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Research Article

Influence Mechanism of Endogenous Abscisic Acid on Storage Softening Process of Hardy Kiwifruit

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Abstract: In order to study the relation of Abscisic Acid (ABA) with other biochemistry factors during hardy kiwifruit softening process. The changing trend of ABA under the fruits storage conditions of 20 and 0°C was analyzed. A conclusion is drawn as below: During storage under 20°C, it shows the highest content of ABA in 4 days to 222.19 µg/L, which reaches the almost same content in 3 and 5 days. The value keeps inclining since 5 days and decline rate is lower in 7 and 8 days. The lowest value is reached to 20.88 µg/L in 10 days. During storage under 0°C, ABA content is at a relatively high level but shows the slow down trend. ABA content falls greatly from 9 to 11 days. After this period, ABA content still follows up-trend and declining then. The peak appears in 15 days to 90.49 µg/L, but it is lower than that in the first nine days. Moreover, peak during storage in environment under 0°C is lower than that during the storage in environment at normal temperature, accordingly delaying fruit softening. As the ABA content rises to the highest level, the fruit hardness drops drastically. When ABA content slightly changes, the hardness decreases gently. ABA content is featured that same changing trend of ethylene content, respiratory intensity, pectase content and amylase content. Moreover, ABA has the same peak appearance time as amylase but it is later than appearance of both pectase and ethylene, they basically match each other. The rule of peak appearance time is not obvious for ABA and amylase. Mutual inhibition exists between peak appearance time of ABA and respiratory intensity. Quick ABA rise is accompanied with slow amylase rise and vice versa.

Keywords: ABA, hardy kiwifruit, softening

INTRODUCTION

Hardy kiwifruit (*Actinidia arguta* (Sieb. et Zucc.) Planch. ex Miq) is also named baby kiwifruit, bower actinidia pear and vine pear. It belongs to *Actinidia* of *Actinidiaceae* as a deciduous vine plant with perennial rootstock. Moreover, it can resist pest, diseases and extreme cold weather and safely overcome the cold winter under -38°C (Bolgingh, 2000). It is distributed in the provinces of Northeast China, North China, Northwest China, Yangtze River Basin and some countries, such as North Korea, Japan and Russia. Three provinces of Northeast China have the most rich kiwifruit resources (Whang, 2000). Berry shape of hardy kiwifruit tends from ball to long and round shape. It is smooth and spotless. Two ends of the kiwifruits are flat. The top of kiwifruit has blunt and short edges, which are smooth and have no fuzz (Kataoka *et al*., 2006). The fruit is famous for its quite high nutritional value and has a large proportion of vitamin C, starch, pectin substance and other substances. The hardy kiwifruit is climacteric fruit. After being collected, hardy kiwifruit will undergo the most obvious change that pulp tissues become soft in a short period, resulting in poor storage property (White *et al*., 2005). Fruit senescence is a quite complex physiological process. People have gained a specific understanding towards fruit ripening after Kidd and West discovered climacterics (Kunsong and Shanglong, 1999) when they studied respiration of apples in the 1930s. According to a large quantity of experimental evidence, fruit softening results from changes of pectin substance of fruit. Pectase (Polygalacturonase for hardy kiwifruit) has mainly induced such change (Peigen *et al*., 1991). During storage, fruits will not only become soft but also release some gas, such as carbon dioxide and ethylene (Lian *et al*., 2008).

Plant hormone is a type of endogenous organic compound with the physiological activity, generated by the plant itself. It only has a quite low content but plays an irreplaceable role in functions of plants. As a key type of ripening hormone, endogenous ABA plays an important role during ripening of climacteric and non-climacteric fruits (White *et al*., 2005). When the concentration of ethylene reaches a specific threshold value during fruit ripening, fruit ripening and senescence are activated. Influences of ethylene on fruits are reflected by speeding up respiration and promoting the amylase rise and PG activity (Guixi *et al*., 1995).

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Three years are spent on the works to study the relation of ABA content with ethylene, respiratory intensity, pectase and amylase during storage of hardy kiwifruits under normal temperature and low temperature. This aims to discuss the mechanism of softening fruits and provide a theoretical basis for the deeply development and utilization of wild kiwifruit resources on storage and process.

