

Accumulation of Sugars and Liquid in Apoplast of Fruit Flesh Result in Pineapple Translucency

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Abstract

Translucency is a recurring problem for pineapple industry. Translucent fruit contained more sucrose, glucose and fructose in apoplast than those in apoplast of normal fruit. There were more liquid in intercellular space of translucent fruit than that of normal flesh. The contents of alcohol and ethylene in translucent fruit were higher than those in normal fruit. Translucent fruit contained less calcium than normal fruit. Electrolyte leakage of translucent flesh was more than that of normal flesh. There were 205 proteins of which the expressions in translucent flesh were higher than those in normal flesh. Calcium-ions-binding protein EF-hand domain-containing protein, ethylene-synthesizing enzyme 1-aminocyclopropane-1-carboxylate oxidase, ROS-producing protein universal stress protein A-like protein were the top three proteins of which the expressions in translucent flesh were higher than those in normal fruit. When much sugar was transferred into fruit pulp and accumulated in intercellular space, water will be absorbed from cells around and translucence formed. The accumulation of sugar and liquid in apoplast were due to that cell wall and membrane were degraded, which was from being attacked by ROS. There might be more and larger pores in cell wall and membranes of translucent flesh. These data played foundations for researching methods for controlling pineapple translucency.

Keywords

Pineapple, Translucency, Accumulation, Sugars, Apoplast

1. Introduction

Pineapple (*Ananas comosus* L. Merr.) is an important crop in tropical- and sub-tropical-countries [1]. Pineapple translucency (or water core) is a recurring problem for marketing fruit [2]. Flesh of translucent pineapple has a wa-

ter-soaked appearance [3]. The intercellular spaces in translucent flesh are filled with liquid [4]. Translucent fruits are fragile and prone to mechanical damage during harvest and postharvest handling [5]. They had poor flavor and significantly lower edible quality. Pineapple fruit translucency affects about 10% of fresh fruit and losses can exceed 30% [6].

Pineapple translucency had close relationship with calcium deficiency, fruit temperature and the size of crown [7] [8]. Fruit translucency index decreased as the amount of calcium applied was increased [7]. It was more severe when maximum and minimum temperature 3 months before harvest were lower than 23°C and 15°C respectively [8]. Covering fruit with clear-plastic during the last 3 weeks of fruit development increased translucency severity [4]. Fruit with larger crowns had lower incidence of translucency [8].

Sugar content is an important component of pineapple quality [5]. Chen and Paull (2000) found that sucrose and fructose in pineapple fruit increased rapidly since 4 weeks before harvest [1]. Removal of 1/3 of the plant leaves 3 weeks before harvest significantly reduced fruit flesh total soluble solids and translucency incidence at harvest [1]. Removal of crown increased translucency [2]. These suggested that sugar accumulation in fruit had relationship with pineapple translucency. Increased apoplasmic solute sugar concentration and water movement into apoplast may lead to pineapple translucency [1]. However, whether these processes did have happened in translucence fruit is to be identified. The mechanism underlying pineapple translucency was still unclear. In this research, sugar contents and volumes of apoplasmic liquid showing water movement in translucent fruit and normal fruit were compared. The activities of cell wall invertase, an enzyme that can transform sucrose into glucose and fructose, were also measured. To understand the mechanism underlying pineapple translucency in whole scale, proteomes in translucent fruit and normal fruit were also analyzed. Results showed that the contents of sucrose, glucose, fructose and total sugar in translucent flesh were similar with those corresponding values in normal flesh. But the apoplasmic contents of sucrose, glucose, fructose and total sugar in translucent flesh were significantly higher than those in normal flesh. The activity of cell wall invertase (CWI) in translucent flesh was higher than that in normal flesh. More sucrose was transformed into glucose and fructose in translucent fruit than normal fruit. Translucent fruit contained more liquid in apoplasmic space than normal fruit. There were 205 proteins of which the expressions in translucent flesh were higher than those in normal flesh. There were 10 proteins whose expressions in translucent flesh versus those in normal fruit (W/C) were more than 5.6. The W/C of 1-aminocyclopropane-1-carboxylate oxidase (ACO) was 11.9. Ethylene in translucent fruit was significantly higher than that in normal fruit. Translucent fruit contained more ethanol than normal fruit. There might be more reactive oxygen species (ROS) in translucent flesh than those in normal flesh, which can attack cell membrane/wall and result in more liquid released from cells. Electrolyte leakage of translucent flesh was significantly higher than that of normal flesh, there might be more pores in cell

