Efficacy of 1-methylcyclopropene (1-MCP) post-cutting treatment on the storage quality of fresh-cut ‘Queen’ pineapple (Ananas comosus (L.) Merr. cv. ‘Queen’)

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Abstract

The efficacy of 1-methylcyclopropene (1-MCP) applied as a post-cutting treatment on fresh-cut ‘Queen’ pineapple was determined in order to assess its potential to maintain the storage quality of fresh-cut ‘Queen’ pineapple, a major Philippine variety. 1-MCP at a concentration of 1 μL L⁻¹ was applied post-cutting by injecting the gas into packed fresh-cut ‘Queen’ pineapples in polypropylene tray overwrapped with LDPE stretchable film. The packed fruits were stored at 5°C and monitored for headspace gas concentrations (ethylene, CO₂, O₂), visual quality deterioration parameters and microbial deterioration indicators. 1-MCP was found to effectively elicit its ethylene inhibiting action as shown by lowered headspace ethylene by about 40% at day 4 storage. Headspace CO₂ levels were likewise lowered by 1-MCP to about 50% at day 2 while higher headspace O₂ levels were generally obtained which had the highest increase at day 2 (about 18%) which created an improved modified atmosphere condition inside the package compared with the control. No significant effects on the visual quality were noted throughout storage. Color differences were however observed, with 1-MCP treatments having significantly higher lightness values and higher hue values at day 2. 1-MCP did not affect the microbial growth (aerobic bacteria, acid-forming bacteria, yeasts and molds, coliforms) on the samples during storage. Aerobic bacteria count was slightly lower than the control at day 3. The fresh-cut pineapple packaged in the manner described had a shelf-life of 3 days based on the microbial limits set by EU countries which is log 7 cfu/mL aerobic plate count. To the best of our knowledge, this is the first study which demonstrated the effects of 1-MCP on fresh-cut pineapple of the ‘Queen’ variety.

Introduction

Pineapple (Ananas comosus (L.) Merr.) is one of the most important tropical fruit crop in the international trade (Hassan and Othman, 2011). It is one of the top agricultural products of the Philippines. Two cultivars are grown in the country, the sweet cayenne also known as the ‘Hawaiian’ variety and the ‘Queen’ or locally known as the Formosa variety. The ‘Hawaiian’ is mostly exported by multinational companies in the processed form while the ‘Queen’ cultivar is cultured to meet the local demand for the fruit. This cultivar is distinct that it has a crispy texture with a peculiar very sweet taste that is highly acceptable in a pineapple fruit. The fruit is primarily marketed in the fresh form as a table fruit (Mabeza and Pili, 2005). According to the Philippine National Standards (2004) at Peel Color Index 2 (PCI 2) the ‘Queen’ pineapple fruit shows yellowing on about 2 to 3 layers of the eyes which is best for fresh consumption.

Keywords

1-methylcyclopropene, Ethylene, Fresh-cuts, Postharvest handling, Post-cutting treatment, ‘Queen’ pineapple

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slices, chunks and cored whole fruit). However, a major problem is its rapid deterioration leading to a very limited shelf-life of approximately 2–3 days. Quality loss is mainly due to pulp browning and accumulation of liquid in the packaging (Antoniolli et al., 2007). Due to slicing, the tissues undergo increased metabolic processes which result in significant changes in their textural, color and flavor properties (Del Nobile et al., 2009). In addition, the shelf life of fresh-cut pineapple is closely tied to its packaging conditions and storage temperature (Soliva-Fortuny et al., 2002).

1-Methylcyclopropene (1-MCP) is an ethylene antagonist which inhibits ethylene action by binding strongly to ethylene receptors in plant tissues (Sisler and Serek, 1997). Aside from its application in intact fruits, 1-MCP has also been applied in fresh cut processing systems and the results are quite variable in terms of whether the 1-MCP treatment provides a benefit, no effect or a negative effect on the shelf life and quality retention of fresh cut product (Toivonen, 2008).

