Characterization of the liquid endosperm attributes in young coconut fruit during storage

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Abstract

Fresh coconut water is consumed as a refreshing soft drink but the quality in young coconut fruit is rapidly changed during storage. Although low temperature is generally applied to most fresh produce for extending the storage life, improper low temperature could accelerate the quality losses. Thus in the present study, attributes of the liquid endosperm of young green coconut cv. 'Nam Wan' were investigated during storage as whole fruit at 4°C, 13°C, and 25°C. Fruit stored at 4°C showed various chilling injury disorders including skin (exocarp) browning and solid endosperm (kernel) splitting from the hard shell (endocarp), whereas the liquid endosperm expressed increasing turbidity and off flavour. The storage life of fruit stored at 25°C could be terminated by day 16 due to dehydrated skin and low quality of the water, whereas storage at 13°C maintained fruit quality throughout 28 days. Lipoxygenase (LOX) activity in the liquid endosperm of fruit stored at 4°C and 25°C immediately increased after storage, related directly to generation of the off flavour. The liquid endosperm comprised high amounts of aliphatic alkanes. When nonane sharply increased, long chain aliphatic alkanes, in contrast, significantly decreased during storage at all temperatures. Interestingly, the liquid endosperm in coconut fruit acquired a pressure of 0.75 Kpa at harvest. The pressure sharply increased in fruit stored at 25°C, consisting with a sharp incline of the dissolving CO_2 concentrations. Furthermore thickness of the solid endosperm of fruit continued to develop during storage.

Introduction

Young coconut (*Cocos nucifera* L.) fruit supply a drink of pleasant and sweet flavour. The water (liquid endosperm) from fresh coconut fruit is consumed as a wonderful refreshing drink (Yong *et al.*, 2009). Coconut fruit is classified as a drupe fruit type, comprising skin (exocarp), fibrous husk (mesocarp), hard shell (endocarp), kernel or white flesh (solid endosperm) and inside water (liquid endosperm). In Thailand, there are 3 major groups of coconut which are 'Nam Hom' (fragrant liquid endosperm), 'Nam Wan' (sweet and non-fragrant liquid endosperm), and the group to be processed for coconut milk. The immature green coconut fruit at approximately 6 to 8 months after anthesis (MAA) of fruit development contains large amount of clear liquid and a jelly-like white flesh which are popular for fresh consumption. The liquid endosperm in the drupe starts to gradually reduce during fruit maturation. At full maturity (9-

10 MAA) the fibrous husk portion is about 31–54% (w/w) by total fruit weight, 12–16% (w/w) of shell, 28–33% (w/w) of kernel and 6–25% (w/w) of liquid endosperm (Perera *et al.*, 2014). The gas in nut starts to appear when the flesh is formed and is found about 25 mL in old nut (Herbert, 1927). In fact, there is a pressure pushing out when the endocarp shell is cracked; so that there might be a relationship between inside-nut pressure and gaseous concentration in the liquid endosperm. Furthermore, the pressure apparently increases during maturation and storage. However, there has recently been no report about the liquid endosperm pressure.

Coconut liquid endosperm containing mainly water (94.99 g/100 g FW), is composed of organic acids (malic acid, and pyridoline), soluble sugars (sorbitol, manitol, sucrose, glucose, and fructose), amino acids (γ-aminobutyric acid, glutamic acid, and lysine) and aroma volatile compounds (2-methyl-1-butyl acetate and terpenes such as D-limonene, α-pinene,

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and 3-carene), contributing the characteristics of fragrant coconut flavour (Yong *et al*., 2009; Jirapong *et al*., 2015). Volatile organic compounds in coconut flesh (solid endosperm) contains mainly δ-lactones, such as δ-octalactone and δ-decalactone (Lin *et al*., 1970) when liquid endosperm exhibits the high proportion of n-alkanes especially of dodecane and tetradecane as the major components (Meethaworn and Siriphanich, 2014; Jirapong *et al*., 2015). Furthermore, coconut liquid endosperm contains high concentrations of middle chain free fatty acids lauric acid ($C_{12:0}$) and myristic acid ($C_{14:0}$) (Jirapong *et al*., 2015). Thus, flavour compounds of the coconut liquid endosperm may be formed by degradating the fatty acids, probably caused by some oxidative metabolisms such as β-oxidation and the lipoxygenase (LOX) pathways. On the other hand, taste of the coconut liquid endosperm is determined by the relationship between soluble solids (SS) and titratable acidity (TA) which getting sourer by a decrease of SS and an increase of TA in long period storage (Changprasert, 2011). From our preliminary study, the quality attributes of the liquid endosperm both visual appearance and flavours were changed quickly in days at room temperature.

