CHAPTER 5
EFFECT OF HIGH PRESSURE ON TEXTURE, MICROSTRUCTURE AND MICROBIOLOGICAL QUALITY

5.1 INTRODUCTION

5.1.1 Texture and Microstructure

Texture is a quality attribute that is critical in determining the acceptability of fruits and vegetables. It is related to the structural, physiological, and biochemical characteristics of the living cells : their changes over time , and their alteration by processes such as cooking and freezing (Abbott and Harker, 2005).

Matz (1962 cited by Waldron *et al.*, 2003) defined texture as the mingled experience deriving from the sensations of the skin in the mouth after ingestion of a food or beverage, as it relates to density, viscosity, surface tension, and other physical properties of the materials being sampled. Bourne (1982) defined the textural properties of a food as the group of physical characteristics that : arise from the structural elements of the food ; are sensed by the feeling of touch ; are related to the deformation, disintegration, and flow of food under force ; and are measured objectively by functions of mass, time, and distance. More recently, in 2002, he stated that texture is a multidimensional attribute, and a collective attribute that encompasses the structural and mechanical properties of a food and its sensory perception in the hand and in the mouth. According to Rojas *et al*. (2002), the textural characteristics of vegetable tissue are governed by their chemical composition and histological structure, while Aguilera and Stanley (1999) similarly reported that texture arises from the arrangement of various chemical species by physical force into distinct micro- and macrostructures, and is the external manifestation of these structures.

The Microstructure features of plant materials influence their textural properties and the final products (Zdunek and Umeda, 2005). They are valuable in revealing possible mechanisms of change in texture during food processing which would help in process optimisation and improvement of product quality to provide greater consumer satisfaction. Otegbayo *et al.* (2005) studied the effects of boiling on

the microstructure of two species of yam, *Dioscorea alata* L., and *D. rotundata* Poir and their association with the textural quality of "boiled yam", arguably the most common food product from yams. Histological studies on raw yams showed parenchyma cells of both species to be three-dimensional and polyhedral in shape, with starch granules loosely arranged in *D. rotundata* but densely packed in *D. alata*. Generally, there was cell separation and rounding off of cells in cooked yams of *D. rotundata* varieties, whereas cell separation was only partial with no rounding off in *D. alata*. Mealy boiled yams showed complete cell separation and the "rounding off" phenomena, whereas the waxy ones showed partial retention of textural cell integrity. These relative changes in microstructure of yam when boiled could serve as indicators of textural quality in boiled yam.

The knowledge of the relationships between texture and structure in fruits and vegetables has been reported to be the key to our ability to understand and control specific textural characteristics in these edible plant materials. More specifically, in fruit and vegetable tissue, firmness is known to be a meaningful attribute to consumers (Ramana *et al.*, 1997 cited by Alvarez *et al.*, 2000a). Several histological factors, such as the size and shape of the cells, the volume of intercellular spaces, the thickness of the cell wall, the cell wall components, and the cell turgor pressure, are related to the firmness exhibited by fruits and vegetables (Thybo *et al.*, 1998 ; Khan and Vincent, 1990).

5.1.1.1 Plant cell wall

The cell wall is the primary structural element in plant tissues and plays important roles in the textural quality of such foods, The plant cell wall is composed of three parts : 1) intercellular cement or middle lamella formed between adjacent cells during cell division ; 2) primary cell wall, a thin and flexible layer which is formed early during cell development and is located between the plasma membrane and middle lamella ; and 3) a secondary cell wall formed during cell maturation, providing strength and mechanical support to the plant cells. Edible plant tissues are usually rich in parenchymatous cells which are thin-walled and nonlignified (Waldron *et al.*, 2003). Figure 5.1 shows a typical plant cell called a parenchyma cell and the components of the plant cell. The major compounds in plant cell walls are polysaccharides, i.e., pectic substances, cellulose and hemicellulose. In most fruits

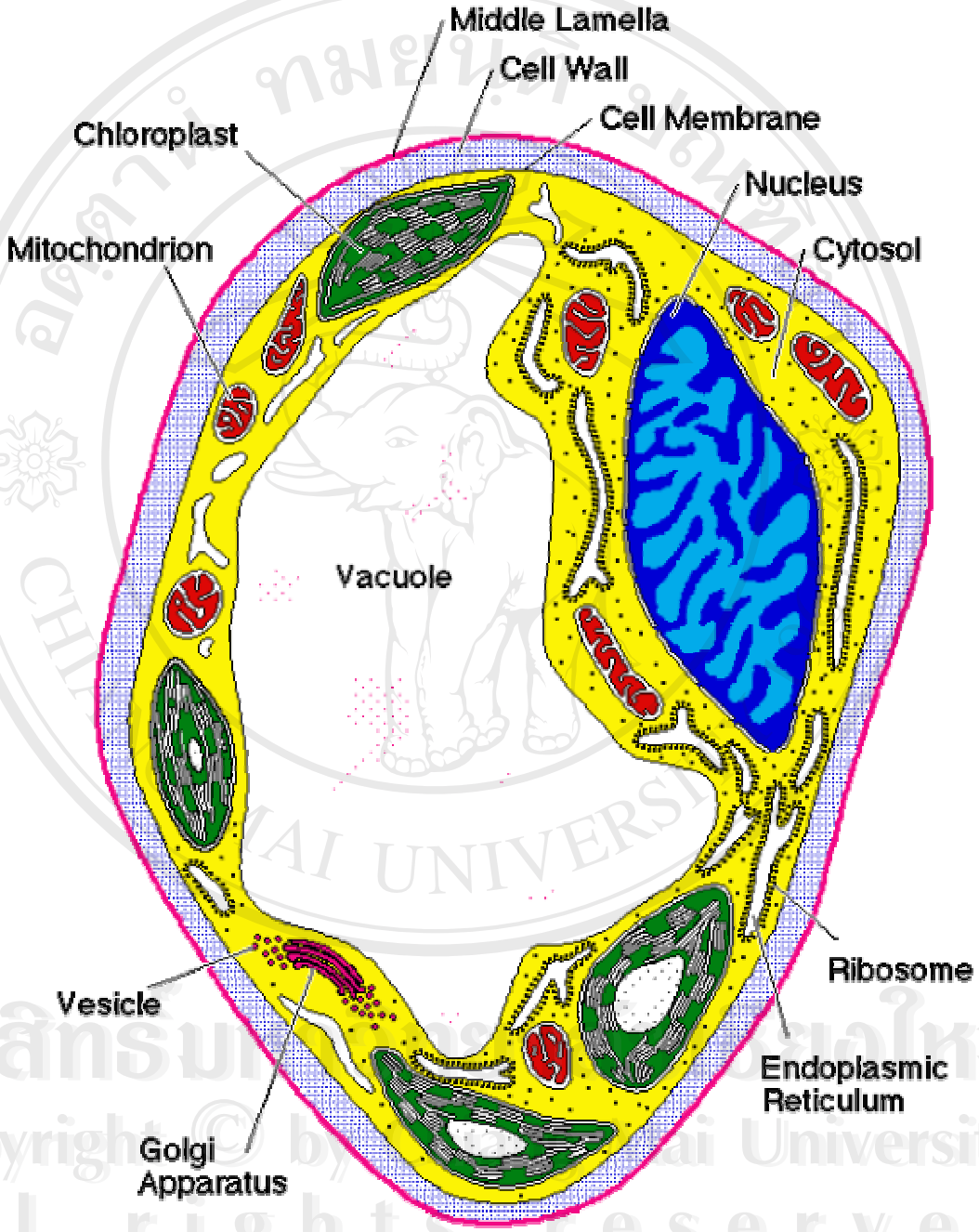

Fig 5.1 A typical plant cell " parenchyma cell" and the component of the plant cell. From Koning (1994).

and vegetables, changes in the cell wall polysaccharides at the cell wall-middle lamella significantly influence the softening of the tissues although softening can also result from loss of turgor. The turgor comes as a result of the interplay of osmotic pressure developed within the vacuole and the pressure exerted by the relatively rigid structure of the cell wall (Tangwongchai, 2000).

5.1.1.2 Components related to texture and cellular structure of plant

5.1.1.2.1 Pectic substances

Pectic substances is a designation for the colloidal polysaccharides in plant, and with cellulose and hemicellulose, are the major constituents of the middle lamella and are structural elements in the primary cell walls of higher plants. They contribute both to adhesion between the cells and to the mechanical strength of the plant tissues. It is believed that these compounds play an important role in the texture of fruits and vegetables.

The main components of pectic substances are D-galacturonic acids, primarily linearly linked by α-(1→4) glycosidic linkages. The galacturonan backbone is interspersed with rhamnose units joined to the reducing end of the uronide by (1→2) linkages and nonreducing end of the next uronide unit by (1→4) bonds, giving the rhamnogalacturonan chain. Side chains, often arabinan, galactan or arabinogalactan, are linked (1→4) to the rhamnose. Other sugars such as D-glucuronic acid, D-glucose, D-mannose and D-xylose are sometimes found in side chains. Many of the carboxyl groups of poly-D-galacturonic acid are esterified with methyl groups to varying degrees. There are a number of constituents under the term pectic substances ; *Protopectin* has been defined as a significant water insoluble portion of the anhydrogalacturonic acid residues in plants. It sticks the cells firmly together and thought to be the parent of other pectic substances. Its precise structure is not known since it partially degrades during extraction from plant cells. The conversion of protopectin into a soluble form by protopectinase leads to cell separation, seen on a macroscopic scale as a loss of rigidity and softening of the tissue. *Pectin* is a water-soluble linear chain of (1→4)-linked α-D-galacto-pyranosyl-uronic acid units of varying chain length, molecular weight and degrees of esterification (DE) with methyl groups. It is classified into two groups; high-methoxy pectin (HMP) in which more than half of the carboxyl groups are methyl esterified ;

and low-methoxy pectin (LMP) in which less than half of the carboxyl groups are in the methyl ester form. It is known to influence the consistency of processed fruit products through its ability to form a gel. Fruit tissues are specially enriched in pectins, being about 7-40% on a dry weight basis. *Pectic acid* is polygalacturonic acids of colloidal nature but essentially all of the carboxyl groups of the acid chain are free of methyl ester groups (Tangwongchai, 2000).

