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The Effect of Harvest Maturity Stage on ACC Synthase Activity and Total Proteins Profile in Kiwifruits during Normal and Controlled Atmosphere Storages

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ABSTRACT

This study indicated that the harvest periods related to soluble solids contents (SSC) content of Hayward kiwifruit significantly affected 1-aminocyclopropane-1-carboxylate (ACC) synthase activity, total soluble protein (TSP) content and protein profile. ACC synthase activity was suppressed, especially in early harvested fruits, by an inhibition of fruit ripening during controlled atmosphere (CA) storage. Separation of TSP using denatured polyacrylamide gel electrophoresis (SDS-PAGE) revealed that one predominant polypeptide with 38 kDa molecular mass that varied among four different harvest periods during normal atmosphere (NA) storage. This polypeptide band was accumulated with increased harvest maturity during NA storage. On the other hand, it was not detected on SDS-PAGE of CA samples. Consequently CA storage suppressed expression of the 38 kDa protein band, which may be responsible for ACC synthesis. SSC of kiwifruits at harvest is an important factor that effects the molecular changes related to ripening of fruits during storage.

Key Words: ACC; *Actinidia deliciosa*; Kiwifruit; Protein; SDS-PAGE; Storage

INTRODUCTION

Kiwifruits are classified as climacteric fruit, since they ripen in response to exogenous ethylene and their ripening is characterized by a period of autocatalytic ethylene production (Whittaker *et al.,* 1997). The plant hormone, ethylene plays an important role in the regulation of many aspects of plant development, including fruit ripening and senescence. It has been established that ethylene is biosynthesized from methionine via AdoMet and 1-aminocyclopropane-1-carboxylate (ACC) (Zeng *et al.,* 2005). ACC synthase and ACC oxidase are two enzymes involved in the ethylene biosynthetic pathway. Various climacteric fruit increase the ACC concentration by expression of ACC synthase gene during the ripening (Jiang & Fu, 2000) Most of the data indicated the existence of multiple ACC synthesis genes, which encode proteins that show diversity in molecular mass as well as in their isoelectric point values. Since ACC synthase proteins induced by different developmental, hormonal and environmental factors differ in their immunological and physicochemical properties it has been suggested that more than one ACC synthase gene is specifically activated (Nakagawa *et al*., 1988). Controlled atmosphere (CA) storage suppressed ethylene effects as indicated by an inhibition of color change, fruit softening and prolongation of the pre-climacteric phase (Arpaia *et al.,* 1984; Gorny & Kader, 1997). Minimum loss of fresh fruits and vegetables is the main objectives of post-harvest technology (Heydari *et al*., 2010). Worldwide consensus recommends that CA storage of kiwifruit should have a mixture of 2% O_2 with 5% CO_2 for a delayed rate of kiwifruit softening, extending storage life up to 3-4 months over NA storage (Arparia *et al.,* 1984; Hasey *et al.,* 1994; Eris *et al.,* 1998). The characteristic of commercial harvest maturity of kiwifruit is determined on the basis of total solids (TS) or soluble solids contents (SSC) (Hasey *et al.,* 1994). Although TS and SSC vary with fruit size, production area, climatic condition and crop load, they are closely linked to the genetically controlled physiological changes (Abdi *et al.,* 2002). There are many cases in which it is desirable to determined relationship among some fruit characteristics (Rashidi *et al*., 2010). For instance, there is close relationship between SSC of kiwifruits at harvest and duration of storage or the maturity of fruits during storage (Hasey *et al.,* 1994). In this respect, this study was undertaken to determine the changes in ACC synthase activities and total soluble protein (TSP) contents and profiles during normal (NA) and controlled atmosphere (CA) storages of kiwifruits harvested periodically according to their SSC in order to determine the best harvest time for prolonged storage.

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MATERIALS AND METHODS

'Hayward' kiwifruits were harvested periodically according to their SSC at levels of 4.5-5.5%, 5.6-6.5%, 6.6-7.5% and 8.5-9.5% from 17 years old plants in Atatürk Central Horticultural Research Institute in Yalova-Turkey (latitude, 40 °39'N; longitude, 29°15'E). SSC was measured by refraktometer (Atago, Japan) in fruit juice from the equatorial zone of the fruit. Harvested fruits were stored in air atmosphere, which is called NA at 0°C and 85-90% Relative Humidity (RH) or CA condition (5% CO_2 + 2% O_2) for 5 months. Harvested fruits were placed in gas tight 100 l. container under static CA (5% CO_2 + 2% O_2) condition at 0°C and 85-90% RH. The concentration of CO_2 and O_2 were daily adjusted and continuously monitored by gas analyzers. The control groups were stored at same temperature and RH but at ambient atmosphere. Initial and monthly sampled (during storage periods) kiwifruit flesh was frozen immediately in liquid nitrogen after peeling and stored at -80°C until used for ACC synthase and protein analyses.

