Study on the feasibility of use of hydrochloric acid as an alternative to sulphur dioxide for preserving longan

Wittaya Apai[1,*], Hatairat Klongdee[1], Nipat Sukhvibul[1], Uthai Noppakoonwong[1], Lim Shwu Shan[2], Luk Seow Cheng[2], Tan Swee Chuan Alice[2], Neo Shan Yu[2], Khoo Gek Hoon[2], Ching Ai Lee[2], Sanong Amareok[3], Sittipong Rattanakam[3] and Vicha Sardsud[4]

Abstract

HCl as possible alternative to SO_2 in fresh longans for export was investigated. Fruit was exported from Thailand and transported to Singapore for fruit quality assessment, i.e. sensory evaluation, HCl acid residue test and shelf life evaluation. The 1st set was treated on 8th July 2013 and had 3 treatments comprising of 4 baskets as replication for each treatment. 11.5 kg of fruits in basket were dipped in a plastic bucket containing 60 L of 6.4% HCl solution+1%NaCl+0.1%Tween 20 (wetting agent) for 5 min and these were allowed to air dry for 2 h. (T1). This was compared with SO_2 (T2) and untreated fruit (T3) treatments. An additional HCl treatment using HCl dipping machine at the same concentration (T4) comprising of 6 baskets was done on 12th July 2013. All 18 baskets from the two sets were transported on 12th July 2013 and arrived at the Post-Harvest Technology Center (PHTC) of Agri-Food & Veterinary Authority (AVA) in Singapore on 19th July 2013. The fruit was stored further at 5°C with 76–96% RH for 20 days. The results found that both HCl treatments (T1, T4) and SO_2 (T2) prevented pericarp browning during storage when evaluated from browning index and pericarp acceptance and fruit decay as compared with untreated fruit (T3). The sensory acceptance of flesh appearance, firmness and taste of HCl (T1, T4) during cold storage showed good result and no significant difference. HCl residue in fruit flesh from two HCl treatments was shown at low value from 0.000 to 0.006% (flesh pH 6.78–7.03) during storage. The fruits dipping in HCl (T1) and (T4), SO_2 (T2) and untreated fruit (T3) could prolong storage life for 25, 27, 31 and 12 days respectively at 5°C, 76–96% RH followed by 3 days ambient storage to simulate display for sale at ambient conditions, thus their shelf life were long enough for export.

Keywords: Longan, hydrochloric acid, HCl residue safety, sensory evaluation

[1] Office of Agricultural Research and Development Region 1, Chiang Mai 50100 Thailand

[2] Agri-Food & Veterinary Authority, 2 Perahu Rd., Singapore 718915 and 10 Perahu Road, Singapore 718837

[3] Agricultural Engineering Research Institute, Chiang Mai 50100 Thailand

[4] Agro-Industry Institute, Maefah Luang University, Thailand

[*] Corresponding author Wittaya Apai, E-mail: wittayaapai@hotmail.com

1. Introduction

Fresh longan is one of the leading exported fruits of Thailand. Sulphur dioxide (SO_2) fumigation is used commercially to extend shelf life of fresh longan for at least 45 days at low temperature storage (Tongdee, 1994). However, there have been numerous reports on the negative effects of its use such as residues in the fruit, asthmatics and reactions in sensitive individuals. Therefore, alternative treatments to SO_2 fumigation are required (Jiang *et al*., 2002; Taylor and Bush, 1986). The most Thai exporters had satisfaction and confidence in SO_2 fumigation to extend longan shelf life, however, the alternative method has to be prepared in the future for protecting the risk of SO_2 residue in fruits. The exporters' specification for alternative to SO_2 has to bleach pericarp to yellow skin color and shelf life extension nearby SO_2.