5.1.1.2.2 Cellulose

Cellulose provides the microfibrillar component in the cell walls of higher plants and consists of β-(1-4)-linked glucose with a degree of polymerisation of between 2,000 and 6,000 in primary cell walls to more than 10,000 in secondary walls. Cellulose comprises the single most abundant polysaccharide component of vegetables. Its glucan chains interact closely through hydrogen bonding, excluding water to produce areas of crystallinity (Waldron *et al.*, 2003). The degradation of cellulose is catalysed by cellulase (Tangwongchai, 2000).

5.1.1.2.3 Hemicellulose

Hemicellulose, together with cellulose, lignin and pectic substances, form the main part of undigested food carbohydrates. According to Waldron *et al.* (2003), hemicelluloses are usually solubilised only by treatments that disrupt the hydrogen bonds which link them strongly to cellulose microfibrils. They are found in both primary and secondary plant cell walls, consisting of xylans, arabinoxylans, mannans, and glucomannans. The degradation of hemicellulose is catalysed by hemicellulase although its catalytic mechanism is poorly understood (Tangwongchai, 2000 ; Waldron *et al.*, 2003).

5.1.1.3 Event which effect texture and microstructure of fruit

There are several well-known treatments which can cause the softening of edible plant tissues. One of these, fruit ripening, is a natural and biochemically mediated process, often considered to be the initial stages of a form of senescence. Another, thermal processing, is a physicochemical process, the extent of which can be modulated by postharvest storage. In both cases, the softening occurs mainly as a result of a weakening of cell-cell adhesion.

5.1.1.3.1 Fruit ripening

Ripening of fruits is a complex process that generally involves tissue

softening. During fruit ripening, cell wall changes include solubilisation and degradation of pectin and a net loss of the non-cellulosic neutral sugars galactose and arabinose, and there may be a decrease in the molecular weight distribution of hemicellulose (Abbott and Harker, 2005). Early histochemical studies on fruit ripening demonstrated dissolution of pectic polysaccharides from the middle lamella of many ripening fruits. In keeping with the long-held assumption that the middle lamella is generally responsible for cell adhesion, particular interest has been taken in enzymes which modify pectic polysaccharides which enrich that part of the cell wall. Enzymes responsible for ripening-related modification of pectic polysaccharides in a range of fruits include endopolygalacturonase (endo-PG), pectinmethylesterase (PME), and β-galactosidase. Other wall-degrading enzymes have also been detected during ripening of various fruits including cellulases, xyloglucan endotrans-glycosylase (XET), and expansins. The proteins of this latter group are unusual in that they exhibit no endo or exohydralase activity. They are thought to act by disrupting hydrogen bonds between wall proteins (McQueen-Mason and Cosgrove, 1994), possibly at the interface between cellulose microfibrils and matrix polysaccharides. It seems a reasonable proposition that textural changes in fruit will involve the activity of 1 or more wall-modifying enzymes from which a number of conclusions can be drawn (Brummell and Harpster, 2001). a) PG is the main enzyme responsible for the depolymerisation and associated solubilisation of pectic polysaccharides. Its activity requires de-esterification of pectic polymers by PME. Suppression of PG reduces fruit softening only slightly (but shelf life is extended). b) A β-subunit protein of PG limits pectin solubilisation. Suppression of its accumulation increases the rate of softening by facilitating cell separation. c) β-galactosidase activity, if suppressed early in ripening, significantly reduces fruit softening, indicating that the removal of pectic galactan side chains is of significance in the ripening process. d) Endo 1,4-glucanase appears to have little impact on fruit softening or the depolymerisation of matrix glycans.

The texture of ripe fruit depends on more than a simple understanding of cell adhesion ; indeed, it is clear that there are many structural factors throughout the hierarchy that contribute to the final organoleptic texture (Waldron *et al.*, 2003). At the level of the cell, the shape and orientation of parenchyma (and other) cells and

intercellular spaces can influence tissue-fracture properties (Khan and Vincent, 1993). At the level of the cell wall, biochemically mediated changes in the cell walls during ripening may be specifically localised in distinct domains, creating considerable structural heterogeneity (Steele *et al.*, 1997). The wall dissolution and associated swelling may also contribute to the fracture properties of the walls. Different internal tissue structures will also be important, as highlighted by the differences in structure and texture between an apple, peach, orange, and banana. Hence, the changes in organoleptic texture of fruits during ripening is dependent on a range of factors throughout the hierarchy of structures and the relative contribution of these changes as ripening progresses (Waldron *et al.*, 2003).

5.1.1.3.2 Postharvest treatments

Postharvest treatments involving dipping or infusing with calcium maintain firmness during storage of a wide range of fruit (Conway *et al.*, 1994). Examination of fracture surface following tensile testing of apple cortex indicated that tissue failure from calcium-treated fruit was due to cell rupture, whereas failure in control apples was due to cell de-bonding. While evidence suggests that calcium influences texture through its interaction with the cell wall (pectin), it may also affect texture through interactions with membranes.

The cell wall may also influence perception of juiciness through its ability to hold and release fluid. In some fruits, the cell wall swells considerably during ripening. It has been suggested that hydrated cell walls and, perhaps, the presence of free juice over the surface of undamaged cells could be responsible for the sensation of juiciness in fruit with soft melting textures. In stone fruit, loss of juiciness is thought to occur when pectates bind water into a gel-like structure within the wall (Ben-Arie and Lavee, 1971). Separation of cells at the middle lamella rather than rupture of cells during chewing is at least partially responsible for the dry, mealy mouth-feel of overripe apples and woolliness of peaches (Abbott and Harker, 2005).

5.1.1.3.3 Precooking effect

Certain edible plant tissues, if thermally treated at 50 to 60 °C, typically for 30 min or more, are much less prone to softening during subsequent high-temperature processing such as canning. This has been attributed to the thermal-stimulation of wall-bound PME, which de-methyl-esterifies the pectic polysaccharides involved in

cell adhesion (Van Buren, 1979). The reduction in methyl ester groups reduces the rate and extent of β-eliminative degradation at high temperatures, and provides a greater opportunity for the pectic polymers to be ionically cross-linked by divalent cations such as calcium. Similar effects can be induced by chemically de-esterifying cell-wall pectic polysaccharides in cold, dilute alkali (Van Buren and Pitifer, 1992).

In some vegetables, such as carrots, the effect of such precooking treatments can be considerable. In some others, potatoes for example, it is often less and it is generally variety dependent. However, the changes in cell-wall chemistry are similar. It is likely that the effect of precooking treatments on thermal stability of cell adhesion relies not only on the PME-derived de-esterification of pectic poly-saccharides, but is also dependent on the availability of divalent cations to produce extra ionic cross-links and the impact of intracellular organic acids which can chelate them. Indeed, the effect on carrots can be reversed by soaking the treated tissue in EDTA. There have also been attempts to relate the kinetics of PME activity to the firming process. However, industrial exploitation of precooking has generally been developed empirically, and is carried out in conjunction with the addition of calcium salts to control texture (Waldron *et al.*, 2003).

5.1.1.3.4 Thermal processing

In general, plant tissue subjected to thermal processing undergoes physical and chemical changes that can be observed by microscopic studies and textural measurements (Thybo *et al.*, 1998). Thermal treatments cause an initial loss of instrumental firmness due to disruption of the plasma-lemma. This allows the free diffusion of water and low-molecular weight moieties, and results in a loss of turgor (Greve *et al.*, 1994). The latter may result in the development of a rubbery character. However, the most significant softening occurs subsequently as a result of an increase in the ease of cell separation in many non-lignified tissues (Van Buren, 1979). This has been of considerable interest for decades, and has been generally attributed to the β-eliminative degradation of pectic polysaccharides which have long been considered to play a major role in cell adhesion. The β-elimination of pectins involves a hydroxyl-ion-catalysed breakage of glycosidic linkages under nonacid conditions, and is typical of thermal processing. Such depolymerisation is strongly influenced by pH, and is enhanced considerably under increasingly alkaline conditions. In addition, it

can be affected by other ions and is enhanced by, for example, Ca, Mg, K ions, citrate, malate, and phytate organic acids (Waldron *et al.*, 2003). In contrast, the softening of thermally-treated acidic fruit tissues has also been attributed to acid hydrolysis of glycosidic bonds in cell-wall polysaccharides, although this has been refuted by Krall and McFeeters (1998).

In addition to causing cell separation in many edible tissues, thermal treatment of plant tissues is often accompanied by swelling of the cell walls. It may be due to the thermal degradation of polymers that stabilise the wall laterally, facilitating disruption, and may also be affected by changes in the ionic composition of the cell wall. Interestingly, the ability of calcium to cross-link pectic polysaccharides and thereby reduce their solubilisation provides a dual role for this ion as a promoter of pectin degradation through eliminative degradation and as an agent that enhances texture through cross-linking (Waldron *et al.*, 2003).

Fruits that are thermally processed, by canning for example, will also be subjected to the effects of ripening as well as those of the processing. In such situations, it is more difficult to maintain and control textural quality, especially when an increase in temperature can stimulate the activity of ripening-related wall-degrading enzymes (Waldron *et al.*, 2003).

5.1.1.3.5 High pressure processing

High pressure treatment is often reported to render fruits and vegetables more pliable and to increase their softness. However, pressures up to 350 MPa can be applied to plant systems without any major effect on texture and structure (Knorr, 1995). Kasai *et al*. (1995, cited by Basak and Ramaswamy, 1998) found the relative hardness (ratio of hardness of pressure-treated vegetables to untreated vegetables) to increase substantially with the magnitude and retention time of the applied pressure. For potato cubes, tissue softening induced by pressure was comparable to that induced by hot water blanching (Eshtiaghi *et al*., 1994).

