EFFECTS OF DIFFERENT VOLUMES OF NITROGEN GAS FUMIGATION ON POSTHARVEST PERFORMANCES MINIMALLY PROCESSED PINEAPPLE (*Ananas Comosus* L.)

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Abstract

Fresh cut pineapple has a high popularity nowadays because of its fresh like quality, ready-to-eat properties and high nutritional value. However, prior to peeling and cutting processes, fresh cut pineapple is usually susceptible to browning incidence, which was catalysed by polyphenol oxidase (PPO) enzyme. Browning incidence generally degraded the overall attributes of fresh cut pineapple, and simultaneously, limited its shelf life. In present study, the effects of different volumes of nitrogen (N_2) gas (0 mL, 5 mL, 10 mL, 15 mL, 20 mL and 25 mL) on fresh cut pineapples were evaluated under 5 ºC storage for nine days. The parameters being evaluated were browning incidence, flesh colour, total phenolic content, ascorbic acid concentration, flesh firmness, soluble solids concentration and titratable acidity. It is shown that N_2 gas fumigation at higher volume had significant effect ($p \leq 0.05$) in reducing browning incidence of fresh cut pineapple. However, no apparent effects were found in other postharvest parameters. In conclusion, fumigation with 25 mL of N_2 gas has a tendency in delaying the occurrence of browning, and maintaining other quality attributes of fresh cut pineapple.

Keywords: polyphenol oxidase, postharvest quality, fresh cut, browning

BACKGROUND

Pineapple or *Ananas comosus* L. is cultivated worldwide with Costa Rica, Brazil, Philippines, Thailand and Indonesia being the leading countries of world pineapple production. In Malaysia, pineapples are mostly cultivated in Johor state because of the enriched peat soil which is suitable for their well-growth. It has been reported that the areas planted with pineapples in Malaysia is 15,649 hectares, with total production estimated at 355,000 metric tons (Thalip et al., 2015). Even though pineapple can be profitable in terms of its total production and utilization, it still experienced a serious postharvest loss due to improper postharvest handling and storage which leads to undesirable physiological disorders incidence. The most common physiological disorder is internal browning or black heart, which is caused by chilling injury (Rohrbach and Phillips, 1990). Another example is sunburn or sunscald that leads to injury and death of fruit's tissue, as a consequence to high temperature exposure (Pegg et al., 1995). All of these disorders have contributed to economic loss and gained appeal towards control practices (Adikaram and Abayesekara, 2012).

In order to reduce postharvest loss of pineapple, it needs to be further processed into end products which makes it durable (Hossain and Bepary, 2015). One of the methods is by introducing the pineapple into fresh cut industry. Fresh cut produces have gained a relatively high popularity among the public today because of fresh like quality, ready-to-eat properties and high nutritional value (Rico et al., 2007). Fresh cut pineapple may seem to be more convenience than a whole pineapple fruit, yet they are highly susceptible to browning incidence, prior to cutting process. Cutting process generally destroyed the cell compartmentalization, allowing the enzymes to come into contact with their substrates (Bretch, 1995). Browning occurred when polyphenol oxidase (PPO) enzyme is in contact with phenolic compounds (Vaughn and Duke, 1984). Browning is truly undesirable following the growing trend in fresh cut industry as it degraded the fruit's appearance, and sometimes altered its flavour, which later decreases the acceptability of fresh cut pineapple. Furthermore, Bretch et al. (2004) have reported that browning is a severe problem that limits the shelf life of fresh cut pineapple, owing to accelerated respiration rate and senescence process. The post cutting life of fresh cut pineapple is only four days at 10 ºC and twelve days at 5 ºC, with visual sign of microbial infection (Marrero and Kader, 2006).

Many treatments have been designed to reduce the chance of browning incidence in fresh cut pineapple. The most common practice is the application of anti-browning agents, for example ascorbic acid. Currently, ascorbic acid is still being commercialized widely as reported by Garcia and Barret (2002). However, a recent study conducted by Wu et al. (2011) have shown that high pressure nitrogen (N_2) gas can be the alternative to ascorbic acid, to delay browning incidence in fresh cut pineapple. It is reported that nitrogen gas can interfere with the activity of PPO enzyme, thus preventing the occurrence of oxidation process. Though, the effects of N_2 gas in delaying browning incidence of fresh cut pineapple still requires further investigation. Hence, this study aims to determine the effects of different volumes of nitrogen gas in reducing the browning incidence and prolonging the shelf life of fresh cut pineapple.

