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EFFECTS OF CALCIUM ASCORBATE AND CALCIUM LACTATE ON QUALITY OF FRESH-CUT PINEAPPLE (*Ananas comosus*)

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Abstract

Due to increasing demand of fresh-cut fruits and vegetables or minimally processed foods that are convenient with fewer preservatives, high nutritive value, and fresh sensory attributes, the fresh-cut produce industry is one of the fastest growing food industries worldwide. This study was conducted to determine the antimicrobial efficacy of calcium ascorbate and calcium lactate on fresh-cut pineapple and evaluate the physicochemical and sensory qualities of the treated and untreated produce. Freshly harvested ripe 'Queen' pineapple fruits (25-60% of the eyes yellow) were obtained from a local plantation, peeled, and sliced as commercially practiced. Calcium ascorbate and calcium lactate were applied at 1.5-2.5% as 2-min dip. Control treatments included 150 ppm chlorine and distilled water as 2-min dip. The product samples were packed in sterile resealable 50 µm-thick polyethylene bag and stored at 7-10 °C for 5 days. Calcium ascorbate and calcium lactate were effective in reducing aerobic and coliform bacteria as well as yeasts and molds at 2.5% concentration. The antimicrobial activities of these organic salts were comparable to chlorine after 3 hours from treatment but were much better than chlorine after 5 days of storage. Meanwhile, the physicochemical properties (color evaluation, titratable acidity, and pH) and sensory attributes (color, aroma, taste, texture, and general acceptability) of fresh-cut pineapple after 5 days of cold storage were not adversely affected using calcium ascorbate.

Key words: Ananas comosus, organic salts, antimicrobials, postharvest quality, pineapple

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INTRODUCTION

Regular consumption of fruits and vegetables has been negatively correlated with the risk of the development of chronic diseases (Liu, 2013). Their consumption has continued to grow rapidly linked to the increased public awareness of their health benefits, even if it remains below the recommended daily intake in many countries, due to barriers such as complacency and lack of willpower to change the diet (Ragaert et al., 2004). In particular, fresh-cut fruits attract consumers because they are fresh, convenient (ready-to-eat or ready-to-serve), low priced, nutritious and with fewer preservatives. Due to increased demand, minimally processed fresh-cut fruits have acquired the swift trend among consumers (Rico et al., 2007). Some examples of minimal processing includes; washing, cleaning, sorting, grading, peeling, cutting and slicing. However, this new trend, has also hiked the chances of outbreaks of food poisoning and food infection related to consumption of fresh-cut fruits and uncooked salads (Bhagwat, 2006). As a consequence, a wide assortment of minimal processing methods has been developed to meet consumers' needs for a fast and convenient products, and to benefit from fruits' healthy image (Ahvenainen, 1996). Furthermore, the hurdle technology looks to the combination of different techniques as a preservation strategy: the intelligent selection of hurdles in terms of the number required, the intensity of each and the sequence of applications to achieve a specified outcome are expected to have significant potential for the future of fresh-cut produce (Corbo et al., 2010).

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Several hurdles were encountered in minimal processing of fresh-cut fruits due to the difficulty in preserving their freshness during prolonged periods. These products, in fact, are characterized by a shorter shelf life than their whole counterparts, because of higher susceptibility to microbial spoilage, increased respiration rate and ethylene production, which is stimulated by wounding of the tissue; in fact, the process operations (i.e. cutting, splicing, etc.) form lesions in the tissue that determine enzymatic browning, texture decay, rapid microbial growth, weight losses and undesirable volatile production, thus reducing highly the shelf life. (Chien et al., 2007).

Antimicrobial treatment is applied extensively in the food industry due to mounting concerns over food microbial contamination and human infection risk (Rico et al., 2007; Vandekinderen et al., 2009). Different washing agents have been studied to determine their efficacy in the inactivation of pathogenic bacteria on fruits and vegetables (Alvarado-Casillas et al., 2009; Ruiz-Cruz et al., 2007). Although, chlorine which is still considered the most commonly used sanitizer due to its simple use, cost effectiveness and its efficacy, future regulatory restrictions will require the development of functional alternatives. Chlorine reacts with natural compounds in water or in food product to form trihalomethanes (THM), highly carcinogenic disinfection byproducts (Bond et al., 2012).

