Effect of Vapor Heat Treatment on Quarantine Disinfestation of Oriental Fruit Fly (*Bactrocera dorsalis*) and Fruit Quality in Passion Fruit

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Key words: Passion fruit, *Bactrocera dorsalis*, Vapor heat treatment, Quarantine, Passion fruit quality.

Abstract

Exporting Taiwan's horticultural commodities have been required pest quarantine treatment by some countries. To reduce the risk of detecting alive pest in these commodities, postharvest quarantine treatment is an important work. The objective of this study was to develop and establish a quarantine disinfestation process for 'Tainung No.1' passion fruit (*Passiflora edulis* Sim.) against oriental fruit fly (*Bactrocera dorsalis*) by vapor heat treatments (VHT). The egg stage of the oriental fruit fly were used in the small and large-scale disinfestation test, when the fruit core temperature reached at 46.2℃ in 20 min, an extremely high the mortality rate achieved more than 99.84%. For enhancing the elimination effect, in the large-scale disinfestation test, the fruit core temperature were raised to 46.5℃ for 30 minutes. The result shown that, mortality rate achieved 100% and in three replicates of large-scale disinfestations tests conducted, the sum of eggs successfully killed was 37,782. This result was in compliance with the Japanese quarantine requirements. Furthermore, after VHT (46.5℃, 30 min) the fruit qualities were examined. The result shown that passion fruit treated by VHT then stored at 9℃ for 7 days following by 3 days shelf-life at 25℃, total soluble solid and titratable acidity content were gradually decreased and peel color no significant change, fruits qualities were acceptable.

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Introduction

Purple passion fruit (*Passiflora edulis* Sim.) belongs to family Passifloraceae that were found by Western explorer in South America (Ozarowski and Thiem, 2013). Purple passion fruit is either egg-shaped or spherical and contains multiple black seeds with attractive aromatic juice inside (Shiomi *et al*., 1996a). Purple passion fruit is rich in many organic compounds such as Vitamin A, C, phenolics, and fiber within its edible seeds (Romero-Rodriguez, *et al*., 1994; Zeraik *et al*., 2011). Therefore, purple passion fruit has become more and more popular in the world. In recent years, the cultivation area of Taiwan passion fruit has increased. In 2015, the total production areas were 627 ha and increased to 680 ha approximately in 2017 (Taiwan Council of Agriculture Executive Yuan, 2017). The 'Tainung No.1' passion fruit produced in Taiwan have good taste, attractive aroma (Lee and Wang, 2007), and was considered to be a potential fruit for the export to many temperate countries. Thus, this study was conducted with the objective to development and establish the suitable quarantine treatment for passion fruit against Oriental fruit fly by vapor heat treatment (VHT). Besides that, the effect of quarantine vapor heat treatment for passion fruit quality were examined.

Materials and Methods

1. Tested fruit material

This study used 'Tainung No. 1' passion fruits (*P. edulis* × *P. edulis* f. *flavicarpa*) as the plant materials which were harvested at Taiwan, Nantou country, Puli Township. Passion fruits then were selected for uniformity of shape, size (75 ± 10 gram), no mechanical injuries, and fruit peel color completely turned into purple in order to conduct in the experiment.

2. Vapor heat chamber description

Vapor heat treatment equipment (steam chamber) description: The vapor heat treatment machine manufactured by Japan (SANSHU SANGYO Co. Ltd., in 2007, Model EHK-1000D) was used for the experiment. The sensor probe was attached to the vapor heat treatment machine is Model PT 100 Ω, which can be connected to the CHINO LE513311S multi-function temperature controller to detect temperature changes during the test. During the experiment was conducted, temperature record was taken each 5 minutes. Fruit and chamber temperatures were monitored using platinum resistance probes calibrated. Fruit probes were inserted into the fruit stem with the tip of the probe located in the core of fruit pulp.