Low temperature is widely used to reduce the rates of chemical reactions and metabolic processes of plant parts such as reduction of respiration rate, ethylene production, secondary metabolic processes, weight loss, and growth of pathogenic microorganisms (Wills *et al*., 1998). The lower temperature is responsible for the longer storage period. However, improper storage temperatures cause damaging disorders called 'chilling injury (CI)' of the stored fruit while most tropical fruits are typically stored at mild temperatures of 10-15ºC. However, Tongdee *et al.* (1991) reported that trimmed young coconut, which most fruit husk are removed, is normally stored at 3-6ºC with 90-95% RH, while the shrinked film wrapped fruit can be held for 3 to 4 weeks. Consignado *et al.* (1976) reported skin browning of whole coconut fruit after 7 days of 0°C storage. Moreover, incorrect temperature management either exceeding high or low is responded for different aroma volatile profiles and flavour perception of fresh produce (Reineccius, 2005). Meethaworn and Siriphanich (2014) reported that significant increases of nonanal and octanal which could be used as indicators of generation of off flavour were found in the liquid endosperm of commercially trimmed young coconuts stored at 4°C after 2 weeks. Thus the present research aimed to investigate quality changes of whole coconut fruit stored at low temperature storage. The attributes and some physico-chemical components in coconut liquid endosperm/solid endosperm were monitored during storage period.

Materials and Methods

Preparation of coconut samples and storage treatments

Young green coconut fruit cv. 'Nam Wan' (sweet with non-fragrant liquid endosperm) at 7 MAA in which the inflorescences were tagged at 50% full bloom of inflorescences, showing the first layer of jelly white flesh stage were obtained from Ratchaburi province in Western Thailand between July 2012. Fruit (4-5 fruit at the middle of each bunch) were selected and brought to the Postharvest Technology laboratory of King Mongkut's University of Technology Thonburi, Bangkok within 2hr by a small truck. Whole coconut fruit were sorted for uniformity of size (ca 2,800-3,000g/ fruit), and skin colour with free from major defects and then stored under continuous conditions of 4°C, 13°C (90-95% RH), and 25°C (65-70% RH) as room storage stimulation.

A subsequent experiment of coconut fruit development after harvest was done in September 2012. Selected fruit were stored at 13°C (90-95% RH), and 25°C (65-70% RH) for short period of 10 days.

Chemicals used in the experiments

Most chemicals used to analyse the qualitative and quantitative parameters in the experiments were provided by Sigma-Aldrich Pte. Ltd., Singapore, unless stated otherwise.

Determination of fruit quality

Colours of the fruit exocarp (skin) and the solid endosperm (flesh) were monitored in the middle of fruit in two opposite places of each fruit using a colorimeter (model CR-300, Minolta Co. Ltd., Osaka, Japan). The solid endosperm thickness was measured at three positions of the stem (half length between the equator and stem end) end, middle (the equator) and bottom ends (half length between the equator and bottom end) using a decimal caliper (Mitutoyo Ltd., Kawasaki, Japan). Percentage of coconut fruit weight loss was measured with a scale of average precision.

The liquid endosperm was subjected to detect the transparency, SS, and organic acids. Liquid transparency was determined of the light transmittance (%T) at the wavelength of 610 nM using a spectrophotometer (UV-1601, Shimadzu Ltd., Kyoto, Japan) as described by Campos *et al.* (1996). Titratable acidity (TA) was determined according

to the AOAC method (2000) while soluble solids (SS) contents were measured using a digital hand refractometer (PAL-1, Atago Ltd., Tokyo, Japan).