METHODS

A total of 40 Josapine pineapples with maturity index of three were obtained from Gong Pauh wholesale market, Terengganu. The experiment was conducted in Post Harvest Technology Laboratory, School of Food Science and Technology, Universiti Malaysia Terengganu.

Before cutting, a total of eighteen plastic containers (20 cm x 20 cm) were sanitized with 75% ethanol, together with the knife and the cutting board. The equipments were then let to air-dry. The peel, crown and 'eyes' of the pineapples were first removed. After that, the pineapples were immediately cut into 252 wedges, with each measuring 2 cm thick and 3 cm tall. A total of 14 pineapple wedges were placed inside the plastic containers, and sealed tightly using parafilm and cellophane tape to prevent the leakage of the gas. Next, the assigned N2 gas (0 mL, 5 mL, 10 mL, 15 mL, 20 mL and 25 mL) were immediately injected into the plastic containers through injection port, and left for 20 minutes to enhance uniform absorption of the gas by pineapple wedges. After 20 minutes, all the pineapple wedges were moved to 126 polystyrene trays, with each polystyrene tray consisted of two pineapple wedges, and later wrapped using cling wrap. The packaged pineapple wedges were then stored at 5 ºC chiller for postharvest quality assessments. The postharvest parameters being evaluated were browning index, total phenolic content, flesh colour, ascorbic acid concentration, flesh firmness, titratable acidity and soluble solids concentration.

The browning incidence of fresh cut pineapple was evaluated visually according to the score given by Ding et al. (2007). The score was being given from 0 to 5, in which 0=None (0-20% of browning), 1= Trace (20-40% of browning), 2= Slight (40-60% of browning), 3= Moderate (60-80% of browning), 4= Severe (80% of browning) and 5= Extremely severe (>100% of browning). The flesh colour was measured using Konica Minolta CR-400 reflectance colorimeter (Minolta camera Co. Ltd., Japan) according to CIELAB colour parameters: L*, chromaticity a* and chromaticity b* (McGuire, 1992). L* represents the lightness coefficient which ranges from 0 (black) to 100 (white). a* ranges from -60 to +60, in which +60 indicates red colour and -60 indicates green colour. b* also ranges from -60 to +60, but +60 represents yellow colour while -60 represents blue colour. a* and b* were further used to calculate chroma [$C^* = (a^{*2}+b^{*2})^{1/2}$] and hue angle ($h^o = \tan^{-1} b^*/a^*$). Chroma (C*) refers to colour intensity while hue angle represents red-purple (0º), yellow (90º), bluish-green (180º) and blue (270º). Total phenolic content of fresh cut pineapple was determined following the method of Singleton and Rossi (1965) with a slight modification, and by referring to the standard curve y=0.0522x+0.0003, R2=0.99. Ascorbic acid concentration in the meanwhile was determined according to AOAC (2004) method using indophenol titration, with standard curve y=11.18x+0.02, R^2=0.99. Flesh firmness was measured using TA.XT plus texture analyzer (Stable Micro Systems, United Kingdom). A probe of P/2 stainless needle was used to penetrate the flesh of the pineapple with a test speed of 5 mm/sec and target distance of 10 mm. Soluble solids concentration was determined using handheld refractometer while titratable acidity were measured using titration method, expressed as % of citric acid.

The data were subjected to the one-way analysis of variance (ANOVA) using GLM (General Linear Models) procedure with SAS 9.1 software package, SAS Institute Inc., Cary, NC, USA. Treatments means were further separated by Tukey (HSD) for least significance at $p \leq 0.05$ (SAS Institute Inc., 1999).