An extensive study on the effect of pressure on the texture of fruits and vegetables has been carried out by Basak and Ramaswamy (1998). They observed the change in firmness of treated samples to be dependent on both pressure level and pressurisation time. In general, the softening curves revealed that texture changes caused by pressure occurred in two phases ; (1) a sudden loss as a result of the pulse

action of pressure, followed by (2) further loss or gradual recovery during the pressure-holding phase. The instantaneous pressure softening was clearly dependent on the pressure level and on the type of product, and the sensitivity ranking was dependent on pressure intensity.

Pear was most pressure sensitive at 100 MPa ; celery, at pressure higher than 200 MPa. At low pressure (100 MPa), slight loss of firmness was caused by compaction of cellular structure without disruption. At higher pressure (greater than 200 MPa), severe texture loss occurred because of rupture of cellular membranes and consequent loss of turgor pressure. Pressure holding time clearly affected the overall texture of processed products. Many fruits and vegetables gradually recovered most of the instantaneous loss in texture after holding times of 30 to 60 min at 100 to 200 MPa, and some became firmer than their fresh counterparts. However, for carrots and green pepper, further texture loss was observed at 200 MPa (Ludikhuyze and Hendrickx, 2001).

In many cases, pressure-treated vegetables did not soften during subsequent cooking, which is attributed to the action of pectinmethylesterase (PME) and disruption of structures, causing enzyme and substrate to come into contact with each other. Firmness effects are thus mainly the result of PME-initiated de-esterification of cell wall pectin, leading to cross-linking with divalent ions. Increased compactness of cellular structures following degassing of the tissue may also be involved. At higher pressure, products may be severely damaged, leading to permanent loss of texture (Ludikhuyze and Hendrickx, 2001).

5.1.1.3.6 Cell Turgor

Plant cells tend to maintain a small positive pressure, known as turgor pressure. This pressure develops when the concentration of solutes inside the cell (more specifically inside the plasma membrane) is higher than outside the cell. The extracellular solution fills the pores of the cell wall, sometimes infiltrates into gas filled spaces, and usually is continuous with vascular (water conducting) pathways of the plant. Differences in solute concentration at the inner and outer surface of the plasma membrane cause water to flow into the cell by the process of osmosis. This net movement of water is halted by the physical constraint of the rigid cell wall and, as a result of this, turgor develops inside the cell (Abbott and Harker, 2005).

The importance of turgor has been demonstrated in a number of ways. The rapid phase of cooking-induces softening of carrot as a result of membrane disruption and the elimination of the turgor component of texture (Greve *et al.*, 1994). Similarly, when produce experience a freeze-thaw cycle the membranes are damaged and the tissues become more flaccid in the case of leafy vegetables and softer in the case of fruits, and often leak much juice upon thawing. Firmness and turgor correlate well in apple (Tong *et al.*, 1999), and turgor is also thought to play a central role in softening and development of mealiness during storage of apples (Hatfield and Knee, 1988).

5.1.1.3.7 Cell-to-Cell De-bonding versus Cell rupture

The strength of the cell wall relative to the adhesion between neighboring cells will determine whether cell rupture or cell-to-cell de-bonding is the mechanism of tissue failure. Cell rupture is generally associated with crisp and often juicy produce, as well as with unripe fruit and raw vegetables. Cell-to-cell de-bonding is frequently associated with a dry, unpleasant texture such as in mealy apples, chilling injured stone fruit and tomato, and juice loss in citrus. However, a dry texture is not always unacceptable to consumers, e.g., banana. In some fruits, cell-to-cell de-bonding does not result in a dry texture ; rather, a layer of juice covers the intact cells exposed following cell separation (Harker *et al.*, 1997). Furthermore, cell-to-cell de-bonding is a common outcome of cooking of vegetables such as potato (Waldron *et al.*, 1997) and carrot (Ng and Waldron, 1997). Tissue collapse can also occur without cell wall breakdown or cell separation. In some tissues, fluids are forced out of cells by compressive forces known as “cell relaxation” (Peleg *et al.*, 1976) or “exosmosis” (Jackman and Stanley, 1995).

5.1.1.4 Determination of cellular structure

New computer techniques of image acquisition and analysis allow geometrical parameters of objects within the image to be obtained almost automatically. The microscopy images can be processed to extract objects of interest and to measure their parameters. However, applying the same procedure to large numbers of images requires a uniform or similar quality that is sometimes difficult to maintain. The success depends on the microscope used, the method of sample preparation and the properties of the material investigated (Zdunek and Umeda, 2005).

A conventional light microscopy, which uses a mixed wavelength light source and illuminates a large area of the sample, will have a relatively great depth of field. Even if not in focus, images of sample from all levels within the field will be visible. These will include sample above, in, and below the plane of focus. As a result the image can be murky, fuzzy, and crowded.

The solution to this problem is the confocal scanning laser microscope (CSLM) or confocal microscope. Fluorescently stained samples are usually examined. A focused laser beam strikes a point in the sample. Light from the illuminated spot is focused by an object lens on to a plane above the objective. An aperture above the objective lens blocks out stray light from parts of the sample that lie above and below the plane of focus. The laser is scanned over a plane in the sample (beam scanning) or the stage is moved (stage scanning) and a detector measures the illumination from each point to produce an image of the optical section. When many optical sections are scanned, a computer can combine them to form a three-dimensional image from the digitised signals. This image can be measured and analysed quantitatively.

The confocal microscope improves images in two ways. First, illumination of one spot at a time reduces interference from light scattering by the rest of the sample. Second, the aperture above the objective lens blocks out stray light as previously mentioned. Consequently the image has excellent contrast and resolution. A depth of 1 μm or less in a thick preparation can be directly observed. Special computer software is used to create high-resolution, three-dimensional images of cell structures and complex samples such as biofilms.

Confocal scanning laser microscopy has certain advantages over other conventional microscopy techniques, including light microscopy, scanning electron microscopy (SEM) and cold-stage SEM (cryo-SEM) (Lapsley *et al.*, 1992). Tissue is viewed fresh and not subject to factors attributed to preparative techniques, and may be viewed at different levels by focusing (optical sectioning). The CSLM technique has already been described exhaustively and although it has been shown to be a powerful tool, offering new possibilities in the microstructural studies of food systems, few studies have been published showing its application to fruit and vegetable tissue. This technique can be successfully applied to establishing the degree of cell adhesion in fresh apple tissue (Alvarez *et al.*, 2000b). However, at present,

image processing is still not fully automatic, and some manual operations are necessary. For example, Konstankiewicz *et al.*(2001 cited by Zdunek and Umeda, 2005) used preliminary manual sketching over the cell wall faces within images taken by tandem scanning confocal light microscope to increase the accuracy of the measurements. The problem of the efficient analysis of any number of images was partially solved by Zdunek *et al.* (2004) for fluorescence images taken by CSLM. The quality of the images and a special computer procedure that allowed a fast analysis of any number of potato and carrot tissue images. The method can be used to obtain the geometrical parameters of the cell wall faces within a large area of plant tissue cross-sections. Their experience showed that confocal microscopes are very useful for high-contrast images with visible cell wall faces. Another advantage is easier sample preparation-sometimes only cutting through raw tissue is enough. Therefore, it is easier and faster to get many images of the tissue, and only minimum image processing is necessary before automatic measurement. However, at present, image processing is still not fully automatic, and some manual operations are necessary.

In 2005, Zdunek *et al.* used CSLM to obtain images of the cell structure of tissue which was applied to assess the mean cell face area and the cell wall volume fraction obtained within the tissue-cross section.

5.1.2 Microbiology

Ultra-high pressure (UHP) treatment has been known as a potential preservation technique for over a century, since Hite demonstrated in 1899 that microbial spoilage of milk could be delayed by application of high pressure (Smelt, 1998). On an industrial scale, high-pressure processing (HPP) of food products is mainly applied for pasteurisation purposes. High-pressure processing may provide distinct product quality merits over conventional heat processing by avoiding the adverse quality effects caused by thermal treatments of long durations. In recent years, researchers have focused mainly on inactivation of vegetative microorganisms by means of HPP (Krebbers *et al.*, 2003). Pressures between 300 and 600 MPa can inactivate yeasts, moulds and most vegetative bacteria including most infectious food-borne pathogens. In general, bacterial spores can only be killed by very high pressure (> 1000 MPa). Bacterial spores, however, can often be stimulated to germinate by

pressures of 50-300 MPa, and then the germinated spores can then be killed by relatively mild heat treatments or mild pressure treatments. However, in most cases a small fraction of spores can survive this treatment (Smelt, 1998) ; thus a combination with other techniques, such as temperature elevation may be required. Meyer (2000) has patented a HPP sterilisation process based on at least two-pressure pulses at a minimum initial temperature of 70°C. This HPP process aims to inactivate vegetative microorganisms and spores, without adversely affecting flavours and minimally changing texture and colour. The aim of the work reported in this chapter is to use HPP process to enhance food safety and shelf life of lychee which will offer a new dimension of Thai fruit and vegetable technologies.

A range of pressure-treated products has been on the United States, European, and Japanese market. Examples of high-pressure processed products commercially available in the United States include fruit smoothies, guacamole, ready meals with meat and vegetables, oysters, ham, chicken strips, fruit juices, and salsa. In Europe and Japan, pressure-treated jams, jellies, fish, meat-products, sliced ham, salad dressing, rice cakes, ham, juices and yoghurts are available. HPP can not yet be used to make shelf-stable versions of low-acid products such as vegetables, milk, or soups because of the inability of this process to destroy spores without the additional use of heat. However, it can be used to extend the refrigerated shelf life of these products and to eliminate the risk of various food-borne pathogens such as *E. coli*, *Salmonella* and *Listeria*. Another limitation is that the food must contain water and not have internal air pockets. Food materials containing entrapped air such as strawberries or marsh-mallows would be crushed under high pressure treatment. Dry solids do not have sufficient moisture to make HPP effective for microbial destruction (Ramaswamy *et al.*, 2005 ; Balasubramaniam, 2005).

