International Journal of Agricultural Technology 2016 Vol. 12(6): 1139-1151
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Effect of Storage Time on some Quality Parameters of Calamondin (*Citrus Madurensis* Lour.) Squash Stored at Room Temperature

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Nonmuang, W., Pongsumran, S. and Mongkontanawat, N. (2016). Effect of storage time on some quality parameters of calamondin (*Citrus madurensis* Lour.) squash stored at room temperature. International Journal of Agricultural Technology 12(6):1139-1151.

Abstract Calamondin is a citrus fruit; known as one of the healthiest fruits suitable to make a drink, not only due to its high vitamin C content, but also its distinctive aroma and test. This study reported for some physical, chemical, and microbiological properties of 43^{o}B and 60^{o}B calamondin squash during 10-week storage at room temperature. The results showed that the L* values of 43^{o}B and 60^{o}B calamondin squash varied between 6.17-9.02 and 3.04-8.76, respectively. On week 6-10, b* values showed significantly lower compared to b* values on week 0. The sediment appeared on top of the bottle during storage. Storage time had no effect on total soluble solid and pH. Titratable acidity and ascorbic acid content declined with increase in storage duration on weeks 2-6. After 10 weeks, total microorganism including yeast and molds of calamondin squash were in the standard level.

Keywords: *Citrus madurensis* Lour., squash, properties, storage time

Introduction

Calamondin or calamansi (*Citrus madurensis* Lour.) is a mandarin-like fruit but quite smaller (2.71-3.62 cm in diameter) weighing about 10-30 g with green-orange peel color that is smooth and very thin (1-2 mm). The Fruit has seeds and orange colored flesh, which has acidic taste and edible peel. Calamondin is commonly grow in sounthern China, Taiwan, Japan, Philippines, Northern India, Vietnam and Malaysia (Ladaniga, 2008 and Chen, 2013).

Calamondin is used for culinary purposes and for making marmalade (Ladaniga, 2008). In Taiwan, It is mainly used for making fruit tea and candied fruits (Chen, 2013). The fruit can be squeezed for juice which has the combination of a sweet mandarin-like aroma with a zesty tase of lime, a slightly

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peel-like note of orange and a hint of acidic astringency (Cheong *et al.*, 2012), low content of sugars (glucose, fructose and sucrose), high level of acids such as ascorbic (44.5 mg/100 g), dehydroascorbic (2.2 mg/100 g) and citric (3.6%) (Nisperos-Carriedo *et al.*, 1992). Calamondin juice is characterized by its high acidity, as reflected by the high citric acid content (Nisperos-Carriedo, 1992). Thus, calamondin drinks are currently popular (Chen, 2013).

Squash is non-alcoholic concentrated syrup used in beverage making. It is usually fruit-flavoured; made from fruit juice, water, and sugar or a sugar substitute (e.g., orange squash, lemon squash, mango squash, etc.). Modern squashes may also contain food colouring and additional flavouring. Some traditional squashes contain herbal extracts, most notably elderflower and ginger. Squash is prepared by combining one part concentrate with four or five parts of water or carbonated water. As a drink mixer, it may be combined with an alcoholic beverage to prepare a cocktail (Desai, 2000 and Squash, n.d.)

Consumers demand for fruit juices have persisted because of their nutritional, health, and wellness benefits. Specifically, citrus juice is valued for its high amounts of vitamin C, carotenoids, natural antioxidants, and its distinctive aroma and taste (Abeysinghe *et al*., 2007; Bull *et al*., 2004; Pala and Toklucu, 2013; Rouseff *et al*., 2009). From microbial safety point of view, given the fact that orange juice is categorized as high acid food (pH<4.6), intensively pasteurized orange juice is stable at room temperature (Silva & Gibbs, 2004). However, the quality of most food products decreases during storage, especially vitamin C. Ascorbic acid or vitamin C is the water soluble vitamin that is essential for human health, highly sensitive to various modes of degradation. Factors that can influence the nature of the degradative mechanism include temperature, salt and sugar concentration, pH, oxygen, enzymes, metal catalysts, initial concentration of ascorbic acid, and the ratio of ascorbic acid to dehydroascorbic acid (Tannenbaum *et al.*, 1985).