1-MCP applied to fresh cuts resulted to reduced ethylene production such as in apples (Jiang and Joyce, 2002) and ‘Hayward’ kiwifruit (Vilas-Boas and Kader, 2007). Softening was also found to be delayed by 1-MCP treatment in ‘Kent’ and ‘Keitt’ mango slices (Vilas-Boas and Kader, 2007), fresh cut ‘Galaxia’ melons (Ergun et al., 2007), and fresh-cut ‘Galia’ melons (Ergun et al., 2008). 1-MCP at a concentration of 1 μL L⁻¹ was also noted.

The mode of application could also play a role in the efficacy of 1-MCP treatment since gaseous 1-MCP is usually applied to intact fruits in an enclosed chamber for 12-24 h to ensure 1-MCP binding. In fresh-cuts however, it would be more practical to apply 1-MCP post-cutting, directly into the packaged fruits in order to minimize further handling which poses risks of microbial contamination and mechanical damage. In this study, 1-MCP was applied as a post-cutting treatment by injecting the gas into the package with fresh-cut fruits. The application of 1-MCP post-cutting on fresh-cut ‘Queen’ pineapple could lead to better storage quality as 1-MCP can better exert its ethylene antagonistic action in the enclosed packaging. This study investigated the efficacy of 1-MCP post-cutting application to fresh-cut ‘Queen’ pineapple to assess its potential as a tool in maintaining the storage quality of the fresh-cut of this major pineapple variety of the Philippines.

Materials and Methods

Samples and Fresh-cut processing

Mature green ‘Queen’ pineapple fruits were procured from Labo, Camarines Norte, Philippines. The pineapple fruits were processed at PCI 2. Prior to processing, the fruits were washed with dilute detergent solution for 10 min, rinsed with tap water and dipped in 200 μL L⁻¹ chlorine solution for 2 min. Fresh-cut processing was carried out in a clean and sanitized minimal processing room. A pineapple fresh-cut processing protocol was followed by first removing the crown and the eyes, then by de-coring and slicing into chunks. The chunks were then dipped in 200 μL L⁻¹ chlorine solution for 1 min, drained and dried using paper towel. Packing of the fresh-cuts was done by weighing 150 g of the fruit chunks into 150 cm x 90 cm clear polypropylene trays, then overwrapping with one layer of LDPE plastic stretchable film. 1-MCP at a concentration of 1 μL L⁻¹ was prepared in an evacuated 1 L volumetric flask by dissolving 1-MCP powder of the brand name AnSIP™ (0.43% 1-MCP) in distilled water. The 1-MCP gas generated was obtained from the flask using a plastic syringe and injected into each of the packaged pineapple fresh-cuts. The packaged fruits were stored at 5°C and 95% RH and monitored for quality parameters throughout storage.

Headspace gas (Ethylene, CO₂, O₂) analyses

Carbon dioxide, oxygen and ethylene headspace concentrations were obtained by gas chromatography using Shimadzu Gas Chromatograph (Model GC-8A) equipped with thermal conductivity detector (TCD) and silica gel column for CO₂ and O₂, while for C₃H₆ analysis, flame ionization detector (FID) and alumina column was used. The GC-FID has the following settings: injection port temperature- 120°C, column temperature - 100°C, column length - 2.0 m, inner diameter: 3.0 mm, and gas flow rate: 35 mL/min. The GC-TCD has the following settings: injection port temperature - 90°C, column temperature - 50°C, gas pressure - 1.25 kg/cm². Amounts of headspace gases were calculated as:

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\text{C}_3\text{H}_6, \text{O}_2, \text{or O}_3 (\text{L}^\circ) = \frac{\text{peak height of sample}}{\text{peak height of standard}} \times \text{standard C}_3\text{H}_6, \text{O}_2 \text{or O}_3 \text{conc (1 L}^\circ) \]

Visual quality rating

Visual quality scores were given by the analyst as follows: 9.8 - excellent, essentially no symptoms of deterioration; 7.6 - good, minor symptoms of deterioration not objectionable; 5.4 - fair, deterioration evident, but not serious, limit of saleability; 3.2 - poor, serious deterioration, limit of edibility; 1 - extremely...
poor, not usable, off-odors, fungal decay.

