CHAPTER 4

EFFECT OF HIGH PRESSURE TREATMENT ON THE LIPOXYGENASE ACTIVITY AND FORMATION OF FLAVOUR COMPONENTS

4.1 INTRODUCTION

4.1.1 LIPOXYGENASE

4.1.1.1 Generalities

Lipoxygenase (E.C. 1.13.11.12, linoleate : oxygen oxidoreductase) is found in a wide variety of plants, particularly the legumes. It has been reported in alfalfa, peas, beans, peanuts, potatoes, and radishes. The highest concentration of lipoxygenase has been reported in soybeans ; urd beans, mung beans and peas contain about 50 % as much lipoxygenase as soybeans, while peanuts and wheat contain 1-2 % of that found in soybeans (Siddiqi and Tappel, 1956 cited by Whitaker, 1985).

Lipoxygenase is of particular importance in food science and technology because of its destruction of the essential fatty acids, linoleic, linolenic, and arachidonic acids, which result in the development of off-flavours and odours, characterised as haylike flavours in underblanched green peas and green beans and beany flavour in soybeans and soybean products. The intermediate free radical hydroperoxides cause damage to other compounds, including carotene,vitamin A , and proteins. Lipoxygenase is involved in flavour formation in plant materials, especially in tomatoes due to the formation of 2-hexenal. The aldehydes formed can be oxidised further by aldehyde dehydrogenase or aldehyde oxidase to carboxylic acids or reduced by alcohol dehydrogenase to alcohols. The carboxylic acids and alcohols then nonenzymatically form esters with characteristic odours (Whitaker, 1985).

The best described lipoxygenase is lipoxygenase-1 from soybean which was crystallised in 1947 (Holman, 1947 cited by Whitaker, 1985 ; Theorell *et al.*, 1947 cited by Wong, 1995b). This enzyme is a metalloenzyme with a nonheme iron as its prosthetic group. The iron is bound directly to the polypeptide backbone and is not readily removed by iron chelators such as bipyridyl and o-phenanthroline (Hammer,

1993). It has a molecular weight of 98.5 kD (Vliegenthart and Veldink, 1982) with an isoelectric point of 5.4 and a single polypeptide chain containing and one iron atom is involved in the active site (Chan, 1973). The pH optimum is 8-9, depending on the presence or absence of detergents added to solubilise the substrates. Soybeans contain three other isoenzymes, all in lesser amounts and with pH optima near pH 6.5 (Whitaker, 1985).

4.1.1.2 Reaction mechanism

Lipoxygenase belongs to the dioxygenase group of enzymes in which both atoms of O_2 are incorporated into the product. It oxidises polyunsaturated fatty acids and esters (at a much lower rate) containing a *cis,cis*-1,4-pentadiene system (Fig 4.1) to form hydroperoxides as the primary products as well as a number of secondary products (Whitaker, 1985). The enzyme attacks the sensitive methylene group of the penta-1,4-diene unit between the two double bonds at the carbon atom 8, counting from the methyl end i.e., n-8 (or ω-8) (Whitaker, 1994). However, the position of the pentadiene group may vary and only one or two of a wide range of possible substrates are preferred for a particular isoenzyme. Linoleic and linolenic acid are preferred as substrates for lipoxygenase-1 while arachidonic acid is claimed to be preferred for lipoxygenase-2, rather than linoleic acid. Lipoxygenase-2 and lipoxygenase-3 from soybean and other higher plant lipoxygenases have approximately equal activities towards free fatty acids, the methyl esters and the triglyceride forms of the substrate (Axelrod *et al.*, 1981). Methyl linoleate was reported as being more preferred for lipoxygenase-2 and lipoxygenase–3 than linoleic acid (Robinson *et al.*, 1995).

There are three steps proposed in the formation of the hydroperoxide products; (1) The activation of the resting enzyme; (2) The removal of a proton from the activated methylene group; and (3) the insertion of oxygen into the substrate molecule with formation of the hydroperoxide (Whitaker, 1991, 1994; Robinson *et al.*, 1995; O' Connor and O'Brien, 1991).

Figure 4.2 shows the oxidation of 8,11,14-(all-*cis*) eicosatrienoic acid. The sequence of chemical events, all done while the enzyme is attached, are as follows: (1) The enzyme forms a stereospecific complex with the unsaturated fatty acid ; (2) soybean lipoxygenase-1 removes only the H_L hydrogen form the

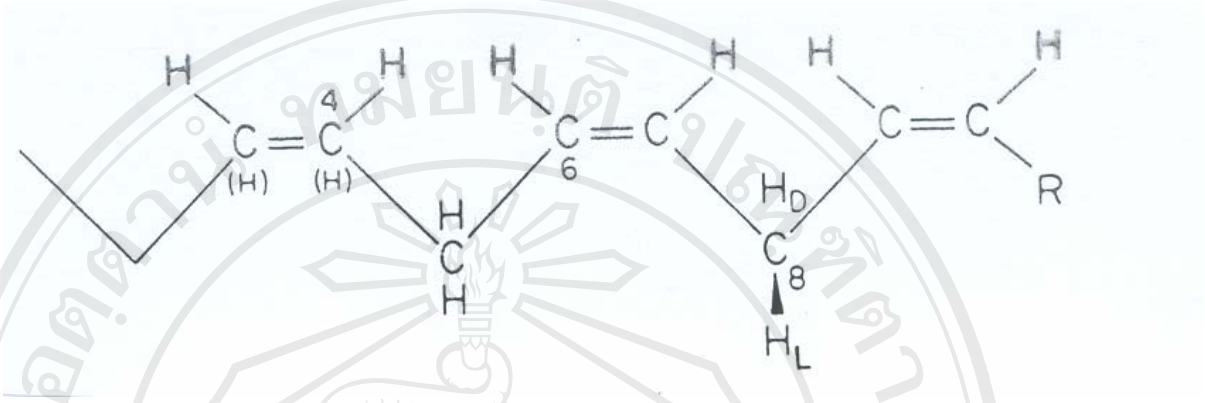

Fig 4.1 The essential part of a fatty acid which permits it to be a substrate for lipoxygenase. Required are *cis* double bonds at ω-6 and ω-9 positions, a hydrogen in the L_S position on ω-8 carbon, and hydrogen at the ω-6 carbon. The ω-3 and ω-4 position may be saturated or unsaturated. From Whitaker (1985).

ω-8 (C-13) carbon (Figs 4.1 and 4.2) while corn germ lipoxygenase removes the HR hydrogen ; (3) the fatty acid free radical isomerises to place the unshared electron at ω-6 (C-15) , causing conjugation and cis → trans isomerisation of the double bond ; (4-5) the O2 bound at this point to enzyme or earlier, adds to the fatty acid radical at ω-6 (C-15) to give the L_s-hydroperoxy radical in the case of soybean lipoxygenase-1 or ω-10 (C-11) in the case of corn germ and potato lipoxygenases. Therefore, the O_2 must enter from the opposite side of the molecule from which the hydrogen is removed in order to produce the *trans*-L_s-hydroperoxy radical in the case of soybean lipoxygenase-1 ; (6) an electron abstracted from the enzyme and a proton from the medium give the product 15-L_s-hydroperoxy-8,11,13-*cis,cis,trans*-eicosa-trienoic acid from 8,11,14-(all-*cis*) eicosatrienoic acid. The corn germ lipoxygenase gives the 9-D_R-hydroperoxy in the case of linoleic acid.

The 15-L_s-hydroperoxy-8,11,13-*cis,cis,trans*-eicosatrienoic acid dissociates from the enzyme under aerobic conditions to regenerate the enzyme, but under

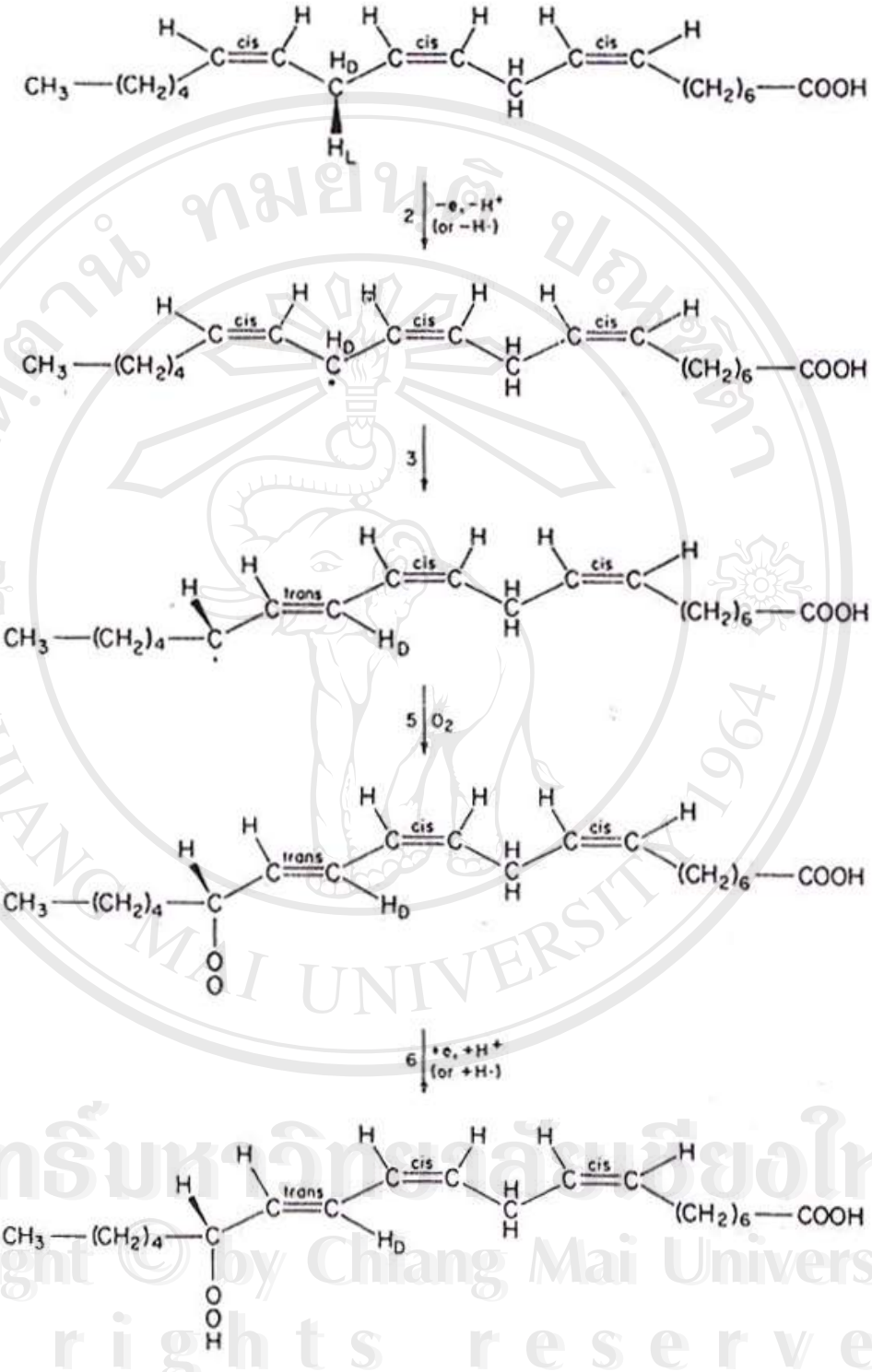

Fig 4.2 Reaction catalysed by lipoxygenase, using 8,11,14-(all-*cis*) eicosatrieonic acid as a typical substrate. From Whitaker (1985).

anaerobic conditions it is further catalysed to a variety of products including dimers, oxodienoic acids, and *n*-pentane. This reaction, which has been studied extensively by adding the fatty acid hydroperoxide to lipoxygenase directly, is catalysed by the enzyme under aerobic conditions, but at a rate approximately one thousandth that seen under anaerobic conditions. Under anaerobic conditions the rate of catalysis of linoleic acid hydroperoxide to further products was shown to be approximately the same as the rate as for formation of the linoleic acid hydroperoxide (Whitaker, 1985).

