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# Influence of Calcium Solutions to Physiological Changes of 'Chiang Mai Pink' Patumma Cut Flower

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## ABSTRACT

This study aimed to evaluate the effect of two calcium formulas ($CaCl_2$ and $CaH_4O_8P_2$) at 0.4 and 0.8% on physical and chemical changes to Chiang Mai Pink patumma. The evaluation which included a comparison with control, included an assessment of weight loss, water absorption, bract color, polyphenol oxidase (PPO) activity, phenolic content, browning incidence and vase life. A completely randomized design was arranged with five treatments: $CaCl_2$ 0.4%, $CaCl_2$ 0.8%, $CaH_4O_8P_2$ 0.4%, $CaH_4O_8P_2$ 0.8% compared with control. The experiment was carried from May to July 2010 with four replications and ten flowers per replication. The results showed that treating with both calcium solutions ($CaH_4O_8P_2$ 0.4%, $CaH_4O_8P_2$ 0.8%) led the flowering stem to absorb less water. At 0.8% $CaH_4O_8P_2$ caused maximal bract color in terms of a* and b*, highest phenolic content and browning severity. These above characteristics brought about the shortest vase life (7.60 days). The Control flower was found to have minimum PPO activity, phenolic substance and browning damage. Thus, applications of $CaCl_2$ and $CaH_4O_8P_2$ at 0.4 and 0.8% were found to be not the appropriate substances for maintaining the quality and prolonging the vase life of the patumma's flower.

**Key words:** $CaCl_2$, $CaH_4O_8P_2$, patumma, browning incidence, phenolic substance

## INTRODUCTION

An important cause of decreased quality and vase life of commercial patumma flower is the rather short storage life and browning incidence found on their bracts. These physiological disorders lead to economic losses (Chutichudet *et al*., 2011a). Some researchers have cited that the occurrence of leaf browning is associated with a calcium deficiency in the plant (Adams and Ho, 1993). This deficiency affected a loss of membrane integrity and promoted enzymatic browning (Franck *et al*., 2007; Chutichudet *et al*., 2009). This damage has long been considered as the main cause of the decrease in market value of ornamental plants in the Zingiberaceae family, such as Siam tulip and patumma because it limits consumer acceptance due to the compromised appearance (Chutichudet *et al*., 2010a; Chutichudet *et al*., 2011a). Therefore, a search for a practical method to decrease this disorder is needed. Some researchers reported that browning appearance in several plants have been linked to calcium deficiency (Cresswell and Weir, 1997). Saure (2005) reported that calcium is known to stabilize cell membranes. In this way, the severity of this physiological disorder would be alleviated. At present, very little information is available on calcium application in order to decrease the browning

damage in patumma flower after cutting. Thus, the aim of this study was to investigate the effects of exogenous different calcium formulas of $CaCl_2$ and $CaH_4O_8P_2$ applied to 'Chiang Mai Pink' patumma flower in an attempt to maintain quality and vase life.

## MATERIALS AND METHODS

Patumma flowers (*Curcuma alismatifolia*) cv. Chiang Mai Pink were harvested at the commercial stage from a commercial garden in Chiang Mai, in the north of Thailand. Each flower was wrapped with a foam sheath and packed carefully in fiberboard cartons then transported in an air- conditioned vehicle to Mahasarakham University. During transport, buckets containing stems were covered with a plastic film shroud to minimize moisture loss. After they arrived at the laboratory, the flowers were selected again for uniformity of size, shape, initial bract color and freedom from external damage. The stem end of each flower was recut with stainless steel scissors into lengths of 30 cm. The experiment was carried out from May to July 2010 at the laboratory of the Division of Agricultural Technology, Faculty of Technology, Mahasarakham University, in the northeast of Thailand. A Completely Randomized Design was arranged and composed of five treatments: calcium chloride ($CaCl_2$) at 0.4 and 0.8%, calcium dihydrogen phosphate ($CaH_4O_8P_2$) at 0.4 and 0.8%, compared with control. Each treatment was carried out in four replicates, ten flowers per replication. Calcium solution was applied to the patumma flower by spraying to the bract and then the flowers were held in the above solution and stored at ambient temperature (27°C, 91% R.H.). The Control flowers were untreated with calcium solution and maintained under identical storage conditions.