MATERIALS AND METHODS

Materials, reagents and equipment:

Materials: The hardy kiwifruit were collected hardly kiwifruits are collected on September 10, 2010, September 2, 2011 and August 24, 2012, respectively in Qianshan Area, Anshan, Liaoning Province, China. Every 10 kg fruits with smooth surface and unified hardness and without mechanical damage are packaged into a plastic box and stored under 20°C±0.5°C or 0°C±0.5°C.

Reagents: Standard ABA sample (99.8%) (Sigma Company), ABA Elisa kit (imported by Shanghai Kanu Biotechnology Co., Ltd.). Other reagents, including 3, 5-dinitrosalicylic acid (DNS), citric acid ($C_6H_8O_7 \cdot H_2O$), sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), (polygalacturonic acid) and PVPP, are analytical reagent.

Equipment: BCD-220 refrigerator, Microplate reader MK3, FHR-5 fruit hardmeter, LP12000S electronic balance, GC-14B gas chromatograph, TU-1810 ultraviolet and visible spectrophotometer, DK-98-Ⅱelectric-heated thermostatic water bath and DNP-9272 electro-heating standing-temperature cultivator.

Methods:

ABA (μg/L): 500 mg hardy kiwifruit were grinded in ice-bath with 500 mg PVPP and 3 mL 80% methanol into homogenate and leach the fruits for 4 h at 4°C. The pulp was centrifuged at the speed of 4000×g at 4°C for 15 min. The upper clean level was collected and added 2 mL 80% extracting solution into the sedimentation, blend the sedimentation, centrifuge the sedimentation for another 15 min at the speed of 4000 g under 4°C and mix it with clean solution of upper level. Use 0.45 μL micro-filtration membranes to filtrate and operate according to steps in the operation instructions of ABA quantitative determination kit (Elisa).

Hardness (kg/cm^2): Use FHR-5 fruit hardmeter to assay 100 fruits hardness, evenly press during assay and measure every fruit twice in parallel and work out the average.

Ethylene production rate (μL/kg/h): Refer to the gas chromatography described by Zaibin (2004), measure twice in parallel and work out the average.

Respiratory intensity (mgCO$_2$/kg/h): Select 1 kg of fruits dealt in different ways, put the fruits into 6 L container for 15 min of hermetic treatment, use infrared ray gas analyzer to directly determine CO_2 concentration in the hermetic container and work out respiratory intensity of the hardy kiwifruit based on conversions.

Calculating formula:

$$CO_2 = X \times K \times V / (M \times H)$$

where,

X : Value determined by gas analyzer, (%)
K : Conversion coefficient of gas analyzer, 1% of determined value = 12500 mg/m^3
V : Residual volume of hermetic container (Holding capacity of hermetic container-volume of pear), mL
M: Mass of fruit, kg
H : Hours of placing the fruit, h

Pectase activity (μg/h/g): Select 100 fruits and determine the activity by referring to DNS colorimetric determination (Jiankang *et al*., 2007). At least two technical replicates were performed for each treatment.

Amylase activity (mg/min/g): Select 100 fruits and determine the activity by referring to DNS colorimetric determination (Jiankang *et al*., 2007). At least two technical replicates were performed for each treatment.

RESULTS AND ANALYSIS

Changes of ABA in hardy kiwifruits during storage: During storage, quality degradations of hardy kiwifruits are reflected by softening, mildew, rot and dehydration. Among these forms of degradation, softening is the most serious problem during storage of hardy kiwifruits. After being soften, fruits will easily get broken. Consequently, pulps are exposed to the air, speeding up pulp rot and degradation. According to results of related study, hardy kiwifruit is climacteric fruit. During storage, it undergoes obvious respiration peak. In the process of ripening and softening, endogenous ABA is indispensable.

Changes of ABA content of fruits during storage are shown in Fig. 1. During storage under temperature of 20°C, ABA content rises at first and declines then. It shows the highest content in 4 days to 222.19 μg/L. three and five days share the almost same content but the content of 5 days is a litter lower than that of 3 days. The value keeps inclining since 5 days. The decrease rate is lower in 7 and 8 days.