Determination of gases in the liquid endosperm

Dissolving O_2 concentration (mg•mL^{-1}) in the liquid endosperm was detected using a Cyber Scan Do 300/310 OD meter (Entech Consultancy Bureau (Madras) Pvt. Ltd., Chennai, India)

Dissolving CO_2 concentration was determined by dropping 3 drops of phenolphthalein in 5 mL of the liquid endosperm. The solution was titrated to the end point (pink colour) by 0.02 N NaOH (Ajax Finechem Pty, Ltd., New South Wales, Australia). The concentration was calculated by

$$CO_2 \text{ (mg•mL}^{-1}) = (A \times N \times 44 \times 100)/ \text{ mL of sample}$$

When A = amount of NaOH used
    N = Naormality of NaOH used
    44 = Molecular weight of CO_2

Dissolving N_2 concentration was measured using the method of Patnaik (2002). Five mL of the liquid endosperm mixed with boric acid and a methylene blue indicator was titrated with 0.02N H_2SO_4 (Avantor Performance Materials, Inc., Centre Valley, PA, USA) compared to the blank (distilled water). The concentration was calculated by

$$NH_3\text{-N (mg•mL}^{-1}) = ((Va\text{-}Vb) \times 280) / \text{ mL of sample}$$

When Va = amount of H_2SO_4 used for sample titration
    Vb = amount of H_2SO_4 used for blank titration

Determination of the liquid endosperm pressure inside drupe

Pressure of the liquid endosperm inside the nuts was determined during storage using modified-pressure gauge equipment. The detection gauge can be provided between 0.00-3.00 Kpa. The inlet stainless steel needle was punctured into the nuts at the geminating pore on the stem end (Supplement). The initial pressure was recorded.

Determination of LOX and POD assay in the liquid endosperm

Twenty mL of coconut liquid endosperm were added with 0.5 g of polyvinylpolypyrolidone (PVPP) and then immediately centrifuged at 11,500 x *g* for 20 mins at 4°C. The supernatant was used as the crude enzyme extract. The protein determinations were carried out using the dye-binding method of Bradford

(1976). A standard curve was constructed using bovine serum albumin in the concentration range of 0–100 μg/mL in which a linear response (r=0.998) was observed.

Lipoxygenase (LOX) activity was monitored spectrophotometrically at 234 nm, according to the method described by Pérez *et al.* (1998) with a slight modification. The substrate solution (25 mL) was prepared by mixing 10 μL of linoleic acid, 10 μL of Tween-20 and 5 mL of deionized water. The solution was clarified by adding 0.1 N NaOH. LOX activity was started by adding 20 μL of crude enzyme extract with substrate solution. One unit of all LOX activity was defined as an increase in absorbance of 0.001 per minute per μg of protein under assay conditions.

Peroxidase (POD) activity was monitored spectrophotometrically at 470nm, according to the method described by Campos *et al.* (1996) with a slight modification. The substrate solution was prepared by mixing 7 mL of 0.2 M sodium phosphate buffer (pH 5.5), 1.5 mL of 0.02 M guaiacol (phenolic substrate) and 0.5mL of 0.02 M hydrogen peroxide. The assay was mixing 25 μL of crude enzyme extract with substrate solution and incubated in a water bath at 35°C. One unit of POD activity was defined as an increase in absorbance of 0.01 per minute per mg of protein under assay conditions.

Determination of non-volatile components in the liquid endosperm

Volatile organic compounds were extracted by solvent extraction technique. A 50 mL sample of coconut liquid endosperm was finely mixed with 20 mL of diethyl ether: n-pentane (1:1 v/v) (Avantor Performance Materials, Inc., Centre Valley, PA, USA) and the mixture was gently shaken in a separation funnel for 10 mins. The water phase was discarded and the organic phase was mixed with 1g of sodium sulphate anhydrous and centrifuged at 9,500 x *g* at 4°C for 20 mins. Gaseous nitrogen was used to evaporate the solvent to 0.5 mL. The condensed extract was kept under N_2 gas and stored at -20°C in a 2 mL vial until analysis.

GC-MS analysis was performed on an Agilent 6850 (GC)/HP 5975 (MS) (Agilent Technologies, Santa Clara, USA). Separation was achieved using helium as carrier gas through a fused silica capillary column (HP-5MS) (Agilent Technologies, Santa Clara, USA). A GC injector was set in the split mode. Oven temperature was programmed starting at 50°C and final temperature at 260°C. Analysis of the spectra was operated in the electron impact (EI) mode with an electron energy of 70 eV; mass range m/z 30-500; and EM voltage, 3000 V. Volatile components were

identified using the ChemStation computer program supplied with the NIST98 library (Mass Spectral Libraries, Agilent Technologies Software).