The following determinations were assessed at 2 day intervals: (1) weight loss of the flowering stalk was calculated as a percentage of the initial weight (%) (2) water uptake by the flowering stalk was measured as mL $day^{-1}$ (3) Bract color was measured by using a Hunter Lab Model No. 45/0-L, serial No. 7092, USA. CIE color values L* (black = -100 and white = +100), a* (redness) (- = green and + = red) and b* (yellowness) (- = blue and + = yellow) were measured to describe the color of the flower's bract. (4) Polyphenol oxidase (PPO) activity determination was carried out according to the method reported by Jiang and Fu (1998). The attained enzyme extracts were measured by spectrophotometer model V-325-XS from China. One unit of PPO activity was defined as the amount of enzyme causing a change of 0.01 in absorbance (420 nm) per 60 sec. (5) Phenolic content was performed as described by Ribeiro *et al.* (2008). Content was expressed as absorption at 765 nm/100 g fresh weight of bract. (6) Levels of browning on bract surfaces were scored by determining visually and expressing as a percentage. (7) Vase life (days) was judged to have terminated when 30% of the flowers had withered. The collected data were statistically analyzed using the SPSS Computer Program, Version 6 (SPSS, 1999).

## RESULTS

The results were collected after spraying calcium in the form of $CaCl_2$ and $CaH_4O_8P_2$ at 0.4 and 0.8% on patumma flowers stored under room temperature. The recorded data demonstrated the following results.

**Weight loss:** The results from Table 1 revealed that treating with calcium in the form of 0.4% $CaCl_2$ showed the lowest flowering stem weight loss of patumma four days after vase life (DAV), while the highest weight loss of flower treated with $CaH_4O_8P_2$ at both concentrations (0.4 and 0.8%) was significantly observed. The fresh weight of flower treated with $CaH_4O_8P_2$ at both 0.4 and 0.8% decreased dramatically throughout the vase life.

Table 1: Weight loss of patumma flower after receiving the different calcium solution

| | Weight loss (%) at different vase life (days) | | | | |
|---|---|---|---|---|---|
| Treatment | 2 | 4 | 6 | 8 | 10 |
| $CaCl_2$ 0.4% | 4.46$^{b}$ | 8.11$^{c}$ | 13.11$^{c}$ | 18.70$^{c}$ | 26.82$^{b}$ |
| $CaCl_2$ 0.8% | 6.66$^{a}$ | 12.20$^{a}$ | 17.44$^{b}$ | 25.90$^{b}$ | 26.54$^{b}$ |
| $CaH_4O_8P_2$ 0.4% | 4.63$^{ab}$ | 8.72$^{bc}$ | 20.90$^{ab}$ | 36.39$^{a}$ | 46.68$^{a}$ |
| $CaH_4O_8P_2$ 0.8% | 3.25$^{b}$ | 11.17$^{ab}$ | 23.85$^{a}$ | 39.97$^{a}$ | 49.88$^{a}$ |
| Control | 4.75$^{ab}$ | 12.04$^{a}$ | 20.86$^{ab}$ | 26.08$^{b}$ | 31.61$^{b}$ |
| F-test | * | * | ** | ** | ** |
| C.V. (%) | 11.67 | 13.00 | 12.52 | 18.62 | 14.16 |
| LSD | 0.84 | 1.05 | 1.43 | 1.73 | 3.75 |

Letters within columns indicate least significant differences (LSD) at p** = 0.01, p* = 0.05

Table 2: Water uptake of patumma flower after receiving the different calcium solution

| | Water absorption (mL) at different vase life (days) | | | | |
|---|---|---|---|---|---|
| Treatment | 2 | 4 | 6 | 8 | 10 |
| $CaCl_2$ 0.4% | 7.50 | 3.10$^{b}$ | 5.20$^{a}$ | 5.20 | 3.10 |
| $CaCl_2$ 0.8% | 6.20 | 2.80$^{b}$ | 2.60$^{b}$ | 4.60 | 3.33 |
| $CaH_4O_8P_2$ 0.4% | 4.60 | 3.00$^{b}$ | 4.30$^{ab}$ | 2.90 | 1.75 |
| $CaH_4O_8P_2$ 0.8% | 6.00 | 2.40$^{b}$ | 2.70$^{b}$ | 3.70 | 4.50 |
| Control | 2.90 | 6.50$^{a}$ | 5.30$^{a}$ | 4.70 | 6.10 |
| F-test | ns | ** | * | ns | ns |
| C.V. (%) | 17.57 | 15.08 | 15.49 | 13.70 | 16.42 |
| LSD | 1.33 | 0.63 | 0.76 | 0.71 | 1.27 |

Letters within columns indicate least significant differences (LSD) at p** = 0.01, p* = 0.05, ns = non significant

**Water uptake:** With respect to water uptake by flowering stem, the results revealed a significantly higher capacity of water uptake from the Control flowers on 4 and 6 DAV, while all flowers treated with both calciums showed a lower water uptake than Control. Afterwards, water uptake from the patumma flowering stem showed insignificant content of 2.90-5.20 and 1.75-6.10 mL at 8 and 10 DAV, respectively (Table 2).