RESULTS AND DISCUSSION

Browning is the main criteria that determine the acceptability of fresh-cut produce since the consumers will only consider it by visual quality. In the present study, the reduced surface browning was recorded in pineapples treated with the highest volume of nitrogen (N_2) gas, 25 mL, which is in agreement with Wu et al. (2011). Besides that, You et al. (2011) and Phonyiam et al. (2016) also reported that anoxic treatment has significantly reduced the browning incidence for both fresh-cut Chinese water chestnut and whole pineapple fruit respectively. Wu et al. (2011) reported that the loss of browning is due to the inhibitory effect of residual N_2 gas in microspores of pineapple wedges, which restrict the intracellular enzymes activity related to phenol degradation. The enzymes activity is hindered as a consequence of anaerobic condition created by N_2 gas by eliminating the oxygen (O_2) gas. In addition, Zhan and Zhang (2005) also reported N_2 gas can form gas hydrate after in contact with water molecules, which in turn reduce the water activity in fruit tissues and influence the structure of enzyme. Thereby, the browning incidence could be prevented.

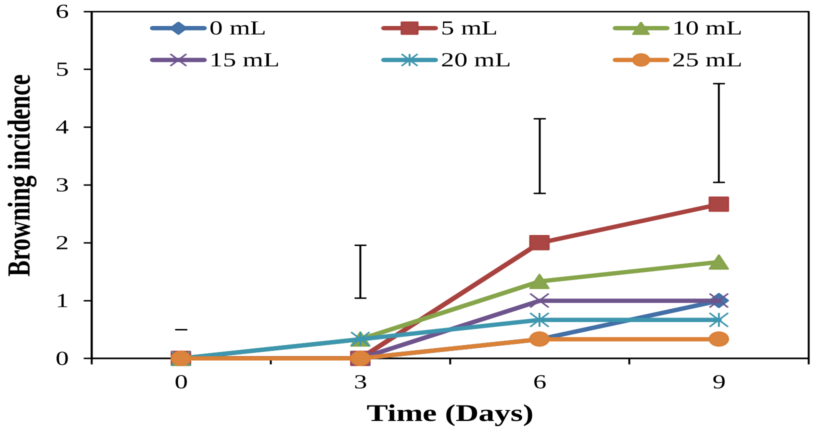

Fig.1:Effects of different volumes of nitrogen gas fumigation on browning incidence minimally processed pineapple. Vertical bars represent HSD at 5% level. (HSD values on Day 0=0, Day 3=0.91, Day 6=1.29 and Day 9=1.71).

Table 1: Effects of different volumes of nitrogen gas fumigation on browning index of minimally processed pineapple. Number in the box denoted to browning score.

	Day 0	Day 3	Day 6	Day 9
0 mL N_2 gas	 0	 0	 1	 1
5 mL N_2 gas	 0	 1	 3	 4
10 mL N_2 gas	 0	 0	 1	 3
15 mL N_2 gas	 0	 0	 1	 2
20 mL N_2 gas	 0	 0	 1	 1
25 mL N_2 gas	 0	 0	 0	 1

In the present study, the pineapples treated with 25 mL of N_2 gas fumigation succeed to maintain the pure yellow colour of flesh throughout the storage time, with only a very mild browning. This is corresponded to the result taken for lightness coefficient (L*) and chromaticity b* (Fig.2 and Fig.3). According to Gonzalez-Aguilar et al. (2004), the degree of browning incidence is determined through the measurements of L* and b*. L* represented brightness and b* indicated browning or loss of yellow colour. In the present study, the high values of lightness coefficient and chromaticity b* for pineapples treated with 25 mL of N_2 gas reflected the lower extent of browning incidence (Table 1; Fig.2 and Fig.3). In contrast, the low values of L* and b* indicated the severity of browning incidence, which was similar to pineapples treated with 5 mL of N_2 gas (Table 1; Fig.2 and Fig.3). Wu et al. (2011) also found that pineapples treated with high pressure (HP) N_2 gas were able to retain high L* and b* values as compared to pineapples treated with normal atmospheric pressure (NAP) N_2 gas. Prior to that, all the pineapple samples have a relatively low values of chromaticity a*, with pineapples treated with 5 mL of N_2 gas recorded the highest reading. Furthermore, the hº for pineapples from the same treatment was also a slight lower compared to other treatments.

According to Wan Zaliha and Koh (2017), on fresh-cut carambola, lower a* value with high hº denoted low browning incidence, and vice-versa.

