CHAPTER 6
CONCLUSIONS

Quality determinations

Fresh lychee and lychee processed in syrup were subjected to pressures of 200-600 MPa and temperatures of 20-60ºC for 10 or 20 min.

High pressure treatment of fresh lychee, decreased the pH, the water activity, and the *a* value, but greatly increased the soluble solid contents. It had little effect on the *L* and *b* values. Lychee processed in syrup (containing citric acid and sugar) had lower pH values and higher soluble solid contents than fresh lychee. With respect to the colour attributes, similar trends to those seen for fresh lychee were seen for the *a*, *b*, and *L* values. Since pressure caused a decrease in the *a* value in both systems it is probable that pressure is an effective means of minimising the pink discolouration of lychees compared to that are thermally processing.

Pink discolouration

Extraction of lychee with *n*-butanol containing 5% HCl and heated in boiling water, resulted in the red solution with maximum absorption at 544 nm. This demonstrated the presence of leucoanthocyanidin in lychee and showed that the pink discolouration in heated lychee may due to the convertion of colourless leuco-anthocyanidin into anthocyanidin when heated under acidic conditions.

Peroxidase (POD)

POD from lychee was extracted with potassium phosphate buffer, pH 6.2 and had a pH optimum between 5.0-8.0 with maximal activity at 6.0. The broad pH optima observed was probably due to the presence of isoenzymes of different pH optima. The loss of activity observed on acidification is attributed to the change in the protein from the native state to a reversible denatured state, brought about by detachment of the heme from the protein.

Application of 200 MPa increased the activity of POD in the non-syruped lychees, the effect appearing more marked at 40ºC for both 10 and 20 min than at 20 and 60ºC. The activation phenomena, related to better availability of the substrate, the only marked inactivation of POD in lychee was at 600 MPa and 60ºC. Similar trends were seen for lychee processed in syrup, but the effects were less marked due to the baroprotective effect of the syrup.

Polyphenoloxidase (PPO)

The pH optimum for PPO was 7.0. At or below pH 4.0, it showed very low activity. On raising the pH from 7.0 to 8.0, a sharp drop in enzyme activity was noticed.

The lychee PPO was subjected to pressures of 200-600 MPa and temperatures of 20-60ºC for 10 or 20 min. At mild temperature (20-40ºC) the degree of inactivation of PPO decreases with treatment time (10 or 20 min) for the three pressure levels. Increasing temperature brought about significant inactivation, with over 90% loss of activity at 600 MPa and 60ºC for both treatment times (10 and 20 min). When processed in syrup the baroprotective effect of the mixture was again seen although there was still significant inactivation under the more extreme conditions. At 600 MPa and 60ºC for both treatment times (10 and 20 min), significant decreases in activity, of over 50% were observed.

Lipoxygenase (LOX)

The pH profile of LOX activity in lychee was bell-shaped with a maximum around 4.0 using linoleic acid as substrate.

The lychee LOX was subjected to pressures of 200-600 MPa for 10 or 20 min at ambient temperature. Pressure treatment led to a reduction in activity of the enzyme for both 10 and 20 min. At 600 MPa for 10 and 20 min, decreases in activity were 86% and 89%, respectively. When processed in syrup the effects were less marked due to the baroprotective effect of the syrup as also found for POD and PPO. At 600 MPa for 20 min at ambient temperature, significant decreases in activity, of over 82% were observed.

This work confirms the ranking of the enzymes according to their pressure induced inactivation in the following order : LOX, PPO and POD.

Flavour components

GC-MS analyses of volatile compounds were obtained from fresh lychee and lychee processed in syrup subjected to pressures of 200-600 MPa at 20-60ºC for 10 or

20 min using solid phase micro-extraction (SPME). Major volatile compounds identified by MS analysis, included 13 hydrocarbons, 9 aldehydes, 6 alcohols, 4 esters, 4 ketones, and 3 miscellaneous compounds.

The hydrocarbons were mainly terpenes, dominated by the monoterpenes, limonene and myrcene. Some of these terpenes contribute to the characteristic aroma of lychee such as *cis*-rose oxide. This compound contributes to the floral green note and also has been described as powerfully fruity. High pressure treatment did not affect the hydrocarbons some of which such as *cis*-rose oxide and limonene are involved in the fruity-floral and citrus notes of lychee.