One of the interesting alternative methods was hydrochloric acid (HCl). Applying HCl as a dipping solution was reported with a very satisfactory result for fresh longan (Apai, 2010; Drinnan, 2004) and litchi (Tongdee, 1994). Therefore, a commercial scale research was needed. The evaluation of different HCl application techniques for fruits was recently studied. Dipping in 6.4% HCl (pH 0.03) for 5 min then draining for 10 min without rinsing in water and stored immediately in cold room provided the best compromise between controlling fruit browning and decay and maintaining eating quality. Dipping in HCl and storage at 3°C was most suitable to store longan for 30–45 days. From the experiment with consumer survey for acceptance testing, the fruits treated with HCl 6.4% (pH = 0.03) for 5 min were 90.3% accepted (Apai *et al*., 2013) In addition, HCl could be incorporated with the other additives for increasing its efficacy and consumer acceptance. This indicated that HCl could possibly be used as an alternative to SO_2. However, a commercial scale research study together with the study on the shelf life of the HCL treated longan in exported country is needed. Singapore is one of the importing countries of Thai longans. At present, the strict standards enforced on fruit imports by the Singapore government agency permits a maximum concentration of SO_2 residue levels of 50 ppm in fresh, whole and unpeeled longan (Agri-Food & Veterinary Authority (AVA), 2012). Therefore, alternative treatment (i.e. HCl treated longan) to SO_2 fumigation was conducted as a joint research with Agri-Food & Veterinary Authority (AVA) to benefit Singapore's consumers and our exporters. In the joint research project, investigation on shelf life of HCl treated longan in Singapore and the HCl residues of the longan upon import and during storage was studied.

2. Materials and Methods

2.1 Materials

During 2013 seasons, mature 'Daw' longan fruit was harvested from a GAP-certified orchard at Lamphun province. The A grade fruit for export with panicle attached was placed in 11.5 kg commercial perforated plastic and transported to Pongcharoen Trading Haadyai packing house. The fruit was stored at 5°C before experiment in the morning. The chemical compounds, i.e. HCl (certified food grade, Siam PVS Chemicals Co., Ltd., Thailand), Tween 20 (Polysorate 20) (certified food grade, Maxway Co., Ltd., Thailand), NaCl (certified food grade, Thai Refined Salt Co., Ltd, Thailand) were used.

2.2 Experiments

Two sets of longans were separately done on different dates, i.e. First and second sets were done on 8th July and 12th July 2013 respectively. The 1st set was 3 treatments comprising 4 baskets as replication for each treatment, i.e. T1 = HCl manual dipping, T2 = SO_2 fumigation and T3 = untreated fruit. Fruit was fumigated with SO_2 at commercial packing house in Lumphun Province in Thailand. All baskets were kept at 5°C in the laboratory at the Office of Agricultural Research and Development Region 1 (OARD1) for one night before dipping. The fruit was washed with clean water and then 11.5 kg of fruits in basket were dipped in plastic bucket containing 60 L of 6.4% (v/v) HCl solution +1% (w/v) NaCl +0.1% (v/v) Tween 20 (polysorbate 20) as wetting agent for 5 min then allowed to air dry for 2 h and transported to packing house in the evening. The fruit was stored at 5°C before transportation with the other baskets on 12th July 2013. The 2nd set had an additional treatment: T4= HCl dipping machine comprising of 6 baskets. The fruit was washed with clean water and then four baskets were dipped in dipping machine containing 1,200 L of 6.4%HCl solution at +1%NaCl +0.1%Tween 20 (polysorbate 20) for 5 min then allowed to air dry for 2 h and then transported to same packing house in the evening. All 18 baskets from two sets were coded with label sticker, arranged in the middle position of container and transported on 12th July 2013 and arrived at AVA on 19th July 2013. The fruit was stored further at 5°C with 76–96% RH for 20 days at Post Harvest Technology Department (PHTC), AVA. The included storage time of 1st and 2nd sets was studied for 31 and 27 days respectively until 7th August, 2013. The sample in the cold storage was taken every 3–4 days for quality evaluation. The treated fruit was then transferred to 25°C and stored for a further 3 days to simulate market conditions.

