Chapter 1

Introduction

1.1 Principles and rationale

Various kinds of mango varieties are cultivated in Thailand, most of which are produced mainly for domestic consumption, while only a few are exported such as 'Nang Krang Wan' and 'Nam Dok Mai'. A large volume of this tropical fruit only leads to low level prices during the peak season. In general, the normal harvest season are short for tropical fruits, such as mango, longan and lychee having harvesting months in April-May, July-August and May, respectively. For this reason, technique for flower induction and related off-season fruit production are being developed to almost routine practice. However, presently applied technique in Northern Thailand involve root treatment of mango is not successful to induce year round flowering and fruiting. These problems together with disadvantage of short storage life are lowering the price of tropical fruits in the market. During the harvesting season, supply has been exceeding market demand and depressing market prices. For that, fruit growers in Thailand have taken a great interest in producing fruits at a time out of harvesting season. Especially, at present, off-season production of mango fruit is made possible by application of the triazol paclobutrazol (PBZ). It can be concluded that the use of paclobutrazol is a well known method for the growers in Thailand in order to produce off-season mango. When it is applied as soil drench, it is more effective than foliar spray. However, there is also a long term residual effect of this chemical in soil (Tongumpai *et al.*, 1997). Since long-distance transport of PBZ is primarily based on the transpiration stream, and therefore does not take place in the phloem (Wang *et al.*,1986), elevated PBZ residues in mango mesocarp after soil application of PBZ for off-season fruit production are not expected. Moreover, there is a public discussion concern about food safety issue in Thailand and its export countries, related to overly intensive use of agrochemicals (Samabuddhi, 2001), especially the consequence is long term application and require

proving this working hypothesis by quantitative residue analysis in off-season mangoes production.

Paclobutrazol is a plant growth regulator registered for the reduction of terminal growth and pruning volume in trees, the inhibition of gibberellin and sterol biosynthesis, and hence the rate of cell division (Fletcher *et al*., 2000). PBZ interferes with gibberellin biosyntheses by inhibiting the oxidation of ent-kaurene to ent-kaurenoic acid through inactivating cytochrome P450-dependent oxygenases (Graebe, 1987). The maximum residue limit (MRL) for paclobutrazol in pomes fruits is at the sub-ppm level, and it has been set up at 0.05 mg/kg in several European countries, such as Spain and Germany, while in others, it is about 10 times higher (between 0.3 and 0.5 mg/kg). When the MRL is established at the low level, a sensitive analytical methodology has to be developed and a limit of quantification (LOQ) of 10 times lower than that of the MRL would be advisable in order to gain reliability in the results obtained (Sancho *et al*. 2003)

Traditionally, gas chromatography (GC) has been the most common technique used for the determination of pesticide residues in fruits but usually includes time-consuming steps, such as solvent extraction and suitable cleanup steps (Stan, 2000). GC-MS multi-methods applied by official food laboratories for pesticide residue analysis allow identification down to at least 0.01 mg/kg in plant foodstuffs (Stan, 2000). HPLC-DAD (Bicchi *et al*., 2001) and GC-MS (Crook, 1999) methods with prior solid phase micro-dispersion or microextraction, respectively, were recommended for specific determination of PBZ residues down to the 0.01 ppm level in plant foodstuffs. By analogy, Crook (1999) suggested that the method is also applied for PBZ residue analysis in soil and water. The solid phase microextraction (SPME), which is a simple, solvent-less technique allowing the extraction and concentration in a single step, was introduced. SPME has been successfully applied to the determination of pesticide residues in various sample matrices, e.g. water, soil and food. Also, SPME provides enhanced sensitivity because the extracted fraction (on the fiber) can be introduced quantitatively into the GC by thermal desorption. Alternatively, the SPME fiber can be desorbed by liquid extraction, and the extract

analyzed by HPLC. Although aqueous samples, e.g. water and beverages, can be analyzed without any further sample preparation by SPME, analysis of solid samples, e.g. vegetables and fruits, is either based on a headspace SPME (HS-SPME) or a solvent extraction of the analytes is performed before direct immersion SPME (DI-SPME) (Sandra *et al*., 2005).

Effects of off-season fruit production on fruit quality are expected to be markedly dependent on climate conditions during cultivation and planned harvest dates, respectively. The paclobutrazol effects of fruits quality have been reported by Salazar-Garcia and Vazquez-Valdivia (1997) indicating that when the quality of paclobutrazol treated 'Tommy Atkins' mango was observed over 3 years, only with low paclobutrazol rate (2.5 and 5 g/tree) affected TSS of fruit juice, but the rates significantly higher at ≥ 10 g/tree. On the contrary, Yadava and Singh (1998) treated 'Dashehari' mango trees with paclobutrazol at the ratio of 2, 4, and 6 g/tree as soil drench. Result showed that paclobutrazol increased number of fruits and yield, maximum effect was found at 4 g/tree. Fruit firmness and pH were little affected by the treatments, but increased TSS and yellowness index. Whereas opposite findings for TSS were reported by Kurian and Iyer (1993) for 'Alphonso' mangoes.

In conclusion, the validation method of paclobutrazol in soil and fruit will be developed and a comprehensive databank on fruit quality of Northern Thai mangoes is aimed, where the influence of maturity, harvesting time and cultivars is also considered.

1.2 Objectives

1. To study the effect of paclobutrazol on physical, chemical properties as well as the ripening index (RPI) of mango fruits.

2. To develop the quantitative technique of paclobutrazol residue determination in fruits (edible part) and in soil by SPME-GC-MS.

1.3 Expected outcome

1. The assessment of quality profile of PBZ treated mango and the ripening index (RPI) will be used as the database for Northern Thai mango cultivars.

2. The method for detection of PBZ residue will be developed particularly for mango pulp, soil which can be applied to other kinds of tropical and sub-tropical fruits. This method used bases on SPME-GC-MS technique.

1.4 Experimental scope

Paclobutrazol determination within the scope of a multi-method for pesticide residue analysis is accessible by gas chromatography with mass spectrometric detection (GC-MS) after solid phase microextraction, which was described by Crook, 1999. This available method has to be validated for routine application in University of Hohenheim. Soil and fruit samples will be collected in Thailand and sent to analyze at University of Hohenheim. Apart of the quality of off-season mango fruits is to be investigated using the full quality profile (Yield, fruit size distribution, mass of whole fruit, colour, mass and yield of flesh, peel and seed, texture of flesh, total soluble solid, titratable acid, sugar-acid ratio, pH). For the study on off-season fruits, the cultivars are presently accessible to off-season production in Chiang Mai province. The analysis of fruit quality is taken place at Agriculture Faculty, Chiang Mai University, Chiang Mai, Thailand.