CHAPTER 2
EFFECTS OF HEAT AND HIGH PRESSURE ON QUALITY DETERMINATIONS

2.1 INTRODUCTION

2.1.1 Effect of high pressure on pH of samples

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.

Since ionisation is enhanced by pressure, variations in the pH of buffer solutions under pressure may occur. Reactions that are pH-sensitive should be performed in a buffer with a small ionisation volume such as Tris since no charges are formed or lost in the systems and hence the pH does not change under pressure. The creation of new charges in the system occurs with water, sodium phosphate, carbonate and acetate buffer leading to a substantial volume changes and therefore pH-changes (Table 2.1).

Table 2.1 Effects of pressure on the pH of several systems

Reaction	ΔV(ml/mol)	ΔpH/100 MPa[i]
$H_2O \leftrightarrow H^+ + OH^-$	-21.3	-0.3[ii]
Tris. $H^+ \leftrightarrow$ Tris + H^+	+1.1	0[iii]
$H_2PO_4^- \leftrightarrow HPO_4^{-2} + H^+$	-25	-0.4[iii]
$CH_3COOH \leftrightarrow CH_3COO^- + H^+$	-12	-0.2[iii]

[i] Decrease in pH for each 100 MPa of applied pressure

[ii] Gross and Jaenicke, 1994

[iii] Heremans, 1995

For fruit juices, which are in general quite acid, a treatment of 500 MPa would cause pH shift of about one unit to the acid side. The effect may be less pronounced since the volume change of ionisation becomes smaller at high pressure (Heremans, 1995).

2.1.2 Effects of high pressure on colour

For many fruit and vegetable products, high pressure treatment largely preserves fresh colour. Chemical and spectrophotometrical analysis of the colour of pressure-processed orange juice showed that colour values were not significantly altered by pressure and during subsequent storage time (Ludikhuyze and Hendrickx, 2001). Colour parameters indicated pressure-treated fruit jam to be superior to conventionally treated jam in brightness (L-value) and redness (a-value) (Watanabe *et al*., 1991). Pressure treatment of strawberries resulted in a high retention of red colour (Ludikhuyze and Hendrickx, 2001). The colour of tomato juice (in terms of a-value/b-value ratio) increased by pressure treatment in comparison to conventional processes ; the highest a/b ratio was observed at pH 4.5, irrespective of the pressure applied. This effect was purely physical in nature (compacting and homogenising effects) and not caused by differences in lycopene content of pressure-treated and heat-treated samples (Poretta *et al*., 1995)

Pressurised guava puree (600 MPa, 25°C, 15min) retained the original colour of freshly extracted puree. During storage at 4°C for 60 days, lightness and greenness fell continuously in all samples, especially in the untreated puree. The colour of pressurised puree samples was much more stable (Yen and Lin, 1996). Avocado puree treated at pressures between 345 and 689 MPa (10 to 30 min) had a colour equivalent to the freshly prepared puree (i.e., a green colour with colourimetric a-value between -7.9 and -7.3)(Mermelstein, 1997 ; Lopez-Malo *et al*., 1998). However, during storage several changes occurred. Less than 1% variation in lightness (L-value) was observed with samples at pH 4.1 treated at 689 MPa for 20 min and stored at 5°C. A higher decrease in lightness was found when puree were treated at lower pressure or higher pH or stored at higher temperature. For hue angle, net colour difference, and a-value, more pronounced changes were observed. During storage, green contribution to colour gradually decreased (i.e., the a-value became positive) because of severe browning as a result of residual PPO activity. The longest acceptability storage time (time to reach the limits of acceptance) could be reached with small initial pH, high pressure resulting in larger PPO inactivation, and lower storage temperature (Lopez-Malo *et al*., 1998). Similar results were obtained for banana puree. High pressure treatment (689 MPa for 10 min) preserved the initial colour of banana puree ;

however, during storage several colour changes occurred because of residual PPO activity that induced enzymatic browning of the puree (Palou *et al*., 1999).