Alternative antimicrobial agents have been identified, natural organic salts have potential for minimally processed products. In fresh-cut eggplant, the antioxidant calcium citrate and ascorbate reduced enzyme activities responsible for browning and softening with (0.4%) and improved consumer acceptance (Bargallo et al., 2012). Manganaris et al. (2007) compared the effect of calcium lactate, calcium chloride, and calcium propionate dipping in peaches. Calcium increased in tissues with no dependence on the source used. Saftner et al. (2003) found treatments with calcium inhibited colour changes and development of tissue translucency in honeydew chunks. Also, the use of phosphorous-free sources of calcium, such as gluconate, citrate, lactate, acetate and carbonate calcium salts, can help to obtain a good balance of calcium and phosphorous in the diet (Cerklewski, 2005). For this reason, the use of natural sources of calcium as preservative with a nutritional fortification effect presents an advantage for the industry and for the consumer. Thus, this study aimed to determine the antimicrobial efficacy of calcium ascorbate and calcium lactate on physicochemical and sensorial quality of fresh-cut pineapple.

MATERIALS AND METHODS

Sample preparation, storage, culture incubation, microbial processing and microscopic examination of fresh-cut pineapple were conducted at the Postharvest Technology Laboratory, Department of Horticulture, Visayas State University (VSU); while culture medium preparation, inoculation and fruit sample storage were accomplished at the Tissue Culture Laboratory, Department of Horticulture, VSU; and Gram staining, microbial enumeration and examination, at the Department of Pest Management, VSU. The experiment was conducted in a completely randomized design (CRD) with three replications. Whole ripe fruits (about 25% shell yellowing) of pineapple cultivar 'Queen' were collected directly from the SAL's pineapple plantation in Ormoc City, Leyte, Philippines. Pineapple fruits were sorted and peeled under sanitary conditions using a sharp clean knife, sliced longitudinally into quarters; each quarter was further cut into about 1.0 cm slices. The slices were immersed as three replication to two concentrations (1.5 and 2.5%) of two organic salts (calcium ascorbate and calcium lactate) for 2 min (Weaver and Shelef, 1993; Aran, 2001), and were compared with 150 ppm sodium hypochlorite and distilled water which were used as control. Samples were then packed in sterile, resealable 50 μm thick polyethylene bags at 7-10°C. Microbial enumeration following the US-FDA (2018) methods; plate count agar (total aerobic bacteria), violet red bile agar (coliform), and potato dextrose agar (yeast and molds) were done after 3 h from treatment and after five days of storage under ambient conditions (27-33°C). Product quality was evaluated in terms of the physicochemical qualities; color using a Minolta CR-13 colorimeter, total soluble solids (TSS) using an Atago N1 Hand Refractometer, pH using an Orion pH meter model 210 A+, titratable acidity (% citric acid) by titration using standard 0.1 N NaOH and 1% phenolphthalein indicator, and for sensory qualities (color, aroma, taste, texture, and aftertaste) using 15 trained panelists and 1-5 rating scale describing the variation in each attribute (Barrett et al., 2010) and 9-point Hedonic scale for acceptability of each attribute; the general acceptability was also evaluated using a 9-point Hedonic scale (Stone and Sidel, 1992). Statistical analysis of results used the CROPSTAT version 7.2 program of IRRI (http://bbi.irri.org/products).

RESULTS AND DISCUSSION

Microbial Load

Calcium ascorbate and calcium lactate at 2.5% concentration significantly reduced total aerobic, coliform, and yeast and molds count after 3 hours from treatment. The effects of all organic salt treatments were comparable to that of chlorine. After 5 days of storage, calcium ascorbate and calcium lactate (1.5-2.5%) remarkably reduced aerobic, coliform, and yeast and molds count relative to water wash and chlorine (Fig. 1 & 2). Aerobic contaminants and Coliform contaminants were mostly Gram-negative long rods and a few short rods. Fungal contaminants were Penicillium sp. and Aspergillus sp. which appeared as white and greenish colonies.

