific effects of factors that influence water activity. Carbohydrates

are generally more protective than salts. At the same water activity, thermotolerance and barotolerance is lower in glycerol than in solutions of monosaccharides and disaccharides. Trehalose seems to be particularly effective (Smelt *et al.*, 2001). There are a number of explanations for the role of trehalose as a particularly effective saccharide against environmental stress. According to Shinsuke *et al.* (1996), macromolecules are stabilised against heat and pressure of thermotolerance and barotolerance seem to be linearly related to the number of equatorial OH groups.

In general, low water activity protects cells against pressure (Oxen and Knorr, 1993), but microorganisms injured by pressure are generally more sensitive to low water activity. For instance, Patterson *et al.* (1995) showed that recovery of pressure-treated cells was much lower when 2% salt was added to the medium. In conclusion, the net effect of lower water activity is not always easy to predict.

1.2.8 Effects of pressure processing on fruit products

The preparation of fresh fruit products is of great interest for the food industry because of consumers' renewed interest in natural and healthy foods. Conventional thermal processing yields products that lose their typical attributes of freshness. In recent years, the use of optimised thermal treatments and the introduction of new ingredients have improved the organoleptic properties of conventional products. This has resulted in products that differ from fresh ones. The short shelf life and low safety level of fresh, unprocessed fruits are significant drawbacks. Crispiness, sharp cutting surfaces, colour, and taste are attributes that any fresh product must keep. High pressure processing can help foods achieve these qualities.

The high rate of microbial inactivation in acid products may correspond to microbial stability. On the other hand, the low enzyme inactivation limits the shelf life of plant products. Nevertheless, measures to control the residual enzyme activity (such as refrigerated distribution or suitable packaging materials) are in line with the high-quality standards required for "fresh, minimally processed" foods.

The remarkable increase in firmness of fruit tissue during storage is most likely due to the selective inactivation of peptic enzymes that cause the complexation of calcium ions with demethylated pectin chains. HPP enhances the rate of this long-term reaction as a consequence of the pressure-induced cell disruption and the loss of

compartmentalisation. However, some times the process may affect the taste and flavour of the product. These changes are the result of the biochemical oxidation of fatty acid traces, a mechanism that involves a sequence of enzymes that are not usually sensitive to pressure. Different ways of solving this problem have been suggested. The most simple measures are in agreement with good manufacturing practices : fast material flow on the processing time, minimal dead times, maximum deaeration, and refrigeration of the raw material before processing. In fact, a low starting temperature and a fast treatment slow down or suppress the lipase activity before the concentration of precursors (free fatty acids) is sufficiently high to initiate the formation of off-flavours. Thus, with proper techniques, natural colours, taste, and typical attributes can be retained in sensitive fruits such as kiwi, strawberries, or raspberries and related products over a comparably long period of time. The proper combination of techniques allows food manufacturers to prepare high-quality conventional foods and to develop completely new products (Rovere, 2001).

Moreover, consumers are more likely to accept these products when the visual freshness is accompanied by a "natural" behaviour. The fact that a fruit salad has an oxidative stability up to several hours after opening the package is important to confirm its natural characteristics and to limit the life of a product formulated without any added preservative. The use of ascorbic acid can help increase the oxidative stability of the product and reduce the technical hurdles to be overcome before packaging.

1.3 THE OBJECTIVES OF THIS STUDY

The aim of this study is to understand the effects of high pressure processing on lychee. It will investigate pigments, flavour components, cell structure, enzymes (peroxidase, lipoxygenase and polyphenoloxidase and also microbiological activities of the products. In addition the pink discolouration which occurs in canned lychee was also studied.

Lychee is an economically valuable product of Thailand. At the peak of the season its price usually reduces a great deal. In order to remedy this situation, lychees are processed into several products including canning process. As with many other fruits and vegetables, a pink discolouration occurs in canned lychee. This phenomena is not only of sensory importance but also leads to nutritional losses causing lower

price of this products. In order to prevent this apparently unavoidable phenomenon, non-thermal processing such as high pressure processing is proposed. For the development of high pressure processed fruits and vegetables, it is essential to study the influence of pressure on pigments, flavour components, cell structure, enzymes such as peroxidase, lipoxygenase and polyphenoloxidase and microbiological activities of the products.

Peroxidase is associated with off-flavours and off-colours in raw and unblanched vegetables. It is often used as an indicator for evaluating the efficiency of blanching processes because of it is the most heat-stable enzyme in vegetables, therefore, it also indicates thermal inactivation of all other vegetable enzymes. Peroxidase is not only heat-stable but also very pressure resistance. Polyphenol-oxidase causes enzymatic browning of damaged fruits and vegetables, and is one of the main causes of quality deterioration (brown colouration and concomitant changes in appearance and organoleptic properties) during postharvest handling, storage, and processing. Lipoxygenase is an enzyme that catalyses the oxygenation of poly-unsaturated fatty acids into the corresponding hydroperoxides. It mediates, either directly or indirectly, a whole spectrum of changes in food quality including colour, flavour, texture, and nutritional quality. Because all of these enzymes represent a major problem in food quality, inactivation of peroxidase, polyphenoloxidase and lipoxygenase are highly desirable.

Because of increasing consumer demand for minimally processed additive-free, shelf-stable products, the food industry is interested in high pressure technology. HPP is gaining in popularity with large food companies because of its capacity to inactivate pathogenic microorganisms with minimal heat treatment, resulting in almost complete retention of freshness, texture, colour, nutrients and flavour during processing. Thus, high pressure treatment of lychee is a feasible and promising technique of preservation which will avoid discolouration without the need to add any chemical substances.

The purpose of this research is to develop novel healthy and wholesome processes for lychee processors and to offer new dimension of Thai fruit and vegetable technologies.