ACC synthase activity: For ACC synthase extraction, frozen tissues of kiwifruits were homogenized in a chilled Waring blender with 1 ml/g of extraction buffer [400 mM potassium-phosphate (pH 8.5), 1 mM EDTA, 5 mL/l 2-mercaptoethanol, and 0.01 mM pyridoxal phosphate] at 4°C. The homogenate was filtered through four layer of cheese cloth and then centrifuged at 16,000 rpm for 30 min. The supernatant was discarded and the pellet was resuspened in extraction media. The resuspended pellet was then centrifuged at 16,000 rpm for 20 min and the supernatant was discarded. The resulting pellet was dissolved in extraction buffer and ACC synthase activity was assayed as described by Gorny and Kader (1997), whose method was modified by Yip *et al.* (1991). ACC synthase activity was measured using a high-resolution gas chromatography (Agilent Hewlet Packard 6890 Series) equipped with ZB-624 capilar colon, at 200°C flame ionization detector.

TSP extraction and SDS-PAGE: TSP was extracted from kiwifruit flesh using the extraction methods described by Arora *et al.* (1992), with a few modifications. Frozen kiwifruit flesh (1 g) was homogenized at 4°C in 10 mL of borate buffer pH 9.0 [50 mM sodium tetraborate, 50 mM ascorbic acid, 1% β-mercaptoethanol, 1 mM phenylmethylsulsonylflouride (PMSF)], with 0.35 g polyvinylpolypyrolidone (PVPP). Samples were shaken on a gyratory shaker for 15 min at 4°C followed by centrifugation at 26,000×g for 1.5 h at 4°C. The resulting supernatant was collected and filtered through 0.4 and 0.2 µm filters. Protein content was measured using the Bradford assay method described by Arora and Wisniewski (1994). Proteins were precipitated according to the method described by Lim *et al.* (1999) by adding trichloroacetic acid (TCA, 10%) to 1.3 mL of sample from the supernatant. Samples were centrifuged at 16,000 *g* for 30 min at 4°C, after 30 min incubation at 4°C. Protein pellets were washed 3 times with cold acetone by centrifugation at 16,000 *g* for 30 min at 4°C. Dried pellets were resuspended in SDS-PAGE sample buffer (65 mM Tris-HCl, 10% (v/v) glycerol, 2% (w/v) SDS, pH 6.8 and 5% β-mercaptoethanol with Bromphenol blue). Discontinuous SDS-PAGE was performed with a vertical electrophoresis unit (Thermo EC 120, NY, USA) using a 4% stacking gel and 12% separating gel. The protein concentrations were determined according to Bradford (1976) assay method. An equal amount of total protein (30 µg) was used for each sample and gels were stained with Coomassie Brilliant blue G-250.

Statistical analysis: Experiments were performed using a completely randomized design with three replicates (20 fruit per replicate). Analysis of variance (ANOVA) was performed using SPSS 11.0 for Windows and the least significant difference (LSD) test ($P < 0.05$) was used for multiple comparisons of the means.

RESULTS

ACC synthase activity: ACC synthase activity was low at harvest time for all harvest periods (Fig. 1). Thereafter ACC synthase activity of kiwifruits showed the ethylene climacteric pattern (Fig. 1). ACC synthase activity increased dramatically during first month of storage especially first harvested fruit in both NA and CA storage (Fig. 1). Indeed this rapid increased of ACC synthase activity was followed by a reduction for the rest of storage period. When we compared to ACC synthase of NA stored fruit was significantly higher than that in CA stored fruits at first month of storage. CA storage suppressed ACC synthase activity.

TSP content and SDS-PAGE profile: In the present study, TSP contents of all harvest periods were decreased at first month of NA storage (Fig. 2). Then sharply increased first and second harvest fruit. The fruits of first harvest showed an exception via keeping the high level of TSP content (~0.20 mg/g fresh weight) after third month of storage. However, TSP contents of first harvest period were almost unchanged during the CA storage, while it sharply increased and reached to the highest level in third month of forth harvest period in CA storage like it was in NA storage. Fruits of fourth harvest period had the highest TSP content (~0.16 mg/g fresh weight) among others in third month of CA storage followed by a sharp decrease to the almost initial level. On the other hand, fruits of second harvest period had the lowest TSP content (~0.01 mg/g fresh weight) among others in second month of storage. The TSP contents of third harvest period had almost the same curves in both NA and CA storages with slight differences. TSP contents of olive increased during full fruit ripening and decreased or remained relatively high throughout fruit softening. There is a close relationship between TSP content and fruit ripening. SDS-PAGE analysis of the samples was repeated at least three times with similar results. Therefore, data from a single, representative SDS-PAGE profile are