3. Insects source

Oriental fruit flies (*Bactrocera dorsalis* Hendel) were used in this study that collected from

infested guava fruits in guava orchards located in central area of Taiwan in 2017. The flies were used in this study had been maintained in the laboratory for 10^{th} to 12^{th} generations and the eggs were collected from 15 to 25 days old female flies.

4. Method for egg collection

The egg collector was a transparent plastic cylinder with lid (diameter 9.5 cm, high 16.5 cm; hand shaker cup). There are 292 pinholes (0.5-0.7 mm) in the cup with a spacing of 1.2 cm. The inner and outer surface of the collector was moistened with fresh guava juice which was the fruit most preferred by several fruit flies (Ekesi *et al*., 2006; Rwomushana *et al*., 2008), and then was placed inside the adult rearing cage. The female flies, lured by the fragrance of guava, inserted their ovipositors through the pinholes and laid their eggs on the cylinder's inner side.

5. Small-scale disinfestation test (Efficacy test)

Forty five fresh passion fruits, uniform in size and maturity, were selected and inoculated with 400 oriental fruit fly eggs each. Five passion fruits were used as control group (non VHT after inoculation). The infested passion fruits were placed in the VHT chamber and subjected to the fruit core temperature reach 46.2℃ by vapor heat. Infested fruits were removed from the chamber when core temperature reached on 46.2℃, remained 10 minutes and 20 minutes later respectively. Treated fruits and control fruits were then placed in 25℃ room for 7 days. The survival of larvae were counted and transformed as mortality rates after incubation. This test was replicated three times.

6. Large-scale disinfestation test (Confirmatory test)

According to the results of the small-scale test, it was determined that the treatment of 46.5 ℃ for 30 minutes was the optimal condition for eliminating the oriental fruit flies in the large-scale disinfestations test.

Passion fruits which uniform in size and maturity, were selected and each inoculated with 400 oriental fruit fly eggs. One hundred and twenty infested Passion fruits were placed in the VHT chamber and subjected to vapor heat treatment. Before steam chamber was operated, all temperature probe sensors were calibrated. The temperature probe sensors were inserted into the core fruits in order to monitor the temperature in fruit center. The fruits were vapor heated for 30 minutes after core fruit temperature reached 46.2℃, and then be moved out the chamber. The treated fruits were kept in the incubation room for 7 days, the estimated time for the oriental fruit fly to develop into 3^{rd} instar larva. Twenty fruits were used as control group, which infested but not treatment and placed in the incubation room for 7 days in order to determine the survival rate. The effects of disinfestation of the oriental fruit fly were then examined. This test was replicated three times.

In order to meet the requirement of probit 9. Japan Plant Quarantine Bureau requirement for large-scale disinfestation test need three replications, and at least 10,000 flies should be treated in

each test; with a total of 30,000 flies being treated. No alive fly can be detected after treatment.

7. VHT quarantine effect on quality attributes of passion fruit

After the large-scale disinfestation test conditions were established, the effects of VHT quarantine on passion fruit quality were carried out with the fresh passion fruits (no hole punch fruits) in the same condition with the large-scale disinfestation test (46.5℃ and remained for 30 min). Sixty (60) passion fruits were used in this experiments in which 15 fruits were done quality before treated and 45 fruits were used for treated. After treated, 15 treated fruits of them was examined quality attributes immediately, the rest (30 fruits) were enclosed in 0.03 mm PE bag, they was then storage in refrigerator at 7℃.

The effect of quarantine vapor heat treatment on quality of Passion fruit were divided into 4 stages that accordance with the actual export procedures as follows (1) before VHT treatment, (2) after vapor heat treatment was conducted immediately, (3) after storage in refrigerator at 9℃, (4) on the 3rd day of rewarmed (shelf life). Passion fruit's peel coloration, total soluble solids and titratable acidity was evaluated at that 4 time points. Each fruit is one replicate, this experiment had 15 replicates.

Peel color

The fruit peel color was measurement by using Hunter Lab Scan colorimeter (MiniScan XE Plus, MSXP-4500S, USA). Measurement of Daylight color mode (D65/10), which expression by the value of L*, a*, b* then the C* value and the h° value were calculated. Each fruit was measured for 3 points on the equator line, and then averaging that 3 values.