**Translucency or water-soaking**

Translucency or water soaking were evaluated by the analyst as follows: 5- translucency absent; 4- ≤ 25% of the exposed pulp; 3- > 25 % but ≤ 50% of the exposed pulp; 2- >50 % but ≤ 75 % of the exposed pulp; 1- ≥ 75% of the exposed pulp.

**Color evaluation**

Color of pineapple fruit slices was measured using a chromameter (Konica-Minolta CR-10) for lightness, L’ and hue, h’. Measurements were done on each side of the fruit slice on the flesh tissue just outside the core area.

**Microbiological analyses (total plate count, acid-forming bacteria count, yeast and mold count, coliform count)**

Twenty five grams of each sample was weighed out aseptically, then added with 225 mL of 0.1% peptone water and homogenized for 1-2 minutes at room temperature. Decimal dilutions in the same diluent was prepared and duplicate 1 mL or 0.1 mL aliquots of appropriate dilutions were mixed by pour plating technique on the following: Plate Count Agar (PCA) for mesophilic aerobic count incubated at 30°C for 24-48 h; Glucose Yeast Extract Peptone Agar (GYPA) Medium plus CaCO₃ for acid-forming bacteria count incubated at 30°C for 24-48 h; acidified Potato Dextrose Agar (PDA) for yeasts and molds incubated at 30°C for 2-4 days; Violet Red Bile Agar (VRBA) for coliforms overlayed with the same medium and incubated at 35°C for 18-24 h.

**Statistical analyses**

Statistical analyses of the results was carried out using SAS (Statistical Analysis System) program. Differences between treatments were tested for significance by ANOVA (Analysis of Variance). Significant difference means (P≤0.05) were separated by using the Tukey’s Test at 5% level of significance.

**Results**

Initial ethylene level in the control was 0.8 uL L⁻¹ which was double than the 1-MCP treatment at 0.4 uL L⁻¹. On the second day however, the same ethylene concentration was achieved corresponding to the peak in ethylene production at 1.6 uL L⁻¹. 1-MCP was noted to have lowered the ethylene levels after day 2 until day 4 to about 40% (Figure 1). Towards the end of storage, starting at day 5, the ethylene levels in 1-MCP treatment appeared to be higher than the control.

Headspace CO₂ concentration was found to be lower in 1-MCP treatment which significantly varied from the control at day 2 (Figure 2). The values of headspace CO₂ ranges from 0.5-1.0 uL L⁻¹ which indicates that CO₂ accumulated within the package throughout storage. At day 2, 1-MCP caused CO₂ levels to decrease by almost 50%, with 1-MCP treatment having 0.5 uL L⁻¹ while the control has 1.0 uL L⁻¹. An almost constant CO₂ level was likewise maintained in 1-MCP treated packages while fluctuating CO₂ levels were noted in the control. On the other hand, headspace O₂ concentration was inversely affected by 1-MCP as higher O₂ levels were obtained in the 1-MCP treatment (Figure 3). 1-MCP was able to maintain a small range of O₂ concentration of about 16.5-18 uL L⁻¹ while the control had fluctuating O₂ concentration which ranged from 14.5-18 uL L⁻¹ throughout storage.

Visual quality was observed to decline during
storage but with no significant difference between the 1-MCP treatment and the control (Figure 4). Visual quality was acceptable until day 5, when the limit of saleability was reached. Translucency or the appearance of water-soaked portions of the fruit slices, was observed to progress during storage with 1-MCP significantly delaying its development at around day 3 until day 5 (Figure 5). The darkening of the tissues were also measured using the color value of lightness (L*-) (Figure 6). Higher L*_ values were obtained in 1-MCP treatments until day 4 which implies less tissue darkening. Another color parameter, the hue, indicates the intensity or saturation of the color, being yellow in the case of pineapple. Higher h*_ values were obtained in 1-MCP treatments until about day 4.