In Thailand, the local research area, Chantaburi province, perfect of calamondin are found. The fruit is used in cooking instead of lemon. Currently, calamondin products are not widely available in any convenience stores and supermarkets. However, its characteristics such as high in vitamin C, sour taste, and distinctive aroma. In addition, calamondin squash is a newly value-added product from calamondin; fruit drink from squash is easy for consumers to prepare just by diluting it with water.

The objectivse of this study were to produced calamondin squash, packed in a bottle and studied on physical, chemical and microbiological properties during storage at room temperature.

Materials and methods

Materials

Calamondin fruit was purchased from a local market in Amphure Kao Kitchakut, Chanthaburi province in Thailand, and were then transported to the laboratory.

Sample preparation

Calamondin fruits were randomly selected in the same size and washed with tap water. The fruits were cut into halfves using a knife and then squeezed with a plain orange juicer. The properties for the obtained juice were presented in Table 1.

Table 1. Chemical properties of calamondin juice

Chemical properties	data±standard deviation (SD)
Total soluble solid (^{o}B)	7.90±0.11
pH	2.57±0.68
Titrable acidity (%)	0.92±0.66
Ascorbic acid (mg/100 ml)	22.58±0.66

Calamondin squash preparation

From a previously popular squash formula, the researchers selected two formula preparations such as 43^{o}B and 60^{o}B. The formula of calamondin squash was exhibited in Table 2.

The squash preparation was prepared as follows. The water was heated and then added with sugar. The calamondin juice was filled and pasteurized at 85^{o}C for 15 minutes. The obtained calamondin squash was filled in sterilized glass bottles (280 ml) under a hot condition. Then, the squashs were cooled down in water. The samples were stored at room temperature (30±5^{o}C) and were determined for their physical, chemical and microbiological properties every 2 weeks of the 10-week storage as described below.

Table 2. The formula of calamondin squash

Treatments	°Brix	Formula		
		Water (gram)	Sugar (gram)	Calamondin juice (gram)
1	43	3,540	3,180	1,260
2	60	1,425	4,320	1,680

Physical properties determination

The color of the samples was determined using a Color meter (Nippon Denshoku, ZE-2000, Japan). The equipment was calibrated with a standard plate. Color measurement was expressed in L* and b* where L* refers to the lightness on a 0 to 100 scale from black to white, while b* (+,-) refers to the yellowness or blueness, respectively.

The stability of calamondin squash was determined through its sedimentation as observed during 10 weeks of storage; height of sediments was measured as well.

Chemical properties determination

The total soluble solid was evaluated using a hand refractometer (Atago, Japan), the pH with a pH-meter (PB-11 Sartorius, Thailand), and titratable acidity and ascorbic acid content were determined according to AOAC (2000).

Microbiological properties determination

Total microorganism, yeast and molds were determined by total plate count on plate count agar (PCA) and potato dextrose agar (PDA), respectively.

Data analysis

Analysis of the abovementioned properties were carried out in three replicates. The data were subjected to analysis of variance (ANOVA) ($p \leq 0.05$). Mean with significant differences was separated by Duncan's multiple range test (DMRT) using computer software.

Results and Discussion

Physical properties of calamondin squash

The color of calamomdin squash was shown in Figures 1 and 2, in terms of L* and b* values. The L* values of 43^{o}B and 60^{o}B calamondin squash were found to vary between 6.17-9.02 and 3.04-8.76, respectively. The increase can be attributed to partial precipitation of unstable suspended particles, but the decrease may be caused by the browning reaction (Tiwari *et al*., 2010 and del Socorro Cruz-Cansino *et al.*, 2015). Elez-Martinez *et al*. (2016) stated that ascorbic acid degradation could be responsible for significant color changes in heat-pasteurized juice stored at 22^{o}C. Ascorbic acid degradation provides reactive carbonyls groups which in turn act as precursors that play a major role in the darkening of citrus juice (Fustier *et al*., 2011 and Yeom *et al*., 2000). The acid-catalysed degradation of sugars and ascorbic acid degradation reactions appeared to be important for browning development in pasteurised orange juice during ambient storage (Wibowo *et al*., 2015).