**Bract colour:**

- **L*:** At 6 and 8 DAV, bract color of the patumma treated with 0.8% $CaCl_2$ showed a significantly higher L* level of 57.86 and 57.27, respectively, while Control flowers showed the least L* values of 53.18 and 50.86 (Table 3)
- **a*:** Most of a* values were unaffected by calcium application. The results from Table 4 showed a similar a* value among treatments during 2 to 8 DAV, excepted for 10 DAV. On 10 DAV, Control flower showed the highest a* value. This data revealed that bract color in terms of redness or a* value of patumma flower was significantly lower when supplementary $CaCl_2$ or $CaH_4O_8P_2$ was applied (Table 4)
- **b*:** The results showed that leaf color, measured by monitoring in terms of b* values, changed significantly on 4, 6 and 8 DAV. From Table 5, bract color of patumma treated with 0.8% $CaH_4O_8P_2$ showed the significantly highest b* of 7.23 which indicated that the bract color of this

Table 3: L* values of patumma flower after receiving the different calcium solution

| | L* values at different vase life (days) | | | | |
|---|---|---|---|---|---|
| Treatment | 2 | 4 | 6 | 8 | 10 |
| $CaCl_2$ 0.4% | 54.87 | 55.74 | 56.05$^{ab}$ | 56.00$^{ab}$ | 58.32 |
| $CaCl_2$ 0.8% | 54.87 | 56.33 | 57.86$^{a}$ | 57.27$^{a}$ | 58.97 |
| $CaH_4O_8P_2$ 0.4% | 56.24 | 57.47 | 56.68$^{ab}$ | 55.05$^{b}$ | 64.76 |
| $CaH_4O_8P_2$ 0.8% | 53.24 | 54.08 | 54.05$^{bc}$ | 52.37$^{b}$ | 67.31 |
| Control | 55.98 | 54.09 | 53.18$^{c}$ | 50.86$^{c}$ | 57.82 |
| F-test | ns | ns | * | ** | ns |
| C.V. (%) | 8.04 | 5.42 | 6.21 | 7.64 | 14.17 |
| LSD | 1.40 | 1.01 | 1.09 | 1.31 | 5.83 |

Letters within columns indicate least significant differences (LSD) at p** = 0.01, p* = 0.05, ns = non significant

Table 4: a* values of patumma flower after receiving the different calcium solution

| | a* values at different vase life (days) | | | | |
|---|---|---|---|---|---|
| Treatment | 2 | 4 | 6 | 8 | 10 |
| $CaCl_2$ 0.4% | 14.76 | 15.16 | 12.46 | 12.40 | 10.72$^{ab}$ |
| $CaCl_2$ 0.8% | 14.73 | 14.65 | 14.48 | 10.96 | 8.06$^{bc}$ |
| $CaH_4O_8P_2$ 0.4% | 14.35 | 14.06 | 13.08 | 8.51 | 6.69$^{c}$ |
| $CaH_4O_8P_2$ 0.8% | 14.34 | 14.86 | 12.66 | 5.52 | 2.30$^{d}$ |
| Control | 14.17 | 13.85 | 13.08 | 11.00 | 11.99$^{a}$ |
| F-test | ns | ns | ns | ns | ** |
| C.V. (%) | 12.21 | 9.92 | 13.05 | 13.75 | 12.99 |
| LSD | 0.55 | 0.44 | 0.54 | 1.07 | 1.28 |

Letters within columns indicate least significant differences (LSD) at p** = 0.01, ns = non significant