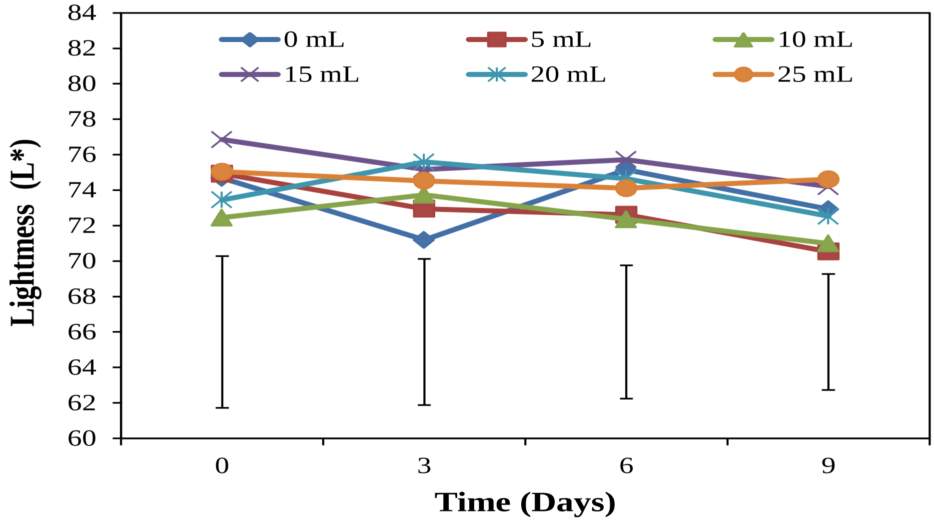

Fig.2: Effects of different volumes of nitrogen gas fumigation on lightness (L*) of minimally processed pineapple. Vertical bars represent HSD at 5% level. (HSD values on Day 0=8.55,
Day 3=8.26, Day 6=7.52 and Day 9=6.54).

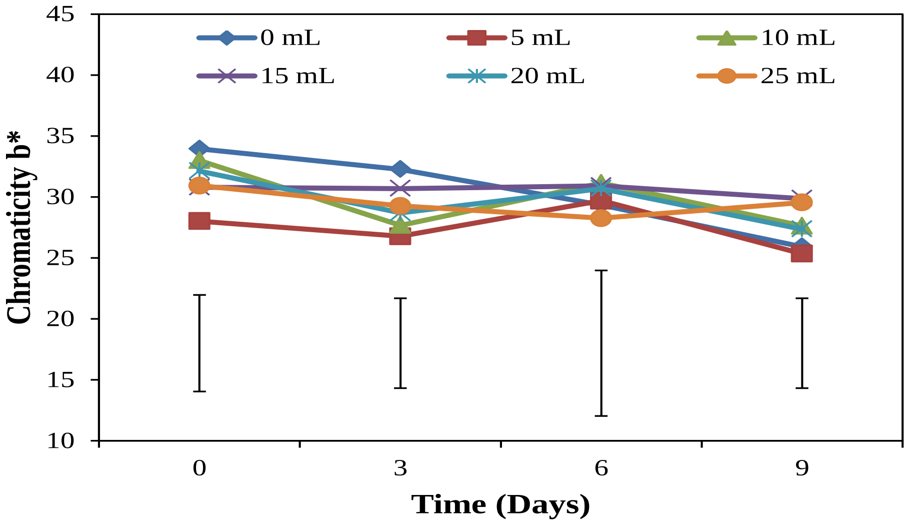

Fig.3: Effects of different volumes of nitrogen gas fumigation on chromaticity b* of minimally processed pineapple. Vertical bars represent HSD at 5% level. (HSD values on Day 0=7.91, Day 3=7.36, Day 6=11.92 and Day 9=7.36).