Total soluble solids (TSS) and Titratable acidity (TA) content

Total soluble solids (TSS) were measured by a digital refratometer, model of Atago PR-32 (Atago Co., Ltd., Tokyo, Japan). The samples juices were prepared as described above. Then drip 2-3 drops of the sample juice for full coverage of the refractometer's prism, TSS was expressed as °Brix.

The TA content in passion fruit was determined by titrating to pH 8.2 with 0.1 N NaOH, using phenolphthalein as an indicator and expressed as g of citric acid per 100 ml of juice or percentage (IAL, 2008). Taking 1 ml of passion fruit juice, then mixing with 9 ml of pure water, finally adding 1 to 2 drops of phenolphthalein. This solution was titrating drop by drop until color changes to pink.

8. Statistical analysis

The experiments results were conducted in a randomized design. The mean values were analyzed by SAS version 9.4 (SAS Institute Inc., Cary, NC, USA, 2018). Analysis of variance was performed; means were compared by the least significant difference (LSD) and t tests at significance level of 0.05.

Results

1. Small-scale vapor heat disinfestation test

Results from the small scale test indicated that mortality increases as the period of vapor heat treatment increases from 0 min to 20 min. More than 93% of eggs were killed when the fruit core temperature reached 46.2℃. When the fruit core temperature reached 46.2℃ and held for 20 min, the mortality rate was extremely high, with values of 99.84 (the first replicate), 99.93 (the second replicate), and 100% (the third replicate) (Table 1.).

2. Large scale vapor heat disinfestation test

Before the large-scale disinfestation test was conducted, the temperature probes were calibrated to 46.5℃ (Table. 2), to ensure that all the temperature probes used in this study may record the temperature accurately.

Oriental fruit fly was most resistant to vapor heat treatment at the egg stage. Therefore, the eggs were inoculated into the passion fruits in the large-scale disinfestation test. In addition, small-scale test indicated that the mortality of the oriental fruit fly eggs reaches at least 99.84% at 20 minutes after the core temperature of the fruit reaches 46.2℃ In order to avoid uneven distribution of temperature in the steamer and to enhance the quarantine disinfestation effective, the infested fruits were kept under the similar vapor heat condition for 30 minutes in the large-scale disinfestation test.

Table 2 showed that in the large-scale vapor-heat treatments, the estimated total number of treated eggs were 13,128, 11,814, and 12,840 in the first, second, and third replicate, respectively and the estimated number of tested eggs was 37,782 in total. No alive larvae was found after treatment and incubation. These results were met with the Japanese quarantine requirement, which specifies that more than three tests involving more than 30,000 insects should be performed and for all the insects tested, no one should remain alive. An average mortality of 73.8% was observed in the control group.

Therefore, in order to ensure 100% mortality of fruit fly with vapor heat treatment, the treatment time obtained from that this study was 30 minutes after the core temperature of passion fruit reached 46.5℃ (Table 2.).

3. Effect of quarantine with vapor heat treatment on the passion fruit quality

Results from the VHT large-scale disinfestation test showed that all the eggs of oriental fruit fly were killed after being exposed to the vapor heat treatment at 46.5℃ for 30 minutes. However, whether or not the treated fruits retain their marketability is another important issue that needs to be considered. The effect of VHT on the passion fruit quality was examined at four time points including before VHT, after VHT, after storage at 9℃ for 7 days, and after 3 days shelf-life at 25 ℃.

Table. 1. Small-scale vapor heat disinfestation of oriental fruit fly eggs in 'Tainung No.1' passion fruit.