Total microbial population in fresh cut pineapple was 10^4 cfu/mL right after the fresh cut processing. The total plate count, acid forming bacteria and the yeast and mold counts followed the same increasing trend in the whole duration of storage (Figure 6). 1-MCP was found to have no effect on the growth of microorganisms in the pineapple fresh-cuts. A slightly lower aerobic plate count was however obtained in 1-MCP treatment at day 3 (Figure 6A). Total coliform count was zero until day 3.

**Discussion**

**Headspace ethylene, CO₂, and O₂ concentrations**

The lower headspace ethylene levels obtained from the 1-MCP treatment is a clear indication of the antagonistic action of 1-MCP on ethylene during the earlier parts of storage (Figure 1A). 1-MCP is known as an inhibitor of ethylene action (Blankenship and Dole, 2003). The action of 1-MCP is mediated through the inhibition of ethylene perception of plant tissues by interacting with the receptor and competing with ethylene for binding sites. Calderon-Lopez (2005) also demonstrated the inhibitory action of 1-MCP on ethylene production in fresh-cut apple slices with more than 50% lowered ethylene production which was almost the same observation herein. The mode of 1-MCP application carried out in this study, which is post-cutting by injecting 1-MCP into the enclosed package, also favored the ethylene inhibiting action of 1-MCP. 1-MCP came into direct contact with the peeled and sliced pineapple flesh which enabled its binding to the receptors on the flesh surface. Thus, the ethylene inhibiting action of 1-MCP occurred more effectively. Also, since the 1-MCP gas was injected directly into the enclosed package and was not removed until the end of storage, longer time for 1-MCP binding was provided which resulted to its enhanced binding. The application of 1-MCP in
fresh-cut processing systems has been approached in three ways: 1) treatment of freshly harvested crop before longer-term storage after which the product is processed, 2) treatment of whole product just before processing, or 3) treatment of fresh-cut product immediately after processing (Toivonen, 2008). The third mode mentioned, which was employed in this study, was shown to be effective in fresh-cut ‘Queen’ pineapple.

However, towards the end of storage starting at day 5, ethylene level in the 1-MCP treatment appeared to be higher than the control. In intact and actively respiring tissues, as time progresses, new ethylene receptors may be formed, and the cells regain sensitivity to ethylene (Sisler and Serek, 1997). But in the case of fresh-cuts, tissues are no longer intact due to the inflicted mechanical damage, thus synthesis of new receptors may not be possible. Hence, the loss of ethylene-blocking effect of 1-MCP may be mainly due to the degradation of receptors and the dissociation of 1-MCP from the receptor (Castillo-Israel *et al*., 2014). Therefore, 1-MCP treatment was effective only at the earlier part of storage. The loss of effect could also be due to the diffusion of 1-MCP through the film. Blankenship and Dole (2003) confirmed that 1-MCP appears to readily pass through plastic bags and fiberboard boxes. It was also pointed out by Blankenship (2001) that the concentration of the 1-MCP gas must be sufficient to saturate the receptors and compete with any ethylene present. The underlying mechanism of which, is system 2 or ethylene autocatalysis, wherein a certain threshold ethylene level in tissues must be attained in order to trigger ethylene biosynthesis. Ethylene seemed to play a role in regulating its own production as proposed by Yang and Hoffman (1984). Since 1-MCP gas had diffused out of the package, 1-MCP gas concentration inside the package decreased and consequently overcome by ethylene which triggered increased ethylene biosynthesis.

The high O$_2$ and low CO$_2$ levels inside the package (Figures 1B and 1C) is an indication that the packaging created a modified atmosphere condition, with 1-MCP enhancing the desirable levels of CO$_2$ and O$_2$ inside the package. 1-MCP seemed to play a role by lowering the CO$_2$ level and eventually maintaining the level of O$_2$ high. 1-MCP also the ability to regulate CO$_2$ and O$_2$ levels in modified atmosphere packaging by regulating the respiration process. Low CO$_2$ is an evidence of decreased respiration rates. High O$_2$ on the other hand, is desirable since anaerobic respiration which leads to fermentation products such as aldehydes and ethanol, is not favored. In fresh-cut banana slices, 1-MCP at 1 uL L$^{-1}$ also caused decreased respiration rates (Vilas-Boas and Kader 2006). The same was observed by Budu and Joyce (2003) using a pre-cutting 1-MCP treatment on pineapple.