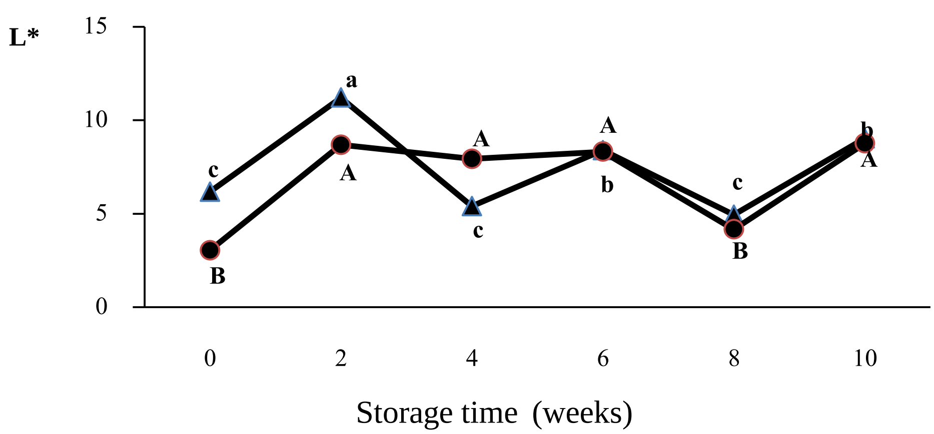

Figure 1. The L* of calamondin squash during storage at room temperature for 10 weeks; lower case letters for 43^{o}B calamondin squash (▲) and upper case letters for 60^{o}B calamondin squash (●) showed the difference among weeks ($p \leq 0.05$)

The b* values of 43^{o}B and 60^{o}B calamondin squash had changed in the same way. At the beginning of storage, b* values were found to vary but on week 6-10 showed constant values and significantly lower compared on week 0. This could be due to the degradation of carotenoids (Khandpur and Gogate, 2016). So, the yellow colour could be decreased.

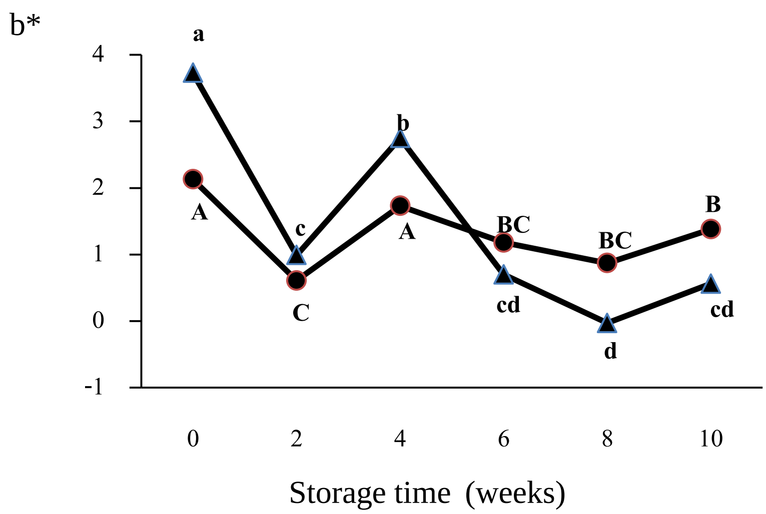

Figure 2. The b* of calamondin squash during storage at room temperature for 10 weeks; lower case letters for 43^{o}B calamondin squash (▲) and upper case letters for 60^{o}B calamondin squash (●) showed the difference among weeks ($p \leq 0.05$)

The sedimentation of 43^{o}B and 60^{o}B calamondin squash were observed during 10 weeks of storage. In 43^{o}B calamondin squash, the sediment was found on top of the squash at week 4, more sediment when stored longer; the height of the sediment was 0.53 cms at week 10. In 60^{o}B calamondin squash, sediment was found on top of the squash at week 2, more sediment when stored longer; the height of the sediment was 1.4 cms at week 10 (**Fig. 3**). To prevent sedimentation and preserve cloudiness, stabilizer e.g. xanthan and carboxymethyl cellulose (CMC) could be added into calamondin squash (Ibrahim *et al*., 2011).

