

CHAPTER 5

CONCLUSION

1. Lime is an important *Citrus* fruit. It is very popular and is used as a necessary adjunct in every day meal. It is generally used for its juice which is taken in various ways. It is regarded as a health-building food of a great value. Lime is excellent source of vitamin C, which is helpful in maintaining the health of body. Recently lime was discovered flavonoids and limonoids, such as limonin, which played chemopreventive properties. This finding, added up the value of lime in disease resistance, and it is more useful if it is proved that these substances in lime can be raised up the immunity. In 2004 Thailand declared to be world's kitchen. Lime juice exists in many dishes of Thai's food to exhibit good taste. And it will be well famous as a safe food for leveling up health, if it is known that mostly Thai's food consist of limonin which is tested as an immunomodulator.

2. In the study, limonin could be extracted from the seeds of lime which is normally discarded. The data indicates that limonin from lime seeds possessed immunomodulatory property by macrophage-stimulating activity *in vivo* by oral administration to mice and measuring the PEC number. The activity was measured by *in vitro* phagocytic activity of PEC both PP and PI. Additionally immunomodulatory properties of limonin can be indicated by the effect on the hematological parameter, and the effect on production of a specific antibody in limonin stimulated mice. In summary, it had been demonstrated that limonin had an increasing effect on innate immunity and specific immunity by oral administration. Maximum PEC numbers was obtained in mice fed with 200 ppm limonin for 6 days, the same as the maximum PP and PI. And it was observed that the more limonin concentration administered to mice, the more PEC number. PP and PI increased. The maximum WBC count was obtained on the 12th day after the mice treated with 200 ppm limonin, as well as the maximum antibody titer of 1,024 was observed in 200 ppm limonin treated mice on the 12th day after immunization.

3. The possible mechanism of limonin's effect on immunological function was the control of cell proliferation both PEC macrophage and lymphocyte. Increased cell proliferation is suggested to play an important role in immune system generation both innate and humoral immunity. Moreover the induction phase, which is the interval during the stimulation of synthesizing activity, and the time for systemic absorption of limonin affect the immunomodulating property of limonin as well.

4. Over all, this present thesis indicated the evidence for the effect of limonin from lime on immunity in mice, both innate and acquired immunity, and limonin could be used for developing functional foods with immunomodulatory activity. But there are still problems to be solved, such as the intensively bitter taste of limonin. The second problem is that limonin is soluble only in organic solvents, so it leads to problems of absorption, and a high dose of limonin therefore gives moderate immunity. So it remains to be further determined or studied, including SAR (structure activity relationship) analysis, pre-clinical efficacy, and mechanistic studies for its immunomodulating property and to understand its mode of action. Additional toxicity and long-term application studies should also be conducted prior to its use.

5. One advantage of limonin as immunomodulating agent in human trials is that it is a naturally occurring compound that is produced endogenously in edible plants and is present in human foods, unlike synthetic substances. Thus it may be have both health and economic benefits.

6. In the study, limonin was completely degraded in pH 10 to 12 aqueous solution. Its degradation follows the apparent first order reaction; it was most stable in pH 5 with the reaction rate constant of $2.0636 \times 10^{-4} \text{ min}^{-1}$, at 45°C with 70%RH, and activation energy of 2.45 Kcal/mole. Solid limonin degradation followed the first order reaction, it was most stable at 45°C.

7. The stability of limonin is examined to provide information for decision making on techniques to avoid the unsuitable decomposition of limonin, and the shelf life or expiratory date could be determined. However it requires more data, if other formulations of limonin are designed, and should

be useful in research and development of the delivery system of limonin. Moreover processing is also important for the application and development of limonin.

8. The processing technique is also important to the application and development of limonin. In the study, untreated lime juice expressed by machine possessed the most limonin content. So it was concluded that processing affects the limonin content in limonin-containing foods, such as lime juice. Even though limonin content in lime juice was much lower than the effective immunomodulating concentration. This indicated that not only limonin in lime juice, but also other biological metabolites, affected the immunopotency in mice, and it had to be further investigated. However it was concluded that processing affected the limonin in food. And the low concentration as well as the stability of limonin in food might be considered and remained investigated. All the data will be used to ensure the limonin containing food is valuable and stable as an excellent economically health food.

9. This finding suggests that limonin can be used for developing physiologically functional foods with immunopotentiating activities for human, now that it may be a health enhancement in food for cancer and immunodeficiency patients in the future. But the problems stated before, as well as the stability of limonin to be evaluated in another designed forms, would limit the types of products that limonin might be incorporated into as a possible food additive or food supplement, but a new delivery system of limonin may be in consideration in the future.