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26 1. Introduction

27 The export of Thai mango (*Mangifera indica* L.) exhibits increasing trend due to the high value
 28 added to the commodity. In 2004, the export value per kilogram of mango increased about 58%
 29 compared to the export value in 2003 (Office of Agriculture Economics, 2005). This increasing trend
 30 initiates the awareness of Thai agro-industries about the regulation of trading countries. One of the
 31 major threats to the premium markets such as Japan and Australia for Thai mango is the presence of
 32 oriental fruit fly (*Dacus dorsalis* H.) inside the fruit. Since cancellation of ethylene dibromide (EDB)
 33 fumigation from the past two decades, non-chemical quarantine treatments, e.g., vapor heat treatment
 34 (VHT) and hot water dipping became the common methods to control this pest. Recently, an interest
 35 of electromagnetic treatments, for instant, microwave and radio frequency, to control oriental fruit fly
 36 in fruits has emerged. Microwave rapidly generates heat in food materials and may be used as an
 37 alternative to conventional VHT which has drawback due to its slow come-up period.
 38 According to Thai regulation for mango export, general guideline to disinfest oriental fruit fly in
 39 mango using VHT was accomplished by monitoring the cold spot of mango at 47°C for 20 min
 40 during VHT. This treatment achieved thermal death time higher than 5D (equivalent to $LT_{99.999}$) for
 41 the first instars (16.45 min) and the egg of oriental fruit fly (<8.91 min) (Jang, 1986), but slightly
 42 lower than 5D for the third instars (22.3 min) (Jang, 1991). An increase in internal temperature of
 43 mango up to 47°C by VHT would take longer than 45 min, hence the fruit would be susceptible to
 44 heat damage. With rapid heating by microwave, a concept of high-temperature-short-time (HTST)
 45 treatment is possible to shorten treatment time while retaining lethality effect higher than 5D. The
 46 HTST concept is extensively used in food processing to minimize thermal degradation of food
 47 quality (Stumbo, 1973; Lund, 1977; and Holdsworth, 1997) and may be applicable for insect
 48 quarantine process. Tang et al. (2000) proposed the HTST thermal quarantine methods using radio
 49 frequency to control codling moth larvae in in-shell walnuts to be a temperature range of 50-54°C.
 50 The third instars codling moth in cherry was also studied (Ikediala et al., 1999). The 915 MHz

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51 microwave treatment on cherries can achieve the fruit temperatures of 45, 50, and 55°C but quality of
52 fruit was promising with treatments of temperature less than 50°C.

53 With regard to mango quarantine treatment, HTST could give several advantages over the
54 conventional heating in term of shortening of come-up time, equivalent or better quality retention,
55 energy minimization, and insect mortality assurance due to high temperature treatment. Varith and
56 Kiatsiriroat (2004) studied the microwave heating on Chokanan mango with 2,450 MHz / 800 W-
57 microwave oven and found an increase of internal temperature up to 46°C within 40 s. Heat
58 distribution inside the fruit depended on fruit orientation, microwave power and treatment time. The
59 horizontal positioned mango treated with 50% microwave power yielded better heat distribution than
60 the vertical one. Continuing to this work, our purpose was to develop a full microwave-vapor heat
61 treatment (MVHT) for oriental fruit fly quarantine process in Namdokmai Si Thong mango which is
62 the export variety. The first experiment explored the lacking information of thermal death and
63 quality kinetics at temperature higher than 48°C. The MVHT process was then developed based on
64 thermal death and quality kinetics. Finally, experiments were conducted to confirm the MVHT
65 quarantine method along with measurement of changes in mango quality after the MVHT process.

66

67 **2. Materials and Methods**

68 **2.1 Materials**

69 Namdokmai Si Thong mangoes freshly harvested from two orchards in Chiang Mai, Thailand, were
70 used in this research. The first orchard provided domestic-graded mango while the second orchard
71 offered export-graded one. The domestic-graded mangoes were primarily used for insect mortality
72 study while the export graded-mangoes were used for a study of changes in quality after MVHT.
73 During fruiting stage, intact export-graded mango was enfolded with horticultural envelope while the
74 domestic graded one was enfolded with the regular white paper. This yielded a major difference in
75 color of skin where domestic-graded mango dominated the green color while the export-graded
76 mango dominated the yellow one (Table 1). Other physical-chemical properties of mango between

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77 two orchards were not different. After harvesting, each lot of mango was stored at 13°C / 90%RH
 78 for 48 h prior to the experiment. The experiment was accomplished within 72 h to avoid effect of
 79 ripening. Each mango weighed between 300-330 g/fruit with maturity of 80% (commercially
 80 estimated). Firmness was characterized with two parameters; failure force at rupture point and
 81 apparent elastic modulus (ASAE Standards, 2003). Details of firmness measurement were discussed
 82 further. Eight hours prior to the test, the samples were equilibrated in room condition of 25°C /
 83 60%RH. Thermo-physical properties of domestic- and export-graded mangoes measured at the room
 84 condition (25°C / 60%RH) are presented in Table 1.