Replicate	Temp.	No. of tested fruit	Total no. of treated eggs	Total no. of survivors	Mortality (%)	Corr. Mort.[z] (Abbot's method(%))
1 (2018.10.15)	Control	5	2,000	426	78.70	-
	46.2℃ 0min	15	6,000	79	98.68	93.82
	46.2℃ 10min	15	6,000	38	99.37	97.03
	46.2℃ 20min	15	6,000	2	99.97	99.84
2 (2018.11.13)	Control	5	2,000	503	74.85	-
	46.2℃ 0min	15	6,000	83	98.62	94.50
	46.2℃ 10min	15	6,000	1	99.98	99.93
	46.2℃ 20min	15	6,000	1	99.98	99.93
3 (2018.11.26)	Control	5	2,000	488	75.60	-
	46.2℃ 0min	15	6,000	5	99.92	99.66
	46.2℃ 10min	15	6,000	3	99.95	99.80
	46.2℃ 20min	15	6,000	0	100.00	100.00

z：Corr. Mort. = (Mort. Treatment - Mort. control)/ (1 - Mort. control)

The fruit peel coloration was described as L*, a*, b*, C*, and h° value for the four time points. Our results indicated that L* value was slightly increased but showed no significant difference as the treatment time point increased, and the C* value was significantly decreased at the end of storage period at 9℃. On the other hand, a* value was significantly decreased. In addition, a* value was significantly decreased besides the b* value was gradually increased as the treatment time point increased. Noteworthy, h° value had approximately increased 3 times after 3 days shelf-life at 25℃ compared with the value before VHT, and showed significant difference (Table 3.).

Total soluble solid and titratable acid were the parameters used for evaluating the fruit quality. In total three time of replicates, the TSS and TA had gradually decreased during the treatment time points, and showed significant difference at the end of storage at 9℃ (Table 3.).

Table. 2. Large-scale vapor heat disinfestation of oriental fruit fly eggs in 'Tainung No.1' passion fruit.

Replicate	Treatment	No. of tested fruit	Total no. of treated eggs	Estimated total no. of treated eggs[z]	Total no. of survivor	Mortality[a] (%)
1 (2018.12.12)	Control	20	8,000	-	2,188	72.7
	46.5℃ 30min	120	48,000	13,128	0	100
2 (2018.12.19)	Control	20	8,000	-	1,969	75.4
	46.5℃ 30min	120	48,000	11,814	0	100
3 (2018.12.26)	Control	20	8,000	-	2,140	73.3
	46.5℃ 30min	120	48,000	12,840	0	100
Total	Control	60	24,000	-	6,297	73.8
	46.5℃ 30min	360	144,000	37,782	0	100

z: Estimated total no. of treated eggs = No. of survivors in non-treated fruit × (No. of treated fruits/ No. of non-treated fruits);

a: No. of corrected treated egg was calculated from Abbott (1925).