For broccoli juice, investigators studied the effect of combined high pressure and temperature on chlorophyll content and green colour (Van Loey *et al*., 1998 ; Weemaes *et al*., 1999a). With regard to green colour, a common change in processed green vegetables is the degradation of chlorophyll (green) to pheophytin (gray-brown), a process associated with quality loss and thus undesirable to consumers. For this reason, minimising chlorophyll degradation and greenness loss is important to food processors. When green vegetables were pressure treated at low temperature, a stability of chlorophyll was observed. This phenomenon has been noticed with other pigments such as annatto, carotene, anthocyanins, and hibiscus extract (Ludikhuyze and Hendrickx, 2001). Even after treatment for 4 hr at 800 MPa and 40°C, no reduction in chlorophyll a and b contents were found. This observation can be attributed to the stability of the covalent structure of chlorophyll to high pressure. Only when high pressure combined with temperatures above 50°C, significant reduction of chlorophyll content became noticeable then chlorophyll a was less pressure-stable and more temperature-sensitive than chlorophyll b. It is worth nothing that the temperature increases for 10°C markedly increased the chlorophyll degradation rate, whereas pressure increases of 100 MPa have only a minor effect (Van Loey *et al*., 1998). Similar results when measuring spectrophotometrically the colour value of broccoli juice after treatment. At low temperature (less than 40°C), no significant reduction of green colour was observed, even after treatment at 800 MPa for 180 min, when high pressure was used at temperatures of 50°C to 60°C, some greenness loss appeared. At temperatures between 70°C and 80°C, greenness loss was noticed at all pressures studied (0.1 to 850 MPa), which indicated both pheophytin and pyropheophytin formation occurred. To minimise chlorophyll degradation and subsequent loss of greenness in broccoli juice during processing, it is recommended to use high pressure treatment below 50°C. In addition, high pressure has been found to induce discolouration in mushrooms and onions, because of the activity of PPO, an enzyme responsible for browning (Ludikhuyze and Hendrickx, 2001). PPO is a soluble enzyme predominantly localised in the cytosol of plant cells or bound to

particulate cell fractions. In intact cells, phenolic compounds are confined to vacuoles and spacially separated from the enzyme. When the cell and tonoplast are disrupted, enzyme and substrate are brought together and phenolic oxidation products are formed, resulting in the formation of brown complexes. Microscopic studies revealed severe damage of the vacuoles of onion epidermis cells treated at 300 MPa and 25°C. At this low pressure, PPO, which is very pressure-resistant, is not inactivated and can catalyse the oxidation of the released phenolic compounds, resulting in enzymatic browning (Butz *et al*., 1994).

2.1.3 Pink discolouration in canned lychee

Besides lychee, the pink discolouration has been found in canned fruits and vegetables such as apple, pear, banana, guava, gooseberry, peach, broad bean, field bean, green bean, black eye pea and cabbage (Ranganna and Parpia, 1974a ; Adams and Blundstone, 1971). It is generally believed that the pink discolouration is caused by the convertion of colourless leucoanthocyanidins into anthocyanidines when heated under acidic conditions. Metals, especially tin have also been suggested as being involved in the pink reaction (Chandler and Clegg, 1970a). Ranganna and Parpia (1974a) found that tin and organic acids are not necessary for colour formation, but if present, the acid increases the intensity of the colour formed in the red region while tin forms an insoluble white precipitate in the cold and shifts the hue on processing to the nonspectral reddish or bluish purple region of the chromaticity diagram.

Luh *et al.* (1960) reported that the pink discolouration of canned Bartlett pears was affected by both processing and cultivation conditions. Pears with low pH and high leucoanthocyanidins content were found to develop a pink colour after canning, especially when excessive heating and delayed-cooling processes were used. The level of applied nitrogenous fertiliser and the degree of exposure to sunlight (causes skin blush) were also found to correlate well with pink discolouration of canned Williams' pears (Czerkaskyz, 1970). Chandler and Clegg (1970b) reported that differences in the variety or maturity of pears can give rise to different degrees of pink discolouration.

Wu (1970 ; cited by Hwang and Cheng, 1986) observed that canned lychees processed at 105°C exhibited a pink discolouration when heated for only 3 min, while

samples processed at 88°C for 25 min did not show any pink colour after 10 months of storage. He also pointed out that besides sterilisation temperature and time, pH value influences the formation of pink colour. The pink discolouration in canned lychee may result from the hydrolysis of condensed tannin into catechin and leucoanthocyanin. Chakraborty *et al.* (1974 ; cited by Hwang and Cheng, 1986) found that a concentrated sugar syrup (35° and 40°Brix) seemed to enhance discolouration and suggested that lychees canned in 30°Brix syrup containing 0.1-0.15 % citric acid and restriction of the processing time in boiling water to less than 10 min can prevent the pink discolouration. Cheng *et al*. (1981; cited by Hwang and Cheng, 1986) observed that a greater citric acid concentration enhances discolouration and also found that canned lychees processed at 100°C, longer heating time resulted in more serious pink discolouration. Blanching the whole fruit prior to removing the skin and seed appeared to be slightly better than those blanched the seedless whole fruit.