The occurrence of browning incidence is closely related to phenolic constituents. The loss of phenolic content usually supports the acceleration of browning incidence. Browning is developed when PPO catalysed the oxidation of phenolic compounds to quinones, which then formed melanins via polymerisation (Marshall et al., 2000). In the present study, the total phenolic content declined with the extent of storage time (Fig.4), which is in agreement with You et al. (2012), for fresh-cut Chinese water chestnut. The reduction is due to the involvement of phenolics as the substrate for oxido-reductase enzyme, PPO (Corzo-Martinez et al., 2012). Similarly, Ke and Saltveit (1989) reported that phenolic compounds in wounded iceberg lettuce were immediately oxidised into brown substances by PPO, even though there is an increased in total phenolic content. Despite of all, it is found that the total phenolic content in present study is not corresponded to browning incidence and colour parameters, and this is in coincident with Rocha and Morais (2001) which stated that no correlation was found between phenolic content and colour parameters (L* and hº) on minimally processed 'Jonagored' apple.

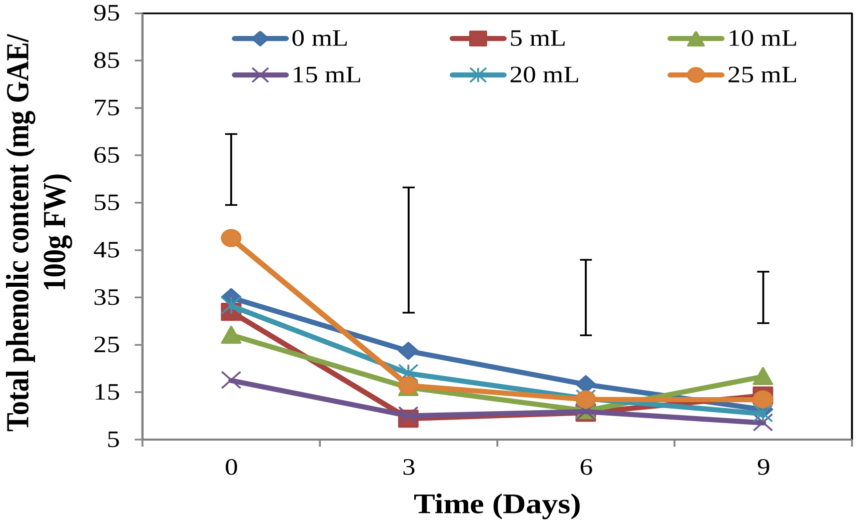

Fig.4: Effects of different volumes of nitrogen gas fumigation on total phenolic content of minimally processed pineapple. Vertical bars represent HSD at 5% level. (HSD values on Day 0=14.97, Day 3=26.44, Day 6=15.94 and Day 9=10.85).

Ascorbic acid or vitamin C is a type of antioxidants which can restrain the oxidation process. According to Hossain and Rahman (2011), pineapple is a good source of antioxidants due to the abundance of phenolics. Beserra et al. (2011) reported that the amount of ascorbic acid present in pineapple is approximately 22.4±0.9 mg/100g, and this is corresponded with the result obtained for all treatments throughout the storage period (Fig. 5). The reduction of ascorbic acid content at the end of study is relevant since oxidative degradation can easily took place with long exposure to oxygen (Davey et al., 2000). Based on the observation, N_2 gas gave no effect to ascorbic acid concentration of fresh cut pineapple. Though, ascorbic acid might play a significant role in reducing browning incidence. Hence, the role of N_2 gas in maintaining ascorbic acid concentration requires further investigation.