UHP has certain advantages over other conventional heat treatment. It causes inactivation of microorganisms, spores and enzymes; retention of fresh-like product flavour, taste, colour, and texture; no nutrient degradation; addition of preservatives and flavours can be avoided or minimised; uniform application of pressure; easy scale-up to commercial production; and clean technology (Balasubramaniam, 2005). Emulsions, which are sensitive to heat can be pressure-treated without affecting the stability of the emulsion. HPP is also used to promote the formulation of small ice

crystals. Under a pressure of 207.5 MPa, water remains liquid at temperatures down to -22 °C. Sudden expansion at that temperature to atmospheric pressure is a means of formation of very small ice crystals provided the latent heat released by freezing can be removed.

The application of high pressure processing in food systems can produce high-value products of superior quality; enhancement food safety and shelf life extension of minimally processed foods; and creation of novel products with unique textures by pressure-induced gelation and denaturing of proteins.

5.2 EXPERIMENTAL

5.2.1 Effect of high pressure treatment on texture and microstructure

5.2.1.1 Materials

Lychees (*Litchi chinensis* Sonn.) were purchased from a commercial orchard at Chiangmai province of Thailand and stored in polyethylene bag at 2°C for 2 to 3 days prior to high pressure and canning processing.

Sucrose, granulated sugar (Savona, UK).

Calcium chloride, anhydrous (Fisher, UK).

Citric acid powder, general purpose reagent (BDH, UK).

Tetraethylrhodamine B (Sigma, USA).

Phosphate buffer saline (PBS), pH 7.3 (Oxoid Dulbecco A BR 14a, UK)

Kew cocktail [IMS*(Tennants Distribution Limited,UK) : 4% Formaldehyde (Sigma, USA) : Deionised water : Glycerine (Sigma, USA) = 2:1:8:1 by volume].

* IMS = Industrial methylated spirit 99

5.2.1.2 Methods

5.2.1.2.1 Canning process

The lychees were canned following the commercial process of the Royal Agriculture Company Ltd, (Chiangmai, Thailand). The lychees were peeled, destoned and soaked in a solution of 1% $CaCl_2$ and 0.1% citric acid for 15 min. The soaked lychees were washed 3 times in deionised water, 120 g of lychees were filled in a plain can with 175 g of syrup, which consisted of 300 g sucrose,1.3 g citric acid and 700 g deionised water. The filled cans were exhausted in steam for 10 min at 80-85°C, then sealed, sterilised in boiling water for 18 min and cooled to 45°C. All canned lychees were stored at room temperature for 2 weeks prior to analysis for texture.

5.2.1.2.2 High pressure processing

The lychees were peeled, destoned and sealed in polyethylene tubing (Cryovac Ltd., UK), care being taken to exclude as much air as possible. Each bag contained 3 lychees so that the total weight was about 45 g. The bags were processed at pressures of 200, 400 and 600 MPa and temperatures of 20, 40 and 60°C for 10 or 20 min in a prototype Stansted "Food-Lab" model 900 high pressure rig (Stansted Fluid Power Ltd., Stansted, UK). Three or four bags were treated at each

pressure/temperature regime.

Further sets of 3 lychees were mixed with the syrup used in the canning experiment in the ratio of fruit to syrup of 1:1 and were subjected to the above pressure/ temperature regimes.

The pressure treated samples (and controls) were stored at 2°C for 2 weeks, and further analysed for textural quality. Some samples were immediately fixed into a Kew cocktail (IMS* : 4% Formaldehyde : Deionised water : Glycerine = 2:1:8:1 by volume) for 3 days, and then preserved in 70 % ethanol prior to studying micro-structure.

5.2.1.2.3 Texture analysis

The firmness of samples was estimated by a penetration test using a TA.XT2 stable Microsystems texture analyser. The lychee fruit was allowed to warm to room temperature and divided longitudinal into two equal segments. The firmness of each segment was tested with a cylindrical probe 4 mm in diameter fixed to a 5 kg load cell. The area under the curve up to the maximum compression of the first bite is the work done by the machine and was used as an index of firmness (g.mm).

5.2.1.2.4 Confocal Scanning Laser Microscopy (CSLM)

Samples which preserved in 70% ethanol were sliced perpendicular to the long axis of the tuber from surface to core using a mechanically guided razor blade. Rectangular specimens (10-13 x 7-8 x 2-3 mm) were placed directly on a glass slide and ethanol was removed by using phosphate buffer saline (PBS), pH 7.3, and then soaked them in a 0.01% tetraethylrhodamine B solution for 20 min. Excess dye was removed by using PBS. The fluorescent dye was protein specific and was excited at 568 nm by the laser light source, and highlighted the cellular structure. Each sample was viewed with an oil immersion 16x0.5 IMMPL/ PLUOT AR objective on a Leica TCS SP microscope. Examination of the structure was possible to a depth of 100 μm (Lapsley *et al.*,1992).

5.2.1.2.5 Experimental Design

To study lychee firmness, after pressure treatment for 10 min or 20 min, two variables were studied, these were 3 levels of pressure (200, 400, and 600 MPa) and 3 levels of temperature (20, 40, and 60°C), i.e., a 3 x 3 factorial in a completely randomised design (CRD). Each treatment was measured four times and all treatments

were carried out in at least 3 replicates. The statistical program, SPSS v 11.5 (SPSS Inc., Chicago, USA), was used for data analysis and Duncan's multiple range test was used for comparing differences between means.

5.2.2 Effect of high pressure on microorganisms

5.2.2.1 Materials

Lychees (*Litchi chinensis* Sonn.) were purchased from a commercial orchard at Chiangmai province of Thailand and stored in polyethylene bag at 2°C for 1 to 2 days prior to high pressure treatment.

Plate Count Agar, PCA (Difco, USA).

Potato Dextrose Agar, PDA (Difco, USA)

Tartaric acid, AR grade (Merck, Geramany).

Bacteriological peptone (Merck, Geramany).

5.2.2.2 Methods

5.2.2.2.1 High pressure processing

The lychees were peeled, destoned and sealed in polyethylene tubing (Cryovac Ltd., UK), care being taken to exclude as much air as possible. Each bag contained 3 lychees so that the total weight was about 45 g. The bags were processed at pressures of 200, 400 and 600 MPa and temperatures of 20, 40 and 60°C for 10 or 20 min in a prototype Stansted "Food-Lab" model S-Fl-850-9-w high pressure rig (Stansted Fluid Power Ltd., Stansted, UK). Six bags were treated at each pressure / temperature regime.

The pressure treated samples (and controls) were stored at 2°C for 0, 1, 2, 3, 4, and 6 weeks, prior to microbiological analysis.

5.2.2.2.2 Microbiological analysis

Reduction in the natural flora was investigated in fresh lychees , processed under the different conditions. Samples were aseptically transferred to a plastic bag, diluted ten times in peptone water (1g/L bacteriological peptone) and homogenised by manually macerating for 1 min. Serial dilutions were made in peptone water, and then plated on plate count agar. The plates were incubated for 2 days at 37°C. In order to determine yeasts and moulds, the rest of the diluted samples from the total aerobic plate count, were plated on potato dextrose agar acidified to pH 3.8 with 10 % sterile tartaric acid. The plates were incubated for 5 days at 25-30 °C.

5.2.2.2.3 Experimental Design

For the samples, pressure treated at 20, 40, and 60°C, two variables were studied, these were 3 levels of pressure (200, 400, and 600 MPa) and 2 levels of time (10 and 20 min), i.e., a 3 x 2 factorial in a completely randomised design (CRD). Each treatment was measured in duplicate for the microorganisms and all treatments were carried out in at least 3 replicates.

5.3 RESULTS AND DISCUSSIONS

5.3.1 Effect of Combined UHP/Temperature treatment on texture

The results of treatment for both 10 and 20 min are summarised in Figs 5.2 and 5.3. No significant difference in firmness was found between untreated (no heat or pressure treatment) and pressurised lychee at room temperature, for both treatment times (10 or 20 min), which agreed with Fuchigami *et al.* (1997) in carrots and Quaglia *et al.* (1996) in green peas. These results are because pressure alone does not degrade pectin (Kato *et al.*, 1997) which plays an important role in the texture of fruits and vegetables (Tangwongchai, 2000).

Increasing the temperature to 40°C showed a tendency for a decrease in firmness although this was only significant at 600 MPa. The effects were more marked at 60 °C where a significant decrease in firmness was seen at 400 MPa. At 600 MPa, 60 °C for 20 min, the firmness was decreased from 119.5 g.mm to 83.0 g.mm (30.5 %). This is most likely due to the heat induced depolymerisation of the pectic substances which results in a high degree of softening during the cooking of fruits and vegetables (Greve *et al.*, 1994). However, Quaglia *et al.* (1996) found that pressure combined with heat did not cause any marked modification in the firmness of pea.

Figs 5.4 and 5.5, show the results for samples treated in syrup and similar trends were seen, with increasing pressures (600 MPa) at both 40 °C and 60 °C decreasing the firmness. At 600 MPa, 60 °C for 20 min, the firmness was decreased from 171.0 g.mm to 120.6 g.mm (29.5%). The lychees processed in syrup were pretreated by dipping or infiltrating with calcium and, not unexpectedly, had a higher firmness than the fresh lychees as calcium influences texture through its interaction with the cell wall (pectin) or cell membranes (Abbott and Harker, 2005). For those lychees canned in syrup, had the lowest firmness of all the treatments. This is because not only of the heat induced depolymerisation of the pectic substances which results in a high degree of softening during the cooking of fruits and vegetables (Greve *et al.*, 1994), but also because of additional softening of thermally-treated acidic fruit tissues has also been attributed to acid hydrolysis of glycosidic bonds in the cell-wall poly-saccharides (Waldron *et al.*, 2003).