To reduce enzymatic reaction prior to canning, can be done by immersing lychee flesh in various solutions for 3 hr (Hwang and Cheng, 1986). Shewfelt (1975) found that lychee flesh immersed in 0.2 % bisulfite solution successfully inhibited the action of polyphenoloxidase and thus prevented pink discolouration in canned lychee. Hwang and Cheng (1986) suggested that shortening the time between peeling and heating and immersing the lychee flesh in sodium bisulfite solution prior to thermal processing could reduce to some extent the discolouration.

The addition of sodium bisulfite to the syrup was effective in inhibiting pink colour formation only when the concentration exceeded 100 ppm but this amount might be imparted after taste (Hwang and Cheng, 1986). It seemed that sodium bisulfite bleached lychee stopped discolouration for the first three months but after six months of storage, the pink colour developed again, probably due to the depletion of bisulfite. Other additives such as ascorbic acid, erythrobic acid, ethylene diamine tetraacetic acid (EDTA), stannous and ferrous ions were found to be effective in controlling the pink discolouration in canned pears (Chandler and Clegg ,1970 c) but were not effective for this purpose in canned lychees (Hwang and Cheng, 1986).

Pink discolouration in canned lychee also depends on the variety or maturity of the fruits. The two common varieties of lychee cultivated in Taiwan are Quey-Wei and Hue-Yeh. The Hue-Yeh variety is more suitable for the canning industry because

the fruit is larger and also less prone to pink discolouration. Ripe lychee fruit, although is more flavour and taste but discolour more easily than unripe fruits (Hwang and Cheng, 1986).

Fruits and vegetables which show pink discolouration during processing often contain leucoanthocyanidins. But, the pink pigment might not be a simple anthocyanin since the syrup of discoloured canned products does not usually contain the pink colour. Along with the formation of anthocyanidin, other reactions may also take place and contribute to the final colour (Ranganna and Parpia, 1974b).

The quantities of leucoanthocyanidin, chlorogenic acid and total phenols in fresh lychees were measured at maximum absorbances of 0.628 (547 nm) and 1.74 (725 nm) for leucoanthocyanidin and total phenols respectively. The chlorogenic acid content was lower than 1 ppm, therefore it should not contribute to the discolouration of canned lychees. Canned lychees processed without blanching contained more leucoanthocyanidin than blanched samples (100°C, 5 min), and the lost leuco-anthocyanidin continuously during storage. Thus enzymatic reactions are likely to occur during heat treatment which can be inhibited by blanching. Moreover, the total phenols showed a similar profile to the leucoanthocyanidin. There was an enzymatic loss of phenolic compounds before canning that could be inhibited by blanching. However, there were only slight loss of total phenols in both blanched and unblanched samples during storage (Hwang and Cheng, 1986).

Besides leucoanthocyanidin and total phenols, tin and iron contents in the fruit also contributed to discolouration (Hwang and Cheng, 1986). The tin content increased with storage time because of detinning from the cans, but lacquer can delay the process. The iron content of canned lychees also increases with storage time. The iron content increased more rapidly during storage in completely-lacquered cans than in end-lacquered cans, probably because of pinholes in the former. Pink discolouration in these two types of containers was not significantly different. Lychees packed in glass containers usually do not show pink discolouration.

Hwang and Cheng (1986) found that the loss of leucoanthocyanin is proportional to the increase of tin in canned lychee and postulated that leucoanthocyanin is first converted to anthocyanin, which then forms a complex with the tin to give rise to the pink pigment. This is in agreement with Chandler and Clegg

