Effect of Controlled Atmosphere Storage (CAS) on Phosphofructokinase Activity in Mango (*Mangifera indica* L.) cv. Keitt

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Abstract. A controlled atmosphere (CA) is an agricultural storage method. It is an atmosphere in which oxygen, carbon dioxide and nitrogen concentration as well as temperature and humidity are regulated. Glycolysis is the basis of both anaerobic respiration and aerobic respiration, and it occurs in nearly all organisms. It is regulated by three enzymes catalyzing nonequilibrium reactions: Hexokinase, phosphofructokinase, and pyruvate kinase.

In this work we studied the effect of controlled atmosphere storage (CAS) of mango at 13°C on the activity of phosphofructokinase (PFK) and on fruit quality. This study was carried out on mango fruits (*Mangifera indica* L.) cv. Keitt. The storage of fruits under the CA retarded ripening and did not affect the quality attributes of Keitt mango. There was a significant decreasing in the activity of PFK as compared with control under (CAS) used in this work. These results indicate a potential for the application of CA for postharvest ripening control of mango. The optimal CA for long-term storage of "Keitt" mango at 13°C is (3% O₂ + 6% CO₂ + 91% N₂); under these conditions the storage life of mango can be extended up to 10 weeks.

Key words: Controlled atmosphere storage, mango, anaerobic respiration, aerobic respiration, fruit quality, phosphofructokinase.

Introduction

Mango, a tropical fruit of great economic importance, is generally harvested green and then commercialized after a period of storage (Elhefny, 2011). Since mangoes are generally harvested at a mature-green stage, post-harvest changes are principally concerned with events associated with ripening and senescence, and with the effects of post-harvest handling techniques (Mathieu and Jacques, 2007 and Pantastico *et al.*, 1984).

A controlled atmosphere (CA) is an agricultural storage method. An atmosphere in which oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) concentration as well as temperature and humidity are regulated (Electronic link). CA can be used as supplemental treatments to keep fresh fruits within their optimum ranges of temperature and relative humidity to extend their postharvest-life (Kader, 1994). Optimal CA conditions lower respiration and ethylene production rates reduce ethylene action, delay ripening and senescence, retard growth of decay-causing pathogens, and control insects. On the other hand, CA conditions unfavorable to a given fruit can induce physiological disorders, enhance susceptibility to decay, and shorten shelf-life following removal from CA conditions. Generally, the longer storage duration either in air or in optimal CA conditions, the shorter shelf-life during subsequent marketing (Kader, 2003). Much research has been directed toward determination of the optimum CA conditions for a large numbers of fruits and vegetables and specific cultivar of each commodity (Richardson and Meheriuk, 1982).

Lowering the O_2 levels around fruits and vegetables reduces their respiration rate in proportion to the O_2 concentration, but a minimum of about 1-3% O_2 , depending on the commodity, is required to avoid a shift from aerobic to anaerobic respiration. Under such respirations, the glycolytic pathway replaces the Krebs cycle as the main source of the energy needed by the plant tissues. Pyruvic acid is no longer oxidized but is decarboxylated to form acetaldehyde, CO_2 and, ultimately, ethanol; this results in development of off flavors and tissues breakdown (Kader, 1986).

Elevated CO₂ concentration also reduces the respiration rate of fresh fruits and vegetables, but above a level of about 20% or higher, depending on the commodity and the O₂ concentration, CO₂ can result in accumulation of ethanol and acetaldehyde within the tissues (Kader, 1986).

Generally, very low levels of O₂ and/or very high levels of CO₂ can induce a shift from aerobic to anaerobic respiration, and can stimulate products of fermentation such as acetaldehyde and alcohol (Yahia *et al.*, 1992).

Glycolysis is the basis of both anaerobic respiration and aerobic respiration, and it occurs in nearly all organisms. It is regulated by three enzymes catalyzing nonequilibrium reactions: Hexokinase, phosphofructokinase, and pyruvate kinase.