Statistical analysis

A completely randomised design (CRD) was used for the experimental management with at four replications (two fruit per replication) per treatment. Quantitative data were subjected to ANOVA, and the means were compared by least significant difference (LSD) test for multi-treatment comparison and by t-test for pair comparison at $P \leq 0.05$ using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Physical changes of whole nut and the solid endosperm

Colours of the outer exocarp and the solid endosperm were fairly maintained in fruit stored at 13°C. At 25°C some dried areas were widely spattered on the exocarp after day 8, whereas fruit stored at 13°C obviously express exocarp shriveling on day 24 (Figure 1A). The fruit weight loss increased constantly during storage at 25°C which could terminate the storage life by 2 weeks. The weight loss was mainly respected by low relative humidity of 60-75% at 25°C storage as low temperature play an additional role of a reduction of respiration of fruits (USDA, 1962). Furthermore, coconut fruit stored at 4°C began to show browning and discolouration on the exocarp after 8 days of storage, related to quick drops of the L^* Hunter scales (Figure 1C) and the hue angles (Figure 1E). This would be related to enzymatically browning generation of chilling injury symptoms developing in many tropical fruits under improper low storage temperature (Patel *et al.*, 2016). Consignado *et al.* (1976) reported that coconut fruit stored at low chilling as 0°C exhibited skin browning after 7 days of storage. In contrast, L^* values of the white flesh of fruit stored at 4°C increased during storage, compared to those of fruit stored at 13°C and 25°C (Figure 1D) while there were no significant differences of the hue angles between treatments (Figure 1F). Furthermore from the observation, the solid endosperm of coconuts stored at 4°C was easily splitted off from the inner hard stone shell when stored for 28 days, compared to those stored at 13°C (Figure 1B).

Physico-chemical changes of the liquid endosperm

Coconut fruit used in the experiment contained the portion of the liquid endosperm at average of 450-500 mL or about 15-17% (w/w) of the fruit
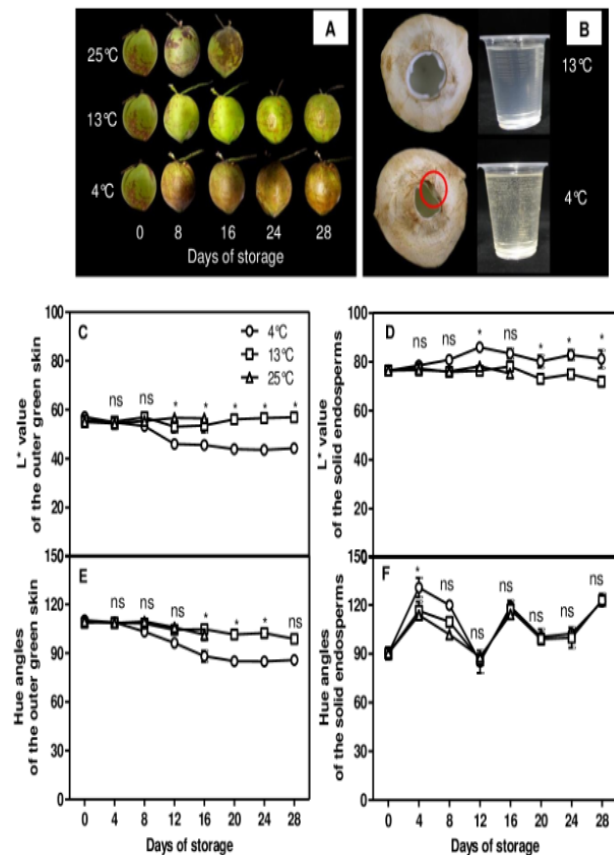

Figure 1. Visual appearance of the fruit skin changes during storage (A) and the solid and liquid endosperm of fruit stored at 4°C and 13°C on day 28 (B), and changes in L^* values (C), hue angles (E) of the outer green skin and L^* values (D) and hue angles (F) of the solid endosperms of whole coconut fruit stored at 4°C, 13°C, and 25°C. Data represent means ± SE of four replications. Asterisks indicate differences between vertical treatments.