2.3 Fruit quality evaluation

2.3.1 HCl and SO_2 residues

HCl and SO_2 residues were analyzed during cold storage every 3 days at Food & Nutrition Chemistry Section, Veterinary Public Health Laboratory Chemistry Department, AVA. The longan sampling using Codex of practice (Department of Agriculture, 2012) from all baskets in each treatment were collected to composite one sample per treatment at about 1 kg. The duplicate determination was done. The pH of the pericarp and flesh ground was measured, using a digital pH meter under continuous stirring (Joas *et al*., 2005). HCl residue in fruits flesh and pericarp) AOAC, 2005a), the HCl residue in pericarp and flesh ground was measured by titration with 0.01 N NaOH solution to pH 7.0, as indicated by autotitrator. Sulfur dioxide residue in fruit was analyzed during cold storage every 3 days. The SO_2 residue in pericarp and flesh was determined using the Tanner method (Kirk and Sawyer, 1991), which was a modification of the Monier-Williams procedure and involves a distillation with phosphoric acid to remove SO_2 from the sample, followed by titration with standardized NaOH solution. Results were expressed as milligrams SO_2 per kg of fresh weight. Total monomeric anthocyanin content in flesh and pericarp tissue extracts of fruit dipped HCl that discolored at day 67^{th} of storage was evaluated using pH-differential method (AOAC, 2005b). Absorbance was measured in a spectrophotometer at 510 nm and at 700 nm in buffers at pH1.0 and 4.5, using A = [(A510 - A700) pH 1.0 - (A510 - A700) pH 4.5] with a molar extinction coefficient of cyanidin-3-glucoside (29600). Results were expressed as milligrams of cyanidin-3-glucoside equivalents per kg of fresh weight.

2.3.2 Sensory evaluation

Sensory evaluation was evaluated during cold storage every 3 days and subsequent display for sale at room temperature storage for 3 days at PHTC. The in-house trained panel consisting of 10 members assessed the samples. Each panelist assessed seven samples. The acceptability of pericarp color both inside and outside, flesh quality, i.e. appearance, aroma, firmness, taste and overall quality using a nine-point hedonic scale where 9 = like extremely, 5 = neither like nor dislike and 1 = dislike extremely.

2.3.3 Visual evaluation

Visual evaluation was evaluated during cold storage every 3 days and subsequent display for sale at room storage for 3 days. Pericarp color, L*, a*, b*, the degree of browning was expressed as L*, a*, b* values. L* value indicated lightness of color wheel, ranged from black = 0 to white = 100, a* value = green color ranged from green = (-) to red = (+) and b* value = (-) blue to yellow (+). Two spots on opposite sides of the fruit were measured and the

mean of the two measurements considered as one reading. The results were expressed as a mean value from four replications of the 10 measured samples.

2.3.4 Percentage of good fruit

Percentage of good fruit was evaluated. Browning index as pericarp browning was separately evaluated at 5 scores of outer and inner pericarp. Fruit was evaluated for changes in pericarp browning visually by using a browning scale (Jiang and Li, 2001) of the pericarp on each individual fruit of ten fruits per replication. The following scale was used: 1 = no browning (excellent quality); 2 = slight browning; 3 = less than 25% browning of the total surface; 4 = 25–50% browning; and 5 = 50% browning (poor quality). The browning index was calculated using the following formula: $\sum$ (browning scale x number of fruit in each class)/total fruit. Fruit having a browning index above 3.0 was rated as unacceptable.

2.3.5 Flesh discoloration

The following scale was used: 1 = normal (excellent quality); 2 = slight flesh discolored; 3 = less than 25% discolored of the total surface; 4 = 25–50% discolored; and 5 = 50% discolored (poor quality). A flesh discoloration index was calculated using the following formula: $\sum$ (discolor scale x number of fruit in each class)/Total fruit. Fruits flesh having a discolor score above 3.0 were rated as unacceptable.

2.3.6 Disease incidence percentage (DI)

Disease incidence percentage was visually assessed by counting the fruits that showed lesions of mycelium or rot on the fruit surface. Storage life was determined from these parameters: Browning index below 3.0 score (Jiang and Li, 2001) Disease incidence (%) below 25% and Sensory evaluation above 5.0 (Lawless and Heymann, 1998).