The conversion of fructose- 6-phosphate (F6P) to fructose-1,6-bisphosphate (F-1,6-P₂) with the concomitant hydrolysis of adenosine triphosphate (ATP) represents the first irreversible step specific for glycolysis. This reaction, catalyzed by 6-phosphofructo-1-kinase (PFK-1; phosphofructokinase-1, EC 2.7.1.11), is subjected to tight control, thus rendering it a critical regulatory point of the glycolytic flux (Hofmann, 1976 and Heinrich *et al.*, 1977). PFK is an important regulatory enzyme and is a key element of control in the glycolytic pathway and is also subject to feedback control. It acts as an indicator of the energy status of the cell.

Temperature management is the most critical factor in the management of ripening in mature-green mangoes. Ripening is retarded when mangoes are held above 30°C. Mature-green mangoes can be held at 10 to 13°C for 14 to 28 days (Paull and Chen, 2004). Ripe mangoes can be held at 10 to 13°C for up to one week. Being a tropical fruit, mangoes are subject to chilling injury if held below 13°C for mature green mangoes, and below 10°C for partially ripe mangoes (Kader and Mitcham, 2008). Ripe mangoes can be held in air storage at 10°C for a few days without chilling injury.

The objective of this work was to study the effect of controlled atmosphere storage of mango at 13°C on the activity of PFK and on fruit quality.

Materials and Methods

Fruit Materials

This study was carried out on mango fruits (*Mangifera indica* L.) cv. Keitt. The fruits were harvested from a private mango orchard located at Cairo–Alexandria desert road El Kilo 84, Egypt. The trees had the same age and were homogenous in their morphology, besides received the standard horticultural care which was adapted in the area. Fruits were picked using a small clipper, and packed in carton boxes. Fruit were harvested at green mature stage and taken directly to post-harvest laboratory in Horticulture Department, Faculty of Agriculture, Zagazig University, it takes 4 hours. All fruits washed with regular tap water and soap and then rinsed with water to remove the residue of soap, dipped in 1000 ppm Imazalil as a disinfectant for 5 minutes, then, air dried. A final sorting was done to recheck the fruits for any defects which were not detected at first sorting.

Controlled Atmosphere Storage

Completely randomized design was used where the four treatments were:

- 1- Storing fruits at 13°C under CA conditions of (3% O₂ + 6%CO₂ + 91% N₂).
- 2- Storing fruits at 13°C under CA conditions of (6% O₂ + 6%CO₂ + 88% N₂).
- 3- Storing fruits at 13°C under CA conditions of (6% O₂ + 9%CO₂ + 85% N₂).
- 4- Storing fruits in PE bags at 13°C (control).

60 uniform fruits were chosen at random for each treatment and packed in poly ethylene (PE) bags and jars. Samples of each treatment were randomly taken at weekly intervals to evaluate the effect of CA on storage period and on the activity of PFK.

CAS chambers were calibrated to establish the specified gas composition by a gas blending flow system. The gas blending system generated CA conditions using external supplies of gases from pressurized gas cylinders fitted with double-stage regulators and outlet controlling devices. These outlets were connected to the inlet flexible

pipes that were inserted into the jars in which the mangoes were stored. The gas mixtures were humidified by bubbling through water before entering the jars containing the fruit.

Fruits were then stored at 13°C, and evaluated every 7 days for total soluble solids (TSS), titratable acidity (TA), pH, weight loss, firmness, and respiration rate. Wedge-shaped slice (approximate 5 g) was removed from each fruit in the replicates and the pooled sample was passed through an electric juicer for the measurement of (TSS) and (TA) by refractometer and titration, respectively. The fruits were weighed before storage to obtain the initial weight, and then weighed after each period of storage as well as after shelf life period. Weight loss was determined as percentage of the initial weight. The pH was measured using a pH meter (style Hanna 8514). Firