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Original Article

The development of 2 acetyl-1-pyrroline (2-AP) in Thai aromatic coconut

Nopporn Jaroonchon[1,2], Krisana Krisanapook[1,2*] and Wachiraya Imsabai[1,2]

[1] *Department of Horticulture, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Kamphaeng Saen, Nakhon Pathom, 73140 Thailand*

[2] *Center for Advanced Studies for Agriculture and Food, Institute of Advanced Studies, National Research University, Kasetsart University, Bangkok, 10900 Thailand*

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Abstract

Coconut water and coconut meat from 1-9 month-old aromatic coconut fruit were used to monitor the development of the aromatic compound; 2 acetyl-1-pyrroline (2-AP) concomitant with the development of each fruit part. 2-AP was also determined in other parts of fruit as well as in other parts of the tree. The aromatic coconut fruit development represented a double sigmoidal curve. The coconut water and coconut meat were detected when the fruit was two and five months old, respectively. Total soluble solids and titratable acidity increased as the fruit got older and reached the optimum taste when the fruit was 6-7 months old. 2-AP in coconut water and meat was found in fruit of 6 months of age at the earliest, then increased with fruit age, and it was found in all parts of the fruit and other parts of the tree in various amounts.

Keywords: *Cocos nucifera*, fruit development, growth pattern, aromatic compound

1. Introduction

Aromatic coconut is an important economic crop in Thailand due to high demand and continuous increase in both domestic and foreign markets. The value of export increased from 33,249 tons in 2010 to 61,539 tons in 2014 (Office of Agricultural Economic, 2014). The unique fragrance of Thai aromatic coconut is an advantage as a niche product for the export market. Unfortunately, the fragrance is sometimes absent or inconsistent and this obstructs the quality control affecting the value of this coconut cultivar. The main aromatic compound that produces this fragrance in aromatic coconut is 2 acetyl-1-pyrroline (2-AP) (Luckanatinvong *et al*., 2011), which can be found in many plants such as mung bean (*Vigna radiata*) (Brahmachary & Ghosh, 2002), green tea (*Camellia sinensis*) (Kumazawa & Masuda, 2002), pandan (*Pandanus amaryllifolius* Roxb.) and jasmine rice (*Oryza sativa* L.) (Buttery *et al*., 1983; Mahatheeranont *et al*., 2001). 2-AP compound is also known as 5-acetyl-3,4-dihydro-2H-pyrroline which is classified in the pyrrole compounds. Although there have been several studies reporting on 2-AP formation in some plants, especially, in fragrant rice (Buttery *et al*., 1983), how 2-AP formation occurs in Thai aromatic coconut has not been revealed. Thus, this study aimed to observe the development of the 2-AP fragrance substance in each stage of Thai aromatic coconut fruit. Better understanding of the 2-AP formation may lead to ensuring the consistency of the fragrance, probably by further study in breeding or finding the genes controlling the fragrance of aromatic coconut.

2. Materials and Methods

2.1 Fruit growth and development

Fifteen aromatic coconut trees of the same size and age (five-years-old) were chosen from an orchard in Ratchaburi province. On each tree, the inflorescences were

* Corresponding author.
Email address: krisana.k@ku.ac.th

tagged to monitor fruit growth and development from 1 to 9 months of age. Due to the different growth rate in each stage of the fruit, the samples were collected differently. Fruit of 1-5 months of age were collected every 15 days with 6-12 fruits at each time, whereas fruit of 6-9 months of age were collected every month with 4-10 fruit at each time owing to the fruit drop during fruit development.

At each stage, fruit size and peel thickness were measured using vernier caliper. Fruit weight was measured by weight scale and water volume was measured by cylinder. The total soluble solid and titratable acidity in coconut water were monitored by refractometer and titration using phenolphthalein as indicator, respectively. (Association of Official Analytical Chemists [AOAC], 2000).

2.2 2-AP content at different stages and parts of coconut fruit

Coconut meat (solid endosperm) and coconut water (liquid endosperm) from fruit in each stage (1-9 months) were used to determine 2-AP content following the method of Luckanatinvong (2015). Detection of 2-AP in parts of the fruit was done in 7 month old fruit. This is because, at 7 months old, every part of the fruit is already developed and it is also the consumption stage. The fruit were separated into 5 parts; green peel (pericarp), white peel (mesocarp), shell (endocarp), coconut meat and coconut water. Each part was chopped into pieces and store at -70°C for the GC-MS analysis. In addition, male flower, female flower, rachillae, leaf, and root were prepared with the same method as done with the fruit samples. All the analyses were done in triplicate of the same samples.