Analysis of variance (ANOVA) and the test of mean comparison according to least significant difference (LSD) were applied with a significance level of 0.05.

3. Results and Discussion

3.1 Effects on pericarp browning, flesh discoloration, disease incidence percentage and sensory evaluation

The results found that both HCl (manual; T1, machine; T4) and SO_2 (T2) treatments prevented pericarp browning during cold storage for 20 days at 5°C and subsequent simulated display for sale at ambient temperature (p<0.05). This results were evaluated from these parameters, i.e. browning index, pericarp color (lightness value; L* and yellow value; b*) and sensory evaluation as pericarp acceptance (Figure 1(a)–(d), 2(a)–(b)). When stored further for 56 days at 5°C and till 67 days for T1–T2 and 63 days for T4 until 12th September 2013, no further browning was observed (Fig. 1a) while untreated fruit turned brown as a result of

chilling injury by 7 days during transportation at 2°C (p<0.05). These results was in accordance with findings from Apai (2010); Apai *et al*. (2013); Drinan (2004). Longan subjected to SO_2 treatment , HCl manual treatment and HCl dipping machine treatment maintained consistently high brightness: L* value as well as yellowness: b* value of pericarp during storage (Figure 1(b)–(c)) as compared with untreated fruit which increased darkening of outer pericarp (p<0.05). The L* and b* results showed negative correlation with browning index. The appearance of pericarp acceptance of longans, as assessed by sensory evaluation during the 20 days storage period, was presented in Figure 1 d.

HCl as food additives is used as acidulate agents in food processing in accordance with GMP standard. It is approved by the Codex and US-FDA (FDA, 1979) and has been commercially used for restoring the red skin color of litchis after fumigation with SO_2 for a long time (Department of Agriculture, 2011; Tongdee, 1994). It helps to lower the pericarp pH (Apai *et al*., 2013) and inhibit polyphenol oxidase (PPO) activity before storage. This suggested that HCl treatments as well as SO_2 could acidify and bleach pericarp to low pH value from 5.49 from untreated fruit to 3.32 or 3.65 for HCl (T1 or T4) and 4.69 for SO_2 on day 4 of storage time and thus maintained low score of pericarp browning throughout the experiment (Table 1 (A), Figure 1(a)). Due to the lowest pericarp pH of HCl manual and dipping machine between 3.23–3.76 and high HCl residue in pericarp (Table 1 (B)), it increased the highest a* value of pericarp skin to closed to orange color (near to red color) while SO_2 and untreated fruit gave a closed to green original color (data not shown). The fruit quality dipping in HCl on 8th July 2013 has less acceptance score in flesh discolouration (Figure 1(g), 2(d) and sensory evaluation as flesh acceptance (Figure 2 f) than fruit dipped in HCl on 12th July 2013. However the others that were flesh firmness, aroma, taste and overall acceptance from HCl manual can be still accepted until day 14th at cold storage and subsequent simulated display for sale 3 days at ambient temperature (Figure 2(h)). In addition to the sensory acceptance (flesh appearance, firmness, aroma, taste) of two treatments (T1, T4) during cold storage for 20 days showed good result and no significant difference (Figure 1(e)). The commercial SO_2 fumigated fruit showed the best fruit quality in sensory acceptance and the least flesh discoloration during storage at 5°C (Figure 1(e), 1(g)) and 25°C (Figure 2(d), 2(f)–(h)). The untreated fruit showed severe flesh discoloration score (Figure 1g) caused by flesh rot (Figure 1(h)), mechanical injury caused by stacking force during transportation and chilling injury (Figure 1 (a)–(c)) and thus affecting the sensory quality during period of time of storage (Figure 1(d)–(e) (p<0.05). When they stored further until 12th September 2013, HCl dipping on 12th July 2013 maintained high significant difference in good fruit percentage as compared with HCl dipping on 8th July 2013 (Figure 1(f)). HCl dipping on 8th July 2013 increased soft fruit when stored for 40 days at

5°C whereas both HCl dipping on 12th July 2013 and SO_2 treatment showed no significant difference in good fruit percentage after the end of storage period (Figure 1(f)).