Organic salts which include calcium-based compounds (e.g. calcium ascorbate and calcium lactate) have been used to extend the shelf-life of fruit and vegetables (Luna-Guzman and Barrett, 2000; Manganaris et al., 2007; Martin-Diana et al., 2005; Soliva-Fortuny and Martin-Belloso, 2003). Calcium helps maintain cell wall integrity by interacting with pectin to form calcium pectate. Different organic salts have been studied for decay prevention, sanitation and nutritional enrichment of fresh-cut fruits and vegetables. Most of these calcium-based solutions were used more when the objective is the preservation and/or the enhancement of the product firmness. In present study, the result showed that both calcium ascorbate and calcium lactate significantly inhibited microbial proliferation, particularly when higher concentration of 2.5% was used. This signifies that these organic salts could be used as an effective alternative to chlorine in decontaminating fresh-cut pineapple.

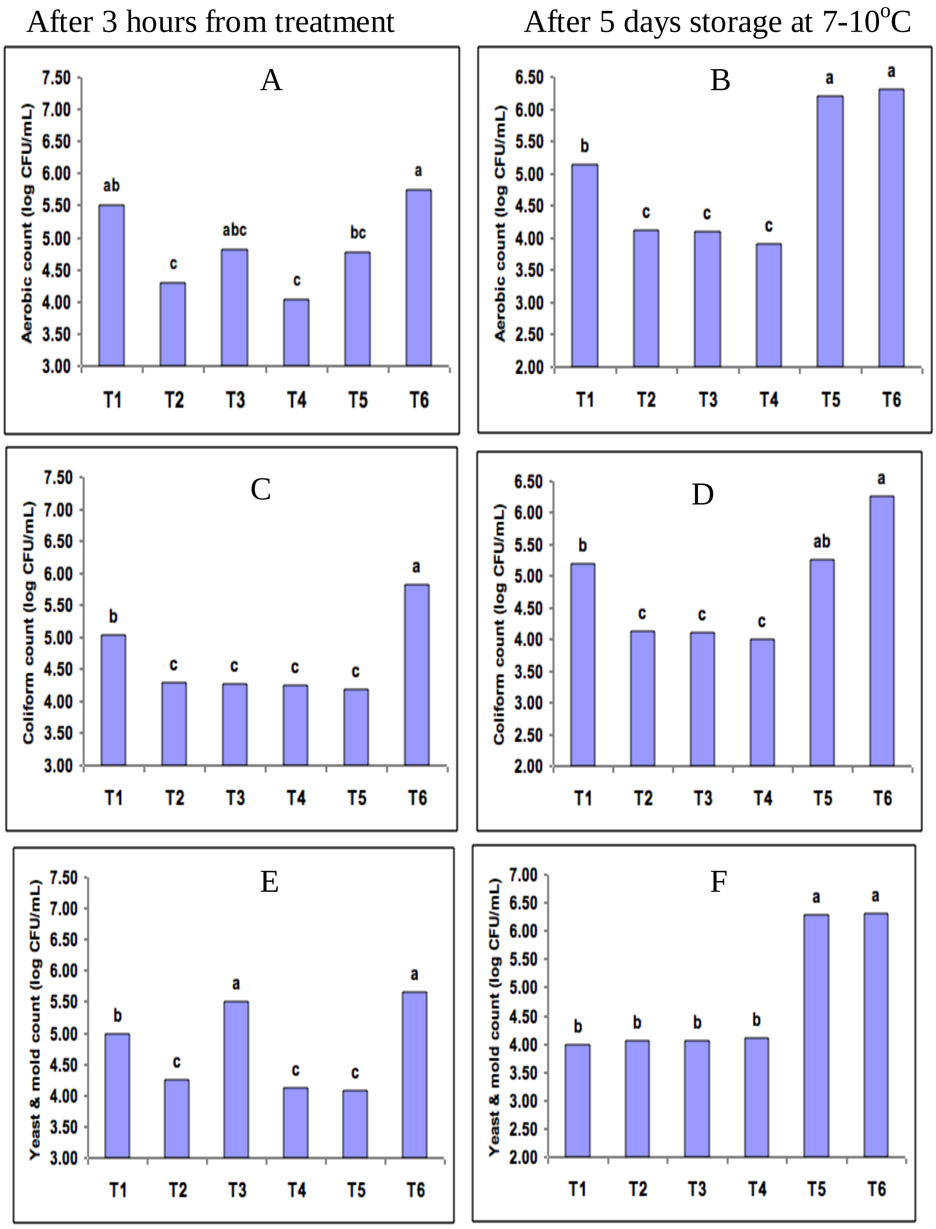

Figure 1. Microbial load on organic salt-treated and untreated fresh-cut pineapple. NOTE: T_1-Ca ascorbate 1.5%; T_2-Ca ascorbate 2.5%; T_3-Ca lactate 1.5%; T_4- Ca lactate 2.5%; T_5-Chlorine; T_6-Water. Treatment means w/ same letter are not significantly different using LSD-5%, % CV: A-11.1; B-7.4; C-7.5; D-12.0; E-5.0; F-2.6)

The antimicrobial effect of organic salts (ascorbate and lactate) has also been obtained in previous studies of Mao et al. (2006) and Aguayo et al. (2008, 2010). The concentrations of the organic salts used as washing treatments were usually within a range of 0.5-3% and dipping time ranges from 1-5 minutes (Martin-Diana et al., 2007). Calcium lactate greatly reduced the growth of aerobic bacteria and coliforms after 3 hours of storage due to the increase in the acidity level of pineapple. Similar results were also observed on the effect on the growth of yeast and molds after 5 days of storage. It was found out that microorganisms were significantly inhibited and enormously reduced its growth and development.

