APPENDIX

Appendix A Preparation of buffers

1. Extraction solution (0.05 M potassium phosphate, 1M KCl, 2%PVPP, pH 6.2 in the section 3.2.2.2

6.81 g of Potassium phosphate (KH_2PO_4 , MW 136.09) was dissolved in 750 ml deionised water and the solution adjusted to pH 6.2 with 1% of NaOH, then added 74.54 g KCl , 20 g polyvinylpolypyrrolidone and made up to 1 litre with deionised water.

2. Sodium acetate buffer (0.01M sodium acetate buffer pH 6.0 containing 0.5 % guaiacol) in the section 3.2.2.2

0.41 g of Sodium acetate anhydrous ($C_2H_3O_2Na$) was dissolved in 450 ml deionised water, then added 2.5 ml guaiacol , and the solution adjusted to pH 6.0 with 1% of acetic acid or 1 of % NaOH and made up to 500 ml with deionised water.

3. Sodium acetate buffer for pH optimum of lychee POD in the section 3.2.2.2

Prepare a solution of 0.02 M sodium acetate (1.64 g $C_2H_3O_2Na$/1 litre) and filled 50 ml of it in each beaker, added 0.5 ml guaiacol and the solution adjusted to pH 2-10.5 (0.5 pH unit intervals) with 1% of acetic acid or 1% of NaOH , and made up to 100 ml with deionised water.

4. Phosphate buffer for pH optimum of lychee PPO in the section 3.2.2.3

Prepare a solution of 0.2 M KH_2PO_4 (27.22g /1 litre) and adjusted 50 ml of this solution to desired pH (1.5-11.0,0.5 pH unit intervals) with 1% phosphoric acid or 1% NaOH, then diluted it to 100 ml with deionised water.

5. 0.1M Potassium phosphate buffer pH 6.5 in the section 3.2.2.3

Prepare a solution of 0.1 M KH_2PO_4 (6.81 g / 500 ml) and 0.1 M Na_2HPO_4 (3.55 g / 250 ml), then mix 300 ml 0.1 M KH_2PO_4 with 0.1 M Na_2HPO_4 until the pH was 6.5.

6. Extraction solution[0.1M Phosphate buffer pH 6.5 containing 1mM EDTA and 0.1%(w/v) Triton X-100] in the section 4.2.1.2.3

Prepare a solution of 0.2 M sodium dihydrogen phosphate (27.6 g of $NaH_2PO_4 \cdot H_2O$ per litre) and disodium hydrogen phosphate (28.39 g of Na_2HPO_4 per litre). Added 0.2923 g EDTA and 1 g Triton X-100 in the mixture of 340 ml 0.2 M $NaH_2PO_4 \cdot H_2O$ and 160 ml 0.2 M Na_2HPO_4 and adjusted the pH to 6.5 by using 1 M HCl or 1 M NaOH. Then diluted the solution to 1 litre with deionised water.

7. Stock linoleic acid substrate in the section 4.2.1.2.3

Prepare a solution of 1% (w/v) linoleic acid in absolute ethanol (linoleic acid 1 g/ 100 ml absolute ethanol). Evaporate the mixture of 0.2 ml tween 20 and 7.1 ml 1%(w/v) linoleic acid under vacuum in a rotary evaporator (set at 60°C,vac 600 mmHg). Dissolved the residual with 100 ml 0.05 M Na_2HPO_4 (7.1 g / 1 litre) and adjusted the pH to 9.0 with 1 M NaOH.

This substrate contained linoleic acid 2.5×10^{-3} M and tween 20 = 0.20%

8. Working linoleic acid substrate in the section 4.2.1.2.3

Diluted stock linoleic acid substrate 20 fold with 0.2 M citrate-phosphate buffer at desired pH (1 ml stock linoleic acid substrate + 19 ml 0.2 M citrate-phosphate buffer). This working linoleic acid substrate contained 1.25×10^{-4} M linoleic acid and tween 20 = 0.01%.

9. 0.2 M Citrate-phosphate buffer pH 4.0 in the section 4.2.1.2.3

Prepare a solution of 0.2 M Na_2HPO_4 (28.39 g / litre) and 0.1 M citric acid (21.02 g $C_6H_8O_7 \cdot H_2O$/ 1 litre). Mixed 370 ml of 0.2 M Na_2HPO_4 and 630 ml of 0.1 M citric acid and adjusted the pH to 4.0 with 3 N HCl (26.73 ml/100 ml) or 3 N NaOH (12 g/100 ml), then diluted to 1 litre with deionised water.