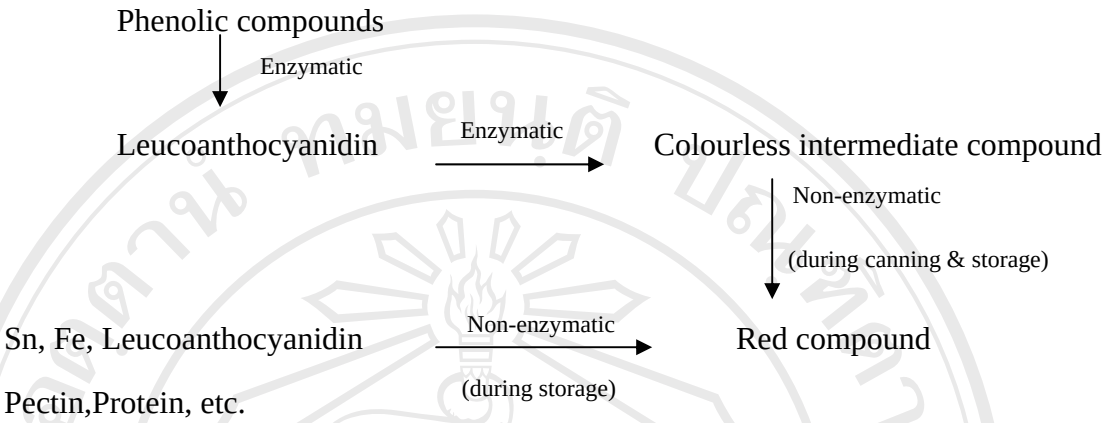
(1970 a) who reported that a tin-anthocyanin complex was responsible for the pink discolouration in canned pears.

Hwang and Cheng (1986) said that the major factors of pink colour formation are ranked in the following order : iron, total phenol, leucoanthocyanin and tin.

Pathway of pink pigment formation

The pathway of pink pigment formation in canned lychees has been postulated by many workers including Wu (1970 ; cited by Wu and Chen, 1999) who found that the pink discolouration in canned lychee may result from hydrolysis of condensed tannin into catechin and leucoanthocyanin. The leucoanthocyanin is further degraded into anthocyanin when the product is heated or stored in acidic conditions (Wu and Chen, 1999). Hwang and Cheng (1986) postulates that the pathway of pink pigment formation in canned lychee shown in Fig 2.1. Phenolic compounds apparently can be enzymatically converted to leucoanthocyanidins and these in turn can be enzymatically converted to a different colourless intermediate compound that changes into a red-coloured compound during canning and storage. Iron and tin are apparently involved in pigment formation either as copigments or as catalysts. In 1992, Wu (cited by Wu and Sheu, 1996) successfully isolated flavanone-3-hydroxylase and dihydroquercetin-4-reductase from lychee flesh and concluded that these two enzymes play a key role in the biosynthesis of leucoanthocyanin. He proposed a pathway for the pink discolouration during canning as follows : after peeling and pitting, the flavonones in mature lychee flesh are first converted into eriodictyol-containing compounds, then hydrolysed by flavanone-3-hydroxylase to dihydroquercetin-containing compounds which are further reduced to leucocyanidin-containing compounds by dihydroquercetin-4-reductase ; when the lychee is heated, the leucocyanidin-containing compounds are converted into cyanidin-containing coloured compounds.

Fig 2.1 Possible pathway of pink pigment formation in canned lychees.



2.2 EXPERIMENTAL

2.2.1 Effect of pressure on quality determinations

2.2.1.1 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).

Standard pH;pH value at 20°C 3.98-4.02, 6.98-7.02, 9.95-10.05 (BDH, UK).

Standard salt for a_w ;SAL-T_{11}, SAL-T_{33}, SAL-T_{53}, SAL-T_{75}, SAL-T_{90}, SAL-T_{98} (Novasine, Switzerland).

2.2.1.2 Methods

2.2.1.2.1 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 and 175 g of syrup, which consisted of 300 g sucrose,1.3 g citric acid and 700 g deionised water, were added. 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 pH, water activity (a_w), soluble solid contents and colour.

2.2.1.2.2 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 pH, a_w, soluble solid contents and colour.

2.2.1.2.3 Quality determinations

The soluble solid contents of all samples were determined using a digital refractometer (Atago,Japan). Results are reported as °Brix at 20°C. For water activity(a_w), the samples were macerated and placed in a cylindrical cup, 4.0 cm diameter x 1.0 cm high, filled approximately 2/3 full. The a_w was measured at 25°C with a Novasine AWC meter (AWC 200, switzerland). For colour, samples were assessed using a Perkin Elmer UV/VIS Spectrophotometer Lambda 20 or Hunterlab Colour Quest Spectrophotometer (because the Perkin Elmer UV/VIS Spectrophotometer Lambda 20 was unavailable.) in the reflection mode. Results were expressed as Hunter *L* (brightness), *a* (green/red) and *b* (yellow/blue) values.