Table 5: b* values of patumma flower after receiving the different calcium solution

| | b* values at different vase life (days) | | | | |
|---|---|---|---|---|---|
| Treatment | 2 | 4 | 6 | 8 | 10 |
| $CaCl_2$ 0.4% | -7.93 | -7.33$^{bc}$ | -2.26$^{a}$ | -3.47$^{c}$ | -2.79 |
| $CaCl_2$ 0.8% | -7.83 | -6.69$^{b}$ | -6.83$^{b}$ | 4.02$^{ab}$ | 2.24 |
| $CaH_4O_8P_2$ 0.4% | -8.69 | -7.87$^{bc}$ | -6.48$^{b}$ | -1.49$^{bc}$ | 1.09 |
| $CaH_4O_8P_2$ 0.8% | -6.44 | -8.34$^{c}$ | -6.16$^{b}$ | 7.23$^{a}$ | 3.90 |
| Control | -6.63 | -5.81$^{a}$ | -5.39$^{b}$ | -2.09$^{c}$ | -2.32 |
| F-test | ns | ** | ** | ** | ns |
| C.V. (%) | 19.65 | 16.41 | 13.32 | 15.88 | 14.36 |
| LSD | 0.70 | 0.38 | 0.61 | 2.00 | 2.24 |

Letters within columns indicate least significant differences (LSD) at p** = 0.01, ns = non significant

treatment became an apparent blue on 8 DAV, while b* values from flower treated with 0.4% $CaCl_2$ and Control still showed the lowest values of -3.47 and -2.09, respectively. These data indicated that calcium treatment in the form of $CaH_4O_8P_2$ affected a change in bract color of the patumma during vase life

**Polyphenol oxidase (PPO) activity:** PPO activity of various treatments obtained in our study on 6 DAV are shown in Table 6. The results revealed that the activity of the PPO from the control

Table 6: PPO activity extracted from patumma flower at 6 DAV

| | PPO activity at different times (sec) | | | | | |
|---|---|---|---|---|---|---|
| Treatment | 0 | 60 | 120 | 180 | 240 | 300 |
| $CaCl_2$ 0.4% | 0.7571 | 0.7521 | 0.7727 | 0.7810 | 0.7714[a] | 0.7683[a] |
| $CaCl_2$ 0.8% | 0.8142 | 0.7772 | 0.7710 | 0.7815 | 0.7845[a] | 0.7822[a] |
| $CaH_4O_8P_2$ 0.4% | 0.7695 | 0.7577 | 0.7442 | 0.7477 | 0.7429[a] | 0.7449[a] |
| $CaH_4O_8P_2$ 0.8% | 0.8135 | 0.7897 | 0.7829 | 0.7818 | 0.7824[a] | 0.7767[a] |
| Control | 0.6741 | 0.7416 | 0.6916 | 0.6742 | 0.6119[b] | 0.4688[b] |
| F-test | ns | ns | ns | ns | * | ** |
| C.V. (%) | 13.82 | 11.26 | 16.59 | 16.84 | 16.96 | 18.67 |
| LSD | 0.0374 | 0.0304 | 0.0442 | 0.0449 | 0.0443 | 0.0468 |

Letters within columns indicate least significant differences (LSD) at p** = 0.01, p* = 0.05, ns = non significant

Table 7: Phenolic content from patumma flower after receiving the different calcium solution

| | Phenolic contents (g 100 $g^{-1}$ FW) at different vase life | | | |
|---|---|---|---|---|
| Treatment | 2 | 4 | 6 | 8 |
| $CaCl_2$ 0.4% | 33.62 | 24.71[c] | 26.72[c] | 39.31[b] |
| $CaCl_2$ 0.8% | 37.60 | 31.46[b] | 28.36[bc] | 26.59[d] |
| $CaH_4O_8P_2$ 0.4% | 33.48 | 29.13[bc] | 32.66[ab] | 34.85[c] |
| $CaH_4O_8P_2$ 0.8% | 36.01 | 40.14[a] | 36.78[a] | 89.46[a] |
| Control | 34.89 | 25.52[c] | 29.02[bc] | 35.88[c] |
| F-test | ns | ** | ** | ** |
| C.V. (%) | 15.42 | 18.37 | 14.29 | 5.34 |
| LSD | 1.9152 | 1.9611 | 1.5520 | 0.8538 |

Letters within columns indicate least significant differences (LSD) at p** = 0.01, ns = non significant

flower differed and was significantly lower than that of the flowers treated with calcium solutions of $CaCl_2$ and $CaH_4O_8P_2$. The greatest activity of PPO was obtained from flowers treated with $CaCl_2$ and $CaH_4O_8P_2$ at 0.4 and 0.8%.

