FRUIT GROWTH, ENDOCARP LIGNIFICATION, AND BORON AND CALCIUM CONCENTRATIONS IN NAM HOM (AROMATIC) COCONUT DURING FRUIT DEVELOPMENT

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ABSTRACT

Fruit growth, endocarp lignification, and boron and calcium concentrations in 'Nam Hom' coconut during fruit development were studied. This study was conducted at Ban Paew district, Samut Sakhon province between January 2014 -September 2015. Whole fruit shape during the first 4 months (mo) after flowering was oval and turned to semi-oval at 8 mo. The shell was a round shape from 5 mo onwards. The endocarp (shell) started to accumulate lignin 2 mo after flowering but accumulation was highest and constant over the 4-8 mo period. Lignin accumulation started from the stylar end and progressed to the stem end. It was completed in the shell by 6 mo. This stage was accompanied by a decrease in water content. The concentrations of cellulose and hemicelluloses in the coconut shell increased rapidly from 2 to 6 mo then remained constant. The amount of fiber in the coconut husk increased with fruit age and was highest at the final harvest. Coconut husk had greater concentrations of both calcium and boron than occurred in the shell. Calcium concentration in the coconut husk decreased only slightly throughout fruit development while calcium concentration in the coconut shell decreased markedly with age. Boron concentration in the coconut husk decreased with fruit age whereas the highest concentration in the shell occurred at 4 mo and then subsequently decreased. These data indicated that the possible role of boron and calcium are likely to be necessary for shell formation and endocarp lignifications of coconut.

Key words: growth, shell, husk, lignin, cellulose, hemicelluloses

INTRODUCTION

'Nam Hom' coconut, or aromatic coconut, is a dwarf type of coconut which assimilates the liquid and solid endosperm at an immature stage (young coconut or tender coconut). Consequently, the coconut bunch can be harvested 6.5-7 mo from flowering. 'Nam Hom' coconut was ranked fourth of all fruit exported from Thailand in 2016. The main export markets for 'Nam Hom' coconut are the USA and China and the quantity and value of these exports have increased continuously over recent years (Office of Agricultural Economic, 2016).

The fruit of coconut, which is a fibrous drupe, consists of the husk (exocarp + fibrous mesocarp), a shell (endocarp) and the endosperm (which is eaten). Fruit development of coconut var. Nana, in terms of dry weight accumulation, was shown to have a sigmoidal pattern (Jayasuriya and Perera, 1985). Jaroonchon et al. (2017) reported that fruit development of 'Nam Hom' coconut followed a double-sigmoid growth curve being characterized by three stages like other drupe fruit such as peach (Chalmers and Ende, 1975; Dardick et al. 2010), Japanese plum (Kritzinger et al. 2017), apricot (Gulsen et al. 1995) and olive (Rapoport et al. 2013). In the first phase, development was characterized during the first 5 mo by rapid growth of the whole fruit and endocarp; in stage II, the

growth rate slowed between 5 to 6 mo of growth; and in the final stage, stage III, which was from 6 mo onwards, the fruit grew steadily until full maturity (Jaroonchon et al. 2017).

During stage II, the endocarp is lignified and its thickness is increased, turning brown and becoming hard (Siriphanich et al. 2011; Jaroonchon et al. 2017). This endocarp hardening of 'Nam Hom' coconut followed the same pattern as that in peach and Japanese plum. In peach fruit during stage II, the mesocarp is relatively quiescent and the endocarp lignifies and hardens, which is the characteristic growth pattern in stone fruit. Fruit hardening is an easy stage to identify in fruit development and endocarp (shell or pit) hardening has become a widely used marker for both biological studies and orchard management (Rapoport et al. 2013). In peach and plum, pit splitting or pit-splitting during development can affect fruit quality. Similarly, with 'Nam Hom' coconut, cracking or splitting during development can affect the quality of the coconut fruit. However, there is very little information on endocarp lignification during fruit development in coconut. Improved knowledge about endocarp hardening would provide options for managing this development disorder.