10. 0.2 M Citrate-phosphate buffer pH 2.0-10.0 (1.0 pH unit interval) for pH optimum of lychee LOX in the section 4.2.1.2.3

Mixed a solution of 0.2 M Na_2HPO_4 and 0.1 M citric acid as below and adjusted to desired pH with 3 N HCl or 3 N NaOH to make 100 ml citrate-phosphate buffer.

Desired pH	0.2 M Na_2HPO_4,ml	0.1 M citric acid,ml
2.2	2.0	98.0
3.0	20.5	79.5
4.0	37.0	63.0
5.0	49.3	50.7

Desired pH	0.2 M Na_2HPO_4,ml	0.1 M citric acid,ml
6.0	62.1	37.9
7.0	82.3	17.7
8.0	95.8	4.2

Appendix B Flavour analysis

1. Calculation of LRI value

The linear retention index (LRI) of each volatile compound was computed by using LRI-calculated sofeware, 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.

An example LRI calculation is shown below.

Neo-allo-ocimene was eluting at 24.436 min. This retention time is between undecane (C_{11}) and dodecane (C_{12}) which have retention time at 22.69 and 26.77 min respectively.

$$\therefore \text{LRI } \textit{neo-allo}\text{-ocimene} = 100 \left(\frac{24.436-22.69}{26.77-22.69} \right) + 100\,(11) = 1143$$

2. Calculation of volatile components

2.1 Relative to % of total volatile compounds

The main volatile compounds appearing in the chromatogram were calculated from the peak areas, compared with that of the total volatile compounds using equation below.

$$\text{Relative to \% total volatile compounds} = \frac{\text{Peak area of individual volatile compound} * 100}{\text{Peak area of total volatile compounds}}$$

An example:

Peak area of hexanal = 144602450

Peak area of total volatile compounds = 1171607478

∴ Hexanal has a relative to total volatile compounds = $\frac{144602450*100}{1171607478}$

= 12.34 %

Total volatile compounds has 12.34% hexanal. Then calculate the mean, SD and %RSD of samples (all treatments were made in triplicate)

$$\%RSD = \frac{SD*100}{mean}$$

2.2 Relative to 100 ng of internal standard injected

Internal standard (1,2-dichlorobenzene 130.6μg/ml of absolute ethanol) was injected 0.1 μL with sample.

1000 μL of internal standard solution has 1,2-dichlorobenzene 130.6 μg or 130.6 ng

0.1 μL of internal standard solution has 1,2-dichlorobenzene 1306 ng

$$\text{Relative to 100 ng of internal standard injected} = \frac{\text{Peak area of individual volatile compound}*100}{\text{Peak area of internal standard}*13.06}$$

An example:

Peak area of hexanal = 144602450

Peak area of internal standard = 18613626

∴ Hexanal relative to 100 ng of internal standard injected = $\frac{144602450*100}{18613626*13.06}$

= 59.48 %

Appendix C Calculation of enzyme activity

1. POD activity

The activity of POD from lychee in the section 3.2.2.2 was calculated by :

$$\text{Unit / ml crude enzyme} = \frac{10 * \Delta A_{470nm} / \Delta t}{V}$$

Δ A = absorbance change

Δ t = time change

$\Delta A / \Delta t$	=	slope
V	=	volume of the crude enzyme (ml)
10	=	conversion factor of the unit of enzyme activity

Unit definition : One unit of POD activity was defined as an increase of 0.1 unit of absorbance per min at 470 nm.

The activity of the enzyme was calculated from the slope of the plot of absorbance against time (Fig A). In this figure the lag phase, to 2 min, can be seen. The slope was calculated from 2 to 5 min.