3.2 Effects of HCl treatment on fruit residue content

Fruit dipped in HCl on 12th July 2013 might have high HCl residue in pericarp ranging from 0.623 to 0.864% (pericarp pH 3.23–3.4) when compared with HCl treatment on 8th July, 2013 ranging from 0.548 to 0.702% (pericarp pH 3.63–3.76) (Table 1(A)–(b)). The high residue dipping in machine was due to imbalance ratio between rate of basket numbers and HCl volume (4 baskets: 1200 L per time). Hence, it should be 10 baskets: 1200 L per time. Both of delaying time after acid dip before exportation for 4 days and acid residual in pericarp (Table 1b) from treatment with HCl manual had resulted in higher percentage of defected fruit as soft fruit (Figure 1(f)) and fresh discoloration (Figure 1(g)). The degradation of HCl residue in pericarp showed no change and consistent value (Table. 1(B)). These results support a reasonable conclusion that HCl is stable in the homogenate of raw longan fruits stored under the conditions used in this study, probably due to the high acidity of this HCl concentration. However HCl residues in fruit flesh from the two HCl treatments showed low value from 0.000 to 0.006% (flesh pH 6.78–7.03) (Table 2(A)–(B) closed to neutral pH of water. This value was less than the 0.5% that exists in human's stomach (pH 1–2). This HCl residue in flesh was decreased because it was neutralized or buffered by the food to which it was added. The small amounts of HCl that may persist in foods or drinks, would, in turn, be neutralized and buffered during ingestion and digestion, or after absorption (FDA, 1979). The addition hypothesized that sugar and acids might be catabolized due to fruit respiration during long storage (Etienne *et al*., 2013). In addition, total soluble solid (%TSS) change among the treatments was not significant during storage as it was a non-climactic fruit (Jiang *et al*., 2002). Nevertheless, fruit treated with HCl manually were also detected with contamination of SO_2 residue in the pericarp at 118 and 91.5 mg/kg on day 4th and 6th during 5°C. SO_2 residues in flesh from all treatments were not detected (data not shown).