Moreover, endocarp hardening in peach is considered to be the result of secondary cell wall thickening and liginification (Ryugo, 1964). Hayama et al. (2006) reported that the cellulose content of the peach endocarp increased rapidly from 48 to 83 days after full bloom (DAFB), then increased slightly thereafter. In *Zinnia elegans*, lignification proceeded after the formation of cellulose microfibrils in the secondary cell wall (Nakashima et al. 1997). In additional, hemicellulose is one of the primary plant cell wall which has cellulose-hemicellulose network (Geitmann 2011), meanwhile secondary cell wall (natural liginocellulose) mainly consist of cellulose, hemicellulose, and lignin in a 4:3:3 ratio and contain a small amount of pectin (Chen 2014). It was considered important, therefore, to understand cellulose and hemicellulose accumulation in endocarp during coconut fruit development.

A number of plant nutrients are important during seed and fruit development of coconut. Boron and calcium have been reported as being necessary for coconut fruit growth. Boron plays very significant role in nitrogen metabolism, protein biosynthesis, cell division and cell wall formation (Chamak and Romheld, 1997). In coconut, boron deficiency causes various malformations and fruit from boron deficient coconut palms are often cracked, have a blackened husk, or lack a shell (Jayasekara and Loganathan, 1988; Kamalakshiamma et al. 2000; Kamalakshiamma and Shanavas, 2002). Calcium is an important nutrient for fruit quality. It is involved in cross-linkages in the middle lamella, which binds cells together, and plays an important role in the stabilization of cell membranes (Stebbins et al. 1972). Determination of boron and calcium concentrations in the husk and the shell during coconut fruit development would help to determine the importance of these particular nutrients for coconut fruit growth.

The research sought to investigate the lignification of the endocarp during shell hardening, including cellulose and hemicelluloses content, and to determine the concentration of boron and calcium in the husk and shell of 'Nam-Hom' coconut fruit during the first 8 mo of development.

MATERIALS AND METHODS

Twenty-four 'Nam Hom' coconut trees of the same size and age (7-years-old) were selected from an orchard in Samut Sakhon province, Thailand. Within each coconut tree, all inflorescences were tagged at flowering to investigate subsequent fruit growth and shell hardening from 1 to 8 mo of age. Coconut bunches were harvested randomly from the selected trees every month, with three bunches at each sampling time.

Fruit weight, fruit size and shell size, husk thickness and shell thickness. Each whole fruit was weighed (g) (Ohaus Adventurer, USA), and height (cm) and diameter (cm) were measured (vernier

caliper, Matui, Japan). Fruit were then cut longitudinally and shell size was measured (height and diameter, cm). Husk thickness and shell thickness were measured at four positions: stem-end position, two positions on each side and at the stylar-end position.

Water content in husk and shell. The coconut husk and shell were separated and chopped into small pieces and their fresh weight (FW) was recorded before drying at 70C in a hot air oven for 72 h or until constant weight. The water content was calculated as follows:

$$\text{Water content (\%)} = \frac{\text{FW-DW}}{\text{FW}} \times 100$$

FW: the initial fresh weight before drying
DW: final dry weight after drying

Fiber in coconut husk. Fiber content of the husk was determined on a 5 g sample using the method of Gould (1977).

Lignin, cellulose and hemicellulose concentrations in the coconut shell. Endocarp tissues were collected monthly. The endocarps were chopped into small pieces and then dried in a hot air oven at 70C before analysis. A 1 g sample of dried endocarp was analyzed for neutral-detergent fiber (NDF), acid-detergent fiber (ADF) and acid-detergent lignin (ADL) concentrations using the detergent method (van Soest et al. 1991). The concentration of lignin is presented as the percentage of ADL. The contents of cellulose and hemicellulose were calculated as follows:

% cellulose = % ADF - % ADL
% hemicellulose = % NDF - % ADF

Lignin staining (lignin accumulation). Longitudinally cut fruit (three fruit per bunch per sampling date) were stained for lignin formation using phloroglucinol-HCl (pink-red color). The lignin staining was modified using the method of Callahan *et al.* (2009) to determine when and where in the endocarp lignification started and where it spread throughout the endocarp. Cut fruit were placed in a phloroglucinol solution [2% (w/v) dissolved in 85% ethanol and mixed with concentrated HCl in the ratio 2:1] for 10 min to indicate the presence of cinnamaldehyde groups in lignin, and then photographed.