membranes of translucent flesh or the diameters of pores in cell membranes of translucent flesh were larger than those of normal flesh. There were much more liquid accumulated in intercellular space of translucent flesh than that in normal flesh, which may results in pineapple translucency. This is the first paper demonstrating the relationship between sugar accumulation in appoplastic space and pineapple translucency. The mechanism underlying pineapple translucency was also proposed.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Pineapple (*Ananas comosus* cv Tainong 17) fruits were cut longitudinally. Pulps of three translucent fruits were mixed as a sample. Normal fruits were treated as the same method. The samples were frozen in liquid nitrogen and stored in -80°C refrigerator.

2.2. Proteome Analysis between Translucent and Normal Fruit

Protein extraction, trypsin digestion, tandem mass tags (TMT) labelling, HPLC fractionation and LC-MS/MS were performed according to the method published [9]. MS/MS data were processed using Maxquant search engine (v1.6.6.0). Tandem mass spectra were searched against *Ananas_comosus_4615_PR_20200330* (23,408 sequences) concatenated with reverse decoy database. Gene Ontology (GO) annotation proteome was derived from the UniProt-GOA database (<http://www.ebi.ac.uk/GOA/>). Functional descriptions of protein domains were annotated by InterProScan (<http://www.ebi.ac.uk/interpro/>). Functional enrichment and clustering were performed according to the paper published [9]. Cluster membership were visualized using “heatmap.2” function (<https://www.rdocumentation.org/packages/gplots/versions/3.0.1>).

2.3. Analysis of Carbohydrate

Two grams of frozen pulp were ground in liquid nitrogen. Eighteen microliters of 90% ethanol were added and homogenated. The mixture was stored in 4°C for 24 hours. The supernatant was concentrated until it was ropy. Pure water was added to the total volume was 10 ml. One milliliter of solution was filtered using filter membrane ($0.45\ \mu\text{m}$). The concentrations of sugar, glucose, fructose in the solution were analyzed using High Performance Liquid Chromatography (Pump: Hitachi pump L-2130, Detector: Hitachi RI Detector L-2490, Column: Water Sugar-Pak I). The concentrations of carbohydrates apoplastic were analyzed using the method published [10].

2.4. Apoplastic Liquid Measurement

Fresh pulp was cut into cubes of which side length was 1 cm. The cubes were washed with pure water to remove outworn tissues and cells. Ten cubes were put

into a 50-ml tube. Pure water was added into the tube until the total volume was 20 ml. The liquid was transferred into another tube and the liquid volume was measured. The volume measured was marked as A. Sample volume was the difference value between 20 ml and A. It was marked as B. Sample was centrifuged for 30 minutes at 300 g, 4°C. Supernatant volume was measured and marked as C. The value of C/B was the volume of apoplastic liquid.

2.5. Determination of Electrolyte Leakage, Cell Wall Invertase (CWI), Ethylene and Ethanol Content

Electrolyte leakage and CWI activity of pineapple fruit flesh were determined according to the paper published [1]. Ethylene content in pineapple flesh was measured according to the paper published [11]. Ethanol content in fruit pulps were measured according to the paper published [12]. NMR was performed using Bruker Avance DPX 300 instrument (operating at 300 MHz for ¹H).

2.6. Calcium Content Measured in Pulp

Pulps were put into liquid nitrogen and transferred into envelope. The envelopes uncorked were put into freeze drier. After water was removed from pulps completely, pulps were ground into powder using liquid nitrogen. One gram of powder was put into pot and put in oven in which temperature was 200°C for 1 hour. And then, they were transferred into 550°C for 2 hours. The samples were put in temperature for 1 hour. Five milliliter 2 M hydrochloric acid was mixed with the sample and shaken for 5 minutes. Twenty milliliter deionized water was added and filtered with Whatman 42# paper. Deionized water was added into the filtrate until the total volume was 25 milliliter. One milliliter 5% lanthanum chloride and 3.9 ml deionized water was mixed with 0.1 ml filtrate. Calcium in the solution was measured using Atomic-Absorption Spectroscopy. Calcium content = Conc measured × dilution factor × 25 ml/dry matter weight.

