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การศึกษาเนื้อมังคุดด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด: การตรวจหาภาพพื้นผิวและธาตุ
Scanning Electron Microscopic Study of Mangosteen Aril: Surface Image and Element Detection

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บทคัดย่อ

มังคุด (*Garcinia mangostana* L.) เป็นราชินีแห่งผลไม้เมืองร้อนที่มีชื่อเสียงในหมู่ผู้บริโภค ในระหว่างการสุกของผลเนื้อผลสีขาวที่แข็งและทึบจะเปลี่ยนเป็นเนื้อสัมผัสที่นุ่มและชุ่มฉ่ำ ในขณะเดียวกันเนื้อมังคุดบางส่วนจะโปร่งแสงและมีเนื้อสัมผัสที่กรอบซึ่งอาจได้รับอิทธิพลมาจากความไม่สมดุลของการได้รับธาตุอาหารบางชนิด (แคลเซียม: Ca และโบรอน: B) ในช่วงก่อนการเก็บเกี่ยว งานวิจัยนี้ตรวจสอบลักษณะของเนื้อมังคุดโดยศึกษาลักษณะพื้นผิวของเนื้อปกติเนื้อแก้วและเนื้อบริเวณใกล้เคียงเนื้อแก้วของเนื้อผลสุกด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) ปริมาณ Ca และ B ในเนื้อมังคุดถูกประเมินโดยโปรแกรม ImageJ (สถาบันสุขภาพจิตแห่งชาติ, สหรัฐอเมริกา) รุ่น 1.62 ผลการวิจัยพบว่าเนื้อแก้วมีพื้นผิวเรียบโดยไม่มีช่องว่างอากาศระหว่างเซลล์ซึ่งแตกต่างจากบริเวณอื่น ปริมาณ B ในเนื้อมังคุดสุกทั้งสามส่วนมีค่าไม่แตกต่างกัน ในทางตรงกันข้ามปริมาณ Ca ในเนื้อบริเวณใกล้เคียงเนื้อแก้วมีค่ามากกว่าเนื้อปกติ นอกจากนี้ยังพบว่าเนื้อแก้วมีปริมาณของลิกนินและความแน่นเนื้อสูงสุดอีกด้วย
คำสำคัญ: แคลเซียม โบรอน มังคุด กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด

Abstract

Mangosteen (*Garcinia mangostana* L.), a queen of tropical fruit, is famous among consumers. During fruit ripening, the aril's rigid and opaque white color turns to a soft and juicy texture. At the same time, some of them turned translucent and had crispy textures that may have been influenced by an imbalance of some nutrient (calcium: Ca and boron: B) management during the preharvest period. This research examined the characteristics of mangosteen pulp by studying the surface characteristics of normal, translucent, and nearby tissue of translucent flesh of ripe aril with a scanning electron microscope (SEM). Ca and B contents in mangosteen pulp were estimated by the ImageJ program (National Institute of Mental Health, USA) version 1.62. The results revealed that translucent aril has a smooth surface without inter-cellular air space, which differs from other areas. The amount of B in the 3 zones of ripe mangosteen pulp was not different. In contrast, the Ca content in nearby translucent flesh was higher than in normal flesh. It was also found that translucent flesh had the highest amount of lignin and firmness.
Keywords: calcium, boron, mangosteen, SEM

Introduction

Mangosteen (*Garcinia mangostana* L.) is classified as a fruit product champion that has been promoted by the government to be commercially grown for export to foreign markets. The important mangosteen growing areas in Thailand are spread out to different regions, such as the eastern region, including Rayong, Chanthaburi, and Trat provinces, and the southern region from Surat Thani, Nakhon Si Thammarat, Chumphon to Narathiwat provinces. The primary market for mangosteen products is China. Besides the good

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taste (sour and sweet), the leading quality of fresh mangosteen that consumers consider, fruit peel, and pulp characteristics are also necessary. Since the mangosteen peel is thick and contains much latex, improper pre- and post-harvest handling promotes "gamboge", where the latex contaminates the flesh, and "yellow latex", where the latex flows out and clings to the skin's surface in spots that unsatisfied the consumer. In addition, a more critical problem is "translucent flesh", a physiological disorder that occurs while the fruit is ripening, giving a crisp and clear texture similar to glass and often found in mature mangosteen fruits that receive rainwater while on the tree (Pankasemsuk *et al.,* 1996). There was an experiment to reduce the symptoms of translucent flesh by providing micronutrients such as B and Ca to the mangosteen tree while the fruit is developing, hoping that those micronutrients will help improve the strength of the cell walls (Pechkeo *et al.,* 2007). Therefore, the objectives of this experiment were to examine the surface image from 3 zones of mangosteen flesh: normal, translucent, and nearby tissue of translucent flesh, and the amount of B and Ca, which are related to the strength of the cell walls.