Boron and Calcium concentrations. A 1 g sample of either dried coconut husk or shell was placed in a porcelain crucible and heated in a muffle furnace from room temperature to 750C for 3 h. The porcelain crucible was then placed at room temperature for cooling. The ash was dissolved in 5 mL of concentrated HCl and 5 mL of concentrated nitric acid, and then adjusted to a volume of 100 mL using distilled water. Boron and calcium were analyzed using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (700-ES series ICP-OES, Agilent Technologies, Australia) at 208 nm for boron and at 393 nm for calcium. The concentrations of boron and calcium in mg/kg were determined using standard curves.

Statistical analysis. The means between fruit age were compared using analysis of variance (ANOVA) and Tukey tests at a significance level of $P \leq 0.05$. Principal component analysis (PCA) was used as a statistical method to evaluate the relationships between fruit age and lignin, cellulose, hemicelluloses, boron and calcium content in coconut shell using PAST version 3.0.

RESULTS AND DISCUSSION

Fruit and shell growth. Fruit fresh weight increased from the 3rd mo until the 6th mo by the similar growth rate. However, the growth rate increased exponentially from 1-5 mo after flowering and then declined with actual fruit weight declining in the final month of assessment (Fig. 1A). The water

content in the coconut husk also declined during the last month of development as the fruit matured (Fig. 3). The decrease of fruit weight after 7 mo was caused by a reduction in the volume of coconut water (Jaroonchon et al. 2017) and also decrease in water content in coconut husk.

Fruit and endocarp (shell) size had similar growth characteristics (Figs 1B and 1C). In first 5 mo, the size of the fruit and the shell rapidly increased, almost linearly. The growth rate then declined. Fruit shape during the first 4 mo was oval and changed to semi-round in the final stages of development (Fig.5). In contrast, the shape of the coconut shell in first 4 mo was oval and then the shell shape changed to round due to the diameter having a higher growth rate (Figs 1C and 5).

A previous study reported that the growth (size) pattern of 'Nam Hom' coconut fruit followed a double sigmoidal curve (Jaroonchon et al. 2017). However, in this research, the results showed a similar pattern of fruit growth in the first and in the second phases, but growth was different in the third phase. Jaroochon et al. (2017) found that the size of both the fruit and the shell slightly increased in phase III but at a slower rate than in the first phase. In our study, although overall fruit size did not increase over the final month of development, both husk thickness and mesocarp thickness at the stem end did increase (Fig. 2A), while shell thickness remained constant (Fig. 2B).

The husk of coconut fruit is morphologically fibrous mesocarp. Fiber content in the coconut husk slightly increased during fruit development from 1 to 7 mo after flowering, and then sharply increased at 8 mo to about 12%, concomitant with the decrease in the water content of the coconut husk (Fig. 3). The fiber and water in coconut husk made the coconut husk flexible which supporting high pressure inside the fruit. Thereafter, a decrease in water content of coconut husk made their flexibility change, when coconut fruit had more mature.