3. Results and Discussion

3.1. Translucent Flesh Contained More Sugars in Intercellular Space than Normal Flesh

Normal pineapple flesh appeared gold and compact (**Figure 1**). There were much liquid in intercellular space of translucent flesh, which showed water-soaked (**Figure 1**). The contents of sucrose, glucose, fructose and total sugar in translucent flesh were similar with those corresponding values in normal flesh (**Figure 2**). However, the apoplastic contents of these sugars in translucent flesh were significantly higher than those in normal flesh (**Figure 3**), demonstrating that more photosynthesis products accumulated in intercellular space of translucent flesh than those in normal flesh. The accumulation of sugars in apoplast of fruit can lead to pineapple translucency.

The activity of CWI in translucent flesh was 15.03 ± 2.47 μ mole hexose/h/gFW, while the corresponding value in normal flesh was only 9.56 ± 2.61



Figure 1. Lognitudinal sections of translucent fruit and normal fruit. The left two sections were normal fruit. The right two sections were translucent fruit.

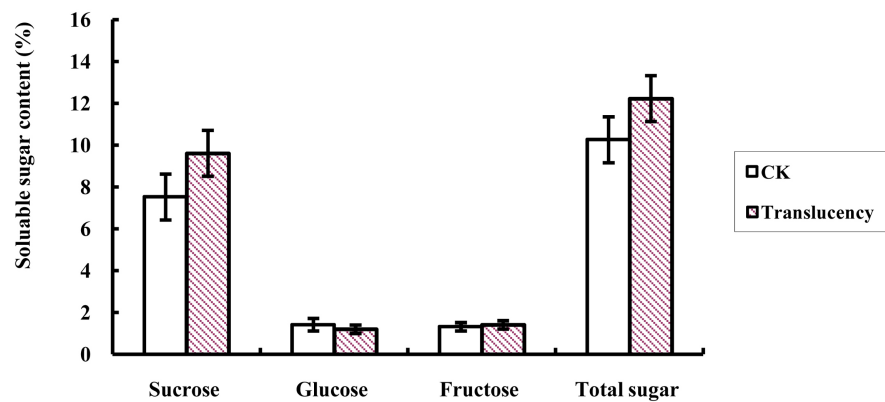


Figure 2. Sugar contents in fruit flesh.

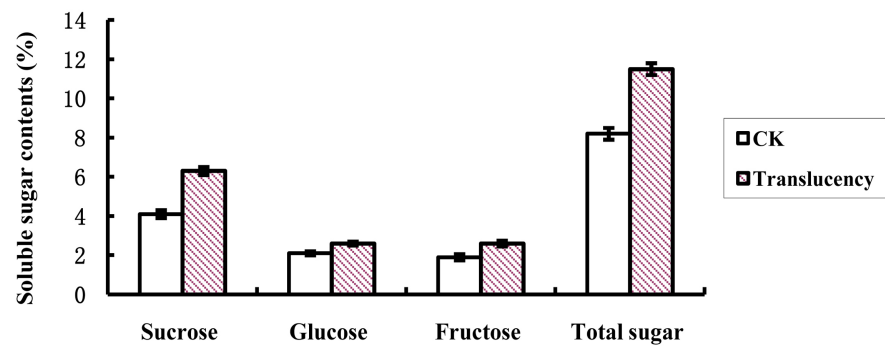


Figure 3. Sugar contents in apoplast.

$\mu\text{mole hexose/h/gFW}$. CWI can transform sucrose into glucose and fructose [13]. The CWI activity in translucent flesh was higher than that in normal flesh, indicating that more sucrose was transformed into glucose and fructose in translucent fruit than that was transformed in normal fruit.

3.2. Proteome Analysis of Translucent Flesh and Normal Flesh

Pulps of translucent fruits and normal fruits were collected for proteome analy-

sis respectively. Five thousands and eighty proteins were identified and 3956 proteins were quantified. The expressions of the proteins identified were determined (**Figure 4**). There were 205 proteins of which the expressions in translucent flesh were higher than those in normal flesh. The top 10 proteins of which the expressions in translucent flesh versus those in normal fruit (W/C) were listed in **Table 1**. EF-hand domain-containing protein can bind with calcium ions [14]. 1-aminocyclopropane-1-carboxylate oxidase (ACO) was an essential enzyme for synthesizing ethylene. The W/C values of EF-hand domain-containing protein and ACO was the highest and the second highest respectively (**Table 1**), suggesting that pineapple translucency had close relationship with calcium and ethylene in fruit.