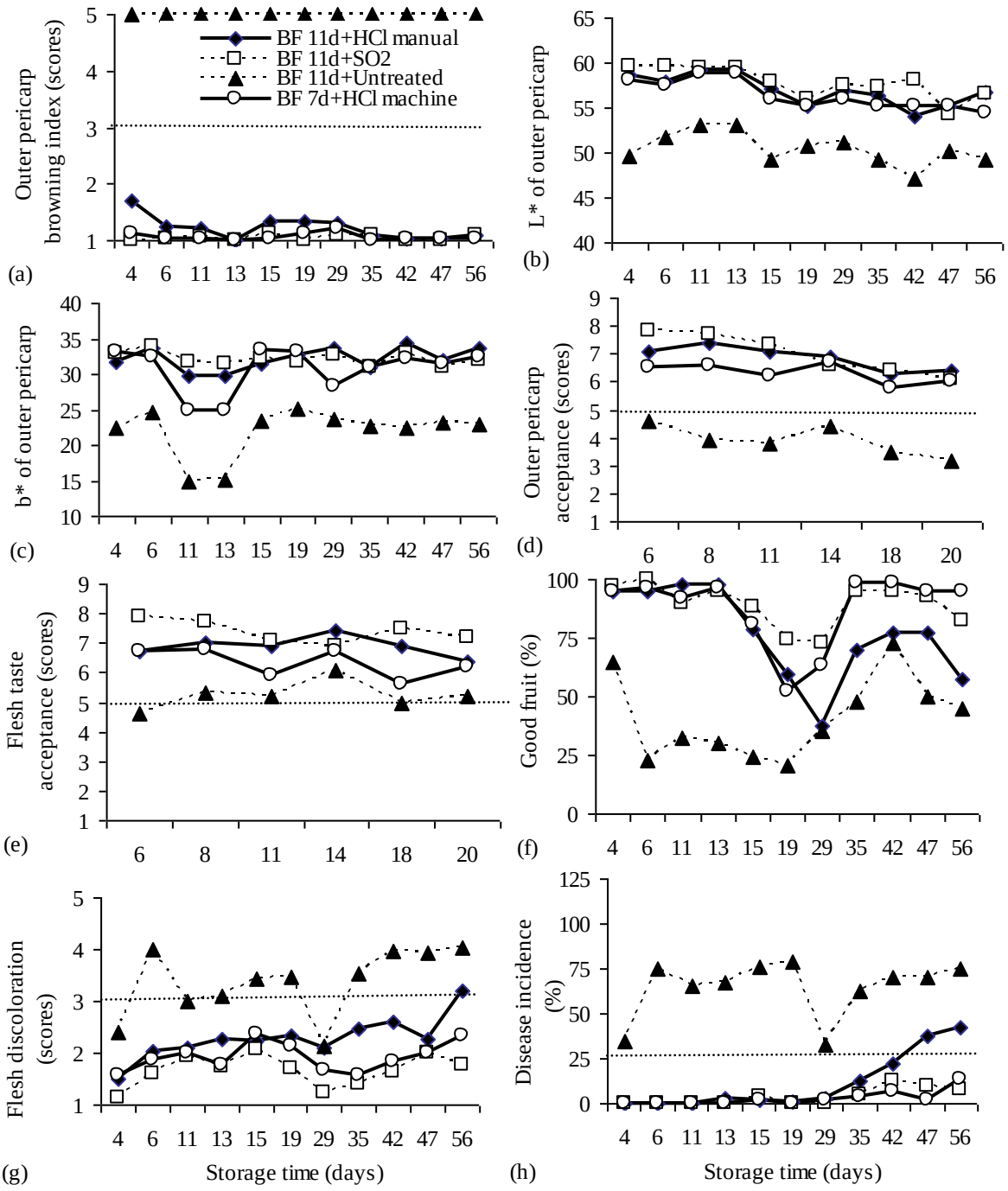

Figure 1 Effects of HCl dipping on fruit quality change in outer pericarp browning

(a) pericarp color: lightness, L* (b) yellowness, b* (c) sensory evaluation as outer pericarp acceptance (d) and flesh taste (e) visual evaluation, good fruit (f) flesh discoloration (g) and disease incidence (h) during storage at 5˚C, 85–90% RH for 56 days, (T1-T3; dipping date was 8th Jul, 2013, BF 11d+56 d = 67 days) and (T4; dipping date was 12th Jul, 2013, BF 7d+56 d = 63 days). Dot line represents limit of acceptance.