4.1.1.3 Structure and active site

Soybean lipoxygenase-1 is composed of a single polypeptide chain of 838 amino acids, with a molecular weight of 94,038. The enzyme contains four cysteine residues but no disulphide bonds. The majority of the tryptophan residues (12 of 13) are located in a hydrophobic region between residues 617-783. Lipoxygenase-2 has a molecular weight of 97,036 with 865 amino acids, and contains 7 cysteine and 15 tryptophan residues. Lipoxygenase-3 consists of 857 amino acids, with a molecular weight of 96,663. There are 5 cysteine and 14 tryptophan residues, but no disulphide bonds in the enzyme molecule. The amino acid sequences of lipoxygenase-1 and lipoxygenase-2 are 81 % identical, whereas those of lipoxygenase-1 and lipoxygenase-3 show 70 % similarity (Wong, 1995b).

There is a lack of clarity regarding the exact structure of the active site of lipoxygenase. All plant lipoxygenase sequences have key histidines and a C-terminal isoleucine which are ligands to the iron and constitute part of the active site (Robinson *et al.*, 1995). According to Whitaker (1991), a cluster of five-conserved histidine residues may be part of the active site responsible for chelating the iron moiety. In addition, the aromatic amino acids, tyrosine and tryptophan, may play a role in the active site. Methionine is also implicated in determining its activity, either directly in the catalytic process or by sterically blocking the active site (Eskin *et al.*, 1977).

4.1.1.4 Determination of enzyme activity

A number of methods have been developed for determining the rate of oxidation of the substrate. Linoleic acid is the substrate most often used for the assay though other polyunsaturated fatty acids such as linolenic or arachidonic acid also

react (Axelrod *et al.*, 1981). The most commonly used are the manometric technique based on the measurement of oxygen uptake by the substrate, and the spectrophotometric assay in which the increase in extinction brought about by the formation of the conjugated diene from the substrate is followed. A third method involves the determination of the peroxide group by a variety of techniques (Tangwongchai, 2000).

4.1.1.4.1 The manometric technique

The manometric procedure has a wide range of applicability, since it may be employed with crude preparations, as well as with purified extracts. However, the method needs a long assay period (up to 30 min) in which initial oxygen consumption can not be measured and the secondary reactions can become important during the assay period. Furthermore, shaking is needed in this procedure which may cause inactivation of the purified enzyme. However, this disadvantage can be overcome by using a high sensitivity oxygen electrode or polarography, which measures the reaction from the beginning and the whole assay may be completed in a few minutes and in which the denaturing effect of stirring or shaking is minimal. In some instances, the oxygen uptake is not directly proportional to the enzyme concentration. In terms of oxygen consumption, one unit of lipoxygenase activity is equal to 1μmole of oxygen consumed per minute (Tangwongchai, 2000).

4.1.1.4.2 Spectrophotometric assay

The conjugated linoleate hydroperoxide has strong absorbance at 234 nm and thus, a direct spectrophotometric assay measuring the increase in absorbance as a function of time is used (Surrey, 1964). This method has the advantage of simplicity and rapidity, but its use requires optically clear solutions for measurements in the ultraviolet. As a rule, this is achieved either by adjusting the pH to 9, where linoleate forms a true solution, or by removing aliquots from the reaction mixture at different time intervals and diluting them with ethanol to obtain clear solutions (Ben-Aziz *et al.*, 1970). One unit of lipoxygenase activity is defined as the amount that will oxidise 1 μmole of linoleate per minute (Tappel, 1962).

4.1.1.4.3 Colourimetric methods

A qualitative colourimetric procedure depends on the conversion of $Fe(CNS)_2$ to the reddish brown of $Fe(CNS)_3$ by reaction with peroxides. The $Fe(CNS)_3$ concentration can be measured spectrophotometrically at 480 nm (Grossman *et al.*, 1971). There are though some disadvantages to this method. For example, it does not provide a continuous assay and the colour is unstable. Furthermore, the relationship between the colour produced and peroxide concentration is not a simple linear one (Eskin *et al.*, 1977).

The rate of hydroperoxide formation can be detected by the oxidation of I^- to I_2 in acid solution. In this assay, aliquots must be removed from the reaction periodically, placed in an acid containing KI, and titrated for hydroperoxide content (Whitaker, 1994). The formation of I_2 will be visible as a pale yellow colour when a concentrated nonacidified potassium iodide solution is used. The addition of 1% starch solution results in a blue-purple colour in the acidified mixture and the intensity of the colour indicates the activity of the lipoxygenase. The enzyme can also be estimated from the bleaching of carotene due to the coupled autoxidation of the carotenoid. However, the disadvantages of this method are: (1) the reaction measured is a secondary reaction and its ratio to the primary reaction is empirical; (2) colloidal substrates are generally used; (3) a linear relationship between enzyme concentration and carotene loss can be obtained only over a narrow range. Since the reaction is not stoichiometric, the reaction needs to be well controlled to achieve reproducible results. Toyosaki (1992 cited by Tangwongchai, 2000) reported that lipoxygenase activity could also be estimated from the time required to bleach methylene blue, estimated by the decrease in absorbance at 660 nm.

The spectrophotometric measurement of an increase in absorbance at 234 nm was used as the enzyme assay in this present study since the method is rapid, convenient and reproducible. Furthermore, the increase in UV-peak absorption is related to the amount of peroxide formed, which is proportional to time and enzyme concentration (Surrey, 1964). A modified spectrophotometric procedure, in which linoleate in the presence of Tween 20 remains soluble over a wide pH range, giving optically clear substrate solutions made it possible to study the stability of the enzyme

over a wide range of pH (Ben-Aziz *et al.*, 1970).

4.1.1.5 Effects of high pressure on Lipoxygenase activity

Pressure inactivation of LOX has been reported to occur between 400 and 600 MPa at room temperature. Unlike PPO, activation of LOX at relatively low pressure has not yet been observed.

Seyderhelm *et al*. (1996) reported LOX to be markedly inactivated by treatment at 600 MPa for 2 to 10 minutes in Tris buffer at pH 7 as well as in milk. Heinisch *et al*. (1995 cited by Ludikhuyze *et al.*, 2001a) studied the inactivation of soybean LOX in borate buffer at pH 9 at pressures up to 650 MPa and temperatures between 0 °C and 75 °C for 5 minutes. Based on the activity loss, these authors constructed a pressure-temperature phase diagram , revealing a slight antagonistic effect at high temperature and low pressure. Such antagonistic effect of pressure and temperature was not observed by Ludikhuyze *et al*. (1998a, 1998b) or Indrawati *et al*. (1999) , who studied the inactivation of soybean LOX in Tris buffer at pressures between 0.1 and 650 MPa at temperatures between -15 °C and 68 °C. Maximal pressure stability of soybean LOX was observed at about 30 °C, indicating that both temperature increase and temperature decrease can accelerate the inactivation at constant elevated pressure.

Furthermore, Ludikhuyze *et al.*(1998a) found that the pressure stability of soybean LOX is largely affected by environmental conditions. Pressure resistance increased with increasing enzyme concentration and decreased with decreasing pH (pH 9 to pH 5.4). In addition, they noted that soybean LOX was much more stable in Tris buffer than in Mcllvaine buffer. Flushing with CO_2 likewise strongly reduced pressure stability, an effect that could be completely attributed to the pH decrease caused by dissolution of CO_2 in the buffer.

4.1.2 FLAVOUR COMPONENTS

Many flavour components in fruits are formed via the enzymatic and chemical reactions that occur when the fruit is damaged or cut. Lipoxygenase (LOX) , which exists naturally in plants, is considered to play a part in the genesis of flavour components in plant products.

Flavour production by LOX in materials ; including raw vegetables, is

triggered by maceration or cell injury, because enzymes are brought into contact with their substrates. However, LOX is also responsible for off-flavour development in foods, e.g., corn, green beans and soybeans, are particularly sensitive during frozen storage (Tangwongchai, 2000).

4.1.2.1 Lychee flavour

Johnston *et al.* (1980) were the first who reported the volatile profile of lychee. These volatile constituents have been investigated by the combined technique of gas chromatography-mass spectrometry with 42 components being identified and confirmed. Of these, 2-phenethylethanol, its derivatives, and terpenoids comprised the major and characteristic portion of the volatiles. According to Grab (1998), 2-phenethylethanol, citronellol, geraniol and linalool are responsible for the floral rosy background ; rose oxide adds an exotic floral rosy topnote. 3-Methyl butyl acetate, 2-methyl-2-buten-1-ol, 1-ethoxy-3-methylbut-2-en and menthol add the fresh, fruity character.

Ong and Acree (1998) extracted the volatile compounds from lychee by using both Freon 113 and ethyl acetate solvents. The odour-active compounds present in the fruit were isolated and characterised using gas chromatography/olfactory (GC/O) and gas chromatography-mass spectrometry (GC-MS). Authentic standards were used to determine mass spectral, retention index, and odour match. GC/O analysis detected at least 60 odour-active volatiles in the fruit extract. More odour-active volatiles were detected in the ethyl acetate extract than in the nonpolar Freon extract. Among the compounds that had significant odour activity, geraniol, guaiacol,vanillin, 2-acetyl-2-thiazoline, 2-phenethylethanol, unknown, (*Z*)-2-nonenal, β-damascenone, 1-octen-3-ol, furaneol, and linalool were found to be the most odour-active. On the basis of their calculated odour activity value (OAV), isobutyl acetate, guaiacol, *cis*-rose oxide, 2-acetyl-2-thiazoline, β-damascenone, furaneol, linalool, (*E*)-2-nonenal, geraniol, and isovaleric acid were determined to significantly contribute to the aroma of this fruit. GC/O analysis confirmed that 2-phenethylethanol was probably responsible for the floral character and that the citrus-fruity aroma is due to the presence of many odour-active terpenes, particularly geraniol. Although *cis*-rose oxide was only 30 % as active in GC/O as the most potent odour, its high OAV indicates its importance to the character of lychee odour. An unknown sesquiterpene-