Materials and Methods

Fully ripe mangosteen fruits were purchased from Talad Thai Market, Lam Luk Ka District, Pathum Thani Province. The normal, translucent, and nearby tissue of translucent flesh, as shown in Figure 1, was cut to a width of 1 cm., length of 1 cm., and thickness of 2-3 mm. Then, the samples were placed on the specimen stub before examining with a scanning electron microscope (SEM: FEI, model QUANTA 450, Germany) to study surface images. After that, the obtained images were used to find the amounts of B and Ca with the ImageJ program (National Institute of Mental Health, USA) version 1.62. Water content, firmness measuring, and lignin determination were done according to the method of Noichinda *et al.* (2017).

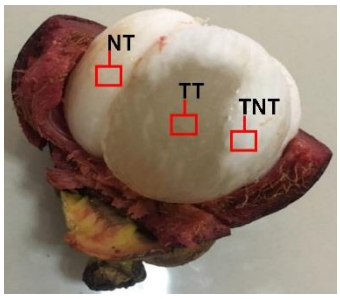

Figure 1 Zones of mangosteen pulp: normal tissue (NT), translucent tissue (TT), and translucent nearby tissue (TNT)

Results and Discussion

It is believed that mangosteen's translucent flesh is caused by the weakening of the cell walls when the fruit absorbs a large amount of water, causing membrane tearing and leakage of the solutes. Therefore, there is a study of applying minor elements such as B and Ca to the trees during the mangosteen fruit development (Pechkeo *et al.,* 2007). It is expected that B and Ca will synergize cell walls to be stronger. However, the SEM study found that normal flesh has a non-smooth surface with many opening air pores at the surface (Figure 2A), while translucent flesh has a smooth surface and very few opening air pores. White stripe layers also covered a large area (Figure 2C). The nearby tissue of translucent flesh had a surface image similar to normal flesh, but white stripe layers appeared in some areas (Figure 2B). The occurrence of mangosteen translucent flesh may result from lignin biosynthesis. This secondary metabolic substance appears in abundance when the fruit is ripe (Wongs-Aree *et al.,* 2021). Lignin is formed from monolignol

produced through the phenylpropanoid pathway during hypoxia and deposited in the layer of the primary cell wall, making it thicker, more dense (stiff structure), and resulting in a white and clear appearance.

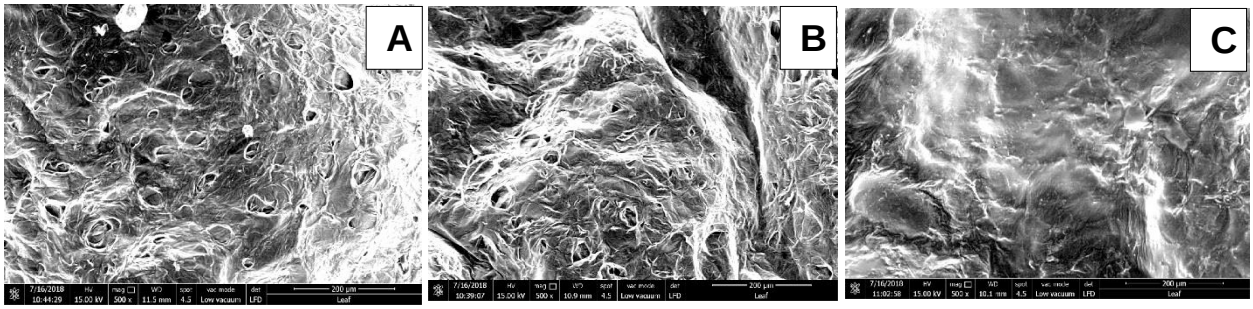

Figure 2 SEM surface images of mangosteen pulp: normal tissue (A), translucent nearby tissue (B), and translucent tissue (C)

Using the ImageJ program to convert the image signal into quantities of various elements revealed that the amount of B in the mangosteen pulp was not different in all 3 zones (Figure 3A). In contrast, the amount of Ca in normal and translucent flesh had no statistical difference (Figure 3B), except that the nearby tissue of translucent flesh appeared to have a different Ca content than normal flesh. Therefore, B and Ca might not directly influence the translucent flesh of mangosteen, though these elements are essential for cell wall strength. Noichinda *et al.* (2017) found that translucent flesh has a relatively higher amount of EDTA-soluble pectin than normal flesh. The chains of this pectin typically have Ca bonding for connection. Therefore, Ca should be used to prevent tissue softening rather than reducing the translucent flesh of mangosteen.