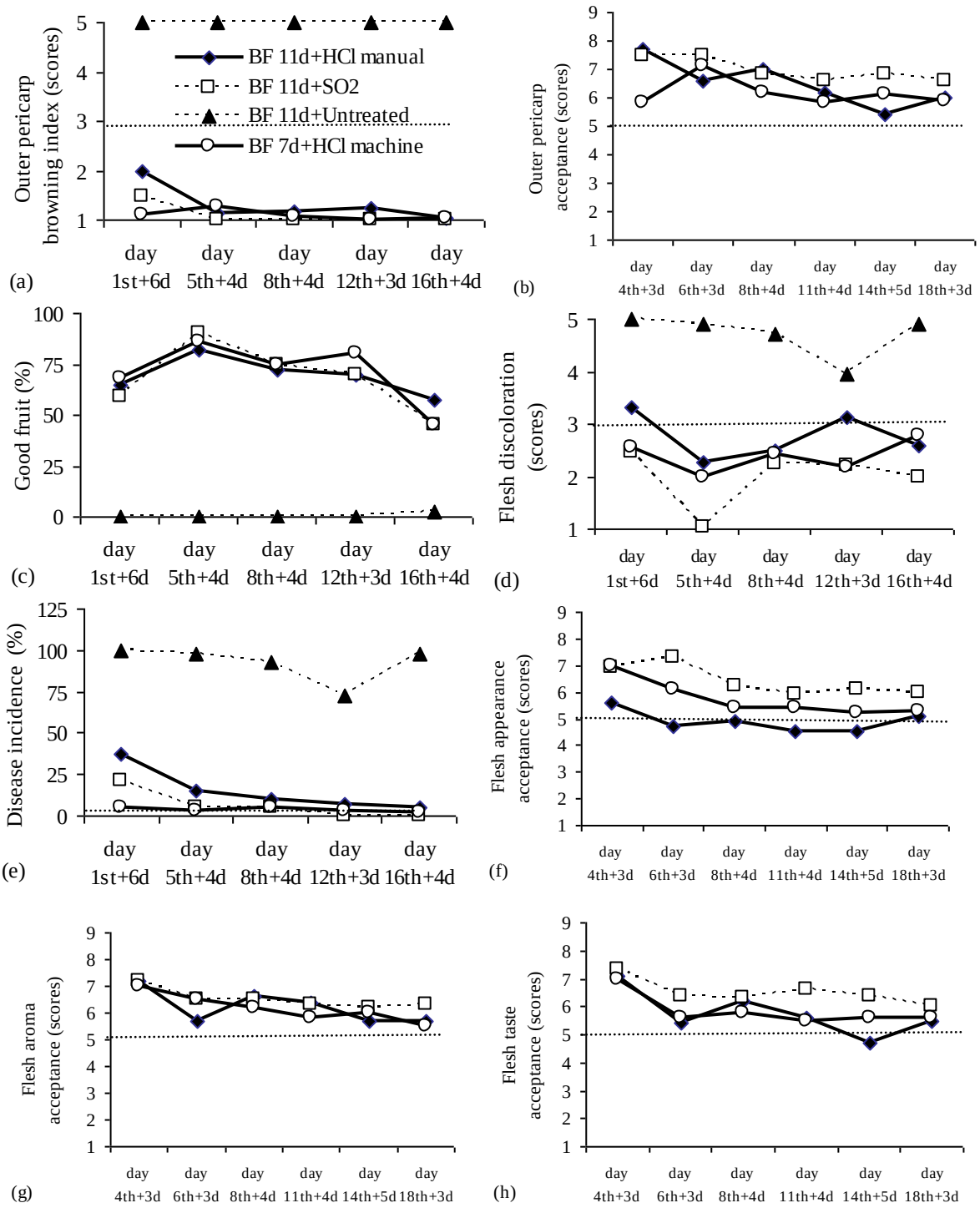

Figure 2 Effects of dipping in HCl on outer pericarp browning index (a) outer pericarp acceptance (b) percentage of good fruit (c) flesh discoloration (d) disease incidence percentage (e) flesh acceptances (f) aroma (g) and taste (h) during simulated display for sale for 3–6 days at room temperature after taken from cold storage at 5˚C, 85–90% RH. Dot line represents limit of acceptance.

Table 1 Effects of HCl on pericarp pH (A) and HCl residue in pericarp homogenate (B) during storage at 5°C, 85–90% RH for 20 days

Pericarp pH (A)	**day 4th**	**day 6th**	**day 8th**	**day 11th**	**day 14th**	**day 18th**	**day 20th**
HCl manual +BF 11d	3.65	3.69	3.69	3.65	3.63	3.72	3.76
SO_2+BF 11d	4.69	4.71	NT	NT	NT	NT	NT
Untreated +BF 11d	5.49	5.32	5.25	NT	5.59	5.48	5.38
HCl machine +BF 7 d	3.32	3.4	3.34	3.23	3.42	3.35	3.39
HCl (%) (B)	**day 4th**	**day 6th**	**day 8th**	**day 11th**	**day 14th**	**day 18th**	**day 20th**
HCl manual +BF 11d	0.618	0.606	0.560	0.573	0.702	0.551	0.548
SO_2+BF 11d	0.261	0.251	NT	NT	NT	NT	NT
Untreated +BF 11d	0.084	0.117	0.127	NT	0.107	0.136	0.127
HCl machine +BF 7 d	0.790	0.724	0.763	0.864	0.623	0.739	0.742