CO$_2$ is also known as an inhibitor of ethylene as it also has the capacity to bind with ethylene receptors but with lesser affinity compared with 1-MCP. CO$_2$ as described by Beyer (1978) has anti-ethylene
properties. The lowered CO₂ levels caused by 1-MCP was also a consequence of the binding of 1-MCP with the receptors, thereby causing less ethylene as well as CO₂ accumulation inside the package since the receptors have already been occupied by 1-MCP. The unbound ethylene and CO₂ just diffused out of the film packaging rather than retained inside.

Changes in atmosphere is an important factor affecting the physiology and biochemistry of fresh-cuts (Watada et al., 1996). Modified atmosphere packaging (MAP) had been used extensively for fresh-cut produce to extend shelf life (Soliva-Fortuny, 2002). 1-MCP treatment and MAP with N₂O had a positive combined effect on the inhibition of respiration and ethylene production of fresh-cut pineapple (Rocculi et al., 2009). In this study, the modified atmosphere was naturally created as the gas transmission through the film achieved equilibrium. The presence of 1-MCP further enhanced the desirable effect of the modified atmosphere packaging by decreasing the respiration rate as evidenced by the low headspace CO₂ concentration.

**Visual quality, translucency and color changes**

Visual quality in fresh-cut pineapple mainly considers the development of discoloration or browning, dullness of color, and appearance of translucent portions. Though 1-MCP was demonstrated to significantly alter the gas concentrations inside the package as discussed earlier, this did not translate to improved visual quality (Figure 2). A negative effect of a modified atmosphere is the accumulation of gases especially CO₂ which can cause damage to quality as evidenced by off-flavour and discoloration (Gil et al., 1998), fermentation, off-odours and colours, carbonation, accelerated deterioration and decay, and water condensation (Zagory, 1988).

The development of translucency in fresh-cuts can be related to texture loss. Translucency as manifested by the alteration of flesh texture to become dark and glassy and the presence of an early over-maturity can cause softening of tissues or decrease of firmness (Artes et al., 2007). Mechanical wounding may promote an increase in ethylene production that can initiate physiological responses like softening related to cell wall degrading enzymes (Vilas-Boas and Kader 2006). Fresh-cut pineapples rapidly develop translucency during storage since the tissues were subjected to extensive mechanical wounding through peeling, eye-removal, de-coring and slicing. The observed lowering of ethylene levels caused by 1-MCP may have led to the delayed development of translucency as the activity of cell wall degrading enzymes are likewise controlled by ethylene.

Rocculi et al. (2009) also observed an increase in L* of the fresh-cut pineapples treated with 1-MCP and N₂O after 6 days of cold storage. High retention of L* values were also observed by Budu and Joyce (2003) on fresh-cut pineapple with pre-cutting 1-MCP treatment. The distinct yellow color of pineapple was maintained by 1-MCP while preventing browning at the same time. Browning had been previously correlated to CO₂. Prolonged exposure to high levels of CO₂ will cause damage to the cells resulting to accelerated browning (Marrero and Kader, 2006). The harmful effects of high CO₂ levels on the tissue physiology, which could be visually assessed through the appearance of accelerated browning and necrosis in the flesh tissue (Gorny et al., 2002) were observed on the control samples. The CO₂ levels coincide with the degree of browning observed. As seen in Figure 3, 1-MCP treated samples had better color retention on the earlier part of storage but abruptly increased in browning and spoilage towards the end of storage.

**Microbial growth**

The growth and metabolism of microbes are key factors known to negatively affect the quality and shelf life stability of fresh cut produce (Zhou et al., 2006). Newly prepared fresh cut pineapple, under careful preparation, could have as much as 10⁶ cfu/g total plate counts of microbial contamination (O’Conner-Shaw et al., 1994). Fresh-cut fruit with very low aerobic plate count (APC) or total plate count (TPC) and especially yeast and mold counts correlate with increased shelf-life (Beaulieu and Gorny, 2004).