**Phenolic content:** The phenolic showed an abundant increase of content in flower treated with 0.8% $CaH_4O_8P_2$ from 4 DAV to 8 DAV. These data indicated that phenolic substance increment was affected by $CaH_4O_8P_2$ application. Highly significant increases in total phenolic content were found in patumma flowers featuring the maximal content (40.14, 36.78 and 89.46 g per 100 g FW) at the vase life of 4, 6 and 8 DAV, respectively (Table 7).

**Levels of browning:** Bract browning was estimated by measuring the extent of the total brown area on bract surface as a percentage. The enzymatic browning in patumma flower was visually detected throughout the vase life period. Throughout the data recording process, the results showed similar trends of PPO activities and levels of browning. PPO activity increased markedly and showed the maximal browning disorder in flowers treated with 0.8% $CaH_4O_8P_2$ in comparison to the control throughout their vase life (Table 8).

**Vase life:** The results showed that applied calcium solution in this experiment affected to shorten the vase life of the patumma flower. A minimum vase life of 7.60 days was observed in treating with 0.8% $CaH_4O_8P_2$ while the Control flower showed the maximal vase life of 14.00 days (Table 9).

Table 8: Level of browning incidence of patumma flower after receiving different calcium

| | Browning incidence (%) at different days | | | | |
|---|---|---|---|---|---|
| Treatment | 2 | 4 | 6 | 8 | 10 |
| $CaCl_2$ 0.4% | 0.60 | 1.30$^{abc}$ | 3.80$^{ab}$ | 9.30$^{b}$ | 23.30$^{b}$ |
| $CaCl_2$ 0.8% | 0.00 | 2.00$^{a}$ | 6.30$^{a}$ | 14.00$^{b}$ | 36.33$^{b}$ |
| $CaH_4O_8P_2$ 0.4% | 0.00 | 0.00$^{c}$ | 1.30$^{b}$ | 12.00$^{b}$ | 26.25$^{b}$ |
| $CaH_4O_8P_2$ 0.8% | 0.20 | 1.50$^{ab}$ | 7.60$^{a}$ | 25.60$^{a}$ | 55.00$^{a}$ |
| Control | 0.00 | 0.30$^{bc}$ | 1.20$^{b}$ | 1.80$^{c}$ | 4.00$^{c}$ |
| F-test | ns | * | * | ** | ** |
| C.V. (%) | 4.75 | 1.48 | 11.97 | 4.40 | 5.89 |
| LSD | 0.2404 | 0.4777 | 1.5303 | 1.7449 | 0.6149 |

Letters within columns indicate least significant differences (LSD) at p** = 0.01, p* = 0.05, ns = non significant

Table 9: Vase life of patumma flower after receiving the different calcium solution

| Treatment | Vase life (days) |
|---|---|
| $CaCl_2$ 0.4% | 10.80$^{b}$ |
| $CaCl_2$ 0.8% | 9.40$^{b}$ |
| $CaH_4O_8P_2$ 0.4% | 9.40$^{b}$ |
| $CaH_4O_8P_2$ 0.8% | 7.60$^{c}$ |
| Control | 14.00$^{a}$ |
| F-test | ** |
| C.V. (%) | 15.18 |
| LSD | 0.4917 |

Letters within columns indicate least significant differences (LSD) at p** = 0.01

## DISCUSSION

With respect to weight loss studies during the vase life of the patumma flower, treating patumma flowers with $CaH_4O_8P_2$ at both 0.4 and 0.8% enhanced flowering weight loss in a same manner. Treating with 0.8% $CaH_4O_8P_2$ caused maximal flowering weight loss from 6 DAV through 10 DAV, while the substances of 0.4% $CaCl_2$ kept the minimum weight loss of flower until the 8 DAV. Halevy (1981) reported that one of the senescence stages in petals is the results of a loss of membrane integrity, causing increased permeability and leakage by placing flower in 0.8% $CaH_4O_8P_2$. Rasmussen *et al.* (1998) also cited that uptake of calcium solution is considered to be passive but it can be depressed by competition with other cations in their solutions. Thus, the most severe flowering weight loss from treating with $CaH_4O_8P_2$ at the high concentration of 0.8% was seen. Unfortunately, this research lacks measurement of membrane permeability as indicated by ion leakage from treating with different calcium solutions to patumma flower during storage. In contrast, Halevy *et al.* (2001) cited that calcium treatments significantly increased the longevity of both Mercedes and Baroness rose flowers. They reported that calcium enhanced the initial fresh weight increment and delayed the later reduction in fresh weight of rose flowers. Furthermore, one possible explanation is that during stress condition, most flowers were induced to lose water and this reason would bring about a premature wilting (Mayak and Faragher, 1986). Similar results were reported by Borochov *et al.* (1982) who cited that the flowers subjected to continuous stress, the rise in flowering weight loss occurred rapidly taken together. These results suggest that treating patumma flower with calcium solutions in terms of $CaCl_2$ and $CaH_4O_8P_2$ at both of 0.4 and 0.8% can be viewed an inappropriate causing water stress and associated with flowering weight loss occurring at a later stage.