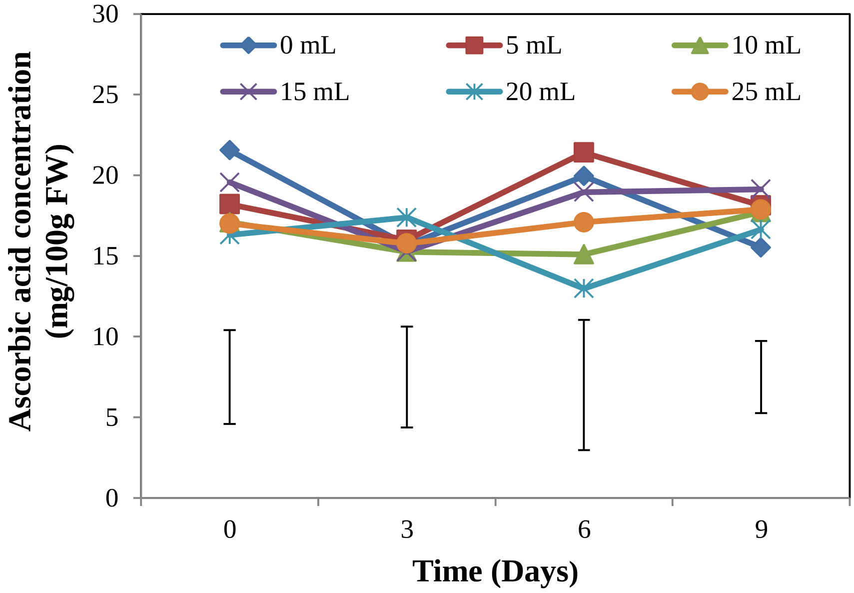

Fig.5: Effects of different volumes of nitrogen gas fumigation on ascorbic acid concentration of minimally processed pineapple. Vertical bars represent HSD at 5% level. (HSD values on Day 0=5.81, Day 3=6.25, Day 6=8.06 and Day 9=4.47).

The firmness of pineapple's flesh in the present study fluctuated throughout the experimental period, though it should decreases with the increasing of storage time. A previous study conducted by Gonzalez-Aguilar et al. (2004) stated that the firmness of fresh cut pineapple decreased to different extent based on the treatments applied (Fig.6). According to Tapre and Jain (2012), the loss of firmness can be related to the conversion of starch to sugar during ripening, breakdown of pectin substances and dehydration of surface water. The dissolution of pectin substances is due to the activation of pectin methyl-esterase and polygalacturonase enzyme which hydrolysed the cell wall structure, prior to maturation stage (Adams, 1991). In this case, the latter reason was most relevant since the pineapple wedges were exposed to chilling temperature that can induce water loss. The treatments applied have no effect on flesh firmness of fresh cut pineapples. The fluctuation in firmness readings might be due to the usage of different fruits, for all treatments throughout the experimental period.

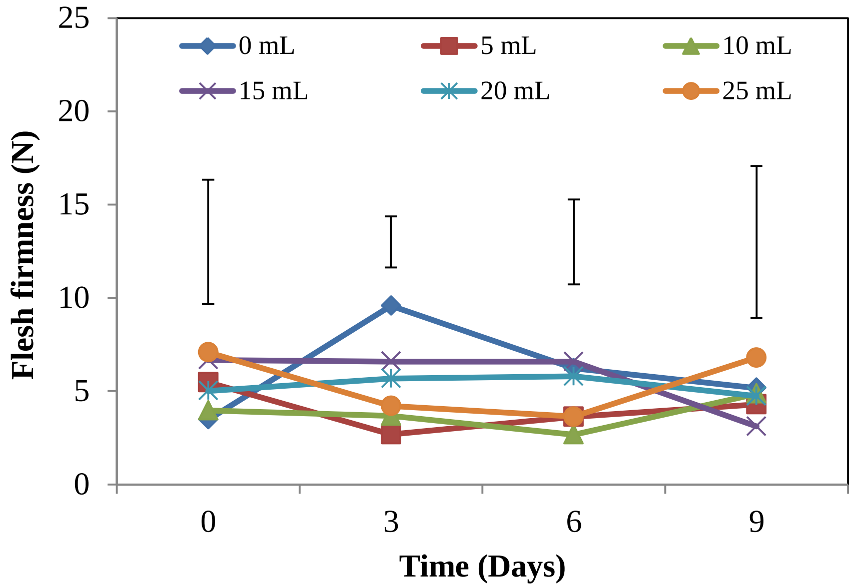

Fig.6: Effects of different volumes of nitrogen gas fumigation on flesh firmness of minimally processed pineapple. Vertical bars represent HSD at 5% level. (HSD values on Day 0=6.68, Day 3=2.73, Day 6=4.56 and Day 9=8.13).

It is obvious that the titratable acidity of fresh-cut pineapples increased with storage time, even though the increase was insignificant (Fig.7). According to Wills et al. (1981), the acids concentration is expected to decline prior to ripening, except for banana and pineapple that achieved highest level at full ripe stage. Marriot (1980) reported that titratable acidity of banana is mostly being influenced by the variations of organic acids prior to ripening since the dominant organic acids in banana are citric, malic and oxalic acid. Similar to banana, pineapple is consisted mainly of citric and malic acid. However, the increase of titratable acidity in the present study could be due to accumulation of ethanol prior to alcoholic fermentation, as the consequence of anaerobic condition (Kelly and Salveit, 1988).

