

## CONTENTS

	<b>Page</b>
บทคัดย่อ	(3)
Abstract	(5)
Acknowledgement	(7)
Contents	(8)
List of Tables	(10)
List of Figures	(12)
Chapters	
1 Introduction	1
Statement of Problems	1
Review of Literature	3
Limonin	5
Nutritive Aspects of Limonin	9
Immunological Activities of Limonin	14
Processing of <i>Citrus</i> Fruits	22

	Deteriorative Factors of Limonin	24
	Purpose of the Study	31
2	Experimental	32
	Materials	32
	Equipment	34
	Methodology and Analysis	36
	Isolation of Limonin	36
	Identification of Limonin	37
	Quantitative Analysis of Limonin	39

## CONTENTS (continued)

	<b>Page</b>
Stability Study of Limonin from Lime Seeds in Aqueous Solution	39
Stability Study of Limonin from Lime Seeds in Solid Phase	41
The Immunological Evaluation of Limonin from Lime Seeds	42
The Effect of Processing Technique on Limonin Content in Lime Juices	51
3 Results	56
4 Discussion	90
5 Conclusion	105
Bibliography	108
Appendix	114
Appendix 1	114
Appendix 2	133
Appendix 3	137

Appendix 4	144
Publications	146
Vitae	147

## LIST OF TABLES

Table		Page
1	Major classes of phytochemicals with antioxidant activity	4
2	Experimental measures of immune status	13
3	Buffer used in the experiments (Citrate-phosphate-borate/ hydrochloride universal buffer)	40
4	Limonin recovery from lime seeds extraction	56
5	<sup>1</sup> H-NMR spectral data of limonin (500 MHz in CDCl <sub>3</sub> )	57
6	Approximate solubility of extracted limonin from lime seeds in pure solvents	58
7	Reaction rate constant (min <sup>-1</sup> ) of limonin decomposition in various pH and temperatures, 70%RH	69
8	Arrhenius activation energy of limonin decomposition at various pH (calculated from equation $E_a = \text{slope} \times 2.303 \times 1.987/1,000$ )	71
9	$k_{\text{observed}}$ ( $k_{\text{obs}}$ ) of reaction affected by extreme pH buffered solution (acid pH range 2, 3, basic pH range 8, 9).	71
10	Reaction rate constant (day <sup>-1</sup> ) of solid limonin in various temperatures 70%RH	75
11	The differential WBC count (in 25 large squares) on the 12 <sup>th</sup> day after	82

administration of untreated, PBS-treated, limonin 100 ppm treated,  
and 200 ppm treated mice

- 12 The antigen-antibody titers of various groups of mice fed with  
different concentrations of limonin on different days after 5 day-  
20%SRBC immunization (n = 6) 84
- 13 Limonin content (ppm) in various processed lime juice. Values with  
different letters are significantly different.  $P < 0.05$ . 85

### LIST OF TABLES (continued)

Table		Page
14	The antigen-antibody titers of various groups of mice fed with differently processed lime juices on different days after 5 day-20%SRBC immunization (n = 6)	88
15	Composition of air dry <i>Citrus</i> seeds	90
16	Phytochemicals in lime juice with antioxidative activity	103

## LIST OF FIGURES

<b>Figures</b>		<b>Page</b>
1	Characteristic structural features of limonin	2
2	Characteristic structural features of limonexic acid isomer	8
3	Characteristic structural features of deoxylimonic acid and limonol	9
4	Characteristic structural features of Limonoic acid and Methyl limonoated-ring lactone	9
5	Standard curve of limonin	59
6	Intra-day validation curve of various concentrations of standard limonin	59
7	Inter-day validation curve of various concentrations of standard limonin	59
8	Degradation curve of limonin in buffered solution at 45°C, 70%RH, plotted between the percentage of the remaining concentration of limonin and time, (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 5, (e) pH 6, (f) pH 7, (g) pH 8, and (h) pH 9 (n = 3)	63
9	Degradation curve of limonin in buffered solution at 45°C, 70%RH, plotted between the natural logarithmic form of the	64



percentage of the remaining concentration of limonin and time, (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 5, (e) pH 6, (f) pH 7, (g) pH 8, and (h) pH 9 (n = 3)

10 Degradation curve of limonin in buffered solution at 70°C, 70%RH, plotted between the percentage of the remaining concentration of limonin and time, (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 5, (e) pH 6, (f) pH 7, (g) pH 8, and (h) pH 9 (n = 3)

65

#### LIST OF FIGURES (continued)

(12)

#### Figures

#### Page

11 Degradation curve of limonin in buffered solution at 70°C, 70%RH, plotted between the natural logarithmic form of the percentage of the remaining concentration of limonin and time, (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 5, (e) pH 6, (f) pH 7, (g) pH 8, and (h) pH 9 (n = 3)

66

12 Degradation curve of limonin in buffered solution at 80°C, 70%RH, plotted between the percentage of the remaining

67

- concentration of limonin and time, (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 5, (e) pH 6, (f) pH 7, (g) pH 8, and (h) pH 9 (n = 3)
- 13 Degradation curve of limonin in buffered solution at 80°C, 70%RH, plotted between the natural logarithmic form of the percentage of the remaining concentration of limonin and time, (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 5, (e) pH 6, (f) pH 7, (g) pH 8, and (h) pH 9 (n = 3) 68
- 14 pH-rate profile of limonin decomposition at 45°C, 70°C, and 80°C 69
- 15 Arrhenius plot of limonin at various pH (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 5, (e) pH 6, (f) pH 7, (g) pH 8, and (h) pH 9 70
- 16 Degradation curve of limonin in a solid state at various temperatures, 70%RH, plotted between the percentage of the remaining concentration of limonin and time (a) 45°C, (b) 70°C, and (c) 80°C 73