During storage under temperature of 0°C, ABA content in fruits is at a relatively high level but shows the trend of slow decline. ABA content falls greatly in the period from 9 to 11 days. After this period, ABA

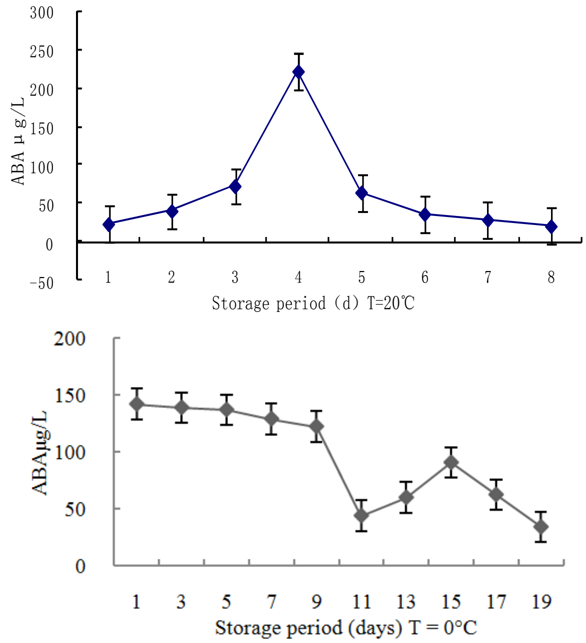

Fig. 1: ABA content of hardy kiwifruits during storage

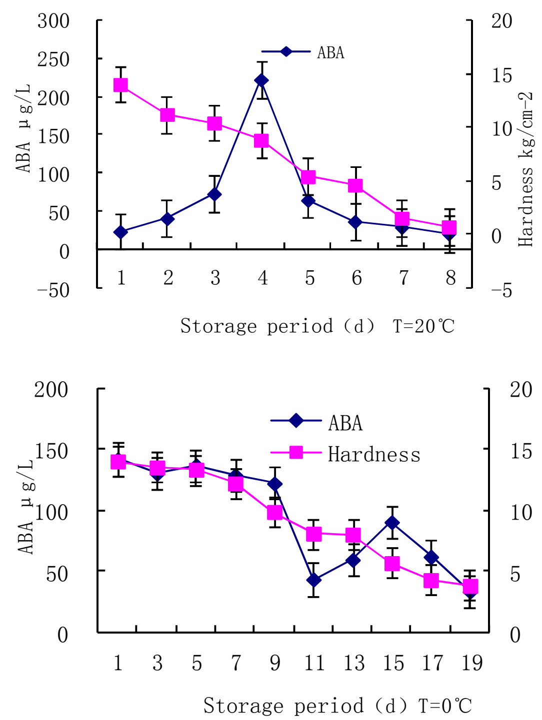

Fig. 2: The relation between ABA and hardness of hardy kiwifruits during storage

content still follows the trend of rising at first and declining then. The peak appears in 15 days to 90.49 μg/L, but it is lower than that in the first 9 days. ABA content declines steadily since then. The reason of these changes is that fruits need to gradually adapt to the cold environment at first. Several days are needed for those

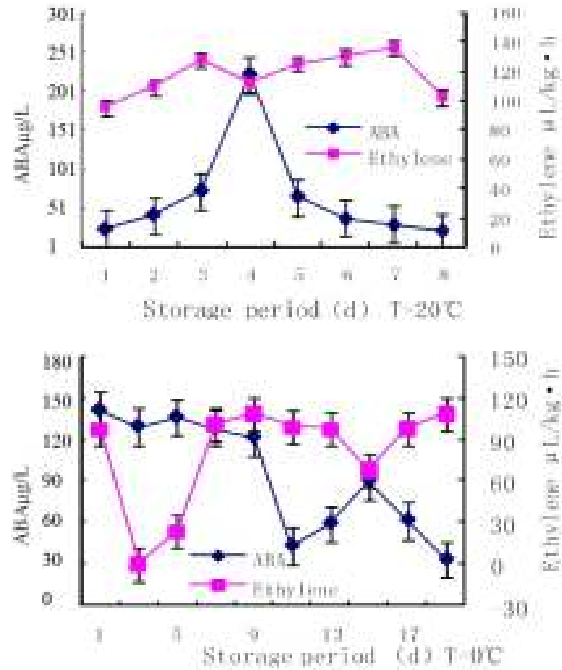

Fig. 3: Relation between ABA and ethylene content of hardy kiwifruits during storage

fruits to fully reach 0°C. Changing trend since then is similar with that of fruits stored under normal temperature. However, appearance time of ABA peak is obviously postponed. Moreover, peak during storage in environment under 0°C is lower than that during the storage in environment at normal temperature, accordingly delaying fruit softening.

