

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Proper method to eliminate natural inhibines in honey was to destroy. Peroxide component by heating at 65°C for 5 minutes in a waterbath and to reduce osmotic pressure by diluting honey 10-30% concentrations with sterile diluted solution.

The optimal medium (Beef extract 0.15%, tryptone 0.6%, peptone 0.5%, soytone 0.03%, glucose 0.2%, starch 0.8%, polysorbate 0.1%, K₂HPO₄ 0.025%, agar 1.5%, starch 0.8%, NaCl 0.05% and bromocresol purple 0.006%) was mixed with deionized water and brought to 100 ml volume and sterilized at 121°C and 15 psi pressure for 15 minutes, 0.4 ml of medium was dropped in polypropylene tube with cap and 0.1 ml of the bacteria suspension was added to be normal test kit that was taken in a lyophilizer to prepare of freeze-dried test kit.

The freeze-dried test kit could effectively test using 30% concentration of honey with 25 µg/kg tetracycline residue, 100 µg/kg oxytetracycline and chlortetracycline residues. Disadvantages from production of test kit from freeze-drying were that addition of 0.5 ml deionized water in test kit was required before dropping the honey sample in the test kit and cost of production of the test kit is high.

The validity study of normal test kit, sensitivity of test kit was 100% and accuracy of the test was 99% from the preparation with or without antibiotic sample within the concentration range that antibiotic could be detected. Specificity of test kit was 98%. The test kit can be used for qualitative test with detection limit for tetracycline group residue of 10 µg/kg. The test was read negative or positive by the change of color. In the absence of antimicrobial substances, the whole solid medium turned into total yellow color. The incubation time was recorded when the negative controls completely turned into yellow color. The medium remained purple in the presence of sufficient concentrations of antibiotics. At intermediate concentrations of

antibiotic, the solid medium turned partly yellow. The positive results were identified if the bottom three-fourths part of the ampoule turned

From the analysis of 120 honey samples collected from bee farms, honey factories and markets during June-August 2010, the samples had mean values of L* (luminosity), a* (redness) and b* (yellowness) values of 48.30 ± 0.23 , 3.31 ± 0.45 and 32.67 ± 0.23 , respectively. It had mean values of pH, total soluble solids and water activity (a_w) of 3.77 ± 0.30 , 80.41 ± 1.15 °Brix and 0.59 ± 0.02 , respectively.

By using the antibiotic residual screening test kit, tetracyclines group residues were not found in 120 honey samples. 30 honey samples (longan (15), bitter bush (4), sunflower (3), forest flower (2), unknown (2), rambutan (1), sesame (1), lychee (1), acacia (1)) were randomly from 120 honey samples found only one chlortetracycline ($8.85 \mu\text{g}/\text{kg}$) from lychee honey (one out of 30 samples) by HPLC technique.

This research had developed a cheap screening test kit of tube diffusion method in microvial polypropylene tube with cap for antibiotic residue detection in longan honey. The test was easy to detect negative and positive reaction. A microbial inhibition assay was carried out, using spores of *Geobacillus stearothermophilus* (DMST 8041) in optimal medium. 0.1 ml of 30 % honey solution was incubated at 65°C for 2-3 hours in waterbath. The shelf-life of the test kit kept at $4-8^\circ\text{C}$ was 9 months. The test kit can detect for flumetrin residues of $50 \mu\text{g}/\text{kg}$. The test kit could not detect at lower than $10 \mu\text{g}/\text{kg}$ for tetracycline group residues, therefore, it has to be confirmed by certain instruments such as HPLC and LC-MS/MS method. Advantages of these tests are that they are inexpensive, are easy to perform, a broad spectrum of antibiotics and reliability

5.2 Recommendation

Further study test kit with other honeys and the test capability between different many batches.

To test other antibiotic and chemical residue in honey after hive treatment and honey product.

To test the test kit with larger number of honey samples from different flora origins and areas to reduce misdiagnosis.