Absorbance at 470 nm

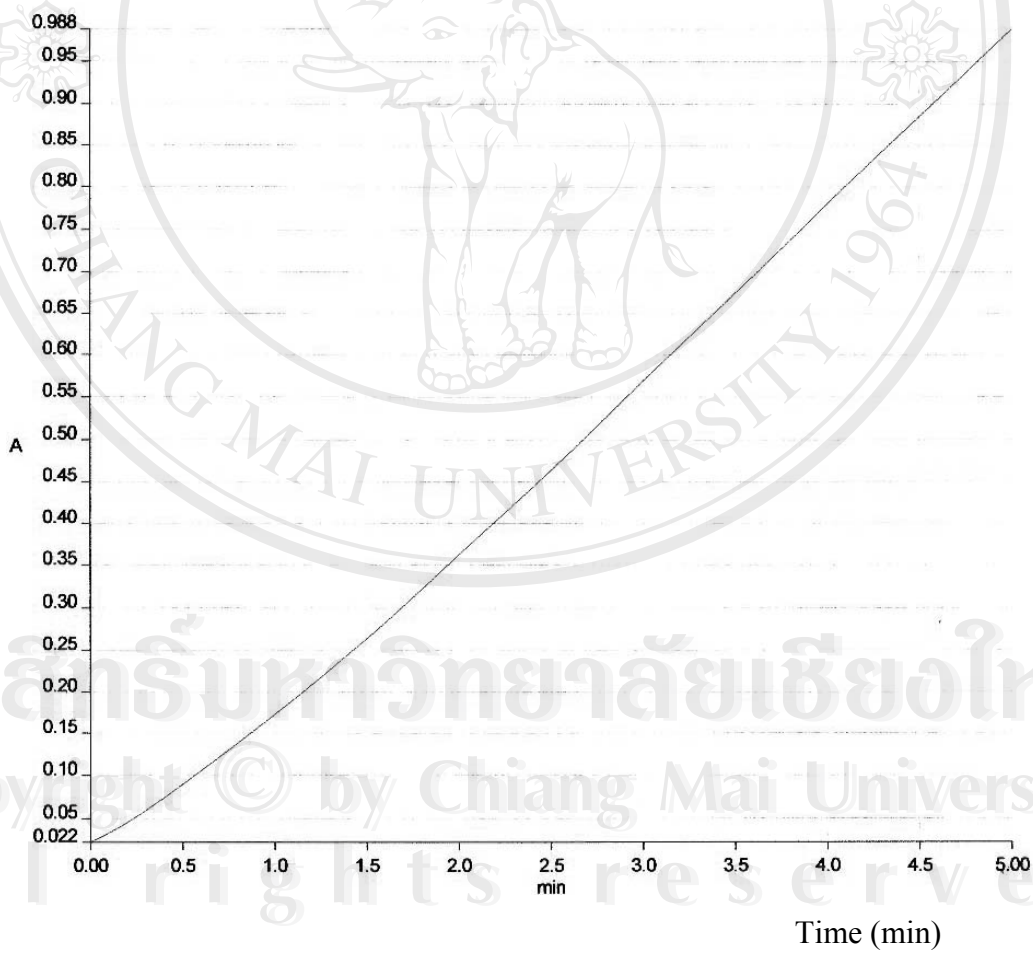

Fig A Absorbance versus time of crude lychee POD assayed with guaiacol. The enzyme was subjected to 400 MPa for 10 min at ambient temperature.

An example : From Fig A

$\Delta A / \Delta t$ = slope = 0.2022

Unit/ml crude enzyme = $\frac{10 * 0.2022}{0.1}$

= 20.22

Reported as % residual activity

% residual activity (relative activity) = $\frac{\text{pressurised sample activity(unit/ml) *100}}{\text{unpressurised sample activity(unit/ml)}}$

An example :

unpressurised sample activity = 20.22 unit/ml

pressurised sample activity = 17.74 unit/ml

% residual activity (relative activity) = $\frac{17.74*100}{20.22}$

= 87.73 %

Residual activity of lychee POD treated at 400 MPa for 10 min at ambient temperature was 87.73 %.

2. PPO activity

The activity of PPO from lychee in the section 3.2.2.3 was calculated by :

unit / ml crude enzyme = $\frac{10 * \Delta A_{420nm} / \Delta t}{V}$

ΔA = absorbance change

Δt = time change

$\Delta A / \Delta t$ = slope

V = volume of the crude enzyme (ml)

10 = conversion factor of the unit of enzyme activity

Unit definition : One unit of PPO activity was defined as an increase of 0.1 unit of absorbance per min at 420 nm.

The activity of the enzyme was calculated from the slope at the linear part of the plot between absorbance against time (Fig B).