Note: T1–T3 was done at 8th Jul, 2013. BF 11 d = before storage at AVA for 20 days at 5°C (stored at packing house at 5°C for 4 days plus transported from Thailand to Singapore for 7 days). **T4** was done at 12th Jul, 2013. BF 7 d = before storage at AVA for 20 days at 5°C (only transported from Thailand to Singapore for 7 days). NT = not determined.

Table 2 Effects of HCl on flesh pH (A) and HCl residue in flesh homogenate (B) during storage at 5°C, 85–90% RH for 20 days

Flesh pH (A)	**day 4th**	**day 6th**	**day 8th**	**day 11th**	**day 14th**	**day 18th**	**day 20th**
HCl manual +BF 11d	7.11	7.02	7.13	7.20	7.09	7.19	7.35
SO_2+BF 11d	6.99	7.13	NT	NT	NT	NT	NT
Untreated +BF 11d	6.94	6.95	6.97	NT	7.13	7.14	7.3
HCl machine +BF 7 d	6.78	6.86	6.88	6.99	6.91	7.09	7.03
HCl (%) (B)	**day 4th**	**day 6th**	**day 8th**	**day 11th**	**day 14th**	**day 18th**	**day 20th**
HCl manual +BF 11d	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SO_2+BF 11d	0.000	0.000	NT	NT	NT	NT	NT
Untreated +BF 11d	0.002	0.001	0.001	NT	0.000	0.000	0.000
HCl machine +BF 7 d	0.006	0.003	0.003	0.000	0.002	0.000	0.000

Note: T1–T3 was done at 8th Jul, 2013. BF 11 d = before storage at AVA for 20 days at 5°C (stored at packing house at 5°C 4 days plus transported from Thailand to Singapore for 7 days). **T4** was done at 12th Jul, 2013. BF 7 d = before storage at AVA for 20 days at 5°C (only transported from Thailand to Singapore for 7 days). NT = not determined.

In Thailand, same treatment set of HCl dipping on 8th July 2013 was also stored for 67 days at 5°C. Results shown that there were some flesh discoloration showing a reddish color which could be due to anthocyanin pigment that renders further confirmation. Positive findings of cyanidin-3-glucoside equivalents in total anthocyanin including has been demonstrated by using pH differential method by spectrophotometer (AOAC, 2005b). Further analysis of the HCl treated longan should be conducted for identifying the anthocyanin type. The pigment substance, cyanidin-3-glucoside, has been detected at low level 0.021 mg/kg in the flesh and not detected in pericarp of longan. It is hypothesized that this is caused by the acid in pericarp or during dip reacted with this substance in aril to become red color during storage. Such similar observation was detected in pericarp in litchi more than aril. This cyanidin-3-glucoside is one of the flavonoid in the phenolic group that is generally detected in other berries and it acts as an antioxidant which is safe for human consumption (Jeon *et al*., 2012). The anthocyanins identification using High Performance Liquid Chromatography (HPLC) should be investigated in the future.

4. Conclusion

The SO_2 treatment (T2) is most effective on longan which resulted in the longest shelf life, i.e. 31 days at 5°C, 76–96% RH. The HCl manual dipping (T1) is slightly less effective on longan which resulted in shorter shelf life than longan subjected to the HCl machine dipping (T4), i.e. 25 days. The HCl machine dipping (T4) is slightly more superior than T1 as the T4 treated longan has 27 days shelf life. This could also be due to the 4 days difference in the treatment date. HCl residue in fruit flesh from the two HCl treatments was found/detected at low values, thus it could be used to replace SO_2 in the future.

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