For water uptake, the results showed that the Control flower tended to have high capacity and absorb the highest amount of water through its flowering stem during storage. The data from Table 2 shows that Control flowers showed the highest water uptake on 4 DAV and 6 DAV. The amount of water uptake declined rapidly by treating with $CaCl_2$ at 0.8%. These detrimental effects of both calcium solutions at 0.8% are somehow associated with a result of severe stress condition that led to damaged cell membrane permeability which in turn led to impede water absorption by the flowering stem. The water stress effect is primarily associated with the induction of an obstruction to water uptake by the flowering stem (Mayak and Faragher, 1986). Similar findings were reported by Halevy and Mayak (1979) who cited that changes in the fresh weight of the flower was mainly due to a change in water content absorbed by the flowering stem. Very limited research about the water uptake through the flowering stem and how it is influenced by these two calcium solutions has been conducted with the patumma flower.

After applying different calcium solutions, color measurement data on patumma bract in terms of L*, a* and b* are shown in Table 3, Table 4 and Table 5. The results revealed that as the vase life progressed on 8 DAV, a highly significant increase in petal blueness (b*) was observed, with the highest mean value (7.23) recorded from flower treated with $CaH_4O_8P_2$ at 0.8%. These results indicated that patumma's bracts were less red and more blue. This was observed from flowers treated with $CaH_4O_8P_2$ at 0.8%. The color of patumma bract cv. Chiang Mai Pink was a result of the anthocyanin pigments, since they were present in such large quantity (Chutichudet *et al.*, 2011b). The reason for this accounting may be due to the patumma flowers in the above treatment losing their physical properties of membrane permeability when holding in the $CaH_4O_8P_2$ solution. The changes in the physical properties of plant membrane lead in turn to overt signs of bract blueing (Barber and Thompson, 1980). These results were in line with Faragher *et al.* (1986) who found that changes in membrane permeability play a critical role in regulation of rose petal color. Faragher *et al.* (1986) also cited that the senescence of flowering bract involves an increase in cell membrane permeability to ions and other small molecules and leads to degradation of pigments and ultimately change to petal color. Zieslin (1989) reported that blueing appearance of some flowers during storage was attributed to an increase in pH (Borohov *et al.*, 1976). Generally, the blueing, a well recognized phenomenon in the senescing of red flowers, was demonstrated to be due to the increase in cell sap pH (Borochov *et al.*, 1978; Halevy and Mayak, 1979). In addition, the formation of anthocyanin-copigment complexes are greatly influenced by pH solution which the flower holds (Asen, 1976). Furthermore, Shibita *et al.* (1949) reported that anthocyanins are also very unstable and lose their red appearance over time because it is a direct variation to pH (Jiang, 2000). Structural differences of anthocyanin affect to the bract color. The increased hydroxylation results in a marked increase in bract blueness (Asen, 1975). Thus, bract color becomes bluer and quickly fades. Thus, the consideration for applying the calcium formula is an important determinant for conserving the bract color of cut patumma. The opposite results were reported by Ortiz *et al.* (2010) who cited that 2% (w/v) $CaCl_2$ solution-treated 'Fuji Kiku-8' apple fruit received the highest color acceptance scores than untreated fruit. However, there is little published information on the changes in bract color of patumma during vase life after apply these two calcium solutions.