fresh weight. SS content of the liquid endosperm was at 6.87% at initial and slightly changed during storage. On the other hand, TA of all samples fluctuated in the region of 0.036-0.047%. Thus SS/TA ratios of the liquid endosperm of fruit stored at all temperatures were varied during storage. The ratios of all treatments apparently increased on day 16 and then continuously decreased through the end of storage (Figure 2A). Taste of coconut liquid endosperm is generally thought of a combination of oily sweet and sour with unique pleasant flavour. The liquid endosperm of all treatments became less sweet because of a slight reduction of the SS/TA ratio at the end.

LOX activities in the liquid endosperm showed significant differences between fruit stored at different temperatures at the first 8 days of storage (Figure 2B). LOX activity in the liquid endosperm of fruit stored at 25°C rapidly increased over 2 units•min^{-1}• µg^{-1} protein after 8 days of storage, whereas interestingly, the activity in fruit stored at 4°C progressively
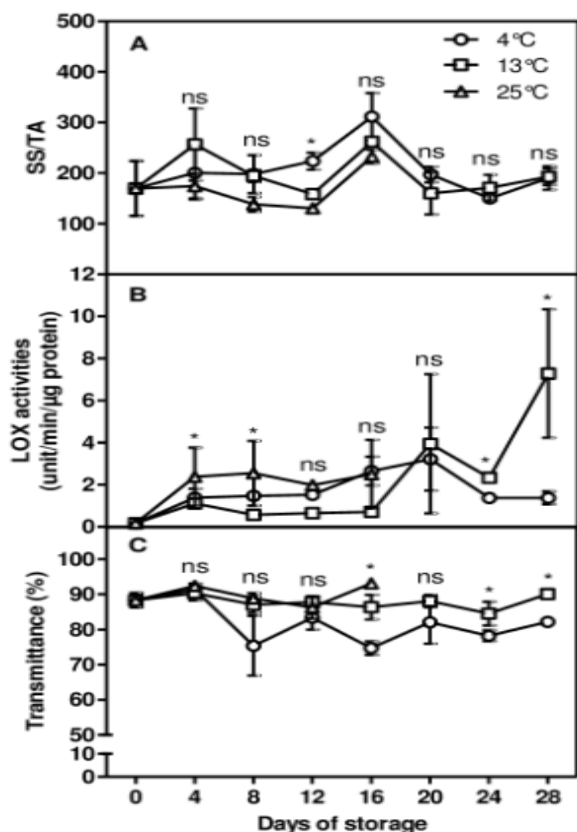
Figure 2. Changes in ratios of soluble solids /titratable acidity (A), LOX activities (B), and light transmittance (C) of the liquid endosperm of whole coconut fruit stored at 4°C, 13°C, and 25ºC. Data represent means ± SE of four replications. Asterisks indicate differences between vertical treatments.

Table 1. Non-volatile components identified in the liquid endosperm of 'Nam Wan' whole coconut fruit (7 months after anthesis) stored at 4°C, 13°C, and 25ºC

		Peak area (x10^5)			
		Day 0	Day 16		
RT	Components		25°C	13°C	4°C
4.30	Nonane (C$_9$H$_{20}$)	6.19	9.00	11.01	11.26
21.07	Docosane (C$_{22}$H$_{46}$)	7.26	0.40	1.45	0.82
24.20	Heptadecane (C$_{17}$H$_{36}$)	2.07	2.10	nd	nd
24.86	Heneicosane (C$_{21}$H$_{44}$)	5.03	2.56	0.09	1.70
25.98	Octacosane (C$_{28}$H$_{58}$)	0.29	-	1.69	0.99
28.59	Eicosane (C$_{20}$H$_{42}$)	1.21	2.52	1.41	3.00
30.04	Pentacosane (C$_{25}$H$_{52}$)	5.58	2.16	2.07	2.48
34.29	Heptacosane (C$_{27}$H$_{56}$)	2.69	3.44	nd	2.13

† Mean values (n = 4) of each compound at each storage temperature were acquired from ion masses ranging between 30 - 500 m/z determined using GC-MS.
‡ nd, not detected

increased during storage proceeded. In fruit stored at 13°C, the LOX activity remained at the first 16 days and then dramatically inclined.