like compound, with a lychee-like odour characteristic of the fresh fruit, was identified by GC/O as being a highly odour potent compound. Taken together, the aroma of lychee was determined to be due to the interaction between compounds with floral, nutty, citrus, and fruity aromas. In 1999, they reported that GC/O analysis of canned lychee indicated that *cis*-rose oxide, linalool, ethyl isohexanoate, geraniol, furaneol, vanillin, (*E*)-2-nonenal, β-damascenone, isovaleric acid, and (*E*)-furan linalool oxide were the most odour potent compounds detected in the fruit extracts. However, on the basis of calculated odor activity values (OAVs), *cis*-rose oxide, β-damascenone, linalool, furaneol, ethyl isobutyrate, (*E*)-2-nonenal, ethyl isohexanoate, geraniol, and δ-decalactone were determined to be the main contributors of canned lychee aroma. When these results were compared with GC/O results of fresh lychees and Gewürztraminer wine, 12 common odour-active volatile compounds were found in all three products. These included *cis*-rose oxide, ethyl hexanoate/ethyl isohexanoate, β-damascenone, linalool, ethyl isobutyrate, geraniol, ethyl 2-methylbutyrate, 2-phenethylethanol, furaneol, vanillin, citronellol, and phenethyl acetate. On the basis of OAVs, *cis*-rose oxide had the highest values among the common odourants in the three products, indicating its importance to the aroma of both lychee fruit and Gewürztraminer wines. Other compounds that had significant OAVs included β-damascenone, linalool, furaneol, ethyl hexanoate, and geraniol. This indicated that while differences exist in the aroma profile of lychee and Gewürztraminer, the common odourants detected in both fruit and wine, particularly *cis*-rose oxide, were responsible for the lychee aroma in Gewürztraminer wine. When headspace SPME was used as a rapid analytical tool to detect the levels of selected aroma compounds deemed important to lychee aroma in Gewürztraminer-type wines, *cis*-rose oxide, linalool, and geraniol were found to be at relatively higher levels in Gewürztraminers. No *cis*-rose oxide was detected in the control wines (Chardonnay and Riesling), while lower levels were detected in the Gewürztraminer-hybrid wine Traminette.

4.1.2.2 Effect of high pressure on flavour components

The potential of high pressure as a new processing technology for the production of fruit juices partly arises from the fact that fresh flavour can be

maintained during pressure treatment. Ogawa *et al.* (1990) and Takahashi *et al.* (1993) reported that Satsuma mandarin juice maintained freshness and original flavour during high pressure processing. Results of extensive sensory testing of orange, grapefruit, and apple juices allowed Mermelstein (1999) to conclude that tasters could not differentiate between the flavour of pressure-processed and unprocessed juice made from the same raw material.

Researchers have shown a particular interest in the effects of high pressure on orange juice. Ogawa *et al.* (1992) stated that high pressure treatment of orange juice does not affect sensory properties. According to Parish (1994), an experienced sensory panel could not perceive differences between orange juice that was pressure treated for 30 seconds at 500 MPa and unpressurised juice. In addition, while the control juice showed signs of fermentation after 2 weeks at 6°C and became organoleptically unacceptable, pressure-treated juice remained free from fermentation for 5 weeks. In a more detailed study, Parish (1998) evaluated the effects of pressure in the range of 500 to 700 MPa on Valencia orange juice. Sensory panels generally judged the flavour of pressure-treated juices to be significantly closer to that of fresh/frozen control juices than thermally treated ones, although some significant differences in flavour between pressurised and control juices were observed. Also during storage, pressure-treated juices maintained their flavour advantages over heat-treated ones. Addition of even minimal heating (50°C to 60°C) was detrimental to overall sensory quality. According to Bignon (1966), sensory qualities of pressure-processed orange juice resemble those of freshly squeezed juice and remain so for more than 30 days. Thus, the retention of fresh flavour is a major advantage of cold, high pressure processing.

For fruit jams (e.g., strawberry jam) high pressure was found to retain fresh flavour much more than traditional thermal processing (Watanabe *et al.*,1991; Kimura *et al.*, 1994)

Unlike orange juice, the flavour of tomato juice and onions is affected by pressure. Sensory panels judged pressure-treated onions to smell less intensely than fresh ones and rather more like cooked or fried onions (Butz *et al.*, 1994). For tomato juice, samples treated at different pressure-temperature-time combinations proved to be inedible owing to a strong rancid taste (Poretta *et al.*, 1995).

In addition to reports on the effect of pressure on overall flavour of fruit and vegetable products, there are reports in the literature containing detailed information about individual aroma compounds. Donsi *et al.* (1996 cited by Ludikhuyze and Hendrickx, 2001) performed a gas chromatographic study on individual flavour components of orange juice. In pressure-treated orange juice, the content of limonene the least stable aroma component, which is drastically changed by heat is similar to that of fresh juice. In addition, pressure treatment did not cause significant modification of other aroma components such as terpinene, cymene, linalool, and myrcene. Headspace gas chromatographic analysis of pressure-treated fruit jam indicated that most original flavour compounds (e.g., *trans*-2-hexenol, linalool, ethyl butyrate, methyl butyric acid) were retained to a much greater degree than with traditional heat treatment. This excellent quality was retained unchanged during storage for 2 to 3 months at 5°C. On the other hand, storage at 25°C resulted in rapid quality deterioration because of dissolved oxygen and remaining enzyme activity (Watanabe *et al.*, 1991 ; Kimura *et al.*, 1994).

For onions, pressure treatment was reported to diminish dipropylsulfide concentration (a compound responsible for pungency and the characteristic odour of fresh onions) and to increase transpropenyldisulfide and 3,4-dimethylthiophene concentrations, leading to a change in flavour that seemed like braised or fried onions. Simultaneously, 2-methyl-pent-2-enal, the main aldol condensation product of propanal resulting from alliinase action, was increased because of pressure-induced cell injury and decompartmentalisation (Butz *et al.*, 1994).

Pressure treatment of tomato juice resulted in a decrease in hexanol and a marked increase in *trans*-2-hexenal and *n-hexanal* , the latter being responsible for fresh tomato flavour when present at a concentration of about 1 to 2 mg/kg. Higher concentrations impart a rancid flavour (Poretta *et al.*, 1995).

In pressurised peach, various alcohols and aldehydes not present in fresh peach were identified, probably produced by enzyme-induced oxidation of unsaturated fatty acids. The concentration of benzaldehyde, in particular, was markedly increased. It is believed that benzaldehyde is enzymatically released from prunasin by disruption of fruit tissue during pressurisation. Indeed, the enzyme activity of the emulsion was found to remain after pressure treatment, and the

adiabatic temperature increase may accelerate the enzymatic reaction. This benzaldehyde largely contributes to the flavour quality of pressure-treated peaches. Further, it was found that the concentration of γ-decalactone, likewise contributing to characteristic peach flavour, was not significantly changed by pressure treatment (Sumitami *et al.*, 1994).

Yen and Lin (1999) studied the changes in the volatile components of guava juice during pressure processing (25°C, 600 MPa, 15 min) and subsequent storage at 4°C and 25°C, using gas chromatography/mass spectrometry. Esters were the most volatile fraction in guava juice and alcohols the second most volatile, with ethyl-1-hexanoate being the most important flavour component. When compared to fresh juice, volatile compounds were not significantly changed by pressure ; however, during storage at 4°C and 25°C, a decrease was noted. Total alcohols and esters as well as the contents of the individual components were relatively close to that of fresh juice after storage at 4°C for 30 days. Pressure-treated juice stored at 4°C maintained flavour stability better than untreated juice, probably because enzymes are partially inhibited. After storage for 60 days, the alcohol concentration was markedly increased, caused not to post-processing contamination but by enzymatic activity. Significant changes were observed when the juice was stored at 25°C for 30 days. Menthol, ethanol, ethyl acetate, methyl-1-propionate, and 2-ethylfurfuran concentration increased while the concentration of other compounds decreased.

4.2 EXPERIMENTAL

4.2.1 Effect of high pressure treatment on the lipoxygenase activity

4.2.1.1 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. Some lychees were kept at -18 °C for up to 7 months for enzyme activity measurements.

Sucrose, granulated sugar (Savona, UK).

Calcium chloride, anhydrous (Fisher, UK).

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

Solution for enzyme assay are as follows :

0.1 M Phosphate buffer (Sigma, USA) pH 6.5 containing 1mM EDTA (BDH, UK), 0.1 %(w/v) Triton X-100 (Sigma, USA).

0.2 M Citrate-phosphate buffer (Sigma, USA). pH 2.0-10.0 (1.0 pH unit interval)

Linoleic acid (Sigma, USA).

Tween 20 (Sigma, USA).

4.2.1.2 Methods

4.2.1.2.1 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 7 months prior to analysis for enzyme activity.

4.2.1.2.2 High pressure processing

The lychees were peeled, destoned and sealed in polyethylene bags (Cryovac Ltd., UK), taking care 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 at room temperature 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/time 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 pressures of 200, 400 and 600 MPa at room temperature for 20 min.

The pressure treated samples (and controls) were stored at -18°C for up to 7 months, and further analysed for enzyme activity measurements.

4.2.1.2.3 Assay for Lipoxygenase(LOX) activity

Ten grams of lychee were homogenised at 4°C with two times (as volume) of 100 mM phosphate buffer, pH 6.5, containing 1mM EDTA and 0.1 %(w/v) Triton X-100 (Smith *et al.*, 1997).The homogenate were centrifuged at 20,000 x g for 40 min at 5°C (Sorvall® RC5 C Plus) and filtered through Whatman No 41 paper, the filtrates were used for the enzyme assays. A 0.1 ml aliquot of crude enzyme extract was added to 2.4 ml of working linoleic acid substrate, containing 1.25 x 10^{-4} M linoleic acid and 0.01 % tween 20 and subsequently its absorbance at 234 nm was followed for at least 5 min with a Perkin Elmer UV/VIS Spectrophotometer Lambda 20.

The pH optima for LOX was determined using the above incubation mixture by adjusting the pH from 2.0 to 10.0 (1.0 pH unit intervals) with 3 N HCl or 3 N NaOH and assayed as described above.

4.2.1.2.4 Experimental Design

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 3x2 factorial in a completely randomised design (CRD). For pressurised syrup lychee, the experimental design was a completely randomised design (CRD). Each treatment was determined twice for the activities 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 used for comparing differences between means.

4.2.2 Effect of high pressure treatment on the volatile components

4.2.2.1 Materials and Equipments

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. Some lychees were kept at -18 °C up to 2-3 months for analysis of the volatile compounds.

Sucrose, granulated sugar (Savona, UK).

Calcium chloride, anhydrous (Fisher, UK).

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

1,2-Dichlorobenzene (Aldrich Chemical, Gillingham, UK)

Solid carbon dioxide (dry ice) (Boc gases, UK).

Helium gas (Boc gases, UK).

Syringe 0.5μL (Supelco, USA).

Solid Phase Micro-Extraction (SPME) fiber assembly 75 μm Carboxen-PDMS [Poly(dimethylsiloxane)] for manual holder (Supelco, USA).

SPME holder (manual) (Supelco, USA).

Glass vial with a Teflon-coated septum, 40 ml (Qm_x Laboratories limited, UK).

4.2.2.2 Methods

4.2.2.2.1 High pressure processing

The lychees were peeled, destoned and sealed in polyethylene bags taking care 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 pressure of 200 MPa at room temperature for 10 min (mildness condition) and 600 MPa at 60°C for 20 min (extreme conditions). Three or four bags were treated at each condition.