For PPO activity, PPO extracted from the patumma bract on 6 DAV exhibited different activity levels as shown in Table 6. PPO activity from flowers treated with $CaCl_2$ and $CaH_4O_8P_2$ at both concentrations (0.4 and 0.8%) increased to high levels more rapidly than the control flowers on 6 DAV. The results indicated that $CaCl_2$ and $CaH_4O_8P_2$ at 0.4 and 0.8% applied to the patumma flowers were shown to have detrimental effects by enhancing the activity of the PPO enzyme. Thus,

both calcium solutions in this experiment may not have potential for controlling browning disorder during the postharvest life of the patumma flower. Generally, enzymatic browning of plant product was characterized by PPO activities (Peiser *et al.*, 1998; Khumjing *et al.*, 2011). Borochov *et al.* (1986) found that the natural senescence of cut flowers is associated with a decline in several parameters of membrane proteins, including PPO which is directly responsible for the browning production. After receiving any stress, decomposition of the structure of cell membranes and changes in their permeability take place (Matile and Winkenbach, 1971). Afterwards, the loss of selective permeability of cell membrane lead to hasten the activity of PPO (Beutelmann and Kende, 1977). Paulin and Droillard (1989) also reported that increase in activity of PPO enzyme during storage can be linked to the membrane degradation. Thus, it may be possible that the ion leakage loss of membrane integrity resulting from the hold flower in solutions of $CaCl_2$ and $CaH_4O_8P_2$. These characteristics led to loss of membrane integrity which is closely linked to PPO activity (Paulin *et al.*, 1986). Further research is needed in order to have a better understanding of the effect of $CaCl_2$ and $CaH_4O_8P_2$ and other possible causes on the browning of patumma bract. On the contrary, Picchioni and Watada (1998) cited that $Ca^{2+}$ pretreatment may prove to be a valuable approach for maintaining quality of fresh-cut carrots, owing to its stabilizing influence on cell membrane systems. Campbell *et al.* (1992) also reported that preharvest foliar treatment of $CaCl_2$ at 1-3% could maintain membrane integrity in processed sweet and sour cherry. These data supported by Picchioni and Watada (1998) found that calcium pretreatment (1% $CaCl_2$) to shredded carrots has been shown to improve storage quality of the tissue. This may partly result from the role of calcium in delaying plant senescence at the membrane level by maintaining the membrane integrity (Fergusan, 1984). The possibility that calcium serves an essential biological function in active membrane repair processes (Chutichude *et al.*, 2010b).

For the results on phenolic content, the results indicated that flower treated with 0.8% $CaH_4O_8P_2$ had the highest phenolic contents since 4 DAV. Generally, the oxidation of phenolic substances was catalyzed by PPO to form browning complex that accumulate during senescence (Chutichudet *et al.*, 2011b). Thus, the oxidation of phenolic compounds is a main cause of browning in fresh products (Benjawan and Chutichudet, 2009). In addition, PPO activity is closely related to the level of these phenolic substances. The results obtained from this study indicate that significant increased levels of phenolic components were activated by calcium solution, especially $CaH_4O_8P_2$ at 0.8%. A probable cause of the increase in phenolic substances from flowers treated with 0.8%$CaH_4O_8P_2$ is the fact that a high concentration (0.8%) of $CaH_4O_8P_2$ may be toxic to plant membrane. Ultimately, it affects to modify the integrity of the plant membrane and its permeability (Barthe *et al.*, 1991). These results are supported by the findings of Spanos and Wrolstad (1990) who claim that the phenolic content of pear depends primarily on the level of stress receipt. In addition, Moe (1975) also found that phenolic substances have a detrimental effect on the longevity of rose flowers. Very little is known about phenolic content in patumma flower after receiving calcium solution.

For degree of bract browning observed by visual evaluation, the results showed that all calcium solutions applied in this experiment increased the browning incidence compared with Control flower. Flower treated with $CaH_4O_8P_2$ at high concentration of 0.8% appeared to have the greatest severity of browning incidence. Benjawan *et al.* (2008) suggested that the browning incidence would result mainly from enzymatic oxidation catalyzed by PPO which involved a loss of compartmentation of enzymes and substrates. Due to cellular breakdown, it leads to a loss of compartmentation of browning-relating enzymes and substrates which results in enzymatic