Relation between endogenous ABA change and fruit hardness of hardy kiwifruits after harvest: As a key type of ripening hormone, ABA plays an important role during the ripening of climacteric and non-climacteric fruits. As shown in Fig. 2, hardness of hardy kiwifruit stored at 20°C presents declining trend that is changing from 14 to 0.7 kg/cm^2. The hardness falls obviously since 3 days, which presents negative correlation for changing trend of ABA. ABA content rises in 3 days but falls since 4 days. As for hardy kiwifruits under 0°C, the hardness also shows declining trend that is changing from. And negative correlation exists between ABA content and fruit hardness. As ABA content rises notably, fruit hardness shows the obvious trend of declining.

Relation between change of endogenous ABA and ethylene of hardy kiwifruits after harvest: According to details on Fig. 3, a specific relation exists between ethylene and ABA content. These two indexes are subject to the almost same changing trend. But appearance time of ethylene peak is earlier than

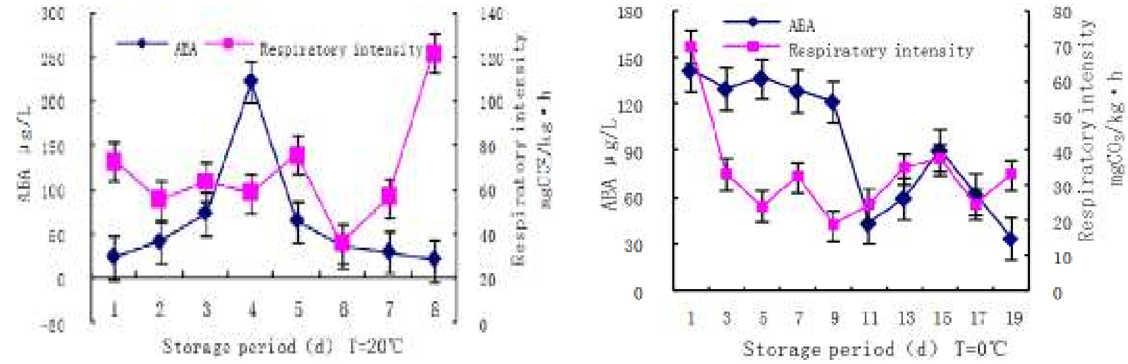

Fig. 4: Relation between ABA and respiratory intensity of hardy kiwifruits during storage

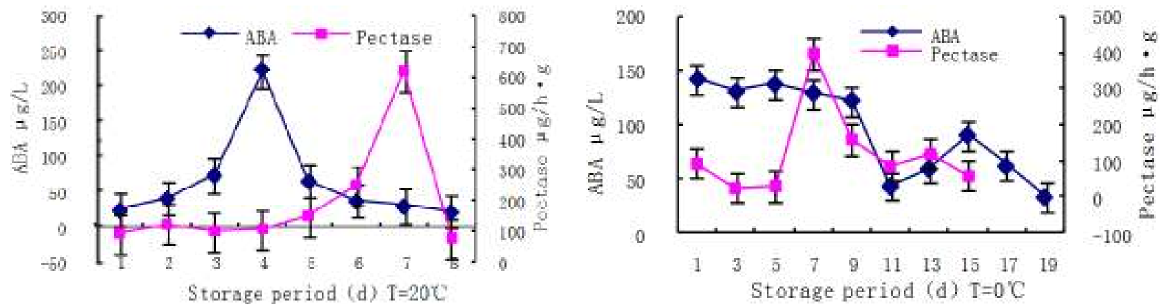

Fig. 5: Relation between ABA and pectase of hardy kiwifruits during storage

appearance time of ABA. Besides, mutual inhabitation exists between ABA and ethylene. When ABA goes to its peak, ethylene goes to its nadir. As ethylene in fruits reaches the maximum, ABA will reach the minimum, thus predicting mutual inhabitation between ethylene content and ABA content. The most obvious inhabitation appears during storage under 0°C. After the peak of ethylene, ABA will be produced in large scale, bringing about the ABA peak. During the whole storage, ABA content undergoes the biggest change. But changes of ethylene content are relatively steady. Thus it can be seen that ABA plays an enormously important role for climacteric fruits.