Inappropriate low storage temperature at 4°C and the high at 25°C induced off-flavour of the liquid endosperm in whole fruit in a short period of time. Low quality of the liquid endosperm was related to the increasing LOX activities. In case of the low temperature storage, this is consistent with Meethaworn and Siriphanich (2014) who reported that off-flavour was presented in trimmed coconuts stored at 4°C for 2 weeks. Furthermore, light transmittance of coconut liquid endosperm as an indicator implement of visual quality was at 88.32%T after harvest (Figure 2C). The liquid endosperm in fruit stored at 4°C was more turbid showing yellowish colour (Figure 1B) and a decrease in the absorbance. When the appearance of coconut liquid endosperm stored at 13°C was still clear, the light transmittance remained stable throughout storage.

Changes of non-volatile components in the liquid endosperm

The major compounds in the liquid endosperm comprised 8 aliphatic alkanes which were between 9 and 28 carbon atoms (Table 1). Docosane, nonane, pentacosane, and heneicosane were high in the coconut liquid endosperm. There have been several studies, reporting majority of a number of alkanes in coconut liquid endosperm (Campos *et al.*, 1996; Meethaworn and Siriphanich, 2014; Jirapong *et al.*, 2015). According to the results, it was not yet clarified whether low temperature affected changes in non-volatile components in the liquid endosperm during storage. However, the proportion of nonane sharply increased while that of other long chain alkanes (Docosane (C$_{22}$H$_{46}$) to Octacosane (C$_{28}$H$_{58}$)) slightly reduced during storage in the liquid endosperm of fruit stored at all temperatures. Nonane (C$_9$H$_{20}$) could be derived from C$_9$ LOX-derived compounds of the fatty acid oxidation when LOX would be induced during storage of fresh produce (Song, 2010). Furthermore, nonane content is related to coconut fruit maturation that increases about 50% in the liquid endosperm from 'Nam Hom' coconut fruit at 6 months to fruit at 8 months after anthesis (Jirapong *et al.,* 2015). The changes of compounds were likely the extensive changes in flavour changes and loss of flavour quality during storage.

Development of coconut fruit after harvest

The postharvest development of the liquid and solid endosperms in fruit were consecutively compared between room temperature and 13°C, the proper storage temperature. Interestingly, we found that the liquid pressure inside the hard shell contained pressure and started at 0.75 Kpa at harvest. The pressure was dramatically increased to 0.5 fold in fruit stored at 13°C and to 1.0 fold in fruit at 25°C during
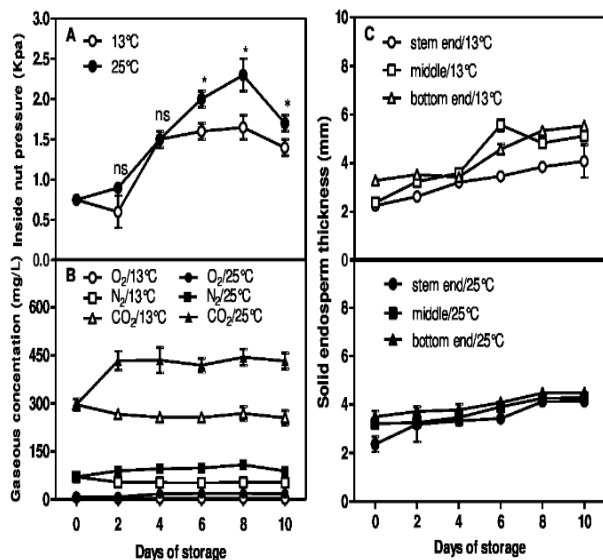
Figure 3. Changes in pressure of the liquid endosperm inside nuts (A), concentrations of gasses in the liquid endosperm (B), and the solid endosperm thickness (C) of whole coconut fruit stored at 13°C, and 25°C. Data represent means ± SE of four replications. Asterisks indicate differences between vertical treatments.