Universal Stress Protein (USP) regulates H_2O_2 level in intercellular space under hypoxic condition and transduces the oxygen-deficient signal into the downstream defense signaling pathway [15]. Phosphofructokinase is an important regulatory enzyme in glycolytic pathway [16]. Pyruvate decarboxylase (AcPVDC) and alcohol dehydrogenase (AcADH) were important enzymes in anaerobic respiration for synthesizing ethanol [17]. These proteins accumulated in translucent fruit, indicating that anaerobic respiration is the main form of respiration in translucent fruit. Translucent fruit might contain less ATP than

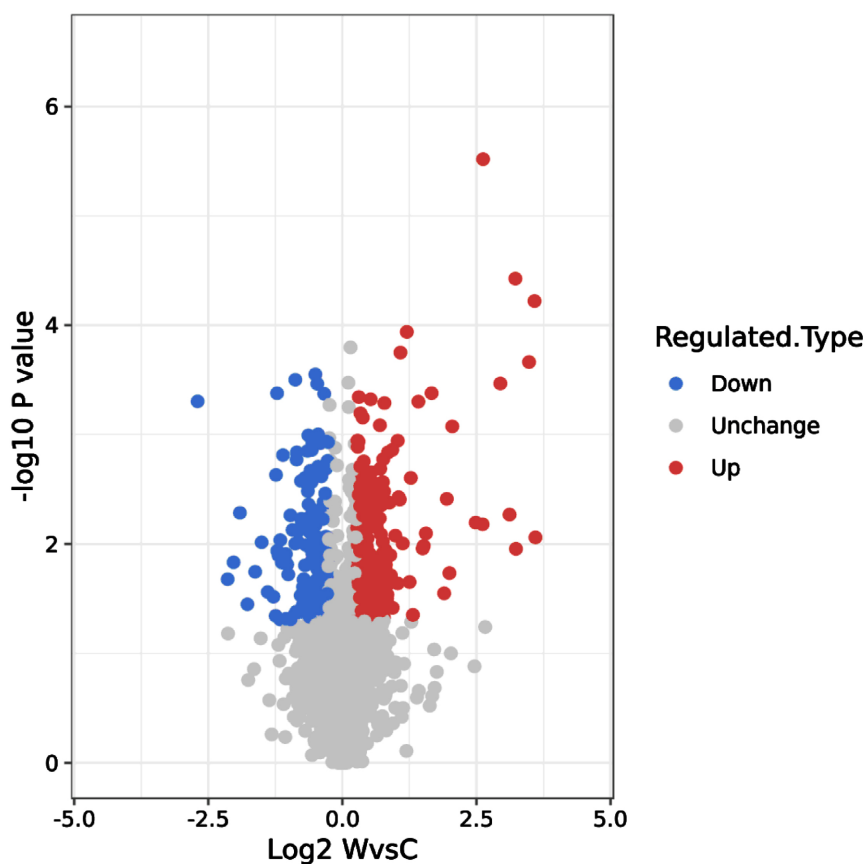


Figure 4. Volcano plot of proteins which are expressed significantly differently between water-core tissues and normal tissues.

Table 1. Top 10 proteins expressing differentially between water-core fruit and normal fruit.

Protein description	Protein accession	Ratio (W/C)	P value
EF-hand domain-containing protein	A0A199UNW3	12.125	0.0087247
1-aminocyclopropane-1-carboxylate oxidase	A0A199W3T2	11.981	0.000060
Universal stress protein A-like protein	A0A199UY58	11.166	0.00021746
Polyphenol oxidase	A0A199VDI5	9.427	0.0111002
LanC-like protein	A0A199V6H8	9.356	0.000037
Pyruvate decarboxylase	A0A199VBL9	8.676	0.005399
Universal stress protein A-like protein	A0A199VJN2	7.7	0.00034
Endoplasmic reticulum oxidoreductin-1	A0A199VV84	6.163	3.029E-06
Alcohol dehydrogenase	A0A199VD83	6.121	0.006618
PLATZ transcription factor family protein	A0A199UDN9	5.604	0.0063832

normal fruit.

For studying the functions of the proteins expressing significantly differentially between translucent flesh and normal flesh, proteins were enriched according to domain classification. Results showed that most of the proteins enriched had Cystathionine b-synthase (CBS) domain, serine carboxypeptidase, universal stress protein family, PBI domain, phosphofructokinase, oxidoreductase NAD-binding domain, and rhodanese-like domain (**Figure 5**). CBS proteins can regulate H₂O₂ content in cell [18]. H₂O₂ can shear polysaccharides and liposome [19]. Cell wall and membranes can be degraded because of these [20]. Proteins containing CBS domains accumulated in translucent flesh, demonstrating that many cells in translucent flesh have been cracked.