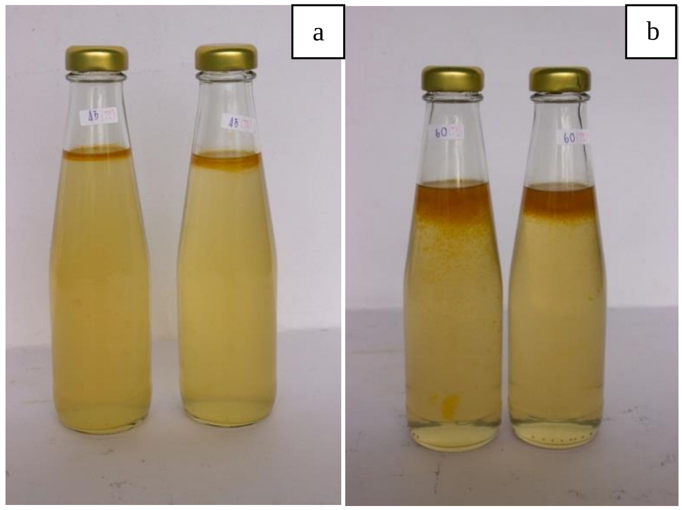

Figure 3. The sedimentation of 43^{o}B (a) and 60^{o}B (b) calamondin squash during storage at room temperature for 10 weeks.

Chemical properties of calamondin squash

Total soluble solid of calamondin squash were exhibited in Figure 4. No significant changes were found. Total soluble solid of 43^{o}B calamondin squash ranged between 43.0-43.1. Total soluble solid of 60^{o}B calamondin squash were 60 throughout the storage of 10 weeks. Value of the total soluble solids was almost constant during the storage period attributed to the lack of microbial growth responsible for altering the Brix value (Yeom *et al.*, 2000). pH of calamondin squash were presented in Figure 5 The numbers of pH were slightly decreased but nonsignificantly different during longer storage time. The pH of 43^{o}B and 60^{o}B calamondin squash ranged between 2.26-2.39 and 2.24-2.40, respectively. This result is similar to pH with orange juice pasteurization at 90^{o}C for 20 seconds and was stable during storage time of 180 days (Agcam *et al.,* 2016). Titratable acidity declined with the increase in storage duration on weeks 2-6 but no significant changes were found in 60^{o}B calamondin squash (Figure 6). No significant changes were found in total soluble solid, pH, and titratable acidity in lycopene fortified guava beverage during storage at room temperature (Pasupuleti and Kulkarni, 2013). The obtained results for the changes in ascorbic acid content were given in Figure 7. It was observed that the ascorbic acid content in 60^{o}B calamondin squash was higher compared to 43^{o}B calamondin squash because the quantity of the calamondin juice used in 60^{o}B was higher than in the 43 ^{o}B. Ascorbic acid content of 43^{o}B calamondin squash and 60^{o}B calamondin squash decreased on weeks 0-4 and showed constancy on weeks 4-10. At week 4, ascorbic acid content of 43°B calamondin squash and 60°B calamondin squash showed loss of ascorbic acid, 7.24 mg/100 ml (89%) and 15.36 mg/100 ml (84%), respectively. Choi *et al.* (2002) found that ascorbic acid loss would be more than 50% for 3 weeks and 100% for 5 weeks during refrigerated storage (4.5°C) of pasteurized juice (at 90°C for 90 seconds). The decrease in ascorbic acid during storage may be explained by oxidative and enzymatic degradation in calamondin squash in which endogenous enzymes are active (del Socorro Cruz-Cansino *et al*., 2015 and Phillips *et al.*, 2016).