2.3 GC-MS analysis

Standard 2-AP was purchased from Toronto Research Chemicals Inc. Dilutions were made using dichloromethane. 2,4,6-Trimethylpyridine (TMP) was purchased from Sigma-Aldrich Co. LLC. and diluted in iso-propanol. Purity of 2-AP was confirmed by gas chromatography mass spectrometer (GC–MS) QP-2010 (Shimadzu, Kyoto, Japan) with HP-5MS capillary column (J&W Scientific, CA, USA) (30 m x 0.25 µm). The mass spectrum showed major ions 28, 43, 69, 83, 111, which were identical to the ions of 2-AP in WILEY 7 library.

Volatiles were extracted and concentrated using preconditioned (at 250°C for 30 min) SPME fibre (PDMS/DVB carboxane) attached to SPME manual holder (57330-U) (Supelco, Bellefonte, PA, USA). The SPME fiber was desorbed for 7 min in GC injector having 250°C temperature in splitless mode. The GC oven program was 2 min hold at 50°C, ramped to 250°C at the rate of 10°C/min

2.4 Quantification of 2-AP content

Quantification of 2-AP was done using the standard 2-AP calibration curve following the method of Wakte *et al.* (2010). Standard 2-AP solutions were prepared in dichloromethane at concentration of 310 ng/µl in different amount of 1550 (5 µl), 3100 (10 µl), 4650 (15 µl) and 6200 ng (20 µl), and subjected to HS-SPME-GC-MS analysis. Calibration curve was developed with a linear correlation coefficient (r value) of 0.9666.

2.5 2-AP extraction

One gram of minced solid samples (coconut meat or other coconut fruit parts) or 5 ml of liquid samples (coconut water) were added with 1 µl of 50 ppm TMP and 1 ml of 0.1 N sodium hydroxide (NaOH) and placed into 50 ml vials with PTFE silicone septa (Chromatography Research Supplies, Louisville, KY, USA). The vials were heated in water bath set at 60°C for 10 minutes. SPME fiber (PDMS/DVB carboxane) (Supelco, Bellefonte, PA, USA) of 1 cm in length attached to SPME manual holder (57330-U) was mounted in the manual SPME holder (Supelco) and inserted through the septum of the sample bottle. The fiber was exposed to the sample headspace for 20 minutes prior to desorption of the volatiles into the splitless injection port of the GC-MS instrument with scan mode.

2.6 Data analysis

Comparisons between the mean value of 2-AP were made using Tukey 's range test. The analysis was performed using SPSS software (Version 17, Chicago, IL, USA).

3. Results and Discussion

3.1 Fruit growth and development

In our observation, the growth pattern of aromatic coconut fruit was a double sigmoidal curve and could be divided into 3 phases like other drupe type fruits such as Peach (Chalmers & Ende, 1975). The first phase occupied the first 5 months, with the rapid growth of fruit size, similar to the shell size (diameter and height) (Figure 1a). The rate increase in height was greater than that in diameter, so the fruit shape was oval in this phase. The second phase was when the fruit was 5-6 months old, which the growth of fruit size and shell size slowed down, the rate of growth in diameter was the same as that height, so the fruit and shell gradually changed to a round shape. After six months, the fruit and shell size slightly increased again but at a slower rate than in the first phase.

In the first phase, the shell was white, soft and quite small when compared with the whole fruit. The peel and shell thickness increased with the fruit age (Figure 1b) until 5 month old. The expansion of peel and shell slowed down for 1 month simultaneously with the hardening of the shell, which may be due to the lignin accumulation (Siriphanich *et al*., 2011). After 6 months the shell thickness was rather steady, turned to black color and became hard, while the peel thickness still increased until 8 months then decreased,