Further sets of 3 lychees were mixed with the syrup used in the canning experiment (section 4.2.1.2.1) in the ratio of fruit to syrup as 1:1 and were subjected to the pressure of 200 and 600 MPa at room temperature for 20 min.

The pressure treated samples (and controls) were stored at -18°C up to 2-3 months, and further analysed for the volatile compounds.

4.2.2.2.2 Canning process

This process was similar to the previous experiment (section 4.2.1.2.1), but stored at room temperature for 2-3 months before analysis.

4.2.2.2.3 Headspace Solid Phase Micro-Extraction (SPME) Analysis

Each lychee fruit was chopped by scissors and blended in mortar after adding an equivalent weight of deionised water for 3 min. Then, twenty grams of homogenate were poured into a glass vial, closed with a Teflon-coated septum and agitated with a magnetic stirrer at 30 °C. The extraction procedure involved the direct exposure of a 75 μm thickness of Poly(dimethylsiloxane)(PDMS) coated fiber to the headspace of each sample in the closed vial (Fig 4.3). Before initial use, the fiber was conditioned for 2 hr at 300°C. The plunger depth was set at 3 cm to allow for maximum desorption into the GC by injecting into the hottest part of the injection port. It was found to be important that the holder be tightly assembled to obtain consistent results (Deibler *et al.*,1999). When sampling, the fiber was injected 1 cm above the solution surface. The fiber was exposed to the headspace for 45 min followed by a 3 min desorption period in the injection port of a GC-MS. At the time to desorb fiber, the loop in front (30 cm) of column was buried with solid CO_2 in

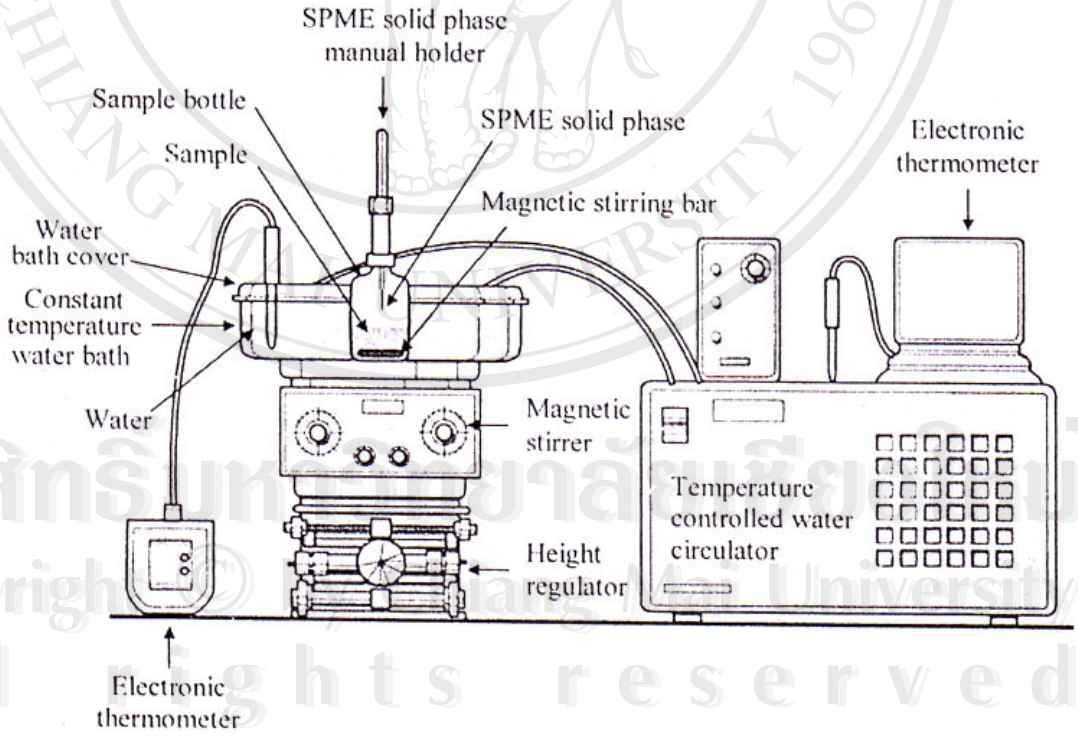

Fig 4.3 New flavour isolation apparatus which can control temperature and magnetic stirring speed of a sample for SPME analysis. From Lee *et al.* (2003).

order to cool the sample for a better resolution of the isolated compound. After removal of the solid CO_2 , the oven was switched on and the start button was pressed. Prior to GC analysis 0.1 µL of an internal standard solution (130.6 µg/mL dichlorobenzene in ethanol) was injected onto the GC column. This permitted the approximate quantities of the components to be estimated by comparison of peaks areas with that for the internal standard. For each treatment three extractions were carried out on different fruits. Fig 4.4 illustrates the sequence of events showing the extraction steps and desorption (injection) steps followed to perform an analysis using SPME.

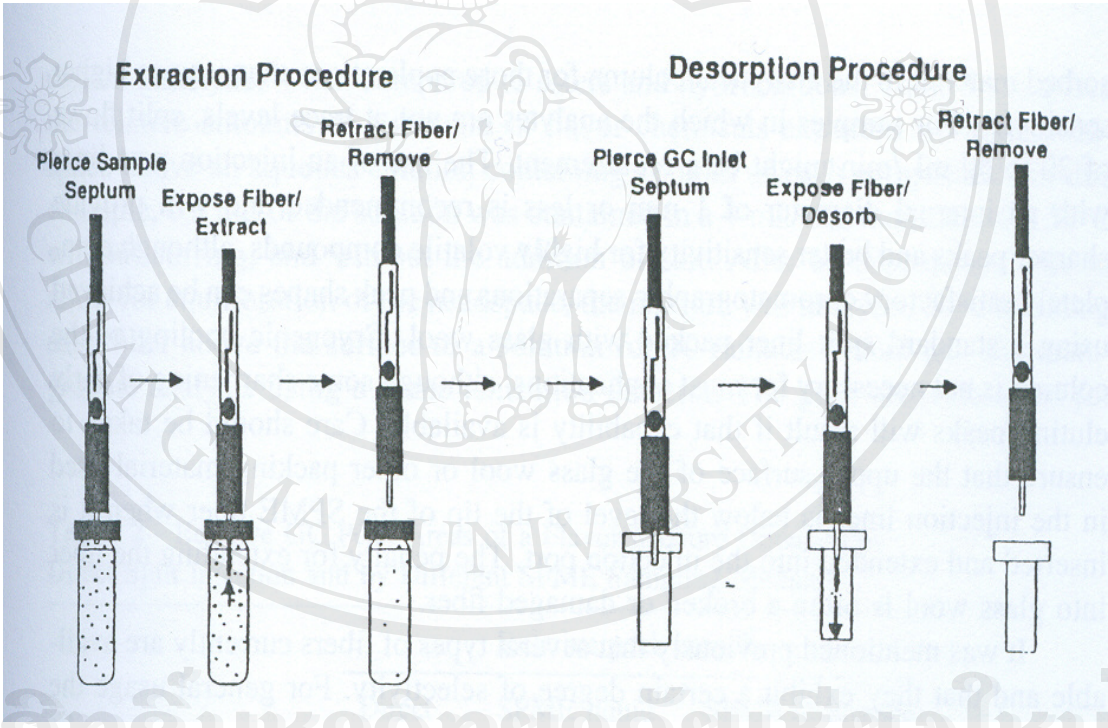

Fig 4.4 Sequence of events showing extraction steps and desorption (injection) steps followed to perform an analysis using SPME. The fiber is inserted directly into a liquid sample with the subsequent absorption of most the analyte molecules (small circles) from the solution. From Harmon (2002).

4.2.2.2.4 Capillary Gas Chromatography/Mass spectrophotometry(GC-MS)

GC-MS HP 5890 and 5972 with a fused silica capillary column Cpsil 8 liquid

phase (0.25µm) with column dimension of 60M x 0.25mmID was used. Carrier gas was helium at 1 ml/min linear velocity. The temperature program was an initial 1 min at 40°C and then a ramp rate of 4°C /min to 250°C and held at 250°C for 10 min(1 injection took 63.5 min). The injector, transfer line and ion source were maintained at 250°C, 280°C and 170°C respectively. The injection split after 30 sec, no solvent delay and splitter 25:1 was used at a split ratio of sample. In all cases, the outlet of the column was directly coupled to the ion source of the quadrupole mass spectrophotometer. In the electron-impact mode (EI), the mass spectrophotometer scanned from m/z 28.5 to 400 at 3.88 scan/sec. The instrument was operated at an ionisation voltage of 70 eV.

4.2.2.2.5 Identification of volatile compounds

The identification of compounds was achieved mainly by comparing their mass spectra with those of known compounds held in the NIST/EPA/NIH Mass Spectral Database (Version 2.0 a , build Jul 1 2002, USA) as well as laboratory database. Identifications were confirmed in most cases by comparing the linear retention indices (LRI) of authentic compounds run under the same condition and on the same (or similar) stationary phase with those of the sample components.

The linear retention index (LRI) of each volatile compound was computed by using LRI-calculated software, according to the equation below.

$$LRI = 100 \left(\frac{t - t_n}{t_{n+1} - t_n} \right) + 100n$$

t = retention time of the compound.

t_n = retention time of the alkane eluting before the compound and possing n carbon atoms.

t_{n+1} = retention time of the alkane eluting after the compound and possing n+1 carbon atoms.

n and n+1 = number of carbon atoms in alkanes eluting before and after the compound respectively.

4.3 RESULTS AND DISCUSSIONS

4.3.1 pH optima for LOX

The pH-optimum of LOX in lychee was 4.0 with linoleic acid as substrate (Fig 4.5). pH-Optimum with linolenic acid as substrate was not examined. This result is in agreement with the value found by Nielsen *et al.* (2004) and Hammer (1993) for LOX from leeks and tomato. Two main isozymes of plant LOX are classified as : Type-1 LOX (e.g. soybean lipoxygenase-1), which has been found in comparatively few plants, and has as optimum pH of 8-9 depending on the presence or absence of detergents added to solubilise the substrates (Whitaker, 1985). The other isozyme, Type-2 LOX (e.g. soybean lipoxygenase-2 and -3) is found widely in plants and has an optimum pH at 6.5 (Robinson *et al.*, 1995). Most studies have found that the pH profile of LOX activity is bell-shaped with a maximum around 7.0-8.0. However, the substrate become more insoluble at pH values below 7. A detergent added to solubilise the substrate at low pH values, so that its can be varied over a wide range, modified the pH profile due to its effect on the lipid micelles (Whitaker, 1991). Surrey (1964) reported that addition of Tween 20 provided uniform distribution of fatty substrate without the formation of an emulsion over the pH range 5-9. It did not interfere with the spectrophotometric determination since Tween 20 at a concentration of 0.25 % at pH 7, did not absorb at any wavelength in the UV.

According to Ben-Aziz *et al.* (1970), the activity maximum of soybean LOX is at pH 9 and addition of Tween 20 does not affect the position of the peak although the shape or slope of the curve on the acid side of the peak is modified. They also found that, at high concentrations, Tween 20 acts as a competitive inhibitor for LOX and suggested that the final concentration of Tween 20 in the typical assay should be at a level of about 0.025 %, so that the LOX was only slightly affected by the detergent.