## LIST OF FIGURES (continued)

Figures		Page
11	Degradation curve of limonin in buffered solution at 70°C, 70%RH, plotted between the natural logarithmic form of the percentage of the remaining concentration of limonin and time, (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 5, (e) pH 6, (f) pH 7, (g) pH 8, and (h) pH 9 (n = 3)	66
12	Degradation curve of limonin in buffered solution at 80°C, 70%RH, plotted between the percentage of the remaining concentration of limonin and time, (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 5, (e) pH 6, (f) pH 7, (g) pH 8, and (h) pH 9 (n = 3)	67
13	Degradation curve of limonin in buffered solution at 80°C, 70%RH, plotted between the natural logarithmic form of the percentage of the remaining concentration of limonin and time, (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 5, (e) pH 6, (f) pH 7, (g) pH 8, and (h) pH 9 (n = 3)	68
14	pH-rate profile of limonin decomposition at 45°C, 70°C, and 80°C	69
15	Arrhenius plot of limonin at various pH (a) pH 2, (b) pH 3, (c) pH 4, (d) pH 5, (e) pH 6, (f) pH 7, (g) pH 8, and (h) pH 9	70
16	Degradation curve of limonin in a solid state at various temperatures, 70%RH, plotted between the percentage of the remaining concentration of limonin and time (a) 45°C, (b) 70°C, and (c) 80°C	73

## LIST OF FIGURES (continued)

Figures		Page
17	Degradation curve of limonin in a solid state at various temperatures, 70%RH, plotted between the natural logarithmic form of the percentage of the remaining concentration of limonin and time (a) 45°C, (b) 70°C, and (c) 80°C	74
18	Arrhenius plot of solid limonin	75
19	PEC numbers of different groups of mice on different days after limonin administration <span style="color: #e91e63;">■</span> untreated, <span style="color: #2e7d32;">■</span> PBS-treated, <span style="color: #fff9c4;">■</span> limonin 5 ppm, <span style="color: #bbdefb;">■</span> limonin 10 ppm, <span style="color: #5d4037;">■</span> limonin 20 ppm, <span style="color: #ff9800;">■</span> limonin 50 ppm, <span style="color: #3949ab;">■</span> limonin 100 ppm, <span style="color: #cfe2f3;">■</span> limonin 200 ppm (n = 4). Values with different letter are significantly different. $P < 0.05$	78
20	PP of different groups of mice on different days after 0.5 ml/day/mouse of limonin administration <span style="color: #e91e63;">■</span> untreated, <span style="color: #2e7d32;">■</span> PBS-treated, <span style="color: #fff9c4;">■</span> limonin 5 ppm, <span style="color: #bbdefb;">■</span> limonin 10 ppm, <span style="color: #5d4037;">■</span> limonin 20 ppm, <span style="color: #ff9800;">■</span> limonin 50 ppm, <span style="color: #3949ab;">■</span> limonin 100 ppm, <span style="color: #cfe2f3;">■</span> limonin 200 ppm (n = 4) Values with different letter are significantly different. $P < 0.05$	78
21	PI of different groups of mice on different days after 0.5 ml/day/mouse of limonin administration <span style="color: #e91e63;">■</span> untreated, <span style="color: #2e7d32;">■</span> PBS-treated, <span style="color: #fff9c4;">■</span> limonin 5 ppm, <span style="color: #bbdefb;">■</span> limonin 10 ppm, <span style="color: #5d4037;">■</span> limonin 20 ppm, <span style="color: #ff9800;">■</span> limonin 50 ppm, <span style="color: #3949ab;">■</span> limonin 100 ppm, <span style="color: #cfe2f3;">■</span> limonin 200 ppm (n = 4) Values with different letter are significantly different. $P < 0.05$	79
22	Total WBC count of different groups of mice on different days after limonin administration (n = 6) at $P < 0.05$	81

## LIST OF FIGURES (continued)

Figures		Page
23	<p>Antigen-antibody titers of different groups of mice fed with different concentrations of limonin on different days after 5 days-20% SRBC-immunization. (■ untreated unimmunized ■ untreated immunized, ■ PBS-treated, ■ limonin 5 ppm, ■ limonin 10 ppm, ■ limonin 20 ppm, ■ limonin 50 ppm, ■ limonin 100 ppm, ■ limonin 200 ppm ) (n = 6) Values with different letters are significantly different. <math>P &lt; 0.05</math></p>	84
24	<p>Total WBC count of different groups of mice on different days after treatment with differently processed lime juices. (■ expressed by machine, ■ expressed by hand, ■ expressed by machine and frozen, ■ expressed by hand and frozen, ■ expressed by machine and heated, ■ expressed by hand and heated, ■ untreated mice) (n = 6)</p>	88
25	<p>Antigen-antibody titers of different groups of mice fed with differently processed lime juices on different days after 5 days-20% SRBC-immunization. (■ untreated unimmunized ■ untreated immunized, ■ lime juice expressed by hand, ■ lime juice expressed by machine, ■ lime juice expressed by hand and frozen, ■ lime juice expressed by machine and frozen, ■ lime juice expressed by hand and heated, ■ lime juice expressed by machine and heated) (n = 6)</p>	89