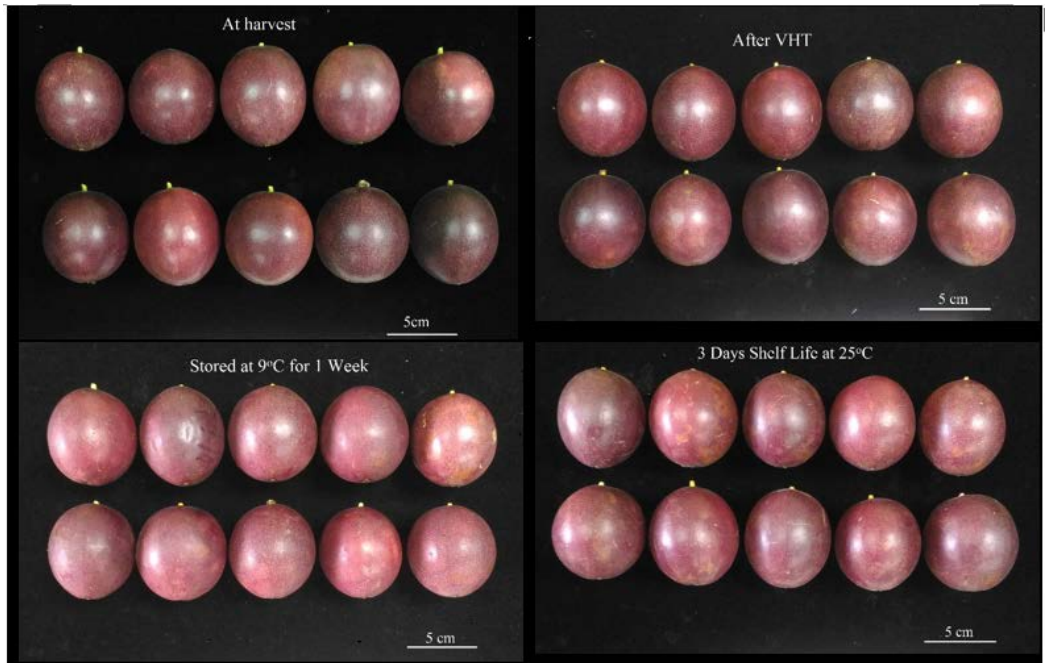

Fig. 1. The appearance of 'Tainung No.1' passion fruit at harvest, after VHT, after VHT followed by storage at 9℃ for 1 week and 3 days shelf-life.

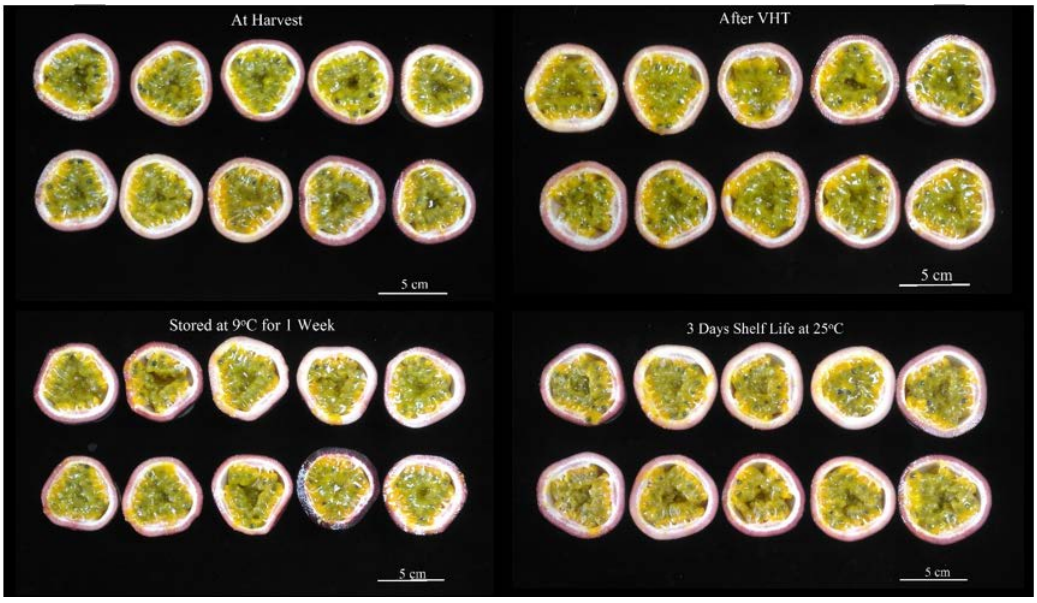

Fig. 2. The sectional view of 'Tainung No.1' passion fruit at harvest, after VHT, after VHT followed by storage at 9℃ for 1 week and 3 days shelf-life.

Table. 3. Effects of vapor heat treatment on the peel color, total soluble solid (TSS), and titratable acid (TA). After VHT, fruits were stored at 9℃ for 7 days and then rewarmed to 25℃ for 3 days.

Treatment time point	L*	a*	b*	C*	h°	TSS (°Brix)	TA (%)
Before VHT	24.4 a	16.3 a	3.1 b	16.6 a	10.1 c	19.2 a	2.8 a[z]
After VHT	25.8 a	16.7 a	3.8 b	17.3 a	12.6 c	18.4 b	2.9 a
Stored at 9℃ for 7 days	25.0 a	12.9 b	3.9 b	13.5 b	16.8 b	17.5 c	2.5 b
3 days shelf-life at 25℃	25.2 a	13.8 b	6.7 a	15.4 ab	26.0 a	17.7 c	2.2 c

z: Mean separation within columns was by LSD at $p \leq 0.05$. Means followed by the same letter are not significantly different by LSD at $p \leq 0.05$.