Water is an essential factor that induces translucent flesh in mangosteen. From the experience of mangosteen growers, they found that after the rain falls for at least 2 hours, these wet mangosteen fruits have translucent flesh after ripening. In this research, translucent flesh had a significantly higher water content than the normal and nearby tissue of translucent flesh (Figure 3C). As we know, water is an essential factor that induces hypoxic stress. Capillary water impedes air movement in intercellular space and causes fermentation. However, the living mature tissue has a high demand for ATP, which results in a higher turnover of fermentation. Cells, therefore, release a lot of reactive oxygen species (ROS), causing membrane deterioration. Thus, ROS elimination is, consequently, necessary. This removal process requires the combined activity of many enzymes to form a precursor for synthesizing many secondary metabolites (Wongs-Aree *et al.,* 2021). Moreover, hypoxia causes the cell to find ATP from other sources, such as the pentose phosphate pathway (PPP). This PPP provides energy and produces a precursor for the phenylpropanoid pathway to produce various substances (Noichinda and Bodhipadma, 2023), i.e., phenolics and monolignol, which cause the cell wall to become thicker and more transparent, also known as translucent flesh.

Mangosteen fruits have different firmness values according to the fruit development stage. The unripe fruit has a high firmness and will gradually decrease as the fruit ripens. Unripe mangosteen pulp had a firmness value of up to 4 Newtons (unpublished data) and decreased to approximately 0.2 Newtons when the fruit was fully ripe (Figure 3D). The translucent flesh had approximately 1.5 Newtons, resulting in a stiff flesh texture that was different from unripe mangosteen pulp with hard crispiness. From the SEM image, it can be seen that the translucent flesh of mangosteen had white stripe layers covering a large area (Figure 2C), which appeared clear and white with the naked eye. This characteristic was consistent with the lignin contents found

to be high in mangosteen translucent flesh (Figure 3E), followed by the nearby tissue of translucent flesh and normal flesh, respectively.

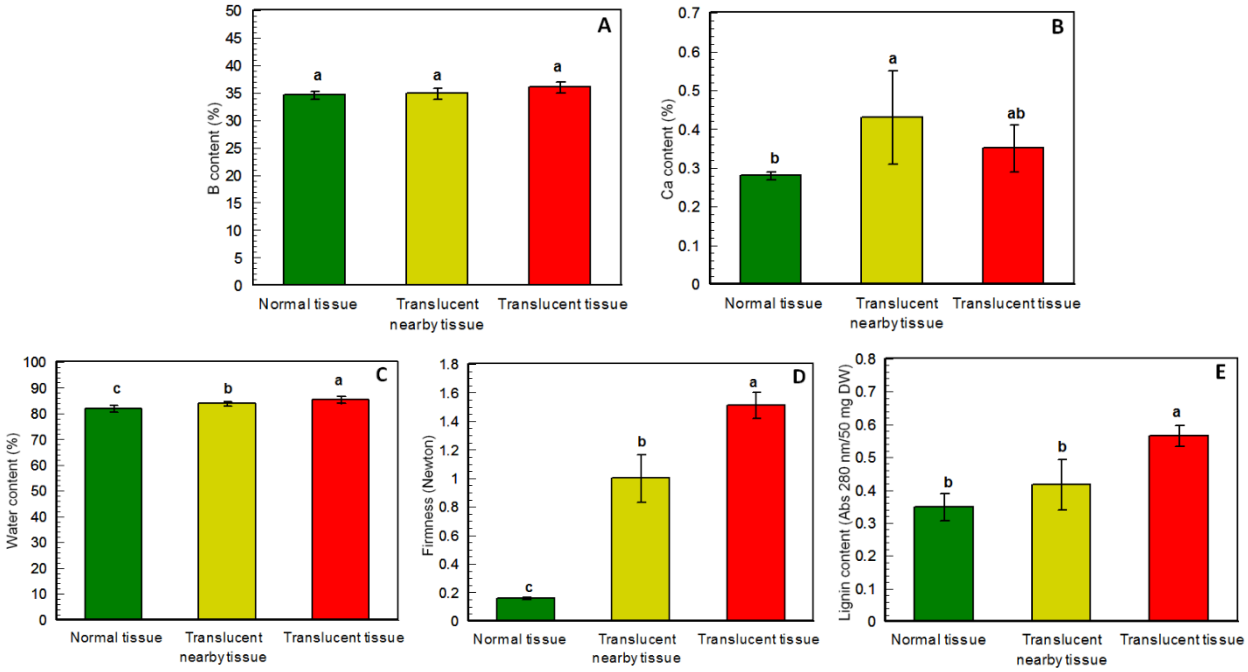

Figure 3 Tissue properties from 3 zones: B content (A), Ca content (B), water content (C), firmness (D), and lignin content (E)

Conclusion

From the surface image of mangosteen translucent flesh under SEM, the white stripe layers of lignin covered a large area to increase the cell wall strength. After Ca and B contents were analyzed, these minor elements in normal and translucent flesh had no statistical difference. Besides, translucent flesh had the highest amount of water content. Therefore, the translucent flesh disorder occurred from the capillary water inside the fruit, which led to hypoxia. In this circumstance, cells must adapt to survive by a defense mechanism, causing lignin production and making crispy textures and transparent like glass.

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