Fig. 1: The effect of harvest maturity stage on ACC synthase activities of 'Hayward' kiwifruits during normal and controlled atmosphere storages
Error bars represent ±SE of three replications

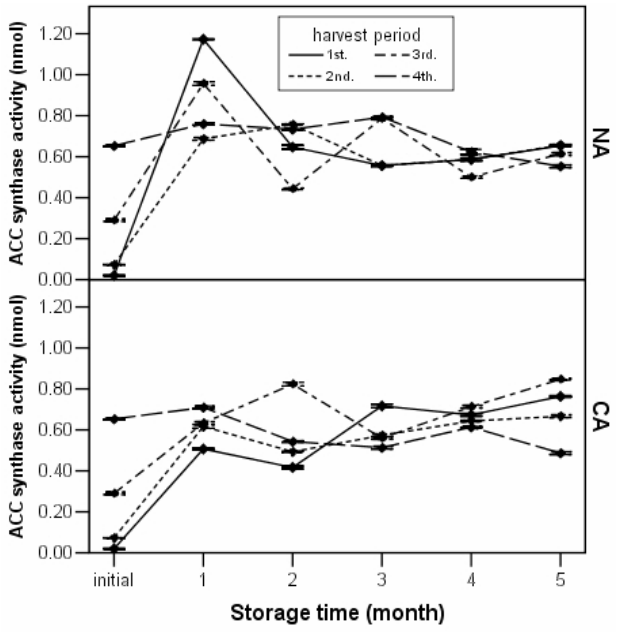
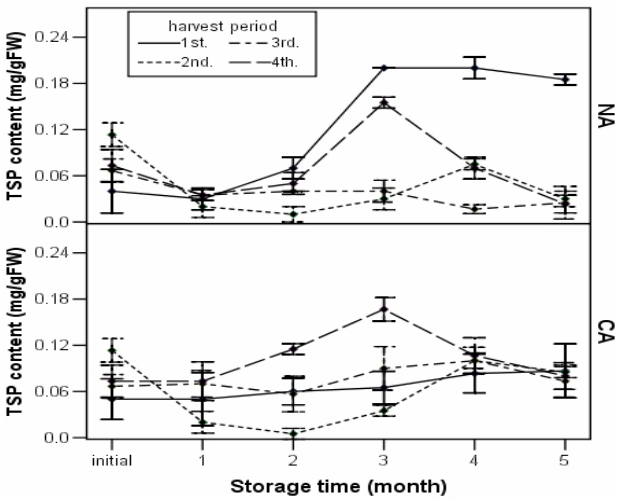


Fig. 2: The effect of harvest maturity stage on TSP contents of 'Hayward' kiwifruits during normal and controlled atmosphere storages
Error bars represent ±SE of three replications



presented here. In SDS-PAGE profiles of the samples harvested periodically and analyzed during NA storage, some differences among the protein bands were detected in the comparison of storage time and harvest periods (Fig. 3). Data indicated that a polypeptide with an estimated molecular mass of 38-kDa accumulated especially in the third, fourth and fifth months of the storage in kiwifruit harvested in the first, second and third harvest periods. The 38 kDa protein band was also seen all over the NA storage in kiwifruit harvested in the fourth harvest period. However, SDS-PAGE profiles of the samples harvested periodically and analyzed during CA storage were identical (Fig. 4). In addition there was no indication of 38 kDa protein band on SDS-PAGE of CA samples. On the other hand, none of the initial sample had 38 kDa protein. Considering SDS-PAGE profiles of samples, some differences were determined depending on the harvest periods and the storage time in NA storage (Fig. 3), while it was identical in all samples of CA storage (Fig. 4).

DISCUSSION

ACC synthase activity of NA stored fruits was significantly higher than that in CA stored fruits at first month of storage. CA storage suppressed ACC synthase activity spread all over the storage period of kiwifruit, which is consistent with the results in apple reported by Gorny and Kader (1996a, b). There are many reports on reduced affinity of ethylene receptor with a synergistic effect of reduced O_2 and elevated CO_2 on ethylene biosynthesis in CA together with having a low SSC and higher fruit firmness (Burg & Burg, 1967: Gorny & Kader, 1996b). It means that kiwifruit harvested at an early stage suppressed ACC synthesis activities more than those harvested late. Furthermore, the need of O_2 for the conversion of ACC to ethylene caused higher ACC synthase activity in NA than CA storage (Adams & Yang, 1979; Gorny & Kader, 1996a).