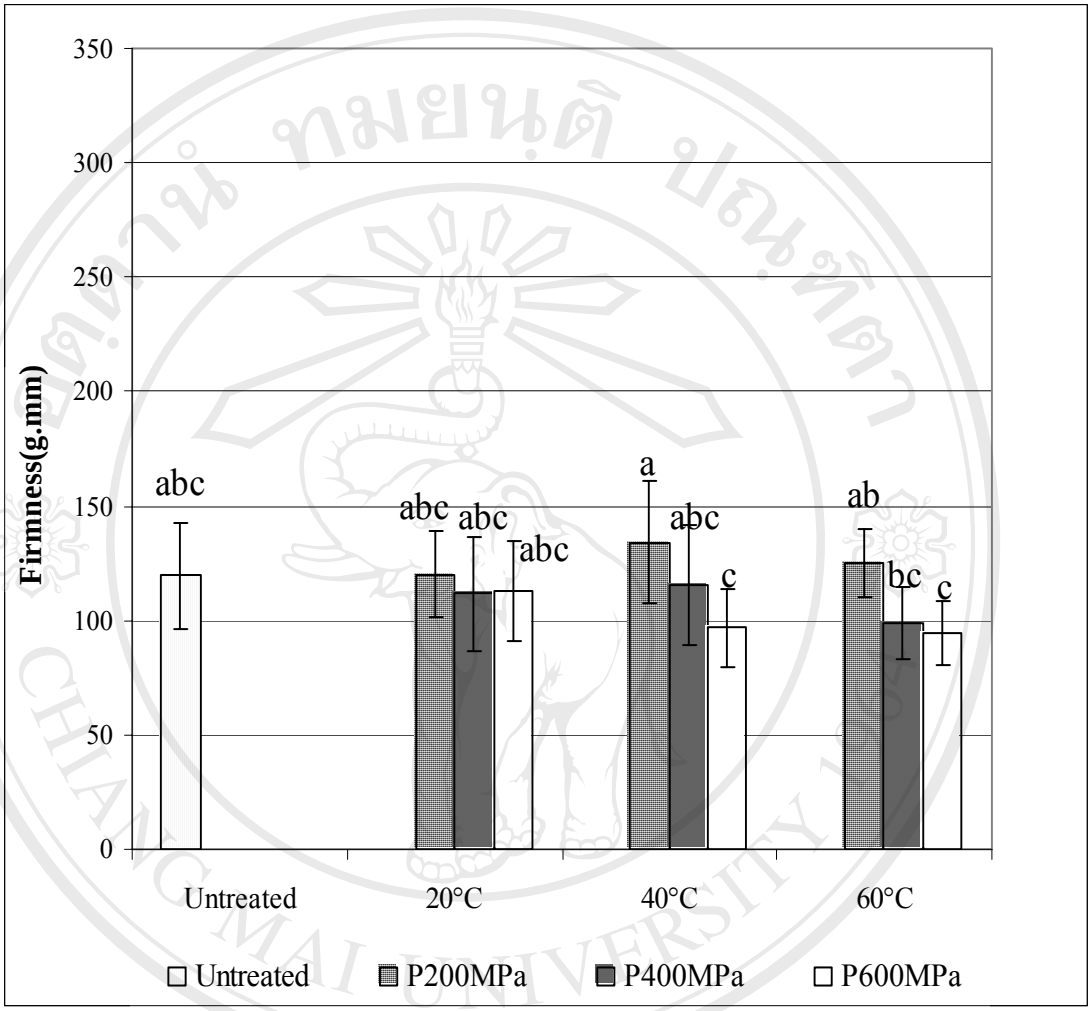

Fig 5.2 Combined effects of high pressure and temperature for 10 min on the firmness of fresh lychees. All values are the means ± S.D. of 12 determinations. Bars with different superscript were significantly different ($p<0.05$).

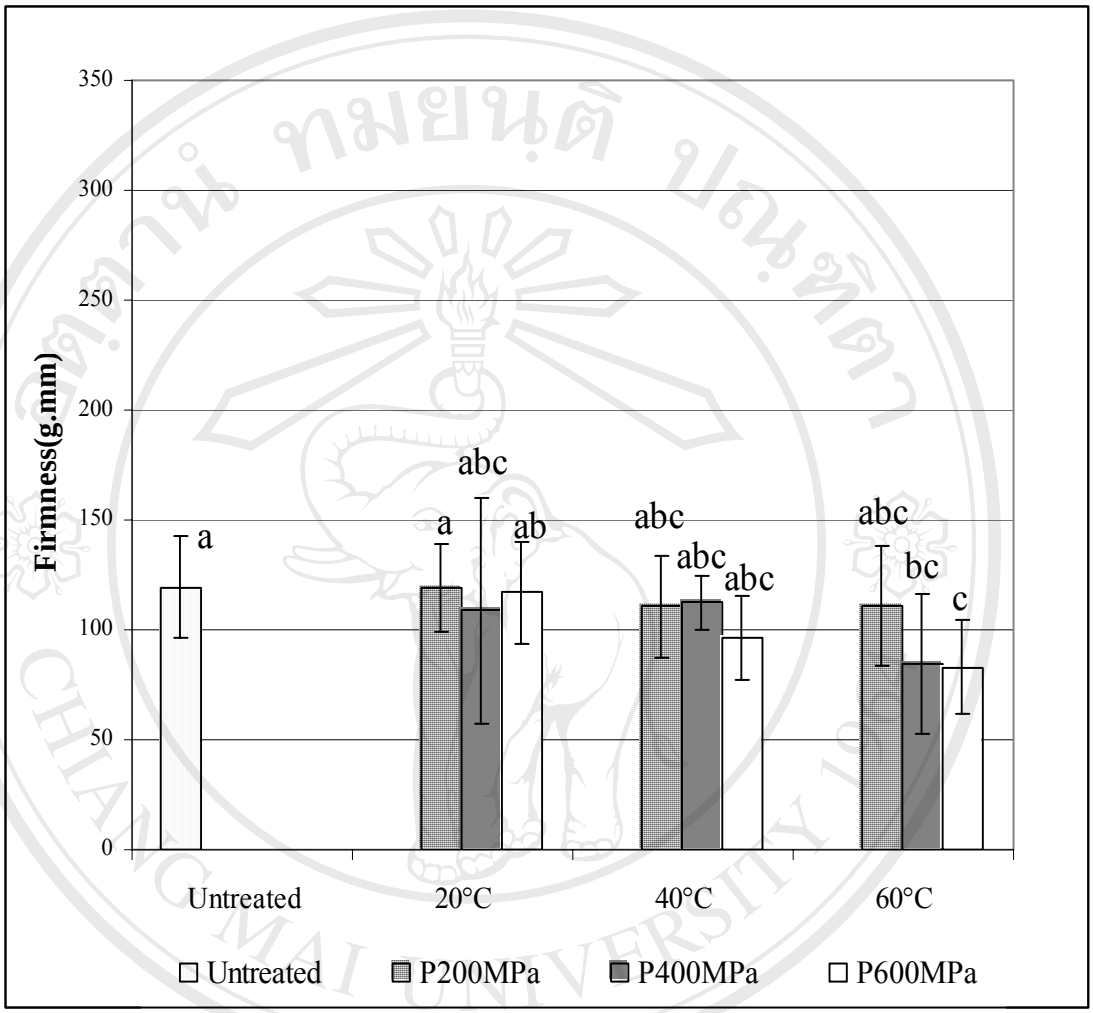

Fig 5.3 Combined effects of high pressure and temperature for 20 min on the firmness of fresh lychees. All values are the means ± S.D. of 12 determinations. Bars with different superscript were significantly different ($p<0.05$).