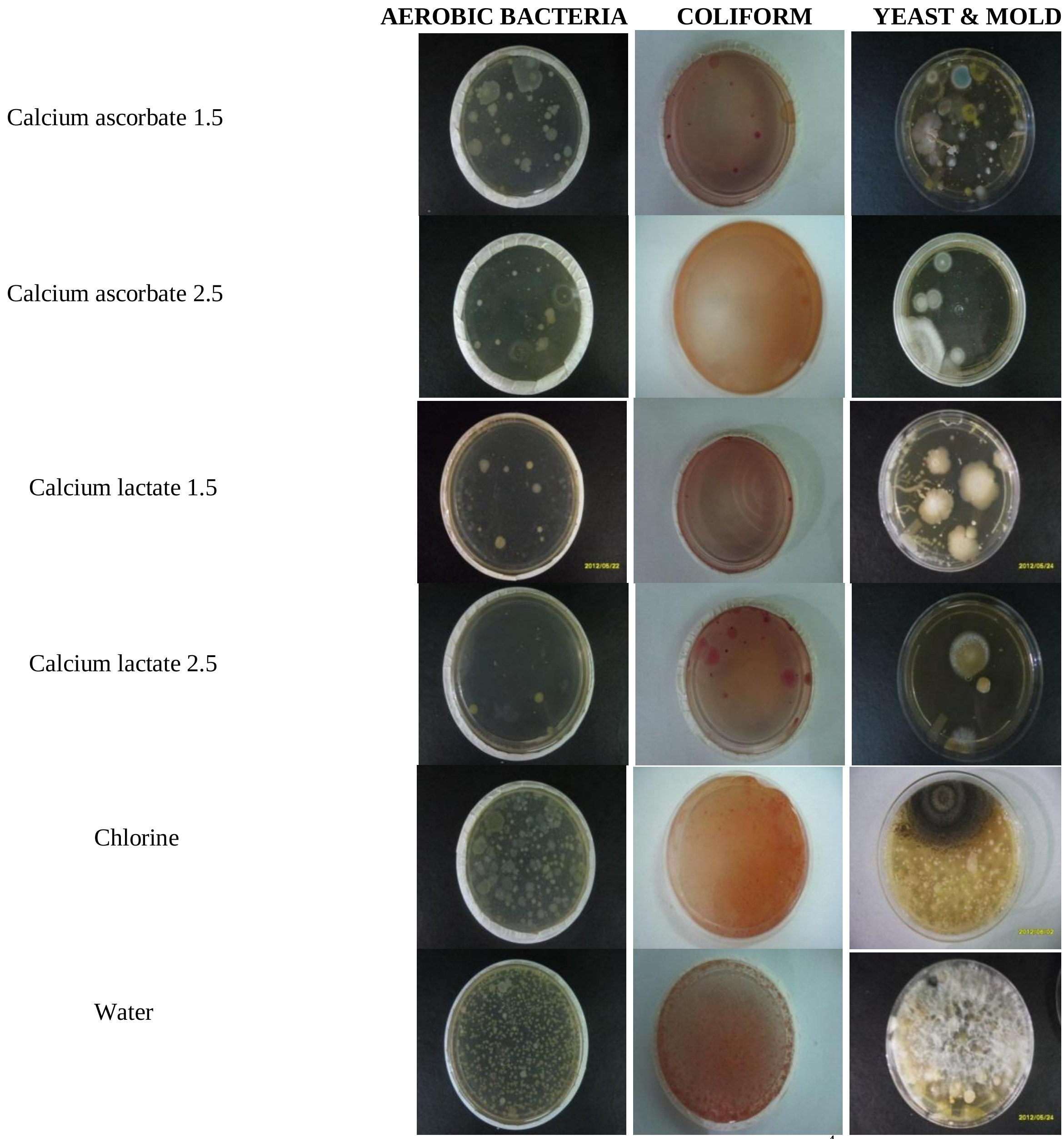

Figure 2. Aerobic & coliform bacteria and yeast & mold colonies from cultured sample 10^{-4} dilution from organic salt-treated and untreated fresh-cut pineapple after 5 days storage at 7-10^{o}C

As days of storage increased, the efficacy of calcium lactate was inevitably stronger that resulted to the inhibitory effect on the growth of microbes. The inhibitory effect of calcium salts on microbial growth has been related to cell wall stability by ionic calcium and polygalacturonic chains, since calcium increases the rigidity of the cell wall and middle lamella and therefore the resistance to microbial enzymes responsible for cell wall degradation and tissue softening (Saftner et al., 2003). Antimicrobial properties of calcium lactate have been reported by Gorny et al. (2002). Calcium lactate was tested on fresh-cut lettuce and carrots and compared with chlorine. As alternative to chlorine, calcium lactate showed no differences in affecting the quality of the product, and both treatments showed similar effectiveness in reducing the microbial load. Calcium lactate is a form of calcium that is produced when lactic acid reacts with calcium carbonate.

Similar to calcium lactate, calcium ascorbate was found to have the same effect as chlorine in reducing microbial load (Gomes et al., 2010). It was revealed that calcium ascorbate reduced microbial counts at a pH range of 3-7 whereas the effect of calcium lactate was pH-dependent, indicating that the anion is responsible for the antimicrobial activity of calcium salts. An interaction between pH and calcium salt for antimicrobial activity is expected when the salt is derived from an organic acid, given the effect of pH on the dissociation of acids. Calcium ascorbate is commonly used as an antioxidant, preservative and source of Vitamin C in foods. In a study of Caballero (2009), calcium ascorbate was sprayed on fresh-cut fruits and vegetables to increase shelf-life and was found to be especially effective at preventing apples from turning brown. The inhibition of aerobic bacteria and coliforms was triggered by some important factors; one factor was the reduction of pH. The inhibitory effect depends on the type of acid and the concentration. Gram negative bacteria such as Pseudomonas and Acinetobacter were inhibited at pH < 5.3 while Gram positive bacteria can be only inhibited at pH < 4.