Absorbance at 420 nm

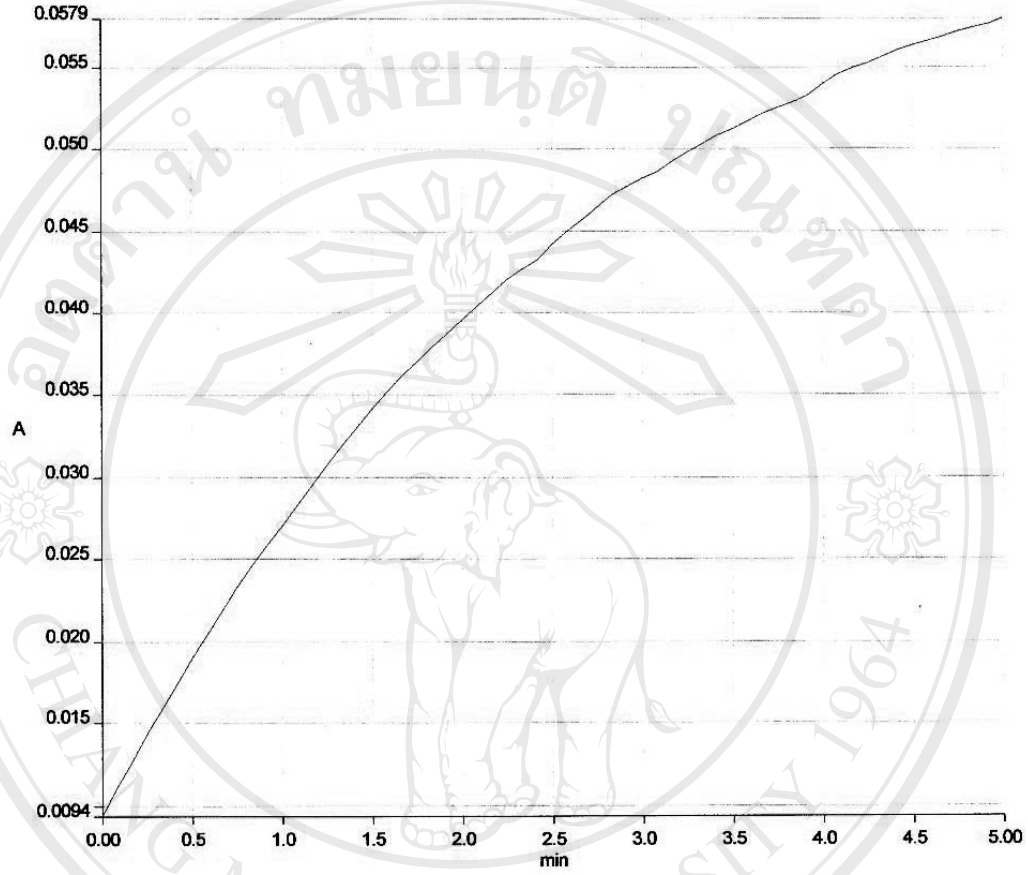

Fig B Absorbance versus time of untreated lychee PPO (control) assayed with catechol.

An example : From Fig. B

$\Delta A / \Delta t$ = slope = 0.0164

unit/ml crude enzyme = $\frac{10 * 0.0164}{0.05}$

= 3.28

Reported as % residual activity

$$\% \text{ residual activity (relative activity)} = \frac{\text{pressurised sample activity(unit/ml) *100}}{\text{unpressurised sample activity(unit/ml)}}$$

An example :

unpressurised sample activity = 3.28 unit/ml

pressurised sample activity = 0.32 unit/ml

$$\% \text{ residual activity (relative activity)} = \frac{0.32*100}{3.28}$$

$$= 9.75\ \%$$

3. LOX activity

The activity of LOX from lychee in the section 4.2.1.2.3 was calculated from the slope (dA/min) at the initial rate of the reaction and reported as % residual activity.

$$\% \text{ residual activity} = \frac{\text{pressurised sample activity(dA/min)*100}}{\text{unpressurised sample activity(dA/min)}}$$

An example :

Pressurised fresh lychee at 200 MPa for 10 min at ambient temperature.

Replication I dA/min = 0.0260

Replication II dA/min = 0.0277

Replication III dA/min = 0.0278

Replication IV dA/min = 0.0281

unpressurised sample activity = 0.0333 dA/min

pressurised sample activity(Replication I) = 0.0260 dA/min

$$\% \text{ residual activity(relative activity)} = \frac{0.0260*100}{0.0333}$$

$$= 78.08\ \%$$

CURRICULUM VITAE

Name Chaveewon Phunchaisri

Date of Birth April 26, 1961

Education

1988 Master of Science degree in Biochemistry, Mahidol University, Bangkok, Thailand

1984 Bachelor of Science degree in Medical Technology, Chiang Mai University, Chiang Mai, Thailand

Experiences

1988-1991 Scientist, Thailand Institute of Scientific and Technological Research, Bangkok, Thailand.

1991-1993 Medical Scientist, Regional Medical Sciences Center, Chiang Mai, Thailand.

1993-now Lecturer, Maejo University, Chiang Mai, Thailand