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Application News

Monitoring Metabolite Changes during Pineapple Ripening

Abstract

The pineapple (*Ananas comosus*) is a non-climacteric fruit with high market value and production volume. Non-climacteric fruits do not ripen after harvest and therefore, the ripening stage at the time of harvest is an important factor that determines sensory quality and shelf life. The ripening stage of pineapple is divided into 5 stages, C0–C4, with the green-ripe fruit at C0 and the full-ripe fruit at C4 based on United Nations Economic Commission for Europe (UNECE) Standard for pineapple (FFV-49) as seen in Fig. 1. This classification is based on the peel color of pineapple, in which C0 stage contains 0 % yellow color, C1 stage contain 0 %–25 % yellow color, C2 stage contains 25 %–50 % yellow color, C3 stage contains 50 %–75 % yellow color, and C4 stage contains 75 %–100 % yellow color. Pineapple is usually exported in the C1 stage, while the fully ripe fruit (C4 stage) is mainly for domestic consumption. To date, there has been no study that analyzed different parts of pineapple including flesh, peel, and crown parts, and incorporating broad coverage of primary metabolites such as sugar, organic acid, amino acid, sugar alcohol, sugar acid, and amine compounds. Hence, this study aimed to investigate the metabolite changes that occurred during the course of ripening in pineapple. Orthogonal projection of latent structures (OPLS) model was constructed using metabolites annotated by GC-MS as an explanatory variable and ripening stages as a response variable. The constructed model from flesh and peel samples indicated several potentially important metabolites that were correlated with the pineapple ripening process.

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Materials

Indonesian pineapples at 5 different ripening stages were used in this study (Fig. 1). Pineapple fruit was cut into three parts, crown, flesh, and peel. Crown part was analyzed by cutting the leaves into a 1x1 cm size before quenching by liquid nitrogen and freeze dried. Peel part was analyzed by scraping the peel into a 1x1 cm size using stainless-steel knife before quenching and freeze drying. Flesh part was analyzed by cutting the fruit into half then the flesh was diced using stainless-steel knife before quenching and freeze-dried.

Fig. 1 Pineapple sample from all ripening stages

Extraction, Derivatization, and GC-MS Analysis

All samples (10 mg; *n*=3) were extracted using 1 mL mixture of methanol, chloroform, and ultrapure water (5:2:2 ratio) containing 100 µg/mL ribitol as internal standard. Mixture solutions were incubated at 37 °C, 1200 rpm, for 30 minutes before centrifuged for 3 minutes at 4 °C. Supernatant (600 µL) was transferred to a new microtube and added with 300 µL of ultrapure water before centrifuged for 3 minutes at 4 °C. Four hundred microliter of supernatant was transferred to a new microtube and subjected to spin-dry for 1 hour and lyophilization overnight. Derivatization process included oxymation using 100 µL methoxyamine hydrochloride (20 mg/mL) with incubation for 90 minutes at 30 °C and silylation using 50 µL N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) with incubation for 30 minutes at 37 °C. Derivatized samples were subjected to GC-MS analysis using a Shimadzu GCMS-QP™2010 Ultra.

Table 1 Measurement conditions

Injection volume	: 1 µL
Column	: InertCap 5 MS/NP Column (30 m, 0.25 mm i.d., 0.25 mm film thickness, GL Sciences)
Split mode	: 25:1 (v/v)
Injection temperature	: 230 °C
Carrier gas	: He
Carrier gas flowrate	: 1.12 mL/min with liner velocity 39 cm/s
Column temperature	: 80 °C for 2 min Increased 15 °C/min to 330 °C 330 °C for 6 minutes
Interface temperature	: 250 °C
Ion source temperature	: 200 °C
Ionization	: Electron Ionization (EI)
Mass range	: *m/z* 85-500
Retention Index	: Standard alkane mixture (C_8– C_{40})

Metabolite Annotation by GC-MS

GC-MS analysis resulted in the detection of 351 metabolite peaks in the crown part, 297 metabolite peaks in the flesh part, and 359 metabolite peaks in the peel part. Among those peaks, 85 peaks in the crown part, 74 peaks in the flesh part, and 73 peaks in the peel part were annotated using MSP Library containing RI and EI-MS from our laboratory experimental data. Metabolites from QC samples with RSD more than 20 % were excluded from the analysis. After exclusion, the number of annotated metabolites in crown part were 56 metabolites, in the flesh part were 47 metabolites, and in the peel part were 54 metabolites. These metabolites were subjected to PCA and OPLS regression analyses.