Discussion

1. Small-scale disinfestation test (Efficacy test)

Results from small-scale disinfestation test showed that the mortality increases as the period of vapor heat treatment increases from 0 min to 20 min. In all there replicate, there was total of 3 individual survivors out of 18,000 eggs treated at 46.2℃ for 20 min. That is extremely low number of survivor. However, in order to guarantee that no survivor in the large-scale disinfestation test to fulfill and meet probit 9 requirement, the treated time was prolonged to 30 minutes.

2. Large-scale disinfestation test (Confirmatory test)

To guarantee the effectiveness of vapor heat treatment in commercial treatment, time and temperature of treatment must exceed the minimum requirement for quarantine security against a treated organism. The mortality rate increased as the expose time to heat temperature and temperature increased (Wang *et al.*, 2002). Moreover, in order to confirm the effectiveness of a pest quarantine treatment, normally large-scale disinfestation test is conducted where thousands

of insects are treated at a specific set of treatment parameters to guarantee that a treatment protocol results in an acceptable level of efficacy (Heather and Hallman 2008).

Vapor heat treatment at 46.5℃ for 30 min (RH > 90%) successfully disinfested passion fruit against oriental fruit fly. Three replicates of over 10,000 insects each with a total of over 30,000 insects showed no survivor after treatment met the requirement set by countries or areas which requires a minimum mortality level of 99.99%. Taiwan 'Irwin' mangoes exported to United States, Japan, Korean, New Zealand are vapor heat-treated at 46.5℃ for 30 min (Yeh and Chen, 2005).

3. Effect of VHT on 'Tainung No.1' passion fruit quality

The goal of the quarantine test is to kill the insects and maintain the quality of the fruit, and the first step of quarantine is to understand the characteristics of the fruit and insects. The quarantine conditions must be formulated with the above two conditions as the basic objectives, and further choose the processing conditions to achieve the best results (Tang *et al*., 2000; Feng *et al*., 2004). The color of the fruit peel was determined by plant pigment composition. Based on a visual aesthetic and on a quality concept perspective, it plays an important role to influence consumer acceptability (Steyn, 2012).

In this study, the passion fruit peel color basically did not change after VHT. However, the fruit peel color was slightly fade and showed some yellowing caused by scald after 7 days of cold storage, particularly after 3 days of shelf-life at 25°C. These results were consistent with Hallman (1990) in which the author reported that carambolas treated at 49-49.3℃ for 45 or 60 min result in expansive scalding after 2 to 3 days treatment and were unmarketable. Application of vapor heat treatment on carambolas at 43.3-43.6℃ for 90 to 120 min and at 46-46.3°C for 60 or 90 min resulted in slightly darker and duller peel color, and rib margins were darker compared with untreated fruits, however, treated fruits were marketable (Hallman, 1990). The vapor heat treatment is to surround the surface of the fruit with saturated water vapor. The effectiveness of heat transfer is better than that of hot air. However, since the water vapor easily condenses on the surface of the fruit to form water droplets during the treatment, it is easy to cause heat damage to fruit peel (Sharp *et al*., 1991). Although vapor heat treatment causes low damage to fruits, it is still easy to cause damage to fruits without resistance to high temperatures such as bananas, avocados, and courgettes (Zhu and Liu, 1990).

Crisosto *et al*., (2003) and Harker *et al*., (2008) showed that the consumers acceptability is based on how the fruit tastes, in which soluble solid content is a dependable tool to determine the index of eating quality in many fruits. In this study, in general, the TSS and TA content had gradually decreased throughout the storage time, particularly after the fruits were moved to 25℃ for 3 days. Nevertheless, the TSS content had some increase after VHT which may due to the difference in the maturity of fruit materials, sine passion fruits used in this study harvested based

on the fruit peel color and may be different in maturity.