85

86 **2.2 Mass rearing of oriental fruit fly**

87 Oriental fruit fly pupae were reared in the Department of Plant Protection, Maejo University, in
 88 screen cages at 25-27°C / 70%RH. Flies that emerged were provided with sugar, hydrolyzed yeast
 89 protein, and water. Eggs were collected from mature females (10-14 days old) during 1-h period.
 90 Eggs used in mortality study were transferred into agar gel box (size of 30x55x20 mm) while those
 91 used in infestation experiment were transferred in the mango fruit.

92

93 **2.3 Preliminary study of mortality rate and quality acceptability**

94 Mortality rate of oriental fruit fly eggs were studied using hot water immersion technique. Two
 95 hundred eggs were incubated on a thin layer of agar gel inside the plastic boxes (fifty eggs each). For
 96 each treatment, four incubated boxes were immersed into water bath model MD16-G (Julabo
 97 Labortechnik GmbH, Germany) with an accuracy of $\pm 0.5^\circ\text{C}$ under the controlled temperature of 46,
 98 48, 51, and 55°C for 2, 5, 12 and 20 min. This resulted in a total of 3200 eggs. It is noted that an
 99 increase in medium temperature within $\pm 0.5^\circ\text{C}$ of the desired temperature required approximately 10
 100 min. Therefore, all mortality treatments were subjected to the additional come-up time of 10 min;
 101 bringing on the actual immersion time of 12, 15, 22 and 30 min. It was also assumed that thermal

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102 lethality effect on insect during come-up period was less significant than that during holding period
 103 and was negligible.
 104 For quality acceptability, four mangoes (domestic graded) per treatment were immersed in hot water
 105 bath under the desired temperatures of 46, 48, 51, and 55°C for 2, 5, 12 and 20 min, equivalent to the
 106 mortality study. The experiment was replicated twice, resulting in a total of 128 fruits. To reach
 107 temperature within $\pm 0.5^\circ\text{C}$ of the designated temperature, the extra five minutes were required so that
 108 the actual elapsed time were 7, 10, 12 and 20 min. After hot water immersion, treated mango was
 109 hydro-cooled with 25°C sprayed water for 30 min. All treated mangoes were then stored at 13°C /
 110 90%RH for 3 days before assessing the heat damage by percent of browning area. Details of heat
 111 damage assessment are discussed in section 2.6.
 112 To quantify the acceptable temperature and time for quarantine treatment, Thermal-Death-Time
 113 (TDT) plot of insect mortality was established, overlaying with Thermal-Quality-Time (TQT) plot.
 114 TDT plot exhibited ranges of insect mortality while TQT plot indicated percent of damage area on
 115 mango peel. Acceptable quarantine process was an area in overlay plot where the insect was 100%
 116 killed and no damage on mango. Treatments of acceptable temperature and time were selected for
 117 further full-quarantine process development in the next experiment.

118

119 **2.4 Development of MVHT Quarantine Process**

120 For MVHT process development on mango, preheat during come-up period was accomplished by
 121 microwave treatment adapted from Varith and Kiatsiriroat (2004) where Chokanan mango treated
 122 with MW power of 400 W for 40 sec increased its internal temperature at the cheek area up to 46°C.
 123 Export-graded mango was treated with MW power of 50% using 2,450 MHz / 800 W microwave
 124 oven model R-254 (Sharp Co., Ltd., Japan). MW preheat treatment was divided into 2 steps. Firstly,
 125 the mango was placed in horizontal position on a polypropylene support at center of the oven and
 126 rotated while being heated with MW power of 400 W for 40 s (Figure 1a). Secondly, the mango was
 127 placed approximately 20 mm away from the exit of waveguide without rotation (Figure 1b). The

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128 25°C water of 1800 cm³ was placed beside the mango to absorb microwave after penetrating through
 129 the mango and to reduce the microwave reflection back to the mango. This step provided a virtual 1-
 130 D radiation focusing at the mango cheek which was the thickest part and subjected to the slowest
 131 heating during conventional VHT heating. Finally, mango was transferred to a vapor (saturated steam)
 132 cabinet for VHT holding process where temperature inside was controlled at 55±1°C using
 133 temperature controller and solid state relay. During treatments, mangoes were sampled in 4-5 periods
 134 and cut in-half for a thermography imaging. Then, thermography images of the sectional mangoes
 135 were quickly taken for step-wised heat distribution during MW and VHT periods using thermal
 136 imaging camera model IR FlexCam™ (Infrared Solutions, Inc., MN, USA). After MW treatment, the
 137 slowest heating spot during MVHT process was identified and used as a reference for holding
 138 temperature during VHT. After preheating and holding period of MVHT, the mango was cooled
 139 down with showering water of 25°C for 30 min. All MVHT preliminary experiments were replicated
 140 six times with six mangoes each. Treated mangoes were stored at 13°C / 90%RH for 24 h for
 141 assessment of percent damage on peel and flesh. Figure 1 schematically demonstrated the steps of
 142 MVHT quarantine treatment on mango.