Chlorine treatment is traditionally applied to decontaminate fresh produce (Watada and Qi, 1999). In the present study, chlorine application on fresh-cut pineapple significantly reduced the microbial population comparable to calcium ascorbate and calcium lactate, after 3 hours from treatment application. However, after prolonged storage (5 days), antimicrobial effectiveness of chlorine depreciated similar to water wash. Beuchat and Bracket (1990) revealed that chlorine wash could reduce microbial load, but not necessarily kills the microorganism such as Listeria on fresh-cut produce. Sanitizers such as chlorine do not eliminate microorganisms inside the tissue. Babic et al., (1996) found no microbial population on the surface of spinach washed with sodium hypochlorite, but when tissues were analysed with low temperature scanning electron microscopy showed a mass of microbes immediately below or adjacent to minute cracks of the epidermis having 10^6 to 10^7 CFU/g population of mesophilic and psychrotrophic aerobic microflora was present in the spinach. Apparently, the microorganisms entered the tissue through the cracks, but the chlorine solution was not able to penetrate these areas sufficiently. In the present study, microorganisms that were not killed during treatment with chlorine may have penetrated the tissue through cracks, since fresh-cut pineapple has many deep cracks and fissures, which resulted to high microbial population after 5 days of storage.

Product Quality

The physicochemical qualities of fresh-cut pineapple treated with organic salts did not significantly affect color (b^* and L^*) and TA (Table 1). Total soluble solids (TSS) generally decreased with storage and the greatest reduction was obtained in the water and chlorine treatments. This was accompanied by significant increases in pH. These results indicate more advanced tissue senescence in the two control treatments as compared to that of the organic salt treatments. For the sensory characteristic, significant treatment differences in color, aroma, taste, texture and general acceptability were obtained (Table 2). In general, these sensory attributes were scored either better than or comparable to that of water or chlorine treatment.

Table 1. Colorimetric b* and L* values, total soluble solids (TSS), pH and titratable acidity (TA) of organic salts-treated and untreated fresh-cut pineapple before and after 5 days storage at 7-10^{o}C

TREATMENTS	b^*	L^* (lightness)	TSS (^{o}Brix)	pH	TA (%)
Calcium ascorbate 1.5%	21.03	65.00	13.87a	3.73c	0.43
Calcium ascorbate 2.5%	25.47	70.33	10.87b	3.75c	0.46
Calcium lactate 1.5%	19.37	65.13	11.60b	3.74c	0.43
Calcium lactate 2.5%	24.03	67.03	10.33b	3.75c	0.40
Chlorine	22.20	72.43	8.20c	3.95b	0.50
Distilled water	17.83	65.10	8.13c	4.10a	0.44
Before storage (initial)	17.60	72.47	14.80a	3.77c	0.54

b* indicates green (lower values) or yellow (higher values). Treatment means with a common letter are not significantly different at 0.05 level LSD.

Table 2. Descriptive (Des.) and acceptability (Acc.) ratings of sensory quality attributes and general acceptability of organic salt-treated and untreated fresh-cut pineapple after 5 days storage at 7-10^{o}C

TREATMENTS	COLOR		AROMA		TASTE		TEXTURE		AFTER-TASTE		GENERAL-ACCEPTABILITY
	Des.	Acc.	Des.	Acc.	Des.	Acc.	Des.	Acc.	Des.	Acc.	
Ca ascorbate 1.5%	2.60a	6.23a	3.40a	6.03a	3.36a	5.23a	3.96a	6.73a	3.27	3.93	6.23c
Ca ascorbate 2.5%	2.67a	6.23a	3.36a	6.13a	2.36b	5.26a	3.13b	6.26b	3.30	3.93	6.83a
Ca lactate 1.5%	2.46b	6.27a	3.33ab	6.03a	2.33b	5.23a	4.07a	6.60a	3.60	3.93	6.63b
Ca lactate 2.5%	2.63a	6.20ab	3.33ab	6.06a	2.40b	5.16ab	3.20b	6.16b	3.23	4.00	6.20c
Chlorine	2.56ab	6.07b	3.20c	5.60b	2.20bc	5.06bc	3.03b	6.03b	3.23	3.93	6.23c
Water	2.10c	4.57c	3.23bc	5.57b	2.06c	4.93c	3.00b	5.96b	3.23	3.93	6.16c