The alcohols were the major volatile fraction in lychee, and esters were the second most important for unpressurised lychee whereas for the pressurised fresh lychee at the higher pressures, the aldehydes were the second most abundant. During high pressure treatment, the aldehydes exhibited marked changes, especially *n*-hexanal which increased 7 and 14 times, after pressure treatment at 200 MPa (20ºC for 10 min) and 600 MPa (60ºC for 20 min) respectively. Pressure treatment enhanced the formation of hexanal by lipid oxidation of polyunsaturated fatty acids which are present at 0.132% in lychee. The acceleration of this reaction by pressure may be due to strong decompartmentation of the enzyme, allowing the reaction to occur. Similar trends were seen in lychee processed in syrup, pressure treatment caused an increases in *n*-hexanal at moderate pressure and very considerable increases at higher pressure, but the effects were less marked than in fresh lychee due to the baroprotective effect of the syrup.

Texture and microstructure

Fresh lychee and lychee processed in syrup were subjected to pressures of 200-600 MPa and temperatures of 20-60ºC for 10 or 20 min. Pressure treatment did not affect the firmness of fresh lychee at room temperature for both treatment times (10 and 20 min). Increasing temperature (40ºC), at 600 MPa caused a decrease in firmness and the effects were more marked at 60ºC where a remarkable decrease in firmness was seen even at 400 MPa. Similar trends were seen in those lychee treated in syrup, with increasing pressure (600 MPa) at both 40ºC and 60ºC decreasing the firmness. Untreated lychee in syrup had a greater firmness than fresh lychee because calcium influences the texture through its interaction with the cell wall or cell membranes. For lychee canned in syrup , the firmness was the lowest of all treatments due to heat induced depolymerisation of peptic substances and also to acid hydrolysis of glycosidic bonds in the cell-wall polysaccharides.

Confocal scanning laser microscopy (CSLM) was used to examine unpressurised lychee and pressure-treated lychee at 200 and 600 MPa and 20 ºC for 20 min. The microscopic appearance revealed that the changes in flesh lychee treated at 600 MPa were more extensive than in those treated at 200 MPa. After treatment at 200 MPa, the 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. After treatment at 600 MPa, the outer layer of flesh appears with collapsed and compacted cell structure, also some trace of migrating fluid through outside. The inner cells having irregular structure and much more densely pack than those treated at 200 MPa, indicate more severely histological damaged.

Microbiological quality

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 (10 or 20 min). High pressure treatment at 600 MPa and 20ºC for 20 min inactivated the microbes in fresh lychees down to a level of 10 cfu g^{-1}. During storage at 3ºC 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. At 60ºC, the microbes counts were reduced to a level below the detection limit (<10 cfu g^{-1}) within 20 min at 200 MPa whereas at 40ºC pressures up to 400 MPa for 10 min were required.

Similar trends were seen for reduction of yeasts and moulds, pressures up to 200 MPa at ambient temperature causing little inactivation. Significant inactivation was observed at pressures in excess of 400 MPa independent of the duration of treatment (10 or 20 min). At higher temperatures (40 and 60ºC), pressures of 200 MPa for 20 min could inactivate these microorganisms in fresh lychees as the final count was down to a level of 10 cfu g^{-1}.

*General conclusio*n

In many situation it is highly desirable that enzymes are inactivated. A prime example of this is polyphenoloxidase which causes enzymatic browning of damaged fruits and vegetables , and is one of the main causes of quality deterioration during postharvest handling, storage, and processing. Others are peroxidase and lipoxygenase. POD is associated with off-flavours and off-colours in raw and unblanched vegetables whereas LOX mediates, either directly or indirectly, a whole spectrum of changes in food quality including ones in colour, flavour, texture and nutritional quality. However, the pressure necessary to bring about inactivation of POD would not be economically realistic. Although, LOX is very pressure sensitive, but it still caused a problem with hexanal formation. In spite of the limited number of samples used, the present studies stress: (i) the high variability in the amounts of lychee fruit volatile flavour components (ii) the problems of lychee flavour stability during post-harvest handling (iii) the need for product quality control to be intensified in future.

However, this work has also shown that under certain pressures, it is possible to use high pressure technology as an alternative to thermal processing. Pressure caused changes in colour, with a decrease in the *a* value indicating that pressure might be an effective means of minimising the pink discolouration of thermally processed lychee. To avoid hexanal formation during high pressure treatment, the lychees could be blanched before high pressure treatment to inactivate LOX. This investigation also suggested that the effects of pressure and temperature were synergistic, resulting in an increased rate of inactivation of microbes and enzymes as both pressure and temperature increase.