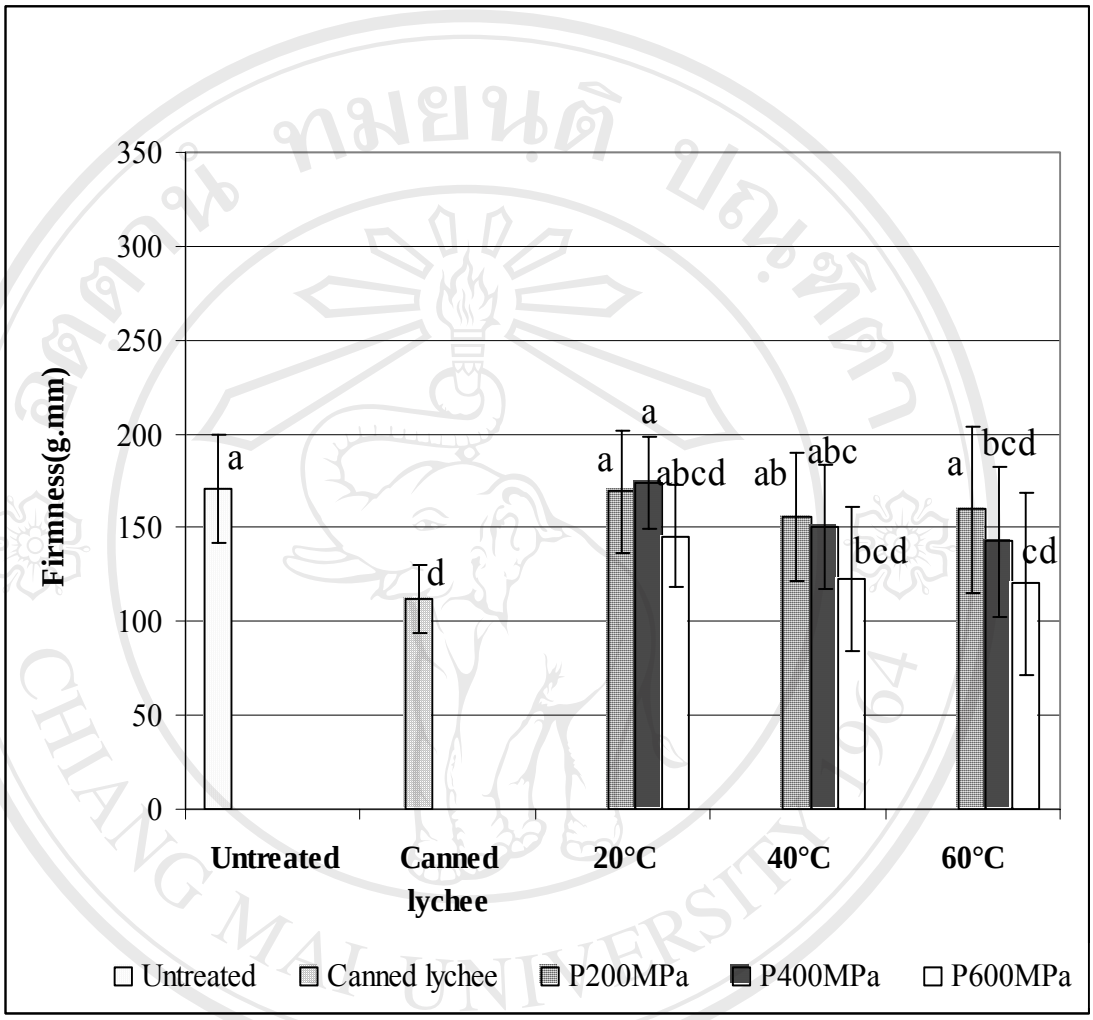

Fig 5.4 Combined effects of high pressure and temperature for 10 min on the firmness of lychees processed in syrup. All values are the means ± S.D. of 12 determinations. Bars with different superscript were significantly different($p<0.05$).

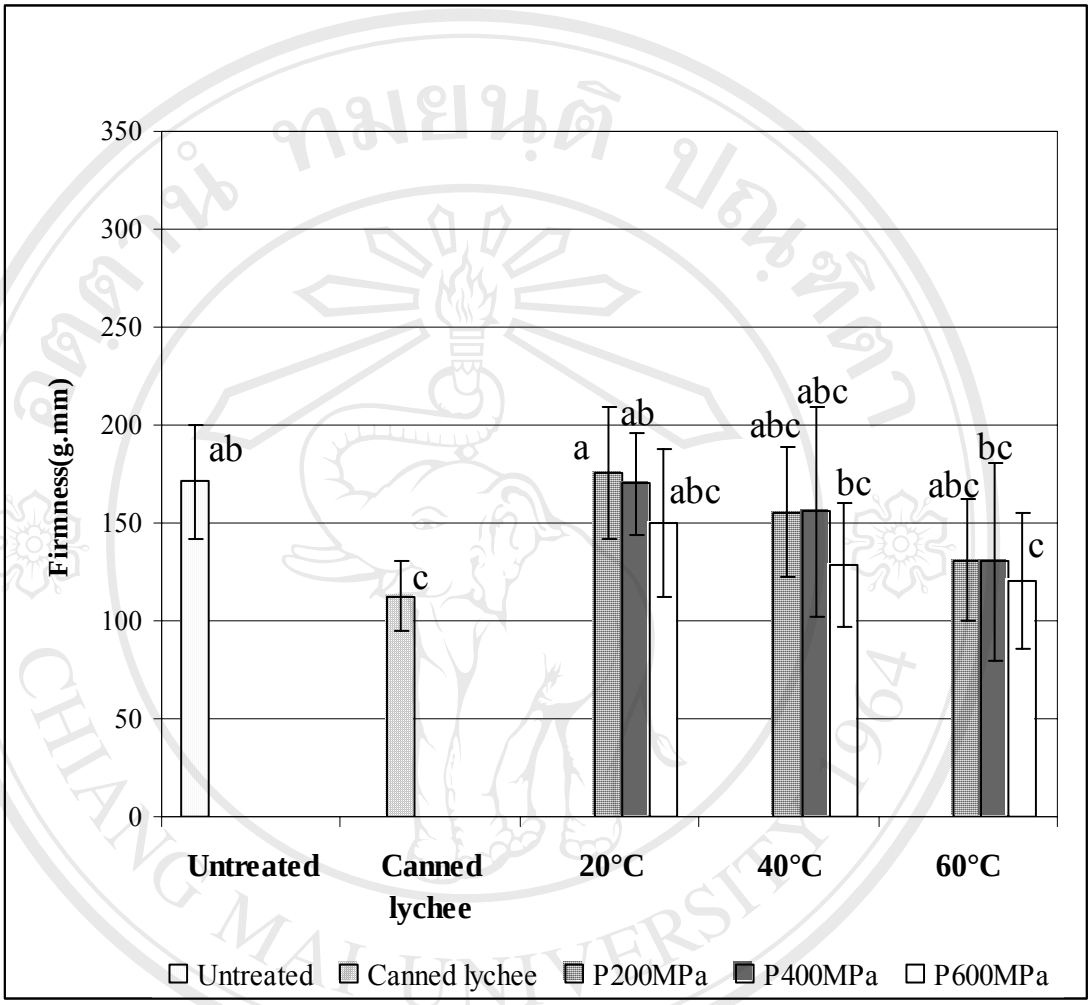

Fig 5.5 Combined effects of high pressure and temperature for 20 min on the firmness of lychees processed in syrup. All values are the means ± S.D. of 12 determinations.Bars with different superscript were significantly different ($p<0.05$).

In conclusion, high pressure treatment at 600 MPa at room temperature did not affect the firmness of fresh lychee. Increasing temperature through caused a decrease in firmness and the effects appeared more marked at the extreme condition (600 MPa, 60°C). This was true for those samples processed either in syrup or not.

5.3.2 Effect of high pressure treatment on the microstructure of lychees

Confocal Scanning Laser Microscopy (CSLM) of 2 to 3 mm flesh lychee cubes combined with optical sectioning produced images taken at a 10 μm depth from the tissue surface. The fluorochrome used, rhodamine B, is protein specific and by staining the membrane, the adjacent cell wall was outlined. Fig 5.6 is fluorescent light images of unpressurised lychee with samples taken progressively from the outside (A) to the inside (D) of the flesh. The images display extra soft, juicy and anisotropic cell structures with varied morphology according to the direction from which the tissue was viewed. In general the cell size increases from the outer to the inner parts. Fig 5.7 shows the images of high pressure treated lychee at 200 MPa and 20°C for 20 min. It can be visualised that this flesh contains large amount of water, with thin and weaken cell walls. Most cells loose their turgid which caused physically changes in cell structure. Outer layer of cells is extremely wrinkled, still some of them maintain their structure, while the inner part collapsed and densely packed with irregular shapes. Fig 5.8 shows images of high pressure treated lychee at 600 MPa and 20°C for 20 min. The outer layer of flesh appears with collapsed and compacted cell structure, also some trace of migrating fluid through outside including salt, vitamin, mineral, enzyme and their substrates. The inner cells having irregular structure similar to those treated at 200 MPa but still collapse and much more densely pack than other treated samples. The most inner cells indicate more severely histological damaged than the others.

Ludikhuyze and Hendrickx (2001) demonstrated that at low pressure (100 MPa), slight loss of firmness in pear and celery was caused by compaction of cellular structures without disruption but at higher pressures (greater than 200 MPa), severe texture loss occurred because of rupture of cellular membranes and consequent loss of turgor pressure. Butz *et al.* (1994) found that pressures above 100 MPa could damage the cell structure of onions whereas Knorr (1995), reported that pressures up to 350 MPa can be applied to plant systems without any major effect on texture and structure.

The high pressure changes cell permeability and enables the movement of water from inside to outside the cell. As a result, the treated lychee tissue had a soaked appearance which was similar to that found for cauliflower and spinach leaves by Prestamo and Arroyo (1998). Although no large changes were observed, such water movement can produce chemical reactions. In spite of these changes, the lychee maintain an acceptable firmness, little changed from the original and with little change in flavour at 200 MPa. This is not true after treatment at 600 MPa.

The plasma membrane encloses the cell defines its boundaries and maintains the essential differences between the cytosol and extracellular environment. Cell membranes are dynamic and fluid structures enabling water and nonpolar molecules to permeate by simple diffusion. The plasma membrane also enables the passage of various polar molecules such as ions, sugars, amino acids, nucleotides and many metabolites. The lipid bilayer provides the basic structure for the membrane and serves as a relatively impermeable barrier for the passage of most water-soluble molecules. Proteins in the plasma membrane are hidden or clothed by carbohydrates, which are in the cell surface. Some plasma membrane proteins serve as structural links connecting the membrane to the cytoskeleton or to the extracellular matrix or an adjacent cell. Other membrane proteins are receptors to detect and transduce chemical signals in the cell environment (Prestamo and Arroyo, 1998).

The plasma membrane has an electric potential and ATP supplies the energy for transferring Na^+ and K^+ ions across it. Na^+ K^+ ATPases have direct functions in regulating cell volume, making a cell swell or shrink. The collapsed appearance of the cells after high pressure treatment was a response to ATPase inactivation (Chong *et al.*, 1985).

In conclusion, the microscopic appearance revealed that changes in flesh lychee treated at 600 MPa were more extensive than in those treated at 200 MPa. The lychees lost their turgor after treatment as a consequence of high pressure changes cell permeability which enabled the movement of water from inside to outside the cell. These affected the cell structure so that the cell looked wrinkled, collapsed, densely packed and cell to cell contacts were more extensive, and somewhat irregular.