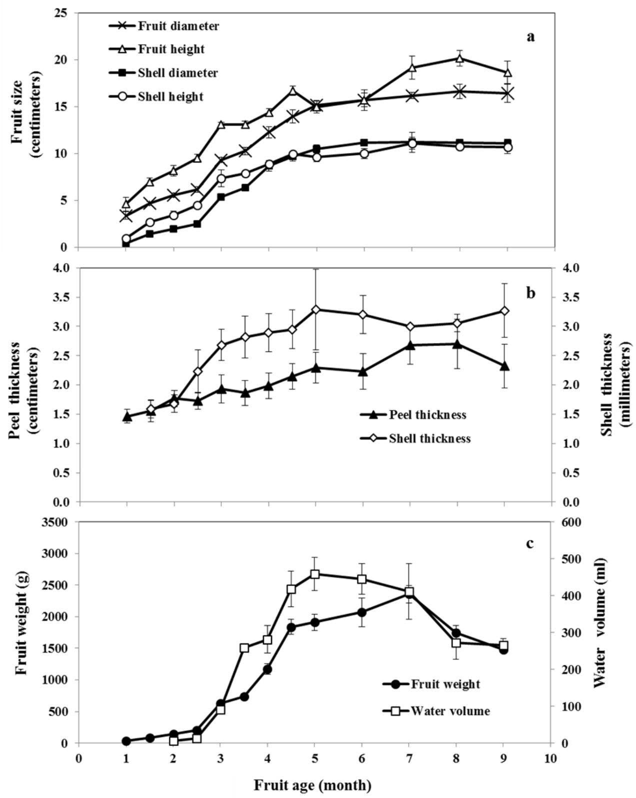

Figure 1. Fruit growth and development of Thai aromatic coconut fruit at each fruit age. (Data are means ± SD)

possibly due to the loss of moisture.

The development of fruit weight and water volume coincided with the fruit size (Figure 1c). The decrease of fruit weight after 7 months may be caused by the reduction in amount of coconut water. At the beginning of fruit growth, the coconut water could be found inside the shell but in the very low amount. After 2 months of fruit age, the coconut water increased rapidly to its maximum at 5 months age (Figure1c) and gradually decreased until 7-months old, then rapidly decreased until the fruit age was about 9 months.

At the beginning of fruit growth, the acidity increased until the fruit was 5 months old and after that became stable until the fruit was 9-months old (Figure 2). TSS in coconut water also increased but it was still low (less than 6%) when fruit age was less than five months, which caused more sour taste than sweet taste of coconut water. Between 5 to 6 months old, the sugar still increased in contrast with the slight reduction of acidity. Thus, the coconut water taste at this age was better than at the younger stage but still not the best time for consumption. After 7 months of growth, sugar in coconut water slightly increased and reached its maximum at 7.5% while the acidity was rather steady.So the taste of coconut water at this fruit age was suitable for consuming. Thus, harvesting time for aromatic coconut is done at this time (between 6.5 and 7 months) from 7-9 month-old, the sugar slightly decreased. Jackson *et al*. (2004) reported that in Green Dwarf variety, after nine months of growth the total soluble solid in coconut water slightly decreased, which is similar to our result.

The development of coconut meat came after the coconut water and started from 5 months old when it began to form as a thin layer of jelly inside of the shell at the basal region (antipodal end) and then developed throughout the cavity of the nut and became thickened as fruit matured. This is similar to the observation in some old studies (Abraham & Mathew, 1963; Prades *et al*., 2012). According to the report of Prades *et al*. (2012) and Jayasuriya & Perera (1985), the development of coconut meat commenced around 5-6 month of fruit growth and continued until full maturity.

3.2 2-AP content in different fruit stages and parts of plants

Since we could detect coconut water when the fruit was in the 2nd month of growth, 2 acetyl-1-pyrroline (2-AP) content was determined at this stage. However, the 2-AP content in coconut water could not be detected until the fruit was six months old, at an amount of 47 ng/g FW (Figure 3). Then it increased with the fruit age and reached the maximum amount of 2,036 ng/g FW when the fruit was at nine months of growth. Thus, the aroma was evident at this older age.

The 2-AP content in coconut meat was higher than in coconut water and could be detected at the 6th- month, in an amount of 1,837 ng/g FW (Figure 3). Then, it also increased

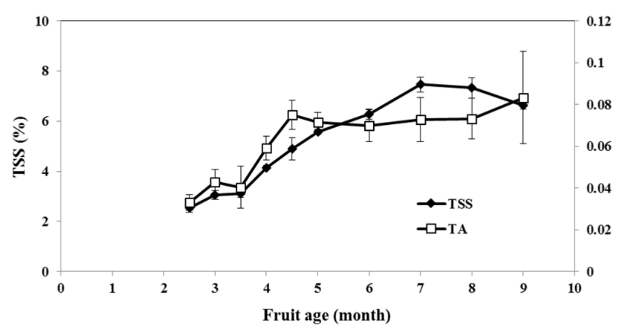

Figure 2. Total soluble solid and titratable acidity development of Thai aromatic coconut fruit at each fruit age. (Data are means ± SD)

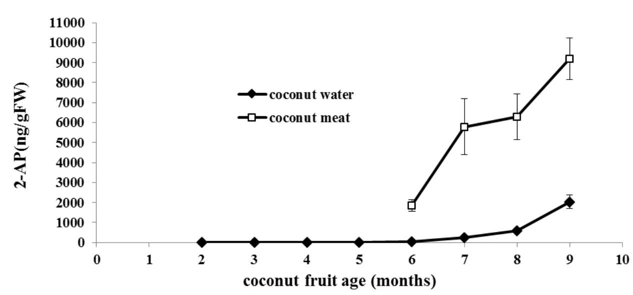

Figure 3. The 2-AP content in coconut meat and coconut water during fruit development from 2-9 months age after pollination. (Data are means ± SD)

with maturity and reached its maximum at the 9th month in an amount of 9,188 ng/g FW. However, it is possible that the 2-AP may still continue to increase. Future work should extend the observation period beyond the 9-month observation period in this study until the 2-AP could not be detected.