4.3.2 Effect of high pressure treatment on LOX activity

Pressure treatment led to a reduction in activity of the enzyme of fresh lychee for both 10 and 20 min (Fig 4.6). A decrease in LOX activity of 86 % was observed after treatment at 600 MPa at ambient temperature (~ 20°C) for 10 min and only 11 % of the original activity remained when treated at the longer time (20 min). This agrees with many investigations on green bean (Indrawati *et al.*, 2000) , peas (Indrawati *et*

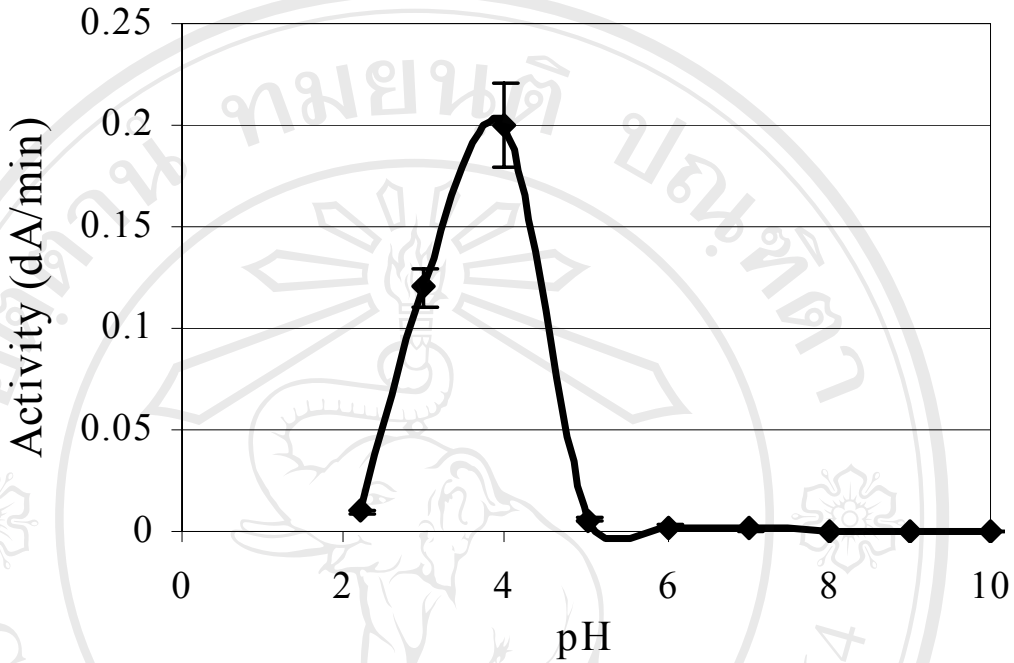

Fig 4.5 The variation of lychee lipoxygenase activity as a function of pH.

al., 2001), and soybean (Ludikhuyze *et al.*, 1998a) who reported that pressure inactivation of LOX occur between 400 and 600 MPa at ambient temperature. Unlike PPO and PME (Pectinmethylesterase), activation of LOX at relatively low pressure has not been observed.

When processed in syrup (Fig 4.7) the effects are less marked due to the baroprotective effect of the syrup as has been found for POD and PPO in the previous study (chapter 3). However, LOX is more sensitive to pressure than POD and PPO. A decrease in LOX activity of 82 % was observed after treatment at 600 MPa at ambient temperature (~ 20°C) for 20 min, whereas, under the same conditions, for POD and PPO, the decrease in activity was 17 % and 43 % respectively. This agrees with Seyderhelm *et al.* (1996) who reported that in buffers it was possible to rank the enzymes according to their pressure induced inactivation in the following order : LOX, lactoperoxidase, PME, lipase, phosphatase, catalase, PPO, and POD.

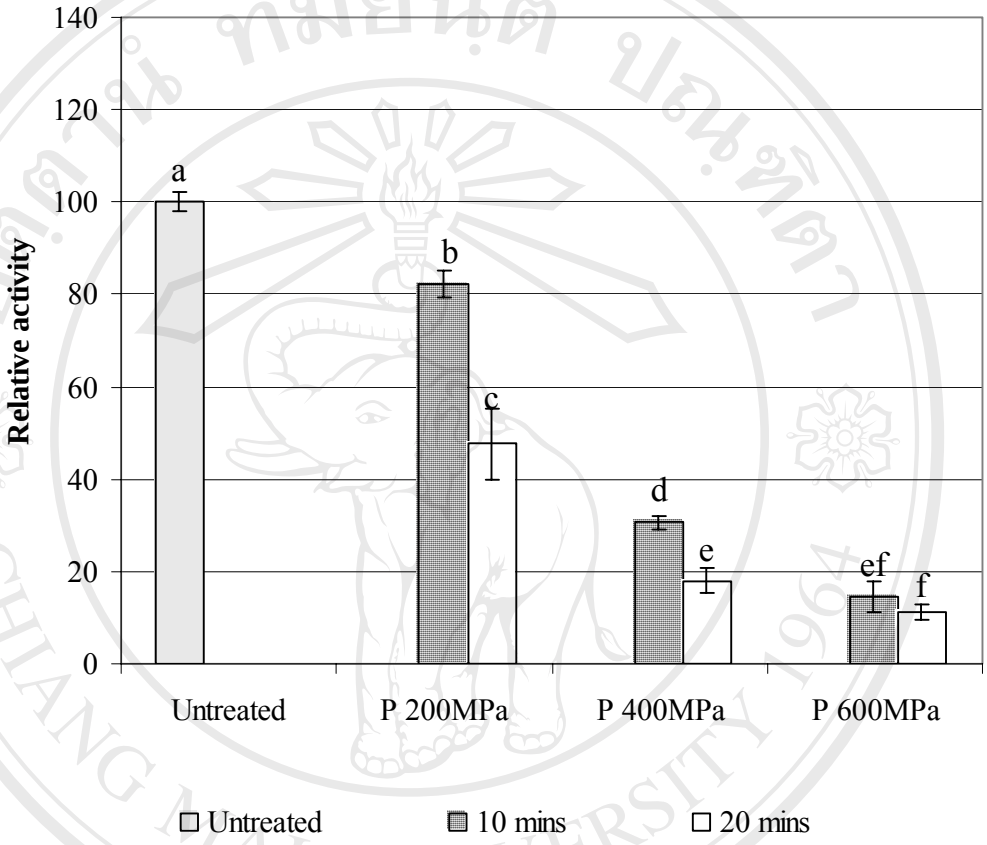

Fig 4.6 Inactivation of LOX activity of fresh lychee as a function of pressures (200-600 MPa at ambient temperature) and times of 10 and 20 min. All values are the means ± S.D. of duplicate determinations on 3 samples. Bars with different superscript were significantly different ($p<0.05$).

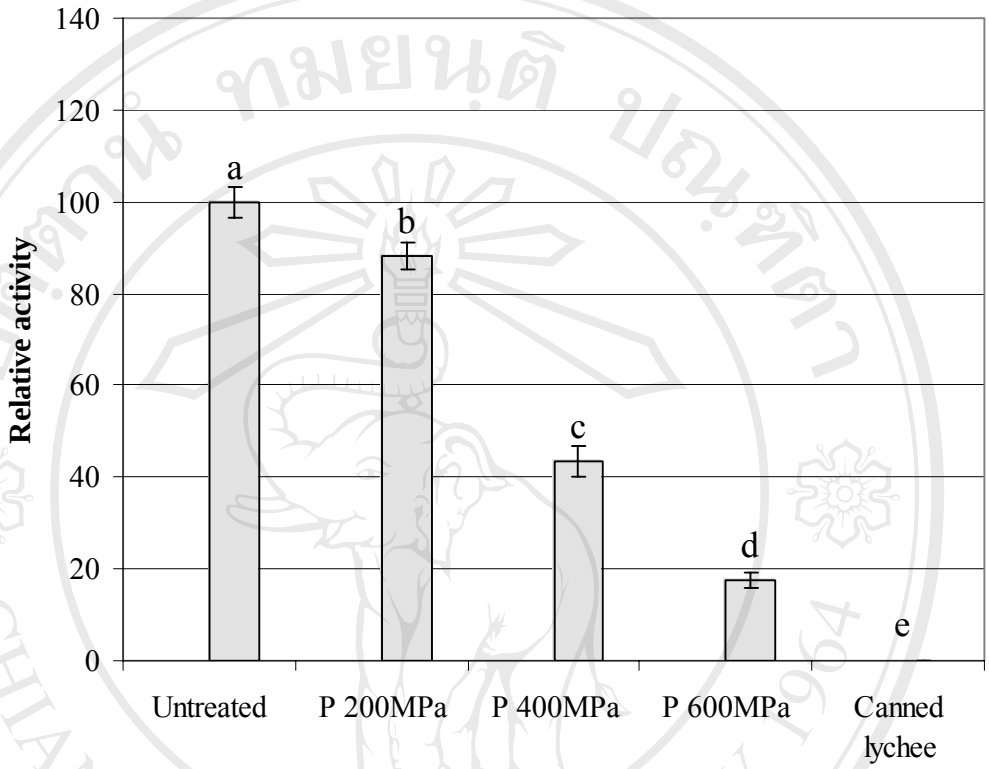

Fig 4.7 Inactivation of LOX activity of lychee in syrup as a function of pressures (200-600 MPa at ambient temperature) and time of 20 min. All values are the means ± S.D. of duplicate determinations on 3 samples. Bars with different superscript were significantly different ($p<0.05$).

4.3.3 Effect of high pressure treatment on the volatile compounds

GC-MS analyses of volatile compounds were obtained from unpressurised and pressurised fresh lychee extracted by the solid phase micro-extraction (SPME) (Fig 4.8). Major Volatile compounds (39) were identified by MS analysis, included 13 hydrocarbons, 9 aldehydes, 6 alcohols, 4 esters, 4 ketones, and 3 miscellaneous compounds (Table 4.1).

The hydrocarbons were mainly terpenes, some of which will contribute to the characteristic aroma of lychee. The terpenoid profile of unpressurised and pressurised fresh lychee is dominated by the monoterpenes, limonene and myrcene (Table 4.1). They are metabolites produced by enzymatic processes. Figs 4.9 and 4.10 show the biochemical pathways that produce the most abundant volatile chemicals found in nature. Figure 4.9 illustrates the enzymatic pathway that generates isoprene, a basic building block for a large variety of acyclic and monocyclic terpenes such as myrcene, ocimene, and limonene. Figure 4.10 shows how isoprene can also be enzymatically converted to acyclic, oxygenated monoterpenes such as geraniol, and alpha terpineol. These biochemical processes can be influenced by many factors (McGee and Purzycki, 2002).

Terpenoids, and mainly the C_{10} (monoterpenes) and C_{15} (Sesquiterpenes) members of this family, have been identified at varying levels in the flavour profiles of most if not all soft fruit. In some species, they are of great importance for the characteristic flavours and aroma. For example, most citrus species are rich in various terpenoid components. Another example is mango (*Mangifera indica*) , in which terpenes comprise the main volatiles of most cultivars studied to date (Aharoni *et al.*, 2004).