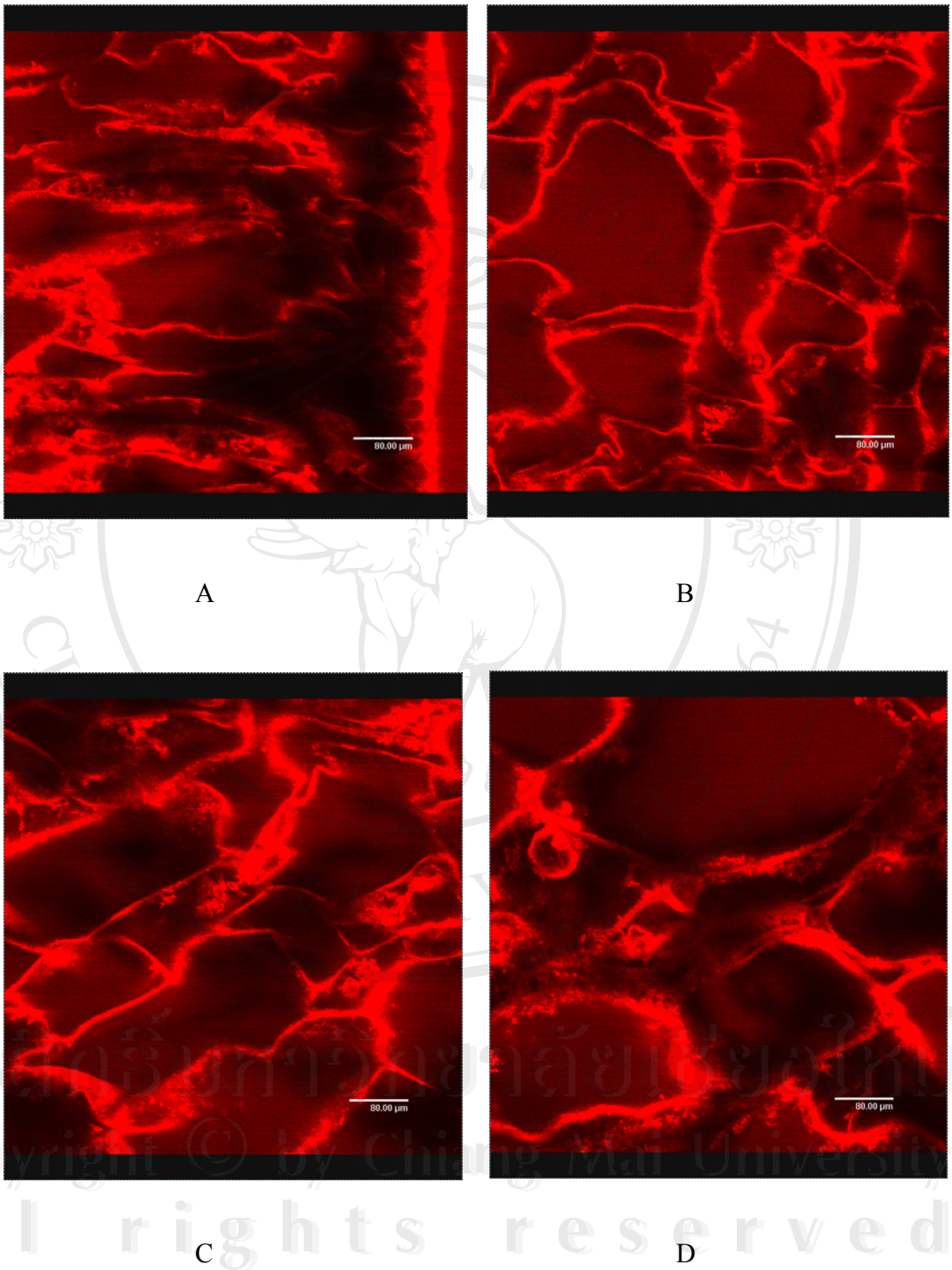

Fig 5.6 CSLM of unpressurised flesh lychee with samples taken progressively from the outside (A) to the inside (D) (each scale bar = 80 µm).

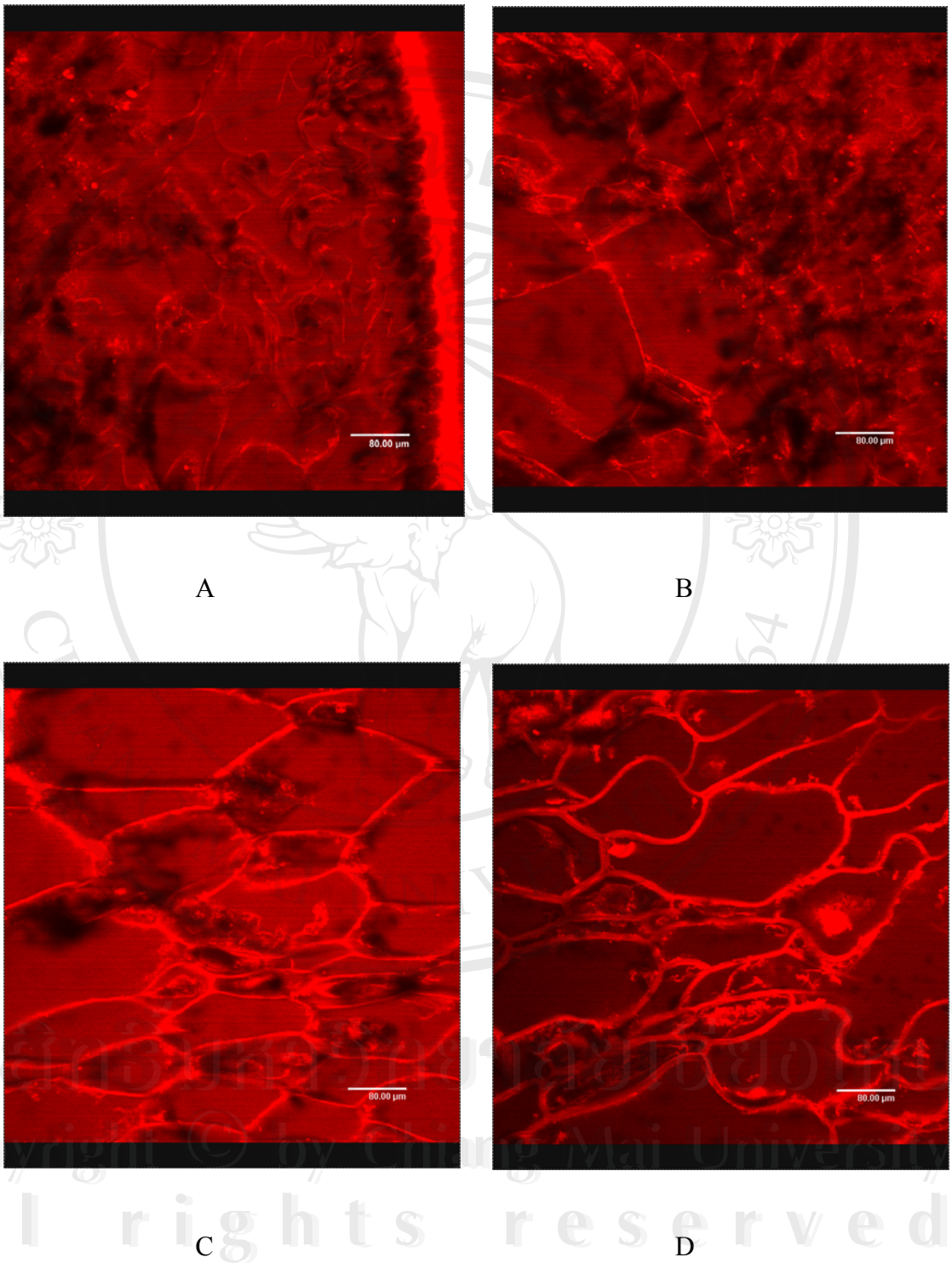

Fig 5.7 CSLM of pressure treated flesh lychee at 200 MPa and 20°C for 20 min with samples taken progressively from the outside (A) to the inside (D) (each scale bar = 80 µm).