Although the 2-AP content in 9th month age of aromatic coconut water and meat was the highest, the proper stage for consumption of aromatic coconut in Thailand is when the fruit is 6.5-7 months old. At this age, it gives the best meat texture and good smell and taste of the coconut water which suitable for fresh consumption or processing. Furthermore, the volume of the coconut water is also highest at this time.

In fact, the 2-AP was detectable in every part of fruit, but the amount of 2-AP in coconut meat was highest in an amount of 5,790 ng/g FW followed by the shell, peel and coconut water, respectively (Figure 4). When we drink the coconut water from the whole fruit, we can smell the stronger aroma than when we drink from the glass, and the aroma is maybe coming from the 2-AP in the coconut meat and husk. Besides, the fragrant substance 2-AP is present in other parts of aromatic coconut tree such as male flower, female flower, rachillae, leaf and root (Figure 5). However, the male flower contained the highest amount of 2-AP (25,039 ng/g FW). In Thailand, the Xenia effect from the cross- pollination between aromatic cultivar and non-aromatic cultivars results in the absence of the aroma has been reported (Petchpiroon, 2006). This suggests that the male flower may play a role in 2-AP formation in the aromatic coconut fruit.

Until now, the 2-AP synthesis pathway in aromatic coconut has not been reported. However, Bradbury *et al.* (2005) reported that the 2-AP synthesis pathway in rice is related to the polyamine pathway, which may be controlled by *Os2AP* gene. The mutation of *Os2AP* gene leads to the loss of function of betaine aldehyde dehydrogenase 2 enzyme (BADH2), then the accumulation of γ-aminobutyraldehyde, which is the substrate of 2-AP, occured. Thus, further work on 2-AP synthesis should be conducted for better understanding of the formation of this substance in Thai aromatic coconut fruits.

In summary, Thai aromatic coconut fruit is characterized by a double sigmoidal curve which can be divided into 3 phases like that found in other drupe type fruit. The first phase was in the first 5 months of growth with rapid growth. In the second phase, the growth rate slowed down between 5 to 6 months of growth. The last phase was from 6 months of growth onwards, when the fruit grew again until full maturity. The coconut water and meat could first be detected at the second and fifth months of growth, respectively. Even though we could detect the presence of fragrant substance 2-AP in coconut water and meat but how this substance conduced is still unknown. Thus, our future study will focus on the molecular study of its synthetic pathway, as well as on cloning the genes that are related to 2-AP biosynthesis in Thai aromatic coconut fruit.

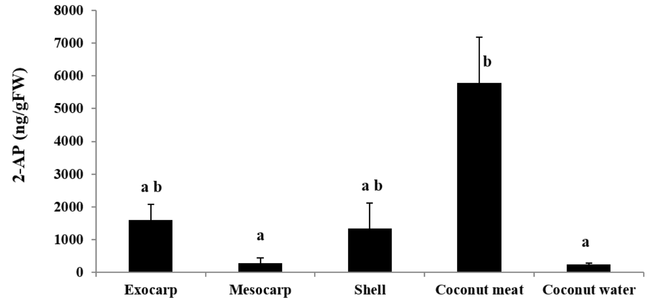

Figure 4. The 2-AP content in each parts of aromatic coconut fruit. (Data are means ± SD)

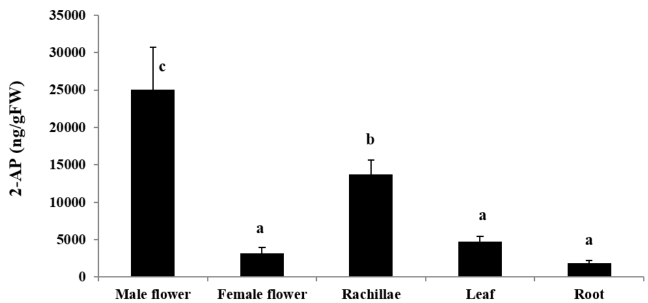

Figure 5. The 2-AP content in other component parts of aromatic coconut. (Data are means ± SD)

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