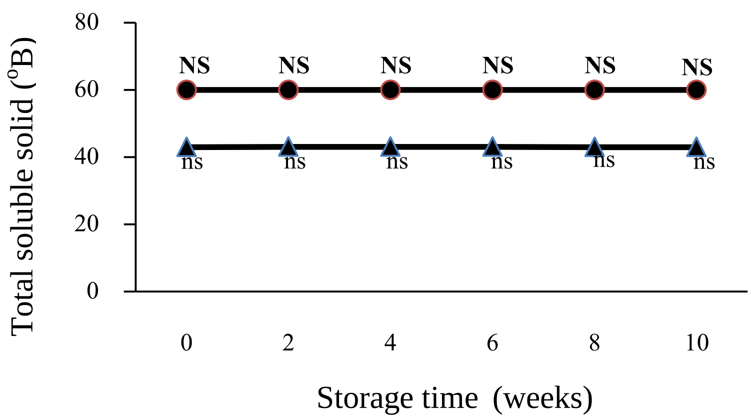

Figure 4. Total soluble solid of calamondin squash during storage at room temperature for 10 weeks; lower case letters for 43°B calamondin squash (▲) and upper case letters for 60°B calamondin squash (●) showed the difference among weeks ($p \leq 0.05$)

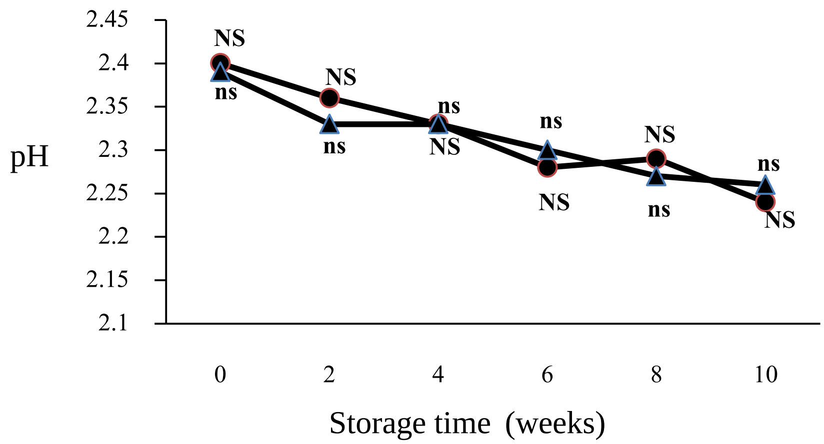

Figure 5. The pH of calamomdin squash during storage at room temperature for 10 weeks; lower case letters for 43^{o}B calamondin squash (▲) and upper case letters for 60^{o}B calamondin squash (●) showed the difference among weeks (p≤0.05)

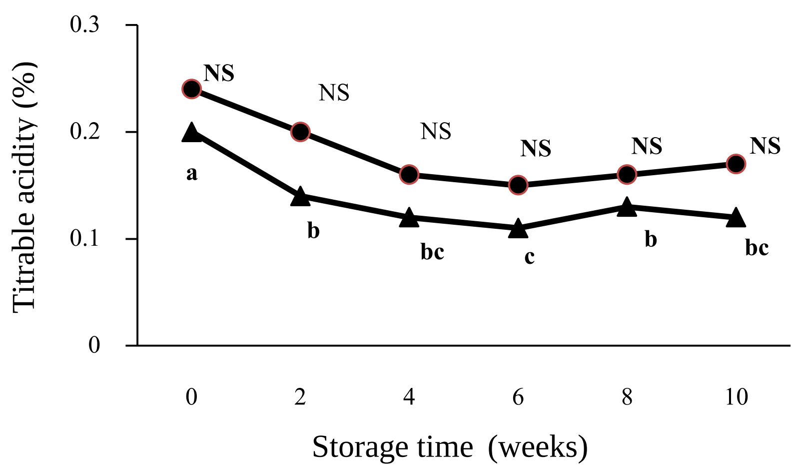

Figure 6. Titrable acidity of calamondin squash during storage at room temperature for 10 weeks; lower case letters for 43^{o}B calamondin squash (▲) and upper case letters for 60^{o}B calamondin squash (●) showed the difference among weeks (p≤0.05)