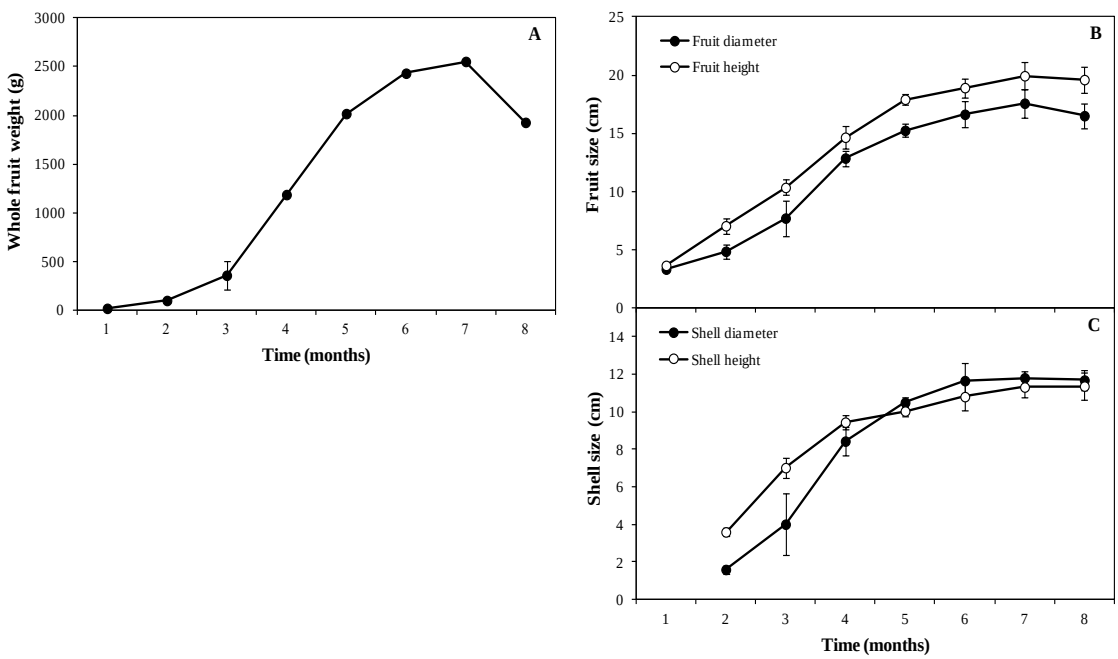

Fig. 1. Change of fresh weight (A), fruit size (B) and shell (endocarp) size (C) of coconut fruit during development. Vertical bars indicate ± SD (n = 5-15 fruit). Some SD values were smaller than the size of the symbols used.

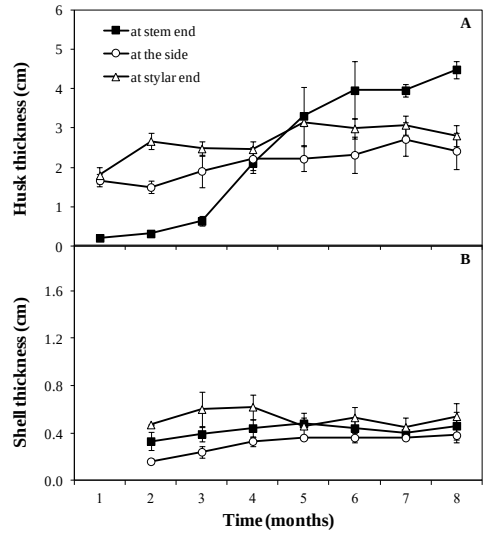

Fig. 2. Change of husk thickness (A) and shell thickness (B) of coconut fruit during development. The thickness was measured in 3 positions; at stem end, at the side and at stylar end. Vertical bars indicate ±SD (n=5-15 fruit). Some SD values were smaller than the size of the symbols used.

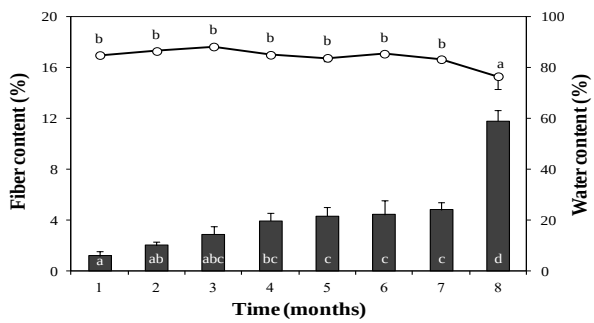

Fig. 3. Fiber content (column graph) and water content (line graph) in coconut husk during fruit development. Vertical bars indicate ±SD (n=3 fruit). Some SD values were smaller than the size of the symbols used. Significant differences ($P < 0.05$) are indicated in lower case letter.

