

CHAPTER 1

INTRODUCTION

1.1 Rationale

Honey bees play a vital role in the environment by pollinating both wild flowers and many agricultural crops as they forage for nectar and pollen, in addition to producing honey and other products. The essential and valuable activities of bees depend upon beekeepers to maintain a healthy population of honey bees because, like other insects and livestock, honey bees can be infected by many diseases and pests. So antibiotics are used in honey bee colonies to combat infection by pathogens. More often, beekeepers prophylactically administer antibiotics to prevent outbreaks of diseases caused by bacterial pathogens such as American foulbrood (Hopingarner and Nelson, 1987; Spivak, 2000), European foul brood (Waite *et al.*, 2003) and others (Thompson *et al.*, 2005). Beekeepers usually administer tetracyclines that have been used since 1967 and are still being used for the treatment and control of a wide variety of bacterial infections (Dinkov *et al.*, 2005). At present, the most commonly applied antibiotic against *Melissococcus pluton* and *Paenibacillus larvae* (causes of the European foulbrood and American foulbrood, respectively) in bees is oxytetracycline, followed by tetracycline, chlortetracycline and doxycycline (Lehnert and Shimanuki, 1980). Oxytetracycline has been used exclusively for the past 22 years to treat European foulbrood in Australia (Hornitzky and Smith, 1999). Australia introduced a temporary maximum residue limit of 0.3 mg/kg for tetracyclines in honey produced at the farm gate, same as in Canada and Korea (McKee, 2003; Dinkov *et al.*, 2005; Jeon and Rhee-Paeng, 2008). In Japan, based on micro-biological research, a value of 0.1 mg/kg was introduced as the allowable residual quantity of tetracycline drugs in honey (Nakazawa *et al.*, 1992). The intensive use of tetracyclines in professional beekeeping in the United States and South America

resulted in tetracycline-resistant *Paenibacillus* strains (Alippi, 2000; Miyagi *et al.*, 2000). In most developed countries, the presence of these antibiotics in food products is not permissible according to the sanitary code (Khismatullin *et al.*, 2003). Many beekeepers usually use antibiotics that are completely prohibited for extra-label use in food-producing animals such as chloramphenicol, nitrofurantoin and tylosin. (The illegal use of antibiotics in honey production has recently been focused). Concerning antibacterial drugs in the European consultation conference on the availability of veterinary medicinal products, it was realized that tetracyclines are used in the treatment of foulbrood. However, although Maximal Residue Limit (MRLs) have been established for all-food-producing species, there are no MRLs for honey (Passantino and Russo, 2008). Furthermore, there is no formulation which is really adapted to the treatment of bees (Mutnelli, 2003). If honey is found contaminated with the prohibited antibiotics, it will be immediately removed from the market. This extensive use of antibiotics in honey bee colonies can cause high levels of residues in produced food products and can become a public health issue. The public has become aware of this issue. The usage of drugs in beekeeping leads to a risk of residue accumulation in honey which may be a cause of allergies in people who consume it, as well as for development of antibiotic resistance in various microorganisms (Bureau of Livestock Standard Certification, 2006). In Thailand, there is no law about the control of drugs in bees and products from bees. The most important contaminants are the substances used for control of bee pests. At present, the most important pests world wide are mites. Nowadays, beekeepers utilize a wide range of different chemical substances, application techniques and methods to keep mite populations under control (Rosenkranz *et al.*, 2010; Ali, 2005).

The surveillance program for antibiotic residue analysis in honey around the world by international standards and the use of antimicrobial substances is strictly regulated in Thailand. It is important, therefore, to have access to rapid, cheap and sensitive testing methods that can detect drug residues in honey. Users look at rapid screening test kits in comparison to traditional testing methods such as Agar Diffusion,

Enzyme-Linked-Immuno-Sorbent Assay (ELISA), High Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS) for determining the presence of the antibiotics in honey. In routine analysis of honey, antibiotics are generally tested by screening determination of positive samples (Primi®test of Netherlands (Stead *et al.*, 2005)), Tetrasensor® of Belgium (Reybroeck *et al.*, 2007), Charm test of Netherlands (Morlot and Beaune, 2003; Reybroeck, 2003) ELISA (Ridascreen®Tetracyclin of Germany (Jeon and Rhee-Paeng, 2008; Pastor-Navarro *et al.*, 2007)) and quantitative determination of positive samples by HPLC (Pena *et al.*, 2005; Solomon *et al.*, 2006; Taokaenchan and Sangsrichan, 2010; Wan *et al.*, 2005) and LC-MSMS (Carrasco-Pancorb *et al.*, 2008) have also been reported.

Honey is mainly produced in the northern part of Thailand. At present, Thai beekeepers are still facing microbiological infection in bees. They solve this problem by using antibiotics because of convenience, cheap and quick results. This problem leads to antibiotic residues in honey and other bee products. Therefore, there is a need for analysis of antibiotic residues. The cost of this analysis using the forementioned techniques is generally too high for the beekeepers and honey processors. The objectives of this research were to develop a cheap screening test kit for antibiotic residue detection in longan honey. This research involved a survey of basic data of longan honey types, elimination of natural inhibitors in honey, investigation of relationship between microbial feed formulations, concentration of indicator substance, various microbial growth and honey sample preparation. Initially, there is only DSM Nutritional Products Company from the Netherlands that has developed Premi®Test to be a commercial growth-inhibitor screening test that detects antimicrobial substances in various applications, such as fresh meat, fish, eggs, honey, urine and feed. Premi®Test facilitates on-site screening for antibiotic residues in the food chain by cattle farmers, slaughterhouses and beekeepers, etc., simply by taking a sample of the fluid of the meat or honey. Premi®Test is much faster than existing test methods: conventional antibiotic residue tests (DSM Nutritional Products Company, 2010), but there has been no report about the application of microbial inhibition assay in

development test kit for detection of antibiotic residues in Thai honey. This research tested only tetracycline, chlortetracycline and oxytetracycline because they are permitted to treat bacteria in bee farms by Department of Livestock Development, Thailand. In addition, tetracyclines are broad-spectrum antibiotics used routinely in veterinary medicine for the treatment and prevention of some infectious animal diseases (Aranda *et al.*, 2006). So, they are bought easily and conveniently from drug shop or animal drug shop.

1.2 Research objectives

1.2.1 To develop a new test kit for detection of tetracycline group residues in longan honey

1.2.2 To study shelf life of the test kit

1.2.3 To validate the test kit with HPLC technique

1.3. Usefulness of the Research

The usefulness of this research can be categorized as follows:

1.3.1 Produce a rapid screening test kit for detection of tetracycline group residues in longan honey for consumer protection

1.3.2 Know shelf life of the test kit

1.3.3 To encourage an increase in analysis of antibiotic residues in longan honey due to its practicality at honey collection centers and bee farms