2.2.1.2.4 Experimental Design

Colour measurement of samples treated at 10 min or 20 min, two variables were studied, these were 3 levels of pressure (200, 400, and 600 MPa) and 2 levels of temperature (20, and 60°C), i.e., using 3x2 factorial in a completely randomised design (CRD). For pH, soluble solid contents and water activity, the variables were the same but using 3 levels of pressure (200, 400, and 600 MPa) and 3 levels of temperature (20, 40 and 60°C) and 3x3 factorial in a completely randomised design (CRD) were applied. Each treatment was determined four time for the measurement of colour and twice for the measurement of pH, soluble solid contents and water activity and all treatments were carried out in 3 replicates. The statistical program, SPSS v 11.5 (SPSS Inc., Chicago, USA), was used for data analysis and Duncan's multiple range test used for comparing differences between means.

2.2.2 Determination of pink discolouration in heated lychee

2.2.2.1 Materials

Lychees (*Litchi chinensis* Sonn.) were purchased from a commercial orchard at Chiangmai province of Thailand and stored in polyethylene bag at 2°C.

n-butanol containing 5% HCl, Merck, Germany.

2.2.2.2 Method

Anthocyanidins were generated from the leucoanthocyanidins of lychee by heating with *n*-butanol containing 5% HCl over a water bath for 10 min (modified from Ranganna and Parpia, 1974b). After cooling, the coloured butanolic layer was centrifuged and filtered through millipore filter (0.45 μm), then their absorbance measured by spectrophotometer from the wavelength of 400 to 700 nm. The spectral data are used to estimate the anthocyanidins which have maximum absorption at 500-550 nm (Musingo and Wang, 2005).

2.3 RESULTS AND DISCUSSIONS

2.3.1 Effect of pressure and heat on quality determinations

High pressure treatment decreased the pH of lychees (originally 4.72) by 0.12 units for those processed under pressure and by 0.60 units for those canned in syrup under commercial conditions (Table 2.2). Not unexpectedly the lychees processed in syrup (containing citric acid and sugar) had lower pH values and higher soluble solid contents than fresh lychees. Soluble solid contents for pressure treated samples were greatly increased but their water activity slightly decreased indicating some loss of water during processing and/or solubilised previously insoluble material. A small amount of fluid was seen in the pressure treated, non-syruped samples (Table 2.2).

Table 2.2 General characteristics of fresh lychee and lychees processed by various means

Treatments	pH	Soluble Solid Contents (°Brix)	Water activity (a_w)
Fresh lychee	4.72	11.2	0.993
Pressurised fresh lychee[a]	4.60±0.13	16.30±1.79	0.967±0.009
Lychee in syrup	4.22	22.6	0.965
Pressurised syrup lychee[a]	4.25±0.10	22.79±0.47	0.961±0.003
Canned lychee	4.12	23.9	0.966

[a] The values quoted are means ± S.D. of all the different pressure/temperature regimes (18) since there was no significant difference between treatments.

With respect to colour attributes it is interesting to note that pressures up to 600 MPa at 20°C for 20 min pressure had little effect on the products. The *L* value increased slightly from for the untreated lychee of 53.94 ±1.12 to 58.22±4.56 for those treated at 600 MPa (Table 2.3). For those pressure treated at 60°C, the *L* value increased more extensively even at 200 MPa (to 62.17±3.72). Further increased pressures only led to slight changes (to 67.98±4.75)(Table 2.4). There were no significant difference between treatment times of 10 and 20 min at 60°C.

Table 2.3 Combined effects of high pressure and temperature at 20°C on the colour attributes of fresh lychees. All values are the means ± S.D. of 12 determinations.

Pressure (MPa)	Time (min)	Colour attributes*		
		L value	*a* value	*b* value
Untreated		53.94±1.12	-0.15±0.31	-3.71±0.40
200	10	56.04±3.50	-0.31±0.39	-2.47±1.13
400	10	60.40±2.21	-0.42±0.37	-3.93±2.02
600	10	58.45±3.18	-0.76±0.80	-0.91±0.62
200	20	ND	ND	ND
400	20	57.69±4.35	-0.82±0.42	-3.77±0.94
600	20	58.22±4.56	-0.55±0.55	-3.18±1.14

ND = Not determination

* Colour measurement with Perkin Elmer UV/VIS Spectrophotometer Lambda 20

Table 2.4 Combined effects of high pressure and temperature at 60°C on the colour attributes of fresh lychees. All values are the means ± S.D. of 12 determinations.