3.3. Translucent Flesh Contained More Ethylene and Ethanol than Normal Flesh

ACO was an important enzyme for synthesizing ethylene in plant [21]. Translucent flesh contained much more ACO than normal flesh, suggesting that pineapple translucence had some relationship with ethylene. Ethylene in pineapple flesh was measured in this study. Results showed that ethylene released from translucent flesh was 0.47 ± 0.02 µl/kg/h, while that corresponding value from normal flesh was only 0.18 ± 0.02 µl/kg/h. Translucent fruit contained more ethylene than normal fruit. The expressions of respiratory burst oxidase can be induced by ethylene [22]. Oxygen molecules can be transformed into reactive oxygen species (ROS) through respiratory burst oxidase [23]. These ROS can attack membranes or cell wall, leading to more pores and larger pores in membranes and cell wall [19]. Electrolyte leakage of translucent flesh will be enhanced. More liquid will flow off from sink cells and accumulate in intercellular space of flesh. The synthesis of ethylene can be induced by cold [14]. This can

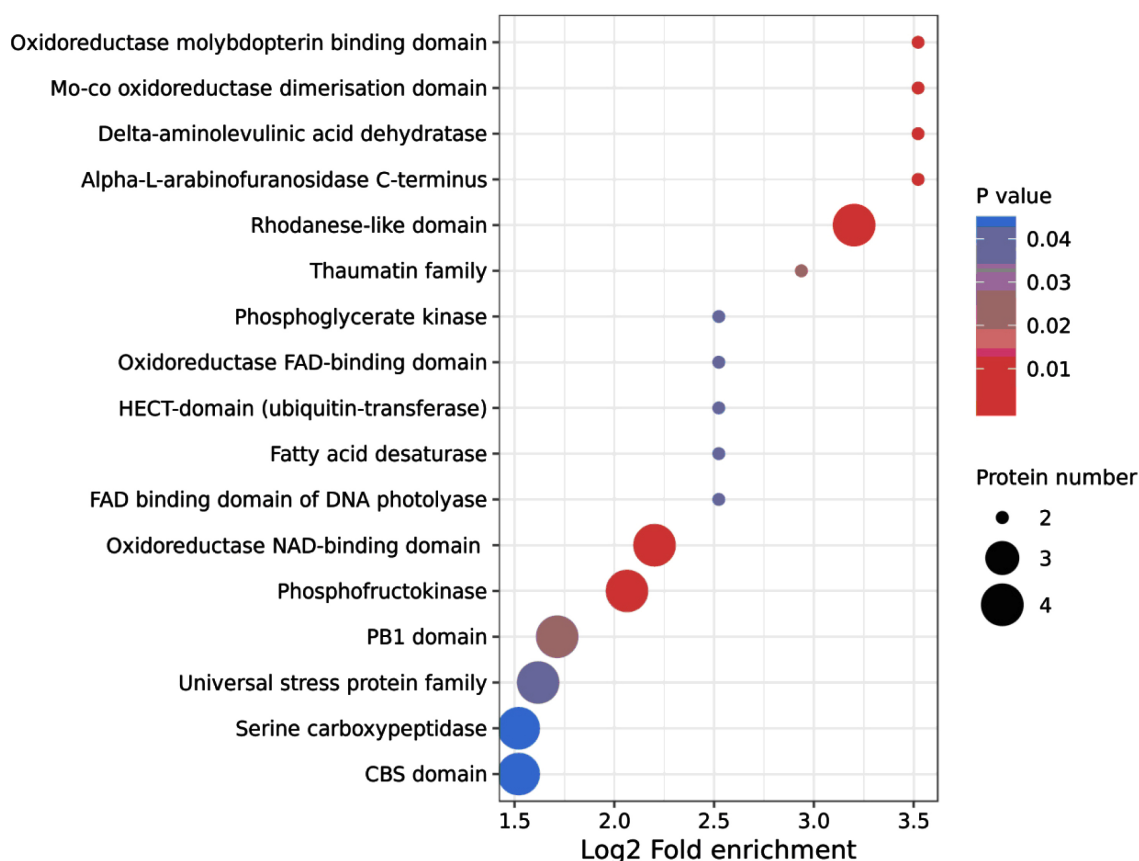


Figure 5. Distribution bubble chart of proteins expressed differentially in domain classification.

explain that fruits on which temperature was lower than 15°C 3 months before harvest had higher incidence of translucence.