144 **2.5 Confirmation experiments on MVHT and VHT quarantine processes: insect mortality and** 145 **fruit quality**

146 Two selected time-temperature combination in acceptable range of TDT and TQT curve were
 147 selected for the infestation experiment. Each domestic-graded mango was infested with fifty eggs
 148 onto the flesh slice, about 23 mm deep from the peel, and sealed with clear tape to prevent leakage of
 149 water into infested area. Two MVHTs and one conventional VHT using 55°C saturated vapor were
 150 selected for quarantine treatments. Experiment was replicated twice which led to a total of 48
 151 mangoes with 2,400 eggs. Treated mangoes were then stored at 25°C / 70% RH to observe an
 152 emergence of larvae. Morality rate of oriental fruit fly egg (reciprocal of percent of egg emergence)
 153 was reported.

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For quality changes over a period of storage time, two selected MVHT and one VHT treatments were performed on 48 export-graded mangoes with two replications of six fruits per treatment. After treatment, mangoes were stored at 13°C / 80%RH for 15 days. Quality measurements, namely, color of peel and flesh, firmness, tritrateable acid (TA), total soluble solid content (TSS) and ratio of TA/TSS were determined on two mangoes from each replication for every third day. Quality parameters were analyzed using Analysis of Variance (ANOVA) using StatView™ version 5.0 (SAS Institute, Inc., NC) with 95% confident interval. Quality parameters were reported significantly different when $p < 0.05$.

162

2.6 Quality measurement methods

Color: Spectroscopy model MiniScan XE (Hunter Associates Laboratory, Inc., Reston, VA) was used to measure color of mango. Measuring condition was set using 10/45° angle with D65 illuminant. Spectroscopy was standardized with white and black plates following the factory protocol. CIE scale (L^* - a^* - b^*) was reported as the color parameters. Measured parameter L^* indicated lightness with scale from 0 to 100; a^* indicated red ($-a^*$) to green ($+a^*$); and b^* indicated yellow ($-b^*$) to blue ($+b^*$) scale.

Firmness: Firmness of mango was determined by two means under penetration test; 1. maximum force at rupture; and 2. apparent elastic modulus followed the guideline of ASAE S386.4 DEC00 (ASAE Standards, 2003). Stainless steel probe with a diameter of 6 mm and radius of curvature of indenter about 66.9 mm was used to penetrate mango flesh to 10 mm in depth. Cross-head speed was set at 1 mm/s. Apparent elastic modulus (E) was calculated using Equation 1 as:

$$E = \frac{0.338 K_u^{\frac{3}{2}} F (1 - \mu^2)}{D^{\frac{3}{2}}} \left(\frac{4}{d} \right)^{\frac{1}{2}} \quad [1]$$

where E = apparent modulus of elasticity (Pa); D =deformation (m); μ =Poisson's ratio (assumed to be 0.45); F =force (N); K_u =dimensionless factor (=1.351 for a flat surface-indenter test); and d =diameter of curvature of the spherical indenter.

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179 *Titratable acid (TA)*: TA was determined by indicator method followed the guideline of AOAC
 180 942.15. (AOAC, 1996).
 181 *Total soluble solid content (TSS)*: TSS was determined by hand refractometer model N-1α (Atego
 182 Co., Ltd, Japan).
 183 *Heat damage assessment*: Heat damaged on treated mango was assessed from percent of browning
 184 area of the peel or whitening area of the flesh. While being placed on a graphical paper, the mango
 185 was captured by a digital camera and then transferred into Adobe® Acrobat Professional® version 6.0
 186 for the area determination using area measuring tool (Figure 2). Percent of damage and non-damage
 187 on each mango was assessed from:

$$188 \quad \% \text{ damage} = \frac{\text{Browning or whitening area of damage}}{\text{Total area}} \quad [2]$$

190 3. Results and Discussion

191 3.1 Preliminary study on mortality rate and quality acceptability

192 Table 2 shows that oriental fruit fly in egg stage was susceptible to heat tolerance at 46°C up to 12
 193 min. Our results agreed with the data presented by Jang (1986) that gave a calculated lethality time in
 194 egg stage of 8.91 min and yielded thermal death rate of $5D$ ($LT_{99.999}$). However, the thermal death
 195 rate data of egg above 48°C were absent. Since our result indicates that the temperature higher than
 196 48°C with holding time at least 2 min yielded 100% mortality, these treatments were promising for
 197 the holding condition for further MVHT quarantine treatment of mango.

198 Table 3 indicates that the mangoes undergoing treatment at 51°C with holding time up to 12 min
 199 were not damage due to heat. Mangoes subjected to heat at 51°C for 20 min and 55°C for 5 min were
 200 slightly susceptible to damage less than 5%. With temperature of 55°C for holding time longer than 5
 201 min, heat damage on mango increased no more than 15%. The TDT and TQT overlay plot (Figure 3)
 202 shows the shaded area of quarantine treatment. Therefore to assure non-damaged quarantined fruit,
 203 the upper boundary of quality acceptance was approximately at 55°C for short period of time for 2