The important characteristic flavour note in lychee is *cis*-rose oxide (Ong and Acree, 1998) which is converted from citronellol (Wüst *et al.*, 1998) and contributes to the " floral green with a clean charp, light, rose green note, diffusive strong" (Matsuda and Yamamoto, 1999) and also has been described as powerfully fruity (Leffingwell, 2005). Although it was only present at low concentration in the fruit, but it is relatively low boiling and will make an important contribution to the aroma. Its odour threshold is 0.5 ppb (Leffingwell, 2005).

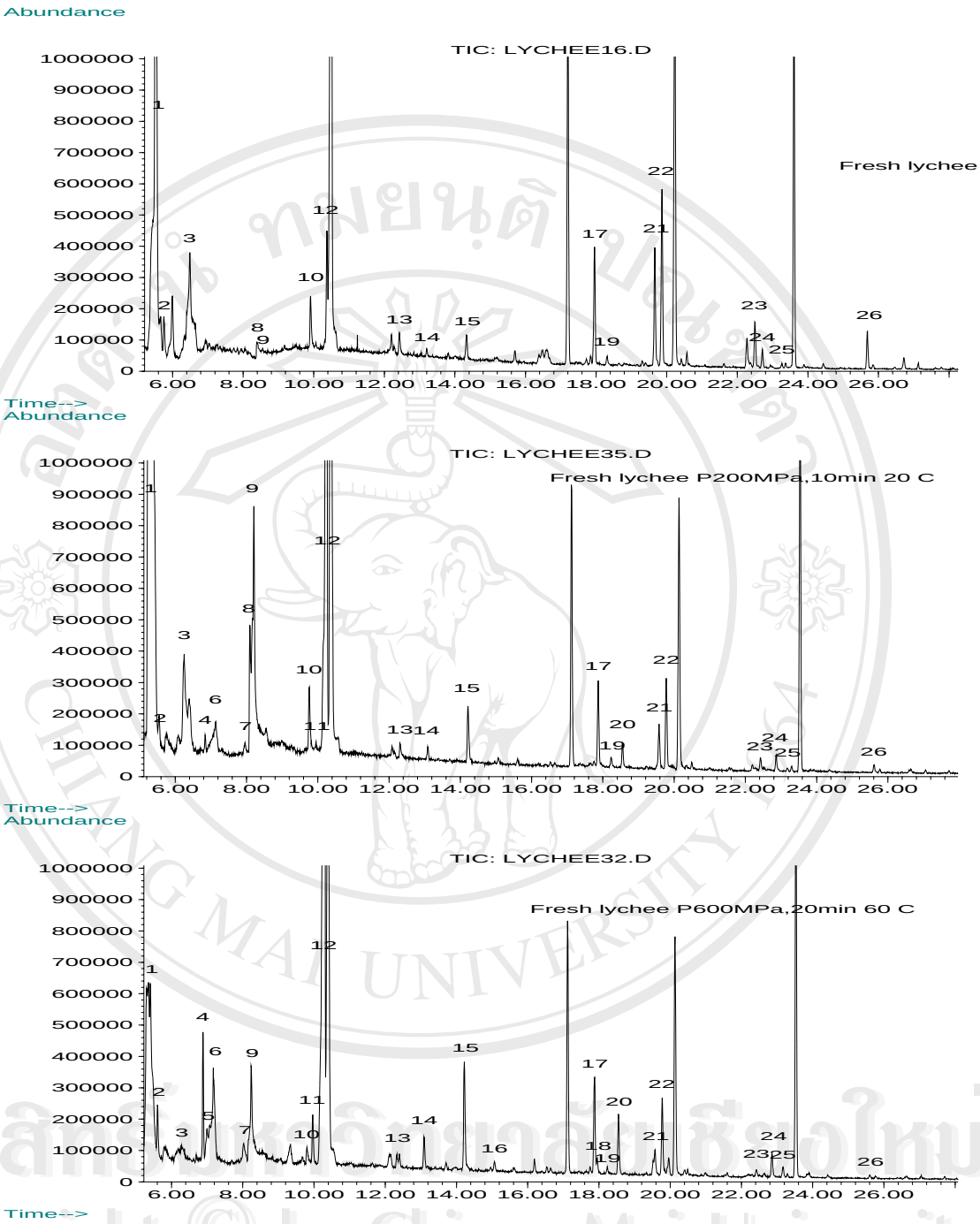

Fig 4.8 GC-MS chromatogram of unpressurised and pressurised fresh lychee; 1= ethyl acetate, 2=2-Methyl-3-buten-2-ol, 3=2-methyl butanal, 4 =heptane , 5=2-ethyl furan, 6= pentanal, 7 = 3-hydroxy-2-butanone , 8= 3-methyl-3-buten-1-ol, 9= 3-methyl-1-butanol, 10 = (E)-2-pentenal, 11= 1-octene, 12 = hexanal, 13 = (E)-2-hexenal, 14=3-methylbutyl acetate, 15=heptanal, 16= methyl hexanoate, 17 = β-myrcene, 18 = 2-pentyl furan, 19 = ethyl hexanoate, 20 = octanal, 21=p-cymene, 22=limonene, 23=p-cymenene, 24=nonanal, 25=*cis*-rose oxide, 26=menthol

Table 4.1 Volatile compounds in unpressurised and pressurised fresh lychee

Class&Compounds	Experimental LRI	Reference LRI*	P 0.1 MPa		P200MPa 10min 20°C		P600MPa 20min 60°C	
			%Rel to IS	%Rel toTV	%Rel to IS	%Rel toTV	%Rel to IS	%Rel toTV
Aldehydes								
(E)-2-butenal	651	641	3.1	1.3	1.5	0.34	1	0.36
2-methyl butanal	661	666	3.86	1.8	12.08	2.89	0.77	0.17
pentanal	701	707	nd	nd	2.17	0.51	4.92	1.48
(E)-2-pentenal	789	754	1.5	0.59	1.84	0.44	0.73	0.22
hexanal	804	806	2.46	1	29.77	7.02	48.53	14.49
(E)-2-hexenal	856	827	0.47	0.2	0.51	0.12	0.41	0.13
heptanal	904	907	2.65	0.22	1.96	0.47	2.66	0.83
octanal	1005	1006	nd	nd	0.91	0.22	1.24	0.38
nonanal	1107	1106	0.22	0.11	0.77	0.18	0.91	0.33
Total			**14.26**	**5.22**	**51.51**	**12.19**	**61.17**	**18.39**
Alcohols								
ethanol	< 600	479	202.09	80.25	280.61	66.83	231.05	68
2-methyl-3-buten-2-ol	618	620*(1)	1.79	0.66	1.51	0.35	1.74	0.54
3-methyl-3-buten-1-ol	734	730	3.14	0.93	4.81	1.16	nd	nd
3-methyl butanol	739	740	4.75	1.5	9.55	2.36	5.94	1.89
menthol	1174	1173*(2)	0.47	0.23	0.43	0.11	0.27	0.08
dihydro carveol	1199	1194*(3)	0.21	0.1	0.23	0.06	0.13	0.04
Total			**212.45**	**83.67**	**297.14**	**70.87**	**239.13**	**70.55**
Esters								
ethyl acetate	607	612	19.71	8.31	53.47	12.69	18.83	6.13
3-methyl butyl acetate	875	877	0.11	0.07	0.46	0.11	1.08	0.41
methyl hexanoate	923	925	nd	nd	nd	nd	0.25	0.08
ethyl hexanoate	997	999	0.28	0.12	0.44	0.11	0.26	0.07
Total			**20.1**	**8.5**	**12.91**	**12.91**	**20.42**	**6.69**

* References LRI are of authentic samples except where shown, (1) Acree and Am (2005) ; (2) Adams (1995); (3) Adams (2001)
IS = Internal standard (2,3-dichlorobenzene)
TV = Total volatile compounds
nd = not determined

Cont. Table 4.1

Class&Compounds	Experimental LRI	Reference LRI*	P 0.1 MPa		P200MPa 10min 20°C		P600MPa 20min 60°C	
			%Rel to IS	%Rel toTV	%Rel to IS	%Rel toTV	%Rel to IS	%Rel toTV
Hydrocarbons								
heptane	700	700	1.08	0.3	0.89	0.21	1.97	0.6
1-octene	795	791	0.14	0.09	0.46	0.11	0.97	0.3
α-pinene	936	937	0.2	0.09	0.33	0.08	0.21	0.06
camphene	954	960	0.12	0.06	0.16	0.04	0.06	0.02
β-myrcene	989	990	1.5	0.68	3.57	0.86	2.59	0.8
α-terpinene	1020	1017*(3)	nd	nd	0.16	0.04	nd	nd
ρ-cymene	1029	1031	1.45	0.67	2.68	0.65	0.95	0.3
limonene	1033	1033	2.33	1.07	4.86	1.18	2.67	0.85
(E)-β-ocimene	1046	1050*(3)	0.14	0.05	0.15	0.03	nd	nd
terpinolene	1090	1089*((3)	0.27	0.14	0.66	0.16	0.12	0.05
ρ-cymenene	1095	1091*(3)	0.69	0.3	0.72	0.18	0.32	0.11
cis-rose oxide	1114	1116	0.1	0.04	0.12	0.03	0.44	0.16
neo-allo-ocimene	1143	1144*(3)	0.12	0.04	nd	nd	nd	nd
Total			**8.14**	**3.53**	**14.76**	**3.57**	**10.3**	**3.25**
Ketones								
2-pentanone	685	687	0.48	0.13	0.3	0.06	nd	nd
2,3-pentanedione	700	698	nd	nd	0.62	0.13	nd	nd
3-hydroxy-2-butanone	728	737	0.35	0.15	0.9	0.2	1.62	0.4
6-methyl-5-hepten-2-one	986	987	0.29	0.1	0.35	0.08	0.17	0.06
Total			**1.12**	**0.38**	**2.17**	**0.47**	**1.79**	**0.46**
Miscellaneous								
2-ethyl furan	694	702	nd	nd	0.68	0.16	1.25	0.38
2-pentyl furan	990	989	nd	nd	0.24	0.06	0.4	0.12
unknown			nd	nd	nd	nd	1.59	0.49
Total			**0**	**0**	**0.92**	**0.22**	**3.24**	**0.99**

* Reference LRI are of authentic samples except where shown, (1) Acree and Am (2005) ; (2) Adams (1995); (3) Adams (2001)
IS = Internal standard (2,3-dichlorobenzene)
TV = Total volatile compounds
nd = not determined

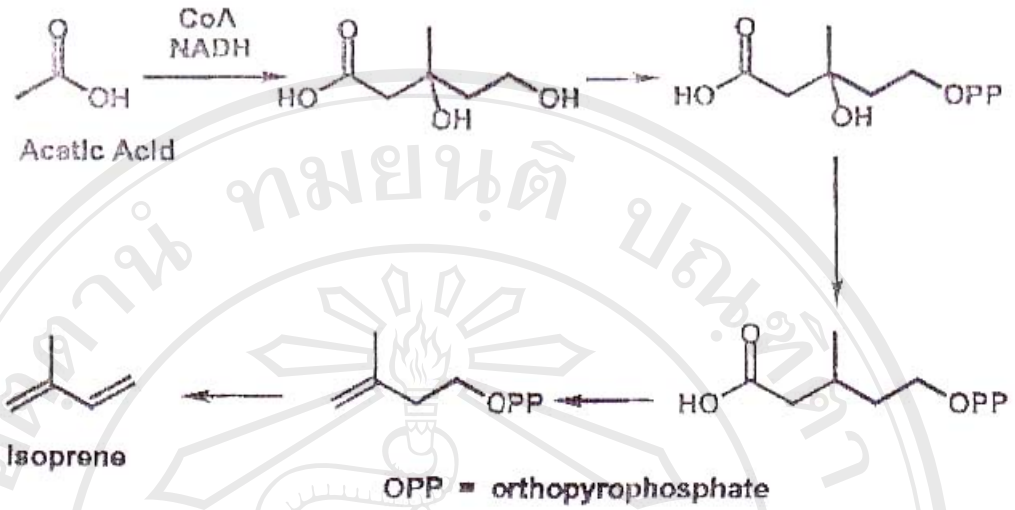

Fig 4.9 Enzymatic pathway to isoprene. From McGee and Purzycki (2002).