Endocarp lignification and shell hardening. Stage II, defined as being the fruit development phase when fruit growth rate slows down, was defined as being 5 to 6 mo from flowering. The shell also attained maximum size at approximately 5 mo, and then remained the same size until harvest (Figs. 1A and 5). The phloroglucinol-HCl test first revealed lignin in the endocarp at 5 mo with accumulation starting at the stylar end. Lignin accumulation then progressed to the stem end and was completed around the entire shell by the 6 mo development stage (Fig. 5). The accumulation of lignin has been shown to be affected by temperature (Kritzinger et al. 2017) so the timing of lignin accumulation may differ from year to year. In this study, chemical determination of the lignin content showed that accumulation started at 4 mo but, in contrast, the lignin staining did not reveal the presence of lignin until 5 mo of development. The possible reason for this difference in timing is that the water content at 4 mo was still high and started to decrease at 5 mo (Figs 4 and 5). Therefore, the concentration of lignin is diluted by water at the 4 mo stage and the concentration of lignin at 4 mo may not be sufficient for staining to occur as the phloroglucinol-HCl test for lignin is not very sensitive. The lignin content in the shell of 'Nam Hom' coconut was about 26% which is considerably less than that previously reported in the shell of tall type coconut (27-50%) (Cagnon et al. 2009).

The concentrations of cellulose and hemicelluloses in the coconut shell were measured during fruit development. The concentration of cellulose was approximately twice that of the hemicelluloses throughout fruit development. Both increased exponentially until 6 mo after flowering and then remained constant until harvest (Fig. 6). This indicates that the endocarp accumulated a large amount of cellulose and hemicelluloses during endocarp hardening, and that the accumulation progresses continuously during stage III, similarly to that in peach (Ryugo, 1962; Hayama et al. 2006). The concentration of cellulose in the shell of the 'Nam Hom' coconut was about 40-43% and, therefore, similar the cellulose content in the shell of tall coconut (van Dama et al. 2004). This indicted that cellulose content is likely to be related to the mechanical strength of the shell during hardening in the 'Nam Hom' coconut (Hayama et al. 2006).

Endocarp lignification proceeded rapidly concomitant with the decrease in water content in the shell and with the increases in the concentrations of cellulose and hemicelluloses (Figs 4, 5 and 6). The coconut shell became a dark color and it was very difficult to cut the shell with a sharp knife by 6 mo after flowering. As the result, the coconut shell lost flexibility and became rigid. In Japanese plum, the creation of more rigid stones (endocarp), made them more prone to breakage (Kritzinger et al. 2017). Consequently, a loss of flexibility in the shell during lignification might enhance the chance of shell cracking or shell splitting in 'Nam Hom' coconut.

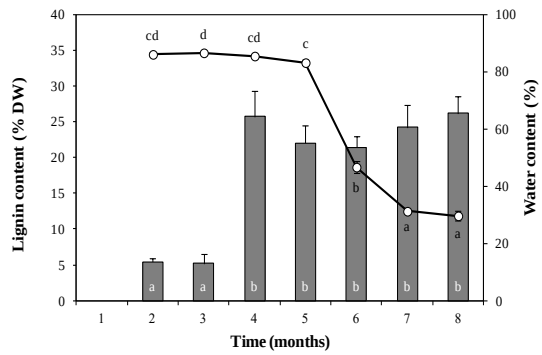

Fig. 4. Lignin content (column graph) and water content (line graph) in coconut shell during fruit development. Vertical bars indicate ±SD (n=3 fruit). Some SD values were smaller than the size of the symbols used. Significant differences ($P < 0.05$) are indicated in lower case letter.

Fig. 5. Time-course of shell hardening as shown by the degree of lignifications using phloroglucinol-HCl stain. Transverse sections of the whole fruit were stained with phloroglucinol-HCl reagent. Bright red coloration is indicative of lignins. Lignin was detected from 5 mo in endocarp tissue (shell) through the period of development tested in this study. Vertical bar indicate scale. The inflorescences of coconut were tagged in January 2014 and January 2015.