The expressions of AcPVDC and AcADH in translucent flesh were more than those in normal fruit, suggesting that translucent fruit synthesized more ethanol. Results showed that ethanol content in translucent fruit with just two eyes yellow was 13.25 ± 0.12 g/L. No ethanol was found in normal fruit with only two eyes yellow. Ethanol content in normal fruits with all eyes yellow was 11.73 ± 0.07 g/L, while the value in translucent fruit with all eyes yellow was 15.37 ± 0.16 g/L. Translucent fruit synthesized more ethanol than normal fruit. Some translucent fruit with heavy symptom have alcohol smell, which might be due to that the expressions of AcPVDA and AcADH were enhanced and more alcohol was synthesized.

3.4. Calcium Content in Translucent Fruit Was Less than That in Normal Fruit

The W/C value of EF-hand domain-containing protein was the highest among the proteins expressing differentially between translucent and normal fruit, suggesting that pineapple translucence had relationship with calcium content. In this study, it was found that calcium content in translucent flesh was $0.0735\% \pm 0.0152\%$. The corresponding value in normal flesh was $0.1533\% \pm 0.0326\%$. Cal-

cium in translucent flesh was lower than that in normal flesh. EF-hand domain-containing protein can bind with calcium ions [24]. If calcium was deficient, pectin molecules cannot be linked [25]. Cell wall tissues cannot be formed [26]. There will be more pores in cell wall of fruit. The diameters of pores in cell wall of flesh will be larger. More liquid will flow out from sink cells of flesh. Pulp cell wall will be degraded [25].

3.5. Electrolyte Leakage of Translucent Flesh Was More than That of Normal Flesh

If cell wall and membrane were attacked by H_2O_2 , or pectin molecules had not been linked, more pores will form in cell wall and cell membrane. More liquid will percolate from sink cells through these pores. For identifying this speculation, electrolyte leakage and liquid content in intercellular space were measured. Results showed that electrolyte leakage of normal flesh was $65.8\% \pm 3.1\%$, while that value of translucent flesh was $75.4\% \pm 2.7\%$. The apoplastic liquid content in normal fruit was $170.1 \pm 11.2 \mu\text{l}/\text{cm}^3$. The corresponding value of translucent fruit was $195.1 \pm 10.5 \mu\text{l}/\text{cm}^3$ (Table 2). Translucent fruit had higher electrolyte leakage and accumulated more liquid in intercellular space than normal fruit. Cells in translucent flesh did have more pores and the pores were larger than those in normal flesh. Translucent flesh was saggy, while normal flesh was compact. That also demonstrates that cell wall and membranes in translucent fruit had been attacked and degraded.

3.6. Possible Mechanism Underlying Pineapple Translucence

Integrating all of the information above, a possible mechanism leading to pineapple translucence was proposed. When much sugar was transferred into fruit pulp and accumulated in intercellular space, water will be absorbed from cells around and translucence formed. If leaves were removed before harvest, less sugar will be synthesized and transferred into fruit. Translucence incidence will be decreased.

When pineapple fruits developed and chilling wave passed, the synthesis of ethylene was induced, resulting in that the expressions of respiratory burst oxidase were enhanced. Oxygen moleculars were transformed into reactive oxygen ROS, which attacks membranes or cell wall, leading to more and larger pores in membranes and cell wall. If less calcium ions were transported into sink cells of fruit, pectin molecules will not be formed. More pores and larger pores will also form in cell wall.

Table 2. Electrolyte leakage and apoplastic liquid in pineapple fruit.

Sample	Electrolyte leakage (%)	Apoplastic liquid ($\mu\text{l}/\text{cm}^3$)
Normal flesh	65.8 ± 3.1	170.1 ± 11.2
Translucent flesh	75.4 ± 2.7	195.1 ± 10.5

4. Conclusion

We conclude that accumulation of sugar and liquid in intercellular space of pineapple flesh led to translucency. The accumulation of sugar and liquid in apoplast was due to that cell wall and membrane were degraded, which was from being attacked by ROS. We revealed that translucent fruit contained more sucrose, glucose and fructose in apoplast of flesh than those in apoplast of normal fruit. Translucent fruit released more ethanol and ethylene and contained less calcium than normal fruit. There might be more and larger pores in cell wall and membranes of translucent flesh. These data will prove useful for researching methods for controlling pineapple translucency in the future.

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Conflicts of Interest

All the authors declare that they have no conflicts of interest.

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