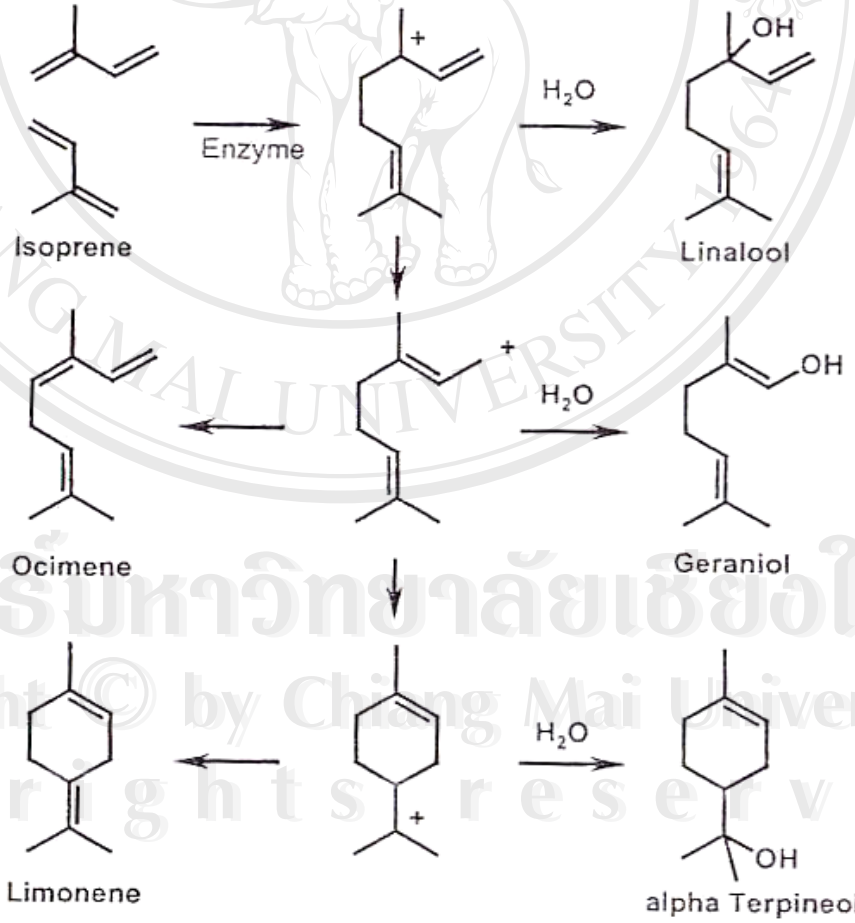

Fig 4.10 Overview of the pathway leading to monoterpene formation in plants. From McGee and Purzycki (2002).

In terms of individual compounds, limonene and myrcene were the main terpene hydrocarbons present in unpressurised and pressurised fresh lychee (Table 4.1). Limonene is a naturally occurring hydrocarbon. It is found in numerous plant oils, including lemon oil and orange oil. Limonene is one of several low molecular weight olefins which has a weak, fresh citrus and orange-like aroma that help form the characteristic odours of fruits and flowers (Selli *et al.*, 2004 ; Reed, 2005). Myrcene is the second most abundant terpene in lychee. It has an "almost citrusy" and a "sweet-balsamic-herbaceous" taste at levels below 10 ppm (Selli *et al.*, 2004).

During high pressure treatment , the hydrocarbons were not changed when compared with the fresh lychee. It seems that high pressure treatment maintains these volatile components in lychee. This is very importance because some components in these groups are involved in fruity-floral and citrus notes of lychee such as *cis*-rose oxide and limonene (Ong and Acree, 1998 ; Johnson *et al.*, 1980).

The alcohols were the major volatile fraction in lychee, and esters were the second for unpressurised whereas for pressurised fresh lychee at the higher pressures, the aldehydes were the second most abundant. Among these components, ethanol presented the largest amount as shown in Table 4.1. During high pressure treatment, the volatile components especially aldehydes were changed when compared with the fresh lychees (unpressurised) and most changed when treated at higher pressures (600 MPa). A remarkable increase in *n*-hexanal (7 times), after pressure treatment at 200 MPa for 10 min at ambient temperature was seen when compared with untreated lychee, a more marked increase in *n*-hexanal was seen after treatment at 600 MPa for 20 min at 60°C (14 times). Ogawa *et al.* (1990) and Takahashi *et al.* (1993) reported that citrus juice maintained its freshness and original flavour during high pressure processing and Kimura *et al.*(1994) on comparing the volatile flavour components of strawberry jams during high pressure processing and pasteurisation found that high pressure processing did not change the volatile components ; however, the concentration decreased drastically during storage at room temperature. The present study corresponds to the investigations of Porretta *et al.* (1995) and Butz *et al.* (1994).

Porretta *et al.* (1995) found that tomato juice showed a remarkable increase in *n*-hexanal content, after pressure treatment at 500 MPa for 3 min, with *n*-hexanal values ranging from 0.3 (± 0.2 s.d.) mg kg^{-1} in untreated samples to 6.4 (± 0.7 s.d.)

mg kg^{-1} in treated ones. Butz *et al.* (1994) found an increase in hexanal in pressurised diced onions. They concluded that pressure treatment enhanced the formation of hexanal by lipid oxidation of linoleic acid. The acceleration of this reaction by pressure may be due to strong decompartmentation of the enzyme, allowing the enzymatic reaction to occur.

Aldehydes are generally formed enzymatically or oxidatively and are seldom considered endogenous in intact fruit. Lipoxygenase (LOX) catalyses incorporation of molecular oxygen into polyunsaturated fatty acids (linoleic acid and linolenic acid) to form hydroperoxides that are cleaved by hydroperoxide lyase (HPL) into various C_6 and C_9 aldehydes such as hexanal, (E)-2-hexenal, (E,Z)-2,6-nonadienal, and (E)-2-nonenal (Lea *et al.*, 2001). Lychee contains 0.351 % lipid with polyunsaturated fatty acids at 0.132 % (USDA, 2005) which can use be as substrates for hexanal formation.

The C_6 –aldehyde products of the HPL reaction depend on the substrate ; hexanal is formed from linoleic acid hydroperoxide whereas *cis*-3-hexenal is formed from linolenic acid hydroperoxide as shown in Figure 4.11 (Bate *et al.*, 1998). The C_6 –aldehydes produced by HPL are released rapidly from disrupted plant tissue and form the basis for the "green note" flavour characteristic of plant tissue. The green note flavour is an important determinant of fresh fruit and vegetable quality, and C_6-volatiles are widely used as a prepared food additive. C_6-volatiles produced from this pathway also have antimicrobial properties, suggesting that they may play a protective role in plants. In addition, C_6-volatiles of this pathway induce phytoalexin accumulation and inhibit seed germination, suggesting that they may also play a signaling role in plants (Bate *et al.*, 1998).

Another aldehyde, which exhibits the fatty and green notes is nonanal, probably derived from lipid oxidation (Ong and Acree, 1998).

Pressurised fresh lychee had more 2-pentyl furan than unpressurised fresh lychee and the higher pressures caused a remarkable increase in 2-pentyl furan because 2-pentyl furan is formed from linoleic acid oxidation (Min *et al.*, 2003).

In pressure processed lychee in syrup, the volatile flavour component profiles were similar to those in pressurised fresh lychee (Fig 4.12), especially the aldehydes with 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

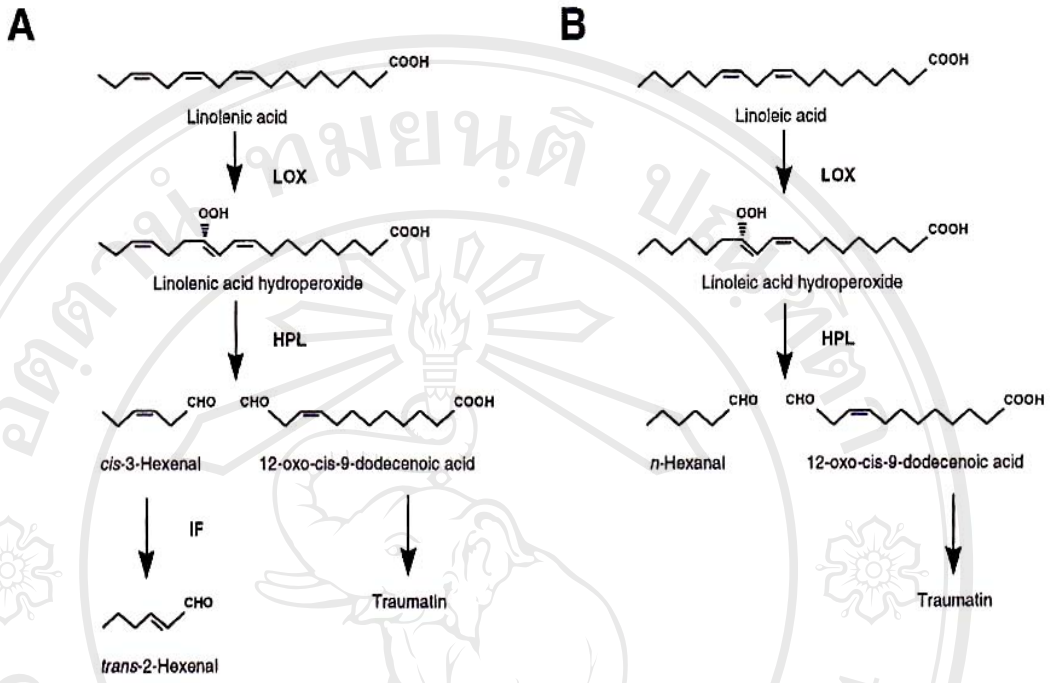

Fig 4.11 Biochemical pathway illustrating the enzymatic activity of LOX and HPL. LOX oxidises linolenic acid and linoleic acid into linolenic acid hydroperoxide and linoleic acid hydroperoxide, respectively. Further cleavage by HPL, forming 12-oxo-*trans*-10-dodecenoic acid and *cis*-3-hexenal or hexanal, respectively. An isomerisation factor (IF) interconverts *cis*-3-hexenal to *trans*- 3-hexenal in vivo. From Bate *et al.* (1998).

baroprotective effect of the syrup. For the hydrocarbon components, the major fractions were limonene and β-myrcene (Table 4.2) but their quantities were increased when compared to unpressurised and pressurised fresh lychee.