Relation between endogenous ABA change and respiratory intensity of hardy kiwifruits after harvest: The relation between ABA and respiratory intensity of hardy kiwifruits during storage is shown on Fig. 4. ABA content in fruits follows almost the same trend of change with respiratory intensity during storage under 20 and 0°C. However, appearance time of ABA peak is earlier than that of respiratory intensity regardless of the storage under 20 or 0°C. In other words, increase of ABA content will promote the respiratory intensity. Meanwhile, the impact of temperature on the respiratory intensity of fruits stored under 0°C is quickly reflected. But impacts of temperature on ABA in fruits are reflected slowly.

Relation between change of endogenous ABA and pectase after harvest of hardy kiwifruits: Pectase is one of important substances for softening fruits, including wild hardy kiwifruits. In light of comparisons on Fig. 5 about changes in ABA content and pectase content in hardy kiwifruits, pectase content in fruits stored under 0°C is one time around lower than that of kiwifruits stored under normal temperature. Pectase and ABA follow almost the same trend and direction of changing. Pectase of fruits stored under 0°C has different peak appearance time with pectase of fruits stored under normal temperature. Peak of ABA content is later than that of pectase content. When pectase reaches its peak, ABA goes to its valley. At normal temperature, ABA may promote formation of pectase. But the relation between ABA and pectase at low temperature should be further studied.

Relation between change of endogenous ABA and amylase after harvest of hardy kiwifruits: According to the details on Fig. 6, the change rule of amylase content during the storage period is approximated to "M" with two obvious peak values. In comparison with trend diagram of ABA, ABA peak is later than that of amylase in fruits stored under 20°C. But appearance time of amylase peak is earlier than appearance time of the first peak of ABA content in fruits stored under 0°C. The time lag is about 15 days. But the second peak

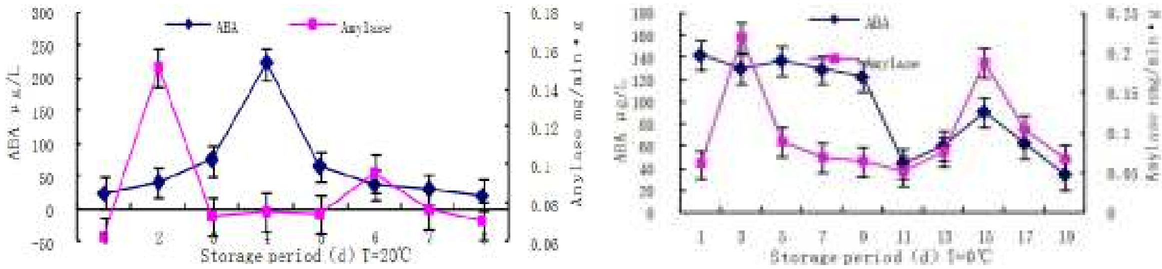

Fig. 6: The relation between ABA and amylase of hardy kiwifruits during storage

appears at the same time. Quick rise of ABA content corresponds to slow rise of amylase content and vice versa.

CONCLUSION

Conclusions are made as follows by fully analyzing relations of ABA content change in hardy kiwifruits during storage with four indexes, including ethylene, respiratory intensity, pectase and amylase related to softening.

Hardness of hardy kiwifruits shows the trend of decline during storage. As ABA content reaches to the highest level, fruit hardness falls drastically. When the content of ABA changes steadily, fruit hardness declines steadily.

ABA content in hardy kiwifruits follows almost the same trend of change with ethylene, respiratory intensity, pectase and amylase.

ABA in hardy kiwifruits has the same appearance time with amylase. But the appearance time of ABA is later than that of pectase and ethylene. But the time lag is quite small. In this view, these indexes have almost the same appearance time. But the rule of appearance time of ABA and respiratory intensity is not clear.

Mutual inhabitation exists between ABA content and ethylene content in hardy kiwifruits, which complies with the conclusion made by Wei and Hui (1989).

High growth rate of ABA content in hardy kiwifruits corresponds to low growth rate of amylase and vice versa.

In conclusion, mutual inhabitation exists between ethylene and ABA in fruits. The increase of ABA content promotes respiratory intensity. At normal temperature, ABA will promote formation of pectase. ABA plays a role of overall regulation rather than simply promoting fruit ripening and senescence and inhabiting synthesis during the storage after harvest of fruits.

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