The total soluble solid content of the yellow passion fruit was ranged from 13 to 18% with an average of 15%. The soluble solids of the purple passion fruit were ranged from 14.4 to 21.9% with an average of 17.3% (Pruthi, 1959). Pruthi (1963) suggested that as the fruit maturity increases, the soluble solid content in the fruit generally increases. Some of the most important factors affecting the flavor of the fruit are the sugar content, acidity and sugar/acid ratio (Liu, 1994). The passion fruit flowers existed on the same vine may be different at flowering time or developmental rate, together with the gradually changes in the environment that may work together to affect be effect on the passion fruit production (Chayut *et al*., 2014). Pruthi (1959) indicated that unripe passion fruit has higher titratable acid content, and the purple passion fruit has titratable acid content ranged from 2.4 to 4.8%. If the passion fruit is stored at ambient temperature for 2 weeks, then titratable acid content in the fruit is decreased.

The total soluble solid content in 'Jen-Ju Bar' guava fruit was highest at harvest, but decreased after VHT at 46.5℃ for 20 and 57 min followed by storage at 5℃ for 7 days, and then move to 25℃ for 3 days (Jhang, 2006).

The citric acid content in 'Tainung No.1' passion fruit accounted for 88.9% of the total organic acid, while the malic acid accounted for 7.6% (Ling, 1983). It may conclude that citric acid is the main organic acid content in passion fruits. Karadeniz (2004) reported that citric acid consumed in respiration. Therefore, gradually decrease trend in acidity content during storage time may be attributed to normal respiratory metabolism process.

Passion fruit juice contains about 17% total soluble solids, mainly sucrose, glucose and fructose (Pruthi, 1963). The composition ratio varies from species to species. Sucrose, glucose and fructose in purple passion fruit juice accounted for 32.4%, 38.1%, and 29.4%, respectively (Chan and Kwok, 1975). Shao *et al*., (2013) reported that heat treatment of loquat fruit at 45℃ for 3 hours results in sucrose degradation by increasing the activity of sucrose-metabolising enzymes, sucrose synthase. Among sucrose-metabolising enzymes, invertases and sucrose synthase are associated with the decomposition of sucrose (Itai and Tanahashi 2008; Li *et al*., 2011). These enzymes function to degrade sucrose levels declined sharply during cold storage time after heat treatment. This may be one of reasons for the gradually decrease of TSS content in passion fruit after VHT followed by cold storage (9℃ for 7 days). In general, the passion fruits quality remained acceptable after vapor heat treatment at 46.5℃ for 30 min.

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百香果蒸熱檢疫處理殺滅東方果實蠅及對果實品質之影響

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關鍵字：百香果、蒸熱、低溫、檢疫處理、東方果實蠅

摘要：台灣園產品出口有些國家需檢疫，為減少出口園產品中檢出有害生物的風險，採收後的滅蟲處理為重要關鍵。本研究利用蒸熱檢疫處理百香果，並評估其殺死東方果實蠅 (*Bactrocera dorsalis*)效果。以東方果實蠅之卵接種於百香果果實內後再進行小規模及大規模殺蟲實驗，使果心溫度升至 46.2℃並持續 20 分鐘，果實蠅的死亡率最低為 99.84%。為了確保完全殺死蟲的效果，在大規模殺蟲試驗中，使果心溫度到達 46.5℃並持續 30 分鐘，在該溫度下亦可達 100%的殺蟲效果。共進行了大規模殺蟲試驗三次重複，成功殺死的總卵數為 37,782。該結果已符合國際間 Probit 殺蟲效果及日本之檢疫處理蟲數標準。此外，蒸熱後調查百香果果實品質，結果顯示，百香果果實蒸熱檢疫處理後於 9℃貯藏 7 日再移至 25℃放 3 日。可溶性固形物及可滴定酸明顯降低，果皮顏色未明顯改變，果實品質具商品價值。

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