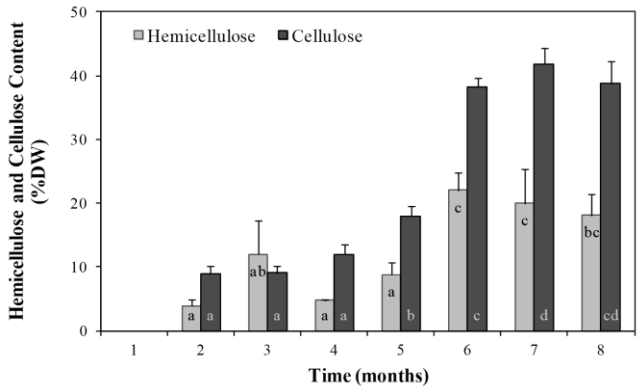

Fig. 6. The content of hemicelluloses and cellulose in coconut shell during fruit development. Vertical bars indicate +SD (n=3 fruit). Significant differences ($P < 0.05$) are indicated in lower case letter. Black lower case letter for hemicellulose and white lower case letter for cellulose.

Boron and calcium concentrations in the husk and shell. Boron and calcium concentrations in the husk and in the shell were studied during fruit development. The coconut husk had greater concentrations of both boron and calcium than those determined in the coconut shell. Boron concentration in the coconut husk decreased with fruit age whereas the highest concentration in the coconut shell occurred at 4 - 5 mo and then subsequently decreased (Fig. 7A). It is noted that boron is necessary for shell formation in peach (Evert et al. 1988). Calcium concentration in the coconut husk declined by only a small amount throughout fruit development, while calcium concentration in the coconut shell decreased with fruit age (Fig. 7B). There was a sharp decrease in calcium concentration in the coconut shell at 5 to 6 mo during the shell hardening phase.

Principle component analysis (PCA) base on a correlation matrix showed that the first component (PC1) with an eigenvalue greater than 1.0 contributed about to 76.26% of the total variation (data not shown). The highest loadings in PC1 (component 1) indicated the importance of this component, with cell wall component, boron and calcium representing the largest portion of those associated with the shell formation in 'Nam Hom' coconut (Fig. 8). PCA analysis revealed that lignin, cellulose and hemicellulose concentrations were strongly associated with lignification of endocarp (Figure 4-6, 8). While, the calcium and boron concentrations were well correlated with the early stage of endocarp development (2-5 mo) (Fig. 7-8).

According to these data indicated that the high calcium and boron concentration in coconut shell of 2-5 mo after flowering play a role in the primary cell wall synthesis. Calcium plays a key function of the cell wall structure (Helper 2005) while boron plays a role in cell wall ultrastructure, which is critical to cell-wall expansion (Hu and Brown 1994). Thus, boron and calcium seem to play important role in the shell formation at early stage. However, coconut fruit at 4-5 mo after flowering revealed the highest boron concentration in coconut shell (Fig. 7A) and was well correlation with this stage (Figure 8) during lignin accumulation and water content decreased (Fig. 4). These indicated that boron might be used as a precursor for the lignin biosynthesis (Lewis 1980). The rapid deposition of lignin coincided with increase in cellulose and hemicellulose (Fig. 6 and 8) and, the growth rate of shell started decrease and the size of shell was constant at the later stage (Fig. 1C). The excess boron limits the growth of tobacco cells, it enhances the strength of the wall (Ghanati et al. 2002). Therefore, the deposition of lignin with in cellulose-hemicellulose net work leads to the secondary cell wall as a natural lignocelluloses (Chen 2014).

The results showed that both boron and calcium were necessary for shell formation and endocarp lignifications in 'Nam Hom' coconut. Therefore, application of both boron and calcium during fruit development should be recommended for reducing some physiological disorders and for improving fruit quality. Boron deficiency causes fruit cracking, a blackening of the husk, or the lack of a shell (Kamalakshiamma et al. 2000; Kamalakshiamma and Shanavas, 2002) and calcium is an important nutrient for fruit quality (Stebbins et al. 1972).