first 8 days (Figure 3A). The portions of dissolving gases in nuts were different to those of the headspace gases which were firstly reported by Herbert (1927). As the hypostasis of a relation between the inside-nut pressure and an accumulation of dissolving gases in the liquid endosperm, N_2, the major in the head space, increases from 81.3% in young nuts to 99.8% when O_2 reduces from 18.7% to 0.2%. CO_2 is found in trace throughout the development. In the liquid endosperm, CO_2 was the major dissolving gas and above 2 times higher than N_2. The liquid pressure was higher in fruit stored at 25°C, related to an increase in CO_2 concentration in the liquid endosperm (Figure 3B). The dissolving CO_2 concentration was at 296.3mg/mL at harvest and inclined to about 435.6mg/mL 4 days after storage at 25°C when it remained stable in fruit at 13°C. The increase in the liquid endosperm pressure was high consistent with the increase in the CO_2 concentration. Furthermore, the dissolving CO_2 could make the coconut water as a carbonated refreshing drink and play a crucial role in the liquid endosperm pressure. Lipid degradation in the liquid endosperm could be responsible from a liquid CO_2 induction since coconut liquid endosperm contains high amounts of medium chain fatty acids (C_{10}-C_{14}) (Jirapong et al., 2015).

Moreover, the solid endosperm had kept the development after harvest as the thickness in the fruit shell increased during storage. Interestingly the thickness steadily increased about 2 folds at both end at the end of the storage when white coconut flesh at the bottom end was thicker than that at the
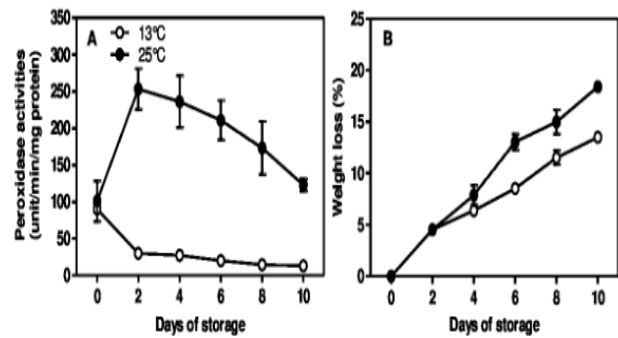

Figure 4. Changes in peroxidase activities of the liquid endosperm inside fruit (A), and weight loss (%) (B) of whole coconut fruit stored at 13°C, and 25°C. Data represent means ± SE of four replications.

stem end about 0.5 folds (Figure 3C). Related to the reduction during storage (Table 1), alkanes in the liquid endosperm may play a role in being a good source for development of the solid endosperm after harvest. POD activity used for glucose oxidation and enzymatic removal of H_2O_2 thus damage of cell was dramatically high in the liquid endosperm of fruit kept 25°C. The POD activity was high 253.29 units/min/mg protein at 25°C and low 29.95 units/min/mg protein at 13°C (Figure 4A). Glucose was one of major sugars in the liquid endosperm followed by fructose and sucrose (data not shown) which is consistent with Yong et al. (2009) who reported high amounts of glucose and fructose in coconut liquid endosperm. As discussed above, differences in the temperature storage inactivation kinetics of POD activities could be due to composition such as sugar contents, salt contents and pH, of the coconut liquid endosperm (Campos et al., 1996). High weight loss from the fibrous husk of fruit occurred during storage at 25°C which the weight loss were 18.4% for storage at 25°C on day 10 (Figure 4B). This was related to visual appearance of skin drying of fruit stored at 25°C in the Figure 1A.

Conclusion

Storage at 4°C induced skin browning of whole fruit after 8 days, whereas storage at 13°C maintained fruit quality throughout 28 days, but the storage life was by 12 days in fruit stored at 25°C. Quality of liquid endosperm was maintained in fruit stored at 13°C when the intense off flavour was high in those of fruit stored at 4°C and 25°C. Light transmittance of the liquid endosperm of fruit stored at 4°C reduced significantly, compared to that at 13°C. Liquid endosperm contained high pressure at harvest and then the pressure increased during storage especially in fruit stored at 25°C, related to high amount of

CO_2 in the liquid. Nonane in the liquid endosperm increased whereas other alkane compounds slightly reduced at all storage temperatures. Thus, among the storage temperatures, 13°C was best to maintain the overall quality of whole coconut fruit as fresh harvest.

Acknowledgements

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