Color descriptive rating: 5-yellow, 4-light yellow, 3-slight brownish yellow, 2-moderate brownish yellow, 1-intense brownish yellow.
Aroma descriptive rating: 5-strong pineapple aroma, 4-moderate, 3-slight, 2-none or absent, 1-others, specify.
Taste descriptive rating: 5-extremely sweet, 4-very sweet, 3-sweet, 2-slightly sweet, 1-bland
Texture descriptive rating: 5-firm and crunchy, 4-moderately firm, 3-slightly firm, 2-soft, 1-very soft
Aftertaste descriptive rating: 5-very perceptible aftertaste, 4-perceptible, 3-moderately perceptible, 2-slightly perceptible, 1-none
Acceptability/General Acceptability rating: 9-like extremely, 8-like very much, 7-like moderately, 6-like slightly, 5-neither like nor dislike, 4-dislike slightly, 3-dislike moderately, 2-dislike very much, 1-dislike extremely

The results indicate that calcium ascorbate and calcium lactate had no adverse effects on the physicochemical and sensory quality of fresh-cut pineapple. The lower levels of the TSS after treatment on fresh-cut pineapple are likely due to reduced ripening. One would expect the total soluble solids would increase as fruit ripened, but this did not occur. These organic salts may inhibit or delay the ripening process of the fresh-cut pineapple. This result was in agreement with the findings of Ngamchuachit et al. (2014) using calcium chloride and calcium lactate on fresh-cut mangos. The effectiveness of calcium ascorbate as an antibrowning agent was found to be due to reduction of 0-quinones back to their precursor diphenols (Toivonen and Brummel, 2008) rather than to direct interaction with PPO (Arias et al., 2007). Moreover, pineapple is a non-climacteric fruit, no dramatic respiratory change occurs with no pronounced ethylene production peak during ripening (Hassan et al., 2011). Organic salt application could regularize the acidity level and phenolic content, thereby maintaining the taste of the produce. Gonzalez-Aguilar et al., (2008) found that dipping treatments with calcium lactate and calcium ascorbate had a positive effect on quality retention of fresh-cut produce. These organic salts has been reported to be a good alternative to chlorine because it avoids the bitterness and off-flavors associated with chlorine and with the promising antibacterial properties of calcium lactate (Hisaminato et al., 2001; Martin-Diana et al., 2005).

CONCLUSIONS

Calcium ascorbate and calcium lactate markedly reduced microbial population of fresh-cut pineapple after 3 hrs and 5 days of storage at 7-10^{o}C. The results obtained in this study demonstrated that the use of these organic salts at 2.5% concentration was needed to elicit the effect, having similar results to those of washing fresh-cut pineapple with chlorine after 3 hrs from storage and showed significant result even after 5 days from storage. Hence, this organic salt treatment does seem to be a promising alternative for improving decontamination efficiency in the sanitation of fresh-cut pineapple. In addition, these decontamination technique have shown an indirect detrimental effect on fresh-cut product quality by affecting plant tissue physiology and structure.

For the sensory attributes, among salts, it was the calcium ascorbate treated fresh-cut pineapple showed no adverse effects, while, others showed poor sensory quality after 5 days of storage.

In general, applying calcium ascorbate and calcium lactate at concentration of 2.5% ensured good quality of fresh-cut pineapple, by reducing microbial load while maintaining good sensory acceptability of fresh-cut pineapple even prolonging storage-life upto 5 days under cold storage. Further research should focus on the optimization of the use of these organic salts in the production process of fresh-cut pineapple.

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