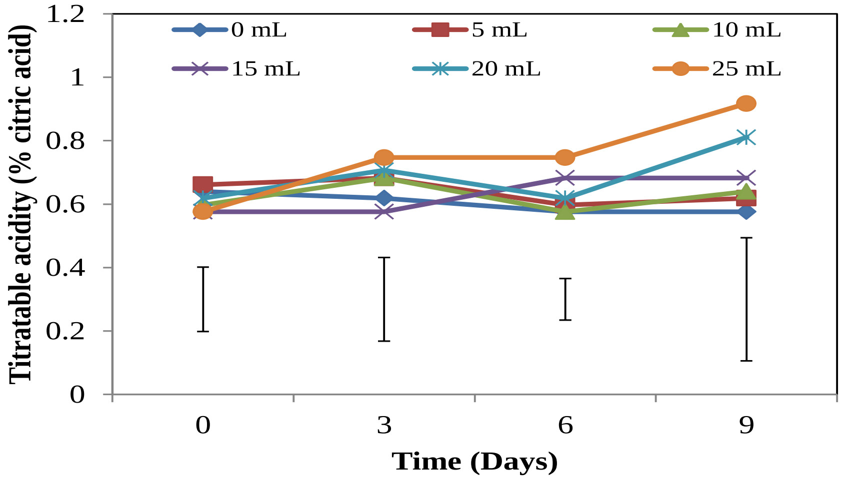

Fig.7: Effects of different volumes of nitrogen gas fumigation on titratable acidity of minimally processed pineapple. Vertical bars represent HSD at 5% level. (HSD values on Day 0=0.20, Day 3=0.26, Day 6=0.13 and Day 9=0.39).

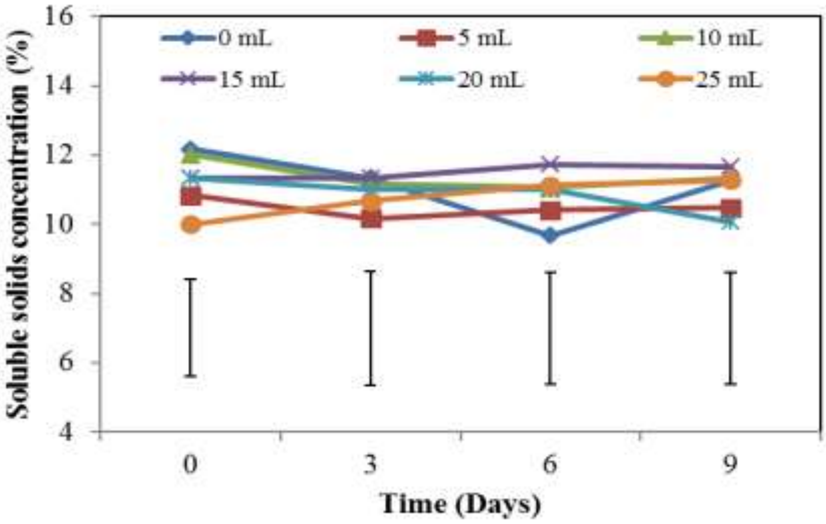

Fig.8: Effects of different volumes of nitrogen gas fumigation on soluble solids concentration of minimally processed pineapple. Vertical bars represent HSD at 5% level. (HSD values on Day 0=2.82, Day 3=3.30, Day 6=3.22 and Day 9=3.22).

For soluble solids concentration, the trends were almost constant for all the treatments and did not differ much. This is because pineapple is a non-climacteric fruit which will not experience any climacteric peak during its storage life, thus the sugar content does not vary extremely (Mantilla et al., 2013).

In conclusion, N_2 gas has a potential in reducing the browning incidence of fresh cut pineapples, especially those treated with 25 mL of nitrogen gas without affecting the overall quality of the fruit. However, it is not enough to prolong the shelf life of fresh cut pineapple since the shelf life of the pineapples is only up to nine days.

ACKNOWLEDGEMENT

The authors wish to thank Universiti Malaysia Terengganu for the grant provided.

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