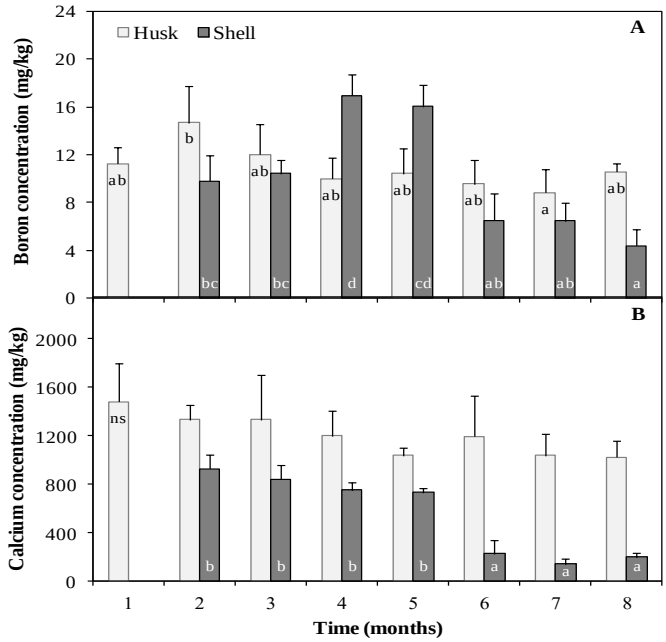

Fig. 7. The concentrations of boron (A) and calcium (B) in the coconut husk and coconut shell during fruit development. Vertical bars indicate +SD (n=3 fruit). Significant differences ($P < 0.05$) are indicated in lower case letter. Black lower case letter for coconut husk and white lower case letter for coconut shell.

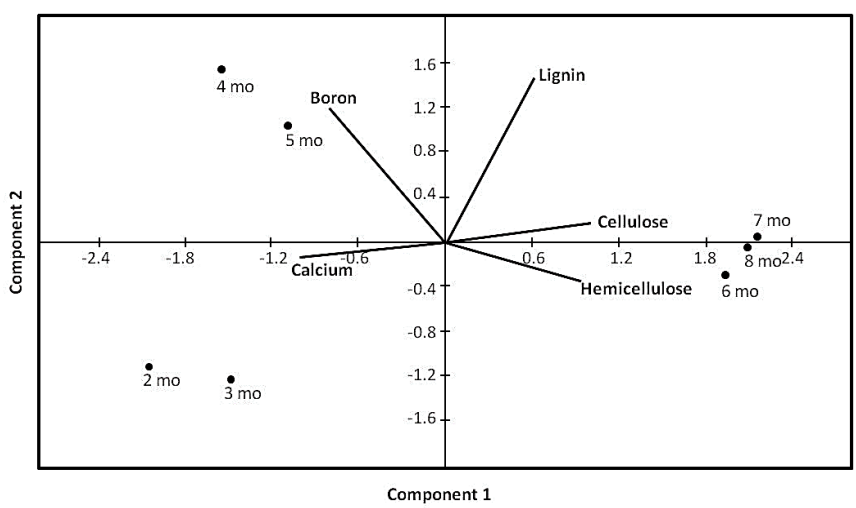

Fig. 8. Principal component analysis (PCA) of the analysis of lignin, cellulose, hemicelluloses, boron and calcium in the coconut shell (indicated by the green lines) and the coconut fruit age: 2 mo, 3 mo, 4 mo, 5 mo, 6 mo, 7 mo and 8 mo (mo = months after flowering).

CONCLUSIONS

The husk and shell of 'Nam Hom' coconut fruit grew rapidly in size during the first 5 mo after flowering and slowed down thereafter. Whole fruit shape during the first 4 mo after flowering was oval and turned to semi-oval at 8 mo. The shell was a round in shape from 5 mo onwards. Lignin accumulation started from the stylar end and progressed to the stem end of the endocarp (shell), and was completed by 6 mo. This stage was accompanied by a decrease in water content. The concentrations of cellulose and hemicelluloses in the coconut shell increased rapidly from 2 to 6 mo and then remained constant. The amount of fiber in the coconut husk increased with fruit age, being highest at harvest. Both boron and calcium concentrations were high in the shell when the fruit was young but decreased with development. Both elements are likely to be important for shell formation.

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