Pressure (MPa)	Time (min)	Colour attributes*		
		L value	*a* value	*b* value
Untreated		50.23±4.69	0.43±0.74	2.05±1.79
200	10	66.81±4.98	0.37±1.10	3.63±2.92
400	10	68.26±5.59	-0.61±1.02	3.92±2.26
600	10	67.50±3.37	-1.24±0.67	4.38±2.49
200	20	62.17±3.72	-0.06±0.64	2.21±1.73
400	20	67.03±3.64	-0.43±1.54	3.72±1.52
600	20	67.98±4.75	-1.84±0.78	2.97±1.91

* Colour measurement with Hunterlab Colour Quest Spectrophotometer because the Perkin Elmer UV/VIS Spectrophotometer was unavailable.

Increasing pressure, at both 20 and 60°C caused a decrease in the *a* value indicating that pressure might be an effective means of minimising the pink discolouration of lychees, since a decrease of *a* value meant less redness (Cheng and Hwang,1984). The *b* value in the pressured samples changed little when treated at 20-60°C and high pressure (200-600 MPa) for 20 min although treatment at 60° C increased the value. Table 2.5 and 2.6, show the results for those samples treated in syrup and similar trends were seen, with increasing pressures at 60°C increasing the *L* value although the effects on the *a* value were less marked. Interestingly the canned samples had lower *a* but higher *b* values than the pressurised samples. These results agreed with those of Yen and Lin (1996) who reported that heating could cause an increase in *L* and *b* values in guava puree.

In conclusion, high pressure treatment caused less loss of visual quality of lychee in both fresh and packed in syrup than those treated under thermal processing.

Table 2.5 Comparison of the colour attributes of pressurised syrup lychee at 20°C. All values are the means ± S.D of 12 determinations.

Pressure (MPa)	Time (min)	Colour attributes*		
		L value	*a* value	*b* value
Untreated		55.03±3.04	-0.61±0.25	-4.34±0.56
200	10	54.69±1.24	-0.48±0.24	-4.31±0.59
400	10	56.46±1.27	-0.61±0.17	-4.81±0.60
600	10	56.71±1.52	-0.78±0.27	-4.64±0.64
200	20	54.56±2.65	-0.59±0.14	-3.70±0.52
400	20	56.01±1.95	-0.74±0.54	-4.19±0.72
600	20	57.92±1.38	-0.75±0.18	-4.36±0.78

* Colour measurement with Perkin Elmer UV/VIS Spectrophotometer Lambda 20

Table 2.6 Comparison of the colour attributes of pressurised syrup lychee at 60°C and canned lychees. All values are the means ± S.D of 12 determinations.

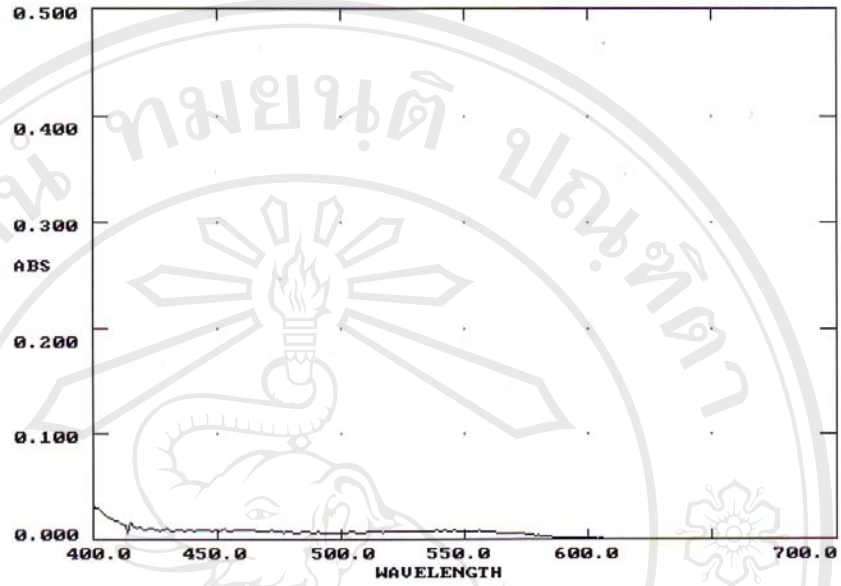
Pressure (MPa)	Time (min)	Colour attributes*		
		L value	*a* value	*b* value
Canned lychee		66.32±3.99	-1.61±1.52	6.21±3.99
200	10	61.58±2.95	-1.42±0.66	1.68±1.89
400	10	61.86±4.69	-1.08±0.52	1.85±1.53
600	10	62.30±4.06	-1.19±0.66	1.74±2.49
200	20	64.23±4.80	-1.01±1.10	1.93±1.47
400	20	64.82±3.63	-1.29±0.45	2.73±1.45
600	20	68.46±5.64	-1.32±0.51	4.20±3.29