The results indicated that a polypeptide with the molecular mass of 38 kDa accumulated generally at third, forth and fifth months of NA storage in kiwifruits harvested at all harvest periods except forth harvest period in NA storage. However, the band was seen all over the storage time in forth harvest period. Consequently, 38 kDa protein band appeared in early stage of storage depending upon maturity of the fruits, which increase during storage. Since the 38 kDa protein was not expressed in any of the initial samples, there is a relationship between fruit maturity and accumulation of 38 kDa protein. In another words, the results revealed a close relationship between detectable stage of a 38 kDa protein and the SSC of fruits at harvest. The high content of SSC in kiwifruit indicates the earlier detection of 38 kDa protein band or visa versa. The data also suggest a relationship between 38 kDa protein and ACC synthase activity since ACC synthase activity related to ethylene biosynthesis and fruit maturity. It is known that there were several isoforms of ACC synthase with different molecular weight. In another words different molecular mass of ACC synthase proteins are responsible for ethylene biosynthesis (Jiang & Fu, 2000). Yip *et al.* (1991) reported two proteins with the estimated molecular masses of 48 kDa and 38 kDa that were related to ACC synthesis even though the relationship between these two proteins could not be explained clearly. On the other hand, in tomato (Satoh & Esashi, 1986) and mung bean (Satoh & Yang, 1988) a 48

Fig. 3: The effect harvest of maturity stage on SDS-PAGE profiles of 'Hayward' kiwifruits during NA atmosphere storages

A total of 30 µg protein was loaded in each lane. Molecular weight markers (MW) and their molecular masses (kDa) are shown on the left side. Arrow to the right mark indicates the position of the 38 kDa protein

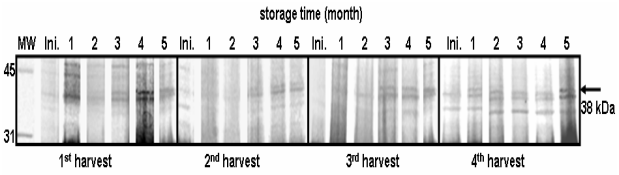
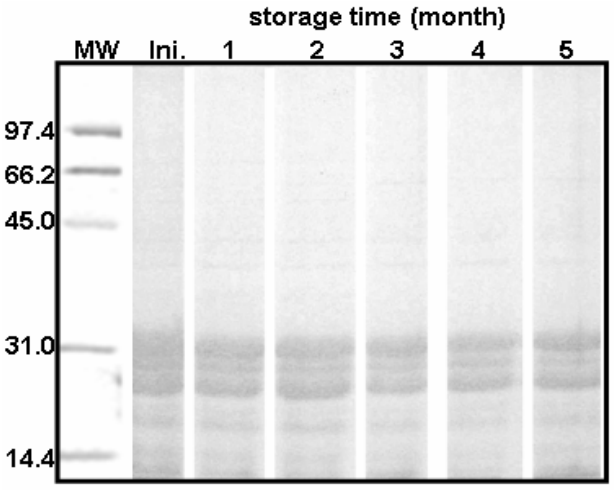


Fig. 4: The effect of harvest maturity stage on SDS-PAGE profiles of 'Hayward' kiwifruits during CA storages

A total of 30 µg protein was loaded in each lane. Molecular weight markers (MW) and their molecular masses (kDa) are shown on the left side



kDa protein was also identified as ACC synthase enzyme. In addition, Blecker *et al.* (1986) reported that in the results of analysis related to the ACC synthase isolated from tomato by SDS-PAGE, monoclonal antibodies against ACC synthase bound specifically to 50 kDa protein. Recently, Zheng *et al.* (2005) suggested that different ACC synthase isogenes were responsible for ethylene biosynthesis in different development stages in persimmon fruits.

CONCLUSION

Harvest time significantly effected ACC synthase activity, TSP content and the protein profile during storage in kiwifruit cv. Hayward. SDS-PAGE indicated a polypeptide, 38 kDa, as a marker among the harvest periods during NA storage, while there was no indication of 38 kDa protein in CA storage. Since CA suppressed the synthesis of 38 kDa protein, which may be responsible for ACC synthase activity. Considering all data in this study, second (5.6-6.5% TSS) or third (6.6-7.5% TSS) harvest period is suggested as the best harvest time for kiwifruit cv. Hayward for prolonged storage in either NA or CA storage conditions.

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