In the heated process, canned lychee, most of the volatile components were lost during sterilisation at 100 ºC for 18 min (Fig 4.12) which agrees with Yen and Lin (1999) who reported that most of the volatile components in guava juice were lost during pasteurisation at 95ºC for 5 min. This was because heating inhibited most of the enzymes and microbes. Surprising the important characteristic flavour note in lychee, *cis*-rose oxide exhibited an increase compared to the untreated and pressure

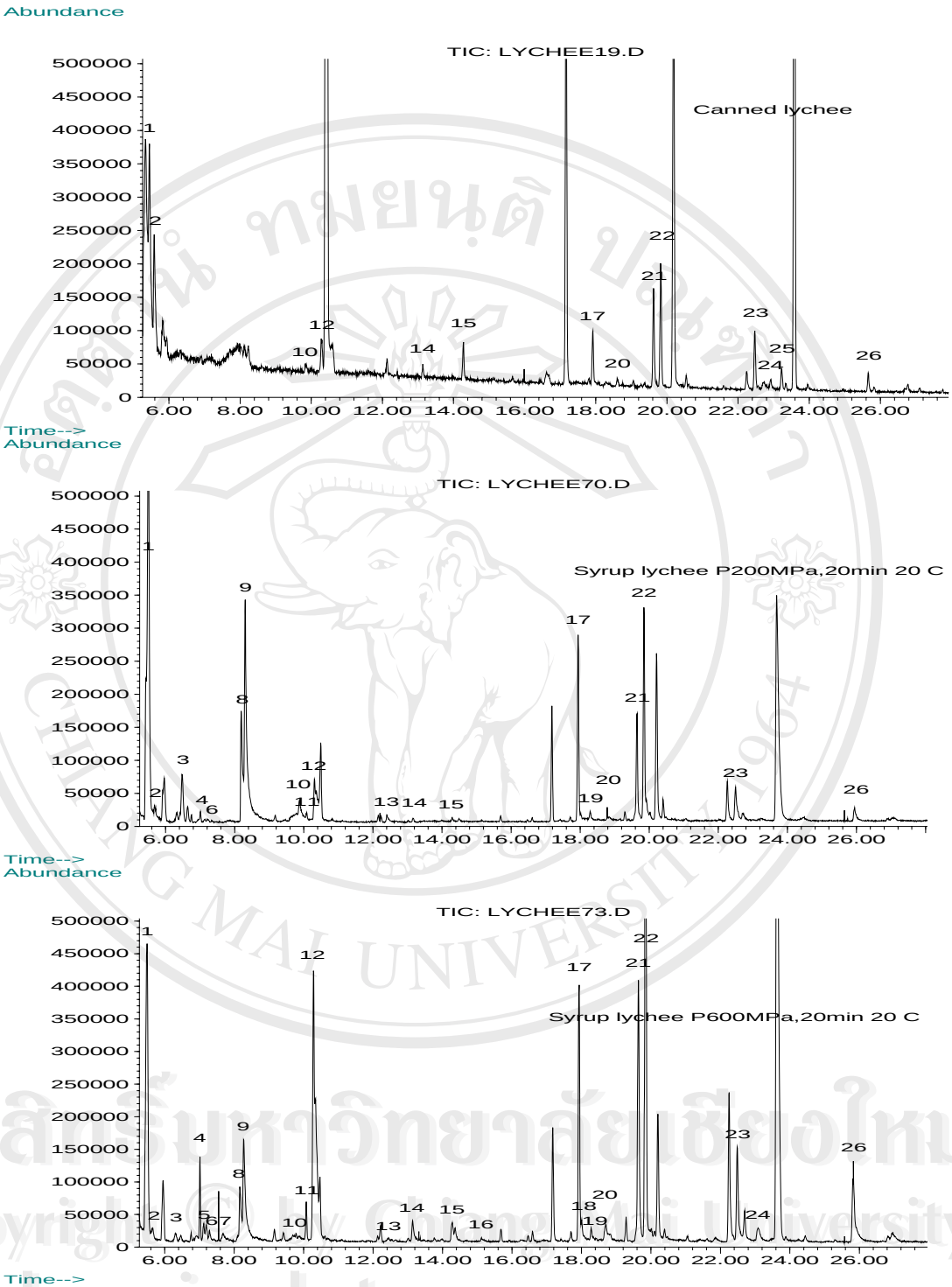

Fig 4.12 GC-MS chromatogram of unpressurised and pressurised syrup lychee; 1= ethyl acetate, 2=2-Methyl-3-buten-2-ol, 3=2-methyl butanal, 4 =heptane , 5=2-ethyl furan, 6= pentanal, 7 = 3-hydroxy-2-butanone , 8= 3-methyl-3-buten-1-ol, 9= 3-methyl-1-butanol, 10 = (E)-2-pentenal, 11= 1-octene, 12 = hexanal, 13 = (E)-2-hexenal, 14=3-methylbutyl acetate, 15=heptanal, 16= methyl hexanoate, 17 = β-myrcene, 18 = 2-pentyl furan, 19 = ethyl hexanoate, 20 = octanal, 21=p-cymene, 22=limonene, 23=p-cymenene, 24=nonanal, 25=*cis*-rose oxide, 26=menthol

Table 4.2 Volatile compounds in canned lychee and pressurised syrup lychee

Class&Compounds	Experimental LRI	Reference LRI*	Canned lychee		P200MPa 20min 20°C		P600MPa 20min 20°C	
			%Rel to IS	%Rel toTV	%Rel to IS	%Rel toTV	%Rel to IS	%Rel toTV
Aldehydes								
(E)-2-butenal	651	641	nd	nd	0.5	0.31	nd	nd
2-methyl butanal	661	666	nd	nd	3.46	2.13	0.4	0.2
pentanal	701	707	nd	nd	0.24	0.14	1.34	0.64
(E)-2-pentenal	789	754	0.21	0.11	1.3	0.84	0.21	0.1
hexanal	804	806	0.62	0.44	4.45	2.65	26.1	12.74
(E)-2-hexenal	856	827	nd	nd	0.26	0.17	0.36	0.18
heptanal	904	907	0.62	0.35	0.46	0.17	1.9	0.94
octanal	1005	1006	0.12	0.07	0.42	0.25	1.42	0.74
nonanal	1107	1106	0.22	0.13	0.41	0.23	1.44	0.7
Total			**1.79**	**1.1**	**11.5**	**6.89**	**33.17**	**16.24**
Alcohols								
ethanol	< 600	479	164.52	87.85	77.3	46.91	64.1	30.64
2-methyl-3-buten-2-ol	618	620*(1)	2.58	1.43	1.26	0.77	0.69	0.32
3-methyl-3-buten-1-ol	734	730	nd	nd	4.83	2.98	3	1.43
3-methyl butanol	739	740	nd	nd	12.95	7.98	8.18	3.9
3-methyl-2-buten-1-ol	773	779*(4)	nd	nd	1.06	0.62	nd	nd
menthol	1174	1173*(2)	0.33	0.19	1.69	0.98	6.32	3.06
dihydro carveol	1199	1194*(3)	0.17	0.09	0.92	0.55	1.22	0.58
Total			**167.6**	**89.56**	**100.01**	**60.79**	**83.51**	**39.93**
Esters								
ethyl acetate	607	612	8.57	5.05	25.46	15.06	14.14	7.03
3-methyl butyl acetate	875	877	0.18	0.1	0.28	0.16	1.31	0.64
methyl hexanoate	923	925	nd	nd	nd	nd	0.22	0.1
ethyl hexanoate	997	999	0.13	0.07	0.36	0.22	0.68	0.32
Total			**8.88**	**5.22**	**26.1**	**15.44**	**16.35**	**8.09**

* References LRI are of authentic samples except where shown, (1) Acree and Am (2005) ; (2) Adams (1995); (3) Adams (2001); (4) Moio *et al.* (1993)
IS = Internal standard (2,3-dichlorobenzene)
TV = Total volatile compounds
nd = not determined

Cont. Table 4.2

Class&Compounds	Experimental LRI	Reference LRI*	Canned lychee		P200MPa 20min 20°C		P600MPa 20min 20°C	
			%Rel to IS	%Rel toTV	%Rel to IS	%Rel toTV	%Rel to IS	%Rel toTV
Hydrocarbons								
heptane	689	700	nd	nd	0.39	0.23	2.82	1.36
1-octene	795	791	nd	nd	0.2	0.12	1.44	0.7
α-pinene	936	937	0.1	0.06	0.24	0.15	0.62	0.3
camphene	954	960	0.22	0.1	0.1	0.06	0.26	0.13
β-myrcene	989	990	0.97	0.54	7.83	4.73	11.84	5.68
α-terpinene	1020	1017*(3)	nd	nd	0.34	0.21	0.92	0.43
ρ-cymene	1029	1031	1.87	1.07	4.94	2.99	13.78	6.53
limonene	1033	1033	2.19	1.24	8.68	5.26	28.54	12.22
(Z)-β-ocimene	1034	1037*(3)	nd	nd	0.77	0.5	nd	nd
(E)-β-ocimene	1046	1050*(3)	nd	nd	0.64	0.39	0.36	0.17
terpinolene	1090	1089*((3)	0.4	0.21	2.49	1.48	7.4	3.46
ρ-cymenene	1095	1091*(3)	1.13	0.64	2.01	1.22	5.64	2.69
cis- rose oxide	1114	1116	0.36	0.19	nd	nd	0.24	0.12
allo-ocimene	1128	1132*(3)	nd	nd	nd	nd	0.23	0.09
neo-allo-ocimene	1143	1144*(3)	nd	nd	nd	nd	0.47	0.2
Total			**7.24**	**4.05**	**28.63**	**17.34**	**74.56**	**34.08**
Ketones								
3-hydroxy-2-butanone	728	737	nd	nd	nd	nd	0.89	0.44
6-methyl-5-hepten-2-one	986	987	0.11	0.06	nd	nd	0.46	0.22
Total			**0.11**	**0.06**	**0**	**0**	**1.35**	**0.66**
Miscellaneous								
2-ethyl furan	694	702	nd	nd	0.19	0.11	0.71	0.32
2-pentyl furan	990	989	0.05	0.03	0.43	0.25	1.19	0.58
unknown			nd	nd	nd	nd	0.51	0.25
Total			**0.05**	**0.03**	**0.62**	**0.36**	**2.41**	**1.15**

* References LRI are of authentic samples except where shown, (1) Acree and Am (2005) ; (2) Adams (1995); (3) Adams (2001); (4) Moio *et al.* (1993)
IS = Internal standard (2,3-dichlorobenzene)
TV = Total volatile compounds
nd = not determined

treated lychee (Tables 4.1 and 4.2).

In conclusion, in spite of the limited number of samples , the present studies stress (i) the high variability in the amounts of lychee fruit volatiles flavour (ii) the problems of lychee flavour stability during post-harvest handling and flavour recovery (iii) the need for product quality control to be intensified in future.