* Colour measurement with Hunterlab Colour Quest Spectrophotometer because the Perkin Elmer UV/VIS Spectrophotometer was unavailable.

2.3.2 Pink discolouration in heated lychee

Extraction of lychee with *n*-butanol containing 5 % HCl did not change the original colour and showed no peak at 544 nm (Fig 2.2 A). Heating the acidified solution in boiling water for 10 min resulted in a darkening of the solution within 5 min and a deep burgendy colour developed within 10 min at which time boiling was stopped. The colour change was also associated with an increase in absorbance at 544 nm (Fig 2.2 B). Leucoanthocyanidin and anthocyanidin are water soluble compounds. The presence of anthocyanidin is indicated in plants by the red colour (Sheridan and Mills, 1998) and have maximum absorption at 500-550 nm (Musingo and Wang, 2005). The red colour change and increase in absorbance at 544 nm in butanolic extracts of lychee after heating with acid demonstrated the presence of leuco-anthocyanidin in lychee (Sheridan and Mills, 1998). It is essential to note that the pink discolouration in lychee may due to the convertion of colourless leucoanthocyanidin into anthocyanidin when heated under acidic conditions as appeared in other fruits and vegetables such as apple, pear, banana, guava, gooseberry, peach, broad bean, field bean, green bean, black eye pea, cabbage and lychee (Ranganna and Parpia,

A



B

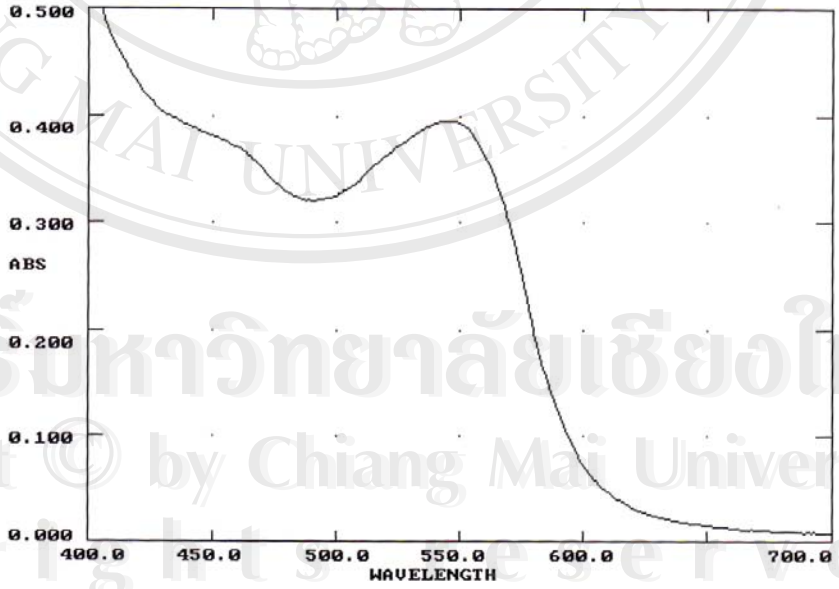


Fig 2.2 Alcoholic extracts of lychee in acid lacking the peak at 544 nm (A), when heated in boiling water the colourless leucoanthocyanidin are converted into anthocyanidin with a peak at 544 nm (B).

1974a ; Adams and Blundstone, 1971 ; Hwang and Cheng, 1986 ; Wu and Chen, 1999).

To sum up, more work is needed to characterise this pink pigment which occurred in heated sample under acidic condition and to clarify the mechanism of pink discolouration which occasionally occurs in canned lychee from fresh lychee to canned product.