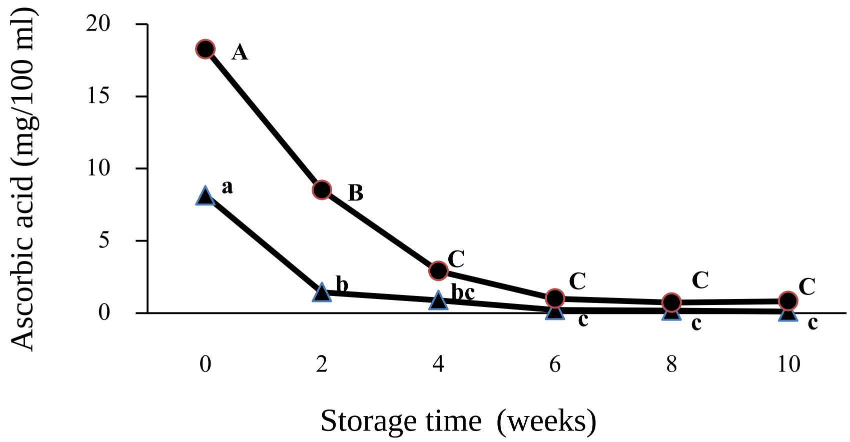

Figure 7. Ascorbic acid of calamomdin squash during stored at room temperature for 10 weeks; lower case letters for 43°B calamondin squash (▲) and upper case letters for 60°B calamondin squash (●) showed the difference among weeks ($p \leq 0.05$)

Microbiological properties of calamondin squash

As seen on Table 3, the microbial growth (total microorganism, yeast and molds) in 43°B calamondin squash and 60°B calamondin squash had the same microbial counts during storage. Total microorganism and yeast and molds showed non detect on week 0 and <30 CFU/ml on weeks 2-10, complying with the Thai community product standards for squash, as it the quash did not exceed 10^4 CFU/g for total microorganism and 10^2 CFU/g for yeast and molds.

Table 3. Total microorganism and yeast and molds of calamondin squash during storage at room temperature for 10 weeks

No. of weeks	**Total microorganism (CFU/ml)**		**yeast and molds (CFU/ml)**	
	43°B	60°B	43°B	60°B
0	nd	nd	nd	nd
2	<30	<30	<30	<30
4	<30	<30	<30	<30
6	<30	<30	<30	<30
8	<30	<30	<30	<30
10	<30	<30	<30	<30

nd, no detection

Conclusion

The evolution of quality parameters of 43°B and 60°B calamondin squash stored at room temperature were studied in this research paper. The results showed that storage time had effect on L* and b* values. The L* values of 43°B and 60°B calamondin squash varied between 6.17-9.02 and 3.04-8.76, respectively. Both 43°B and 60°B calamondin squash showed low b* values on prolong storage. During storage, the sediment appeared on top of the bottle. There was less sediment in 43°B calamondin squash than in 60°B calamondin squash. Storage time had no effect on total soluble solid and pH. Titratable acidity declined in the early storage duration. The ascorbic acid content in 60°B calamondin squash was higher than 43°B calamondin squash. Ascorbic acid content of 43°B and 60°B calamondin squash declined sharply on weeks 0-4. After 10 weeks, total microorganism and yeast and molds of calamondin squash showed <30 CFU/ml. This research is a good preliminary study on the quality of the new calamondin squash product during storage. Squash should be consumed within two weeks because of the ascorbic acid content which remains high even after long time storage duration.

Acknowledgement

The authors would like to thank the Rajamangala University of Technology Tawan-ok for the financial support.

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(Received: 27 October 2016, accepted: 5 November 2016)