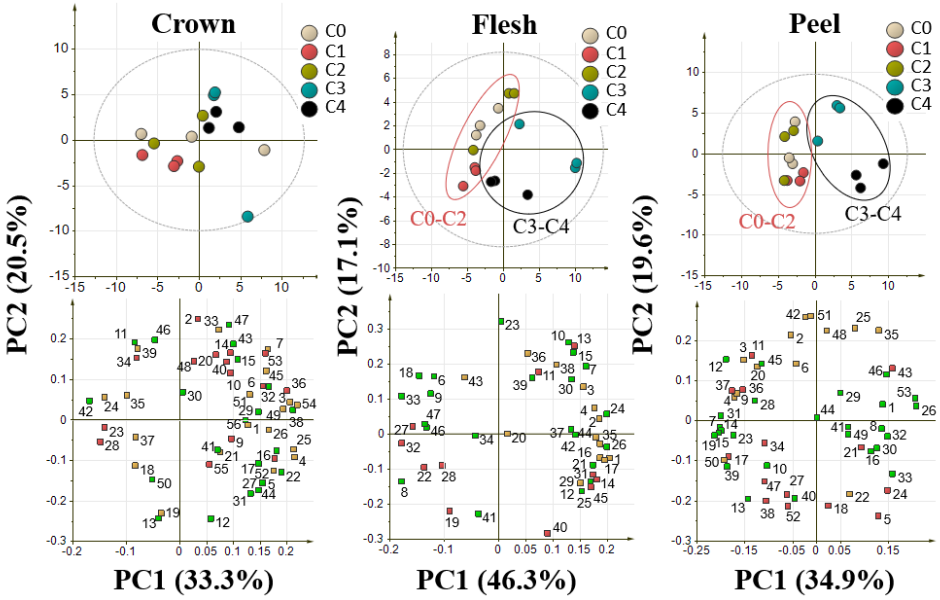

Fig. 2 PCA results from crown, flesh, and peel parts of pineapple from different ripening stage
Legends represent the samples and colored as follows: brown: C0 stage, red: C1 stage, green: C2 stage, blue: C3 stage, black: C4 stage.
Upper part show score plot; Bottom part show loading plot.
Loading plot was colored based on metabolite classes: green: sugars; red: organic acids; yellow: amino acids and amines

PCA and OPLS

PCA results showed that less ripe samples (C0–C2) were clustered together and ripe fruit samples (C3 and C4) formed a separate cluster. This trend was explained by 63.9 % and 53.3 % variance in the flesh and peel part, respectively. The crown part did not show any trend related to ripening and therefore was excluded for further analysis. OPLS regression analysis was used to identify metabolites that were highly influenced by the process related to the ripening stage as the response variable. Statistically important metabolites for the models were indicated by the score of variable influence on projection (VIP).

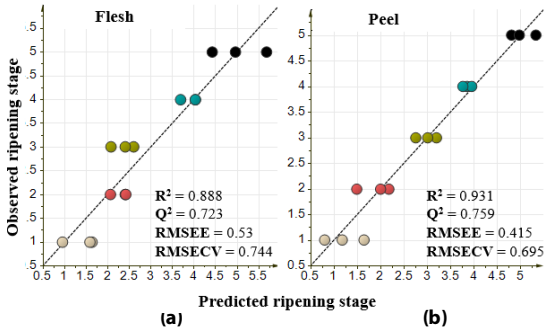

Fig. 3 OPLSR results from flesh (a) and peel (b) part of pineapple. Explanatory variables are annotated metabolites with response variable is ripening stage

Variable Influence on Projection (VIP)

Metabolites with a VIP score of more than 1 considered important for the model. Table 2 shows the five highest VIP metabolites from OPLSR analysis. Based on previous literature, these metabolites might play a role in regulating fruit firmness or fruit cell wall thus affecting shelf life of pineapple fruit. For future applications, these VIP metabolites could be added exogenously to regulate specific effects, for example, the addition of polyamine and ascorbic acid to regulate the shelf life of fruit. In addition, influencing the level of the metabolites through post-harvest treatment, such as regulating inositol, galactose, and raffinose by cold or heat treatment, may also contribute to improving the shelf life of pineapple. This study helps resolve post-harvest issues faced by the pineapple industry by inferring the metabolites critical in the ripening process.

Table 2 VIP metabolites from flesh and peel

Flesh	Coefficient	Peel	Coefficient
Melezitose	+	Inositol	-
Inositol	-	Mannose	-
Xylonic acid	+	Galactose	-
Gluconic acid	+	Sucrose	+
Raffinose	-	Aspartic acid	-

<References>
Previously published in Ikram MMM, Ridwani S, Putri SP, Fukusaki E. GC-MS Based Metabolite Profiling to Monitor Ripening-Specific Metabolites in Pineapple (*Ananas comosus*). Metabolites. 2020;10(4):134. Published 2020 Mar 31. doi:10.3390/metabo10040134

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