oxidation and eventually causes intense browning of tissue (Ju and Zhu, 1988). The maximal browning incidence of this treatment possibly arose from the increased phenolic content which is a precursor for browning production as a consequence of increased PPO activities. It was observed that the browning of patumma flower is associated with enhanced PPO activity (Chutichudet *et al.*, 2011a). These rapid increases in PPO activity reflect the more rapid onset of browning occurrence in patumma bract during storage. This is in agreement with Brecht *et al.* (1993) who reported that similar browning damage of raw fruits and vegetables occurs during postharvest life as a result of receiving stress. Halaba and Rudnicki (1986) also claimed that the structure of plant membranes may be destroyed by water stress and osmotic stress. In addition, both phenolic contents and PPO activity were found to be closely correlated to the degree of browning (Lee *et al.*, 1990) because phenolic compounds were the major browning substrates present in plant tissues (Sun *et al.*, 2006). However, little is known about the role of calcium treatments on the attributes influencing browning disorder of patumma flower. In addition, Conway *et al.* (1995) established a close relationship between fruit calcium levels and this physiological disorder. For example, internal breakdown (Bangerth *et al.*, 1972) has been reduced significantly by postharvest calcium treatment. The relationship between calcium ions and the cell wall may partially explain the maintenance of membrane integrity, probably through the cooperative binding of calcium ions. If the extreme calcium ions are applied, it could lead to the dissociation of membrane and result in increased sensitivity to browning disorder. Similar effects were observed by Conway *et al.* (1995) who revealed that further increasing the concentration of the $CaCl_2$ solution results in a greater increase in total calcium but a significantly lower increase in cell wall bound calcium and may result in injury to the flower such as the loss of flowering cell wall degradation. The additional calcium probably remained in the intercellular spaces or negatively affected the cell membrane, resulting in flower injury (Conway *et al.*, 1995). Thus, it may be possible that the patumma flower may be susceptible to browning incidence after receiving 0.8% $CaH_4O_8P_2$ due to an increase of both PPO activity and phenolic content and these facilitate the appearance of the browning disorder. This is consistent with the observation of Munoz-Munoz *et al.* (2009) who found that some phenolic compounds have been shown to be good substrates involved in the oxidation of polyphenols by PPO enzymes (Ke and Saltveit, 1989). The similar results were reported by Chisari *et al.* (2010) who cited that a general increase of phenolic content was noticed in correspondence of maximum values of PPO activity. Thus, these coincidence of high PPO and phenolics enabled the patumma flower to become more susceptible to enzymatic browning. Khumjing *et al.* (2011) reported that there was an apparent beneficial effect of $CaCl_2$ at 2% for controlling browning disorders in 'Grand Rapids' lettuce while calcium application, in terms of $Ca(NO_3)_2$ at 2%, had a detrimental effect on browning appearance in lettuce by increasing the browning appearance to the highest degree of 25.33%. However, the appropriate calcium concentration for controlling the browning incidence of patumma flower has not been elucidated.

For vase life, the results showed that patumma's vase life was reduced by treating with these two calcium solutions. The shortest vase life of patumma flower was observed from treating with 0.8% $CaH_4O_8P_2$ (7.60 days). While the Control flowers showed the longest vase life of 14.00 days. Marschner (1995) found that since calcium transport in the phloem is extremely low. These results corresponded to Yang and Jie (2005) who reported that when calcium moving into the xylem in flowering stem for absorption, $Ca^{2+}$ is transported with the xylem stream via mass flow of water. Thus, high transpiring tissues having high influx of calcium via xylem transport also can be sensitive to calcium excessive. In addition, Yanga *et al.* (2011) cited that the calcium-excessive

disorders are mostly caused by fast absorption and sudden transport and distribution in the plant. Thus, the inappropriate calcium solution absorbed by the flowering stem induced toxic change in the osmotic potential of the flower tissues which caused a detrimental effect to plant tissue (Altunkaya and Gökmen, 2008). The shortest storage life of flower treated with 0.8% $CaH_4O_8P_2$ may be accompanied by the maximal traits of weight loss, phenolic contents corresponding with the occurrence of the highest severity of browning incidence which accelerated flower senescence. These results were in agreement with Lester (1995) who reported that muskmelon fruit senescence is affected by calcium application. Calcium chloride applied at 0.04 M could delay the fruit aging. While the spray calcium chloride at concentration of 0.16 M hastened the senescence of muskmelon fruit by promoting the rate of plasma membrane degradation and possibly the osmotic toxicity of the cell. However, the disadvantage of $CaCl_2$ and $CaH_4O_8P_2$ at 0.4 and 0.8% involvement in the browning damage and shortening vase life of patumma flower is not fully understood and requires further investigation.

## CONCLUSION

In conclusion, application calcium solutions in the form of $CaCl_2$ and $CaH_4O_8P_2$ at 0.4 and 0.8% may not be the appropriate substances for applying to patumma flower. The results showed that flower treated with $CaH_4O_8P_2$ at 0.8% had the highest significant increases of flowering weight loss, phenolic content, browning occurrence and the shortest vase life.

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