Initial microbial counts of 10⁴ cfu/mL (Figure 4) indicated that despite the sanitation protocols, spoilage microorganisms still proliferate during the storage of the fresh cut pineapple samples. This is due to the innate microflora present in the samples. Coliforms were not detected until day 3 of storage which indicates efficiency of the processing sanitation protocol employed.

Microbial growth also affects the levels of O₂ and CO₂ in the package. Jacxsens et al., (2002) reported that spoilage microorganisms proliferating on the produce during storage consumed O₂ inside the packaging. In effect, the rise in microorganism count also contributes to the increase of CO₂ accumulation inside the package. A rise in count of all microorganisms were observed on day 3 which correlates with the increase in CO₂ observed at day 4.

The microbiological shelf life of the fresh cut commodities is affected by composition and physicochemical properties of the raw fruit condition. However, the fresh cut processing protocol is crucial
because it determines the sources of spoilage during storage. In this regard, the native microflora of fresh-cut fruits, is usually substituted by bacterial strains (Soliva-Fortuny et al., 2002). Ahvenainen (1996) emphasized the great importance of the application of correct hygiene procedures to prevent the risk of microbiological contamination in fresh-cut pineapples.

Similarly, Ergun et al. (2007) observed that 1-MCP-treated fresh-cut ‘Galia’ melon fruit exhibited slightly reduced populations of both total aerobic organisms and Enterobacterium, but likewise, did not incur a difference in the keeping quality between 1-MCP-treated and control fruits. Studies by Roccucci et al. (2009), Budu and Joyce (2003) and Rupasinghe et al. (2005) also revealed that fresh-cut pineapples with 1-MCP treatment did not show any beneficial effects in terms of microbial growth inhibition. It was also suggested that 1-MCP may in fact increase decay and microbial growth in fresh-cuts. Ku et al. (1999) postulated that 1-MCP knocked out the ethylene-associated wound response important for disease resistance mechanisms. Zhou et al. (2006) concluded that 1-MCP alone had no direct effect on the growth of microbes but could indirectly affect it by counteracting the ethylene-induced population increase of aerobic and lactic acid bacteria as well as yeasts and molds. Results of previous studies of 1-MCP application on a variety of fresh-cut fruits and vegetables suggest that there are other factors which significantly affect microbial deterioration rather than 1-MCP.

Aerobic plate count and acid forming bacteria count reached log 7 cfu/mL at day 3, which is the maximum limit of mesophilic aerobic total plate count as proposed by Raybaudi-Massilia et al. (2007). This set limit is also followed by Spanish legal authorities and other EU countries (Sperber and Doyle, 2009). Therefore, based on microbial safety, fresh-cut ‘Queen’ pineapple packaged in the manner described in this study, should be consumed on or before 3 days of storage.

Conclusion

The post-cutting application of 1-MCP on fresh-cut ‘Queen’ pineapple packaged in polypropylene trays overwrapped with LDPE stretchable film resulted to an improved modified atmosphere package with lower ethylene and CO₂ levels, and generally higher O₂ levels throughout storage compared with the untreated fruits. 1-MCP was able to effectively exert its ethylene inhibiting action by slowing down ethylene production and respiration rate. Color changes such as development of browning discoloration, fading of the yellow color as well as translucency were likewise delayed by 1-MCP during days 2 and 3 of storage. However, the general visual quality was not affected by 1-MCP. Microbial growth was neither affected by 1-MCP. The fresh-cut ‘Queen’ pineapple packaged in the manner described has a shelf-life of 3 days based on microbial safety limits. 1-MCP post-cutting application on fresh-cut ‘Queen’ pineapple can be an effective tool in maintaining the storage quality by controlling the ethylene-mediated deterioration since 1-MCP was demonstrated to effectively control ethylene production. The manner by which 1-MCP was applied in this study was also proven to create a desirable modified atmosphere inside the package.

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