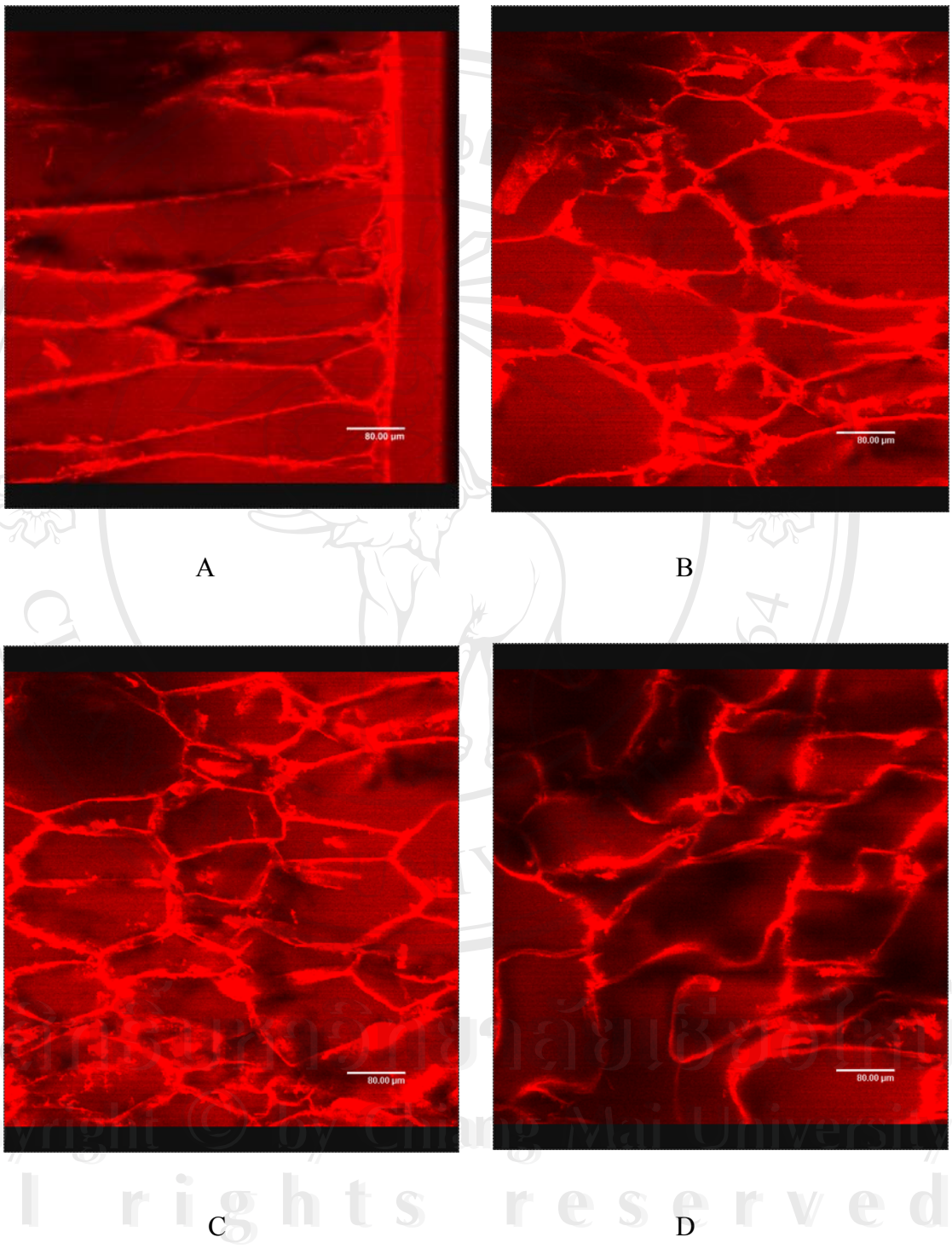

Fig 5.8 CSLM of pressurised flesh lychee at 600 MPa and 20 °C for 20 min with samples taken progressively from the outside (A) to the inside (D)(each scale bar = 80 µm).

5.3.3 Effect on inactivation of microorganisms

5.3.3.1 Reduction of total bacterial count

The effects of high pressure under various process conditions on the natural flora compared to raw fresh lychee are shown in Tables 5.1, 5.2 and 5.3. High pressure pasteurisation up to 200 MPa at ambient temperature caused little inactivation of the naturally occurring microorganisms. Significant inactivation was observed at pressures in excess of 400 MPa, independent of the duration of treatment (Table 5.1). However, such treatment did not destroy all microorganisms, which was also found in apple juice treated at 500 MPa 5 min (Tonello *et al.*,1997). On the contrary, high pressure processing for 5 min at 450 MPa of orange juice and grapefruit was shown to destroy the total microflora (Tonello *et al.*,1997). High pressure treatment at 600 MPa and 20°C for 20 min inactivated the microorganisms in fresh lychees down to a level of 10 cfu g^{-1}, which is similar to the levels found by Yen and Lin (1996) in guava puree. After storage at 2 °C for 4 weeks, unpressurised fresh lychee and those pressure treated at 200 MPa both had microbial levels above 2.5 x 10^6 cfu g^{-1}, but the count for those treated at higher pressures (400 and 600 MPa) remained similar to the count at the beginning and was relatively constant throughout 6 weeks of storage. A small number of microorganisms were able to survive after pressurisation, but did not multiply during storage at 2°C. These bacteria were not yeasts or moulds (Table 5.4) because their populations were reduced to less than 10 cfu g^{-1}. It is assumed they were other resistant vegetative bacteria or most probably aerobic bacterial spores. These results are similar to those found for fresh-cut pineapples by Aleman *et al.* (1994) and precut mangoes and carambolas by Boynton *et al.* (2002).

When treated at higher temperatures, remarkably increased inactivation was seen (Table 5.2 and 5.3). At 60 °C and pressure up to 200 MPa, microbial counts were reduced from 6.3 x 10^3 cfu g^{-1} to a level below the detection limit (<10 cfu g^{-1}) within 20 min, whereas at 40°C microbial counts were reduced from 6.3 x 10^3 cfu g^{-1} to 1.7 x 10^3 cfu g^{-1} and remained stable during 6 weeks of storage. Unlike those pressure-treated at 200 MPa and 20°C, no growth of the microorganisms occurred. For this treatment at 40°C and 60°C, in the most extreme conditions, microbial populations were below the detection limit, suggesting that they were unable to repair injuries

produced by the high pressure treatment (Parish, 1998). Fresh lychees treated by high pressure were microbiological stable agreeing with results reported by Ogawa *et al.* (1989), Donsi *et al.* (1997), and Parish (1994) in citrus juice, orange juice and orange juice, respectively.

Table 5.1 Microbiological level (cfu g^{-1}) of pressure-treated lychee at 20°C and unpressurised lychee stored at 2°C for 6 weeks.

Treatment	Storage Time(wk)					
	0	1	2	3	4	6
Untreated	$6.3x10^3$	$1.1x10^4$	$4.6x10^4$	$3.5x10^5$	TNTC	TNTC
P200MPa,10min	$1.7x10^3$	$7.9x10^3$	ND	$2.4x10^5$	TNTC	TNTC
P200MPa,20min	$1.6x10^3$	$2.5x10^3$	$1.7x10^4$	$2.5x10^5$	ND	TNTC
P400MPa,10min	20	58	40	65	40	188
P400MPa,20min	20	38	33	40	260	30
P600MPa,10min	10	10	20	35	17	13
P600MPa,20min	10	10	45	30	15	15

ND = Not determined

TNTC = Too numerous to count (>2.5 x 10^6 cfu g^{-1})

5.3.3.2 Reduction of yeasts and moulds

The effects of combined pressure and temperature on the reduction of yeasts and moulds are shown in Table 5.4. When treated at ambient temperature, pressures up to 200 MPa caused a little inactivation of yeasts and moulds. At 200 MPa and 20°C or 40°C for 10 min had little effect on these microorganisms whereas at 60°C there was at least a 1 log cfu g^{-1} reduction compared to the unpressurised fresh lychees. High pressure treatment of fresh lychees at 400 MPa inactivated yeasts and moulds to levels of less than 10 cfu g^{-1} as was found for the total bacterial count on fresh lychees. These results are in agreement with those of Smelt (1998) who reported that pressures between 300 and 600 MPa can inactivate yeasts and moulds and most vegetative bacteria, including most infectious food-borne pathogens. Butz *et al.*

Table 5.2 Microbiological level (cfu g^{-1}) of pressure-treated lychee at 40°C and unpressurised lychee stored at 2°C for 6 weeks.

Treatment	Storage Time(wk)					
	0	1	2	3	4	6
Untreated	$6.3x10^3$	$1.1x10^4$	$4.6x10^4$	$3.5x10^5$	TNTC	TNTC
P200MPa,10min	$1.3x10^3$	$1.6x10^3$	$4.0x10^3$	ND	$9.1x10^2$	$1.0x10^3$
P200MPa,20min	$1.7x10^3$	$3.0x10^3$	$3.4x10^3$	$1.4x10^3$	ND	$1.2x10^3$
P400MPa,10min	<10	<10	<10	<10	10	10
P400MPa,20min	<10	<10	<10	<10	10	10
P600MPa,10min	<10	<10	<10	<10	ND	<10
P600MPa,20min	<10	<10	<10	<10	ND	<10

ND = Not determined

TNTC = Too numerous to count (>2.5 x 10^6 cfu g^{-1})

Table 5.3 Microbiological level (cfu g^{-1}) of pressure-treated lychee at 60°C and unpressurised lychee stored at 2°C for 6 weeks.

Treatment	Storage Time(wk)					
	0	1	2	3	4	6
Untreated	$6.3x10^3$	$1.1x10^4$	$4.6x10^4$	$3.5x10^5$	TNTC	TNTC
P200MPa,10min	6.0x10	$4.0x10^2$	$6.0x10^2$	ND	$9.3x10^2$	$8.6x10^3$
P200MPa,20min	<10	<100	40	<10	50	<10
P400MPa,10min	<10	<10	<10	<10	ND	<10
P400MPa,20min	<10	<10	<10	<10	ND	<10
P600MPa,10min	<10	<10	<10	<10	<10	<10
P600MPa,20min	<10	<10	<10	<10	<10	<10

ND = Not determined

TNTC = Too numerous to count (>2.5 x 10^6 cfu g^{-1})

(1994) demonstrated that in diced onions treated at 300 MPa and 40°C, yeasts and moulds were reduced by five orders of magnitude within 30 min.

Table 5.4 Combination effects of high pressure and temperature on the reduction of yeasts and moulds on fresh lychee.

Treatment	Yeasts & Moulds count (cfu g^{-1})		
	20 °C	40°C	60°C
Untreated	4.0×10^2		
P200MPa,10min	1.8×10^2	3.2×10^2	4.0 x 10
P200MPa,20min	7.1×10^2	<10	<10
P400MPa,10min	<10	<10	<10
P400MPa,20min	<10	<10	<10
P600MPa,10min	<10	<10	<10
P600MPa,20min	<10	<10	<10

In conclusion, the important naturally occurring microorganisms on fresh lychee are yeasts and moulds. On high pressure treatment of fresh lychees at ambient temperature significant inactivation was only observed at pressures in excess of 400 MPa whereas at higher temperatures (60°C) pressures as low as 200 MPa for 20 min was sufficient to inactivate them. It is advisable to use ultra-high pressure replacing thermal process. Since this study shows that pressure could inactivate microorganisms to a satisfactory level within the Thai recommendation for minimal process ($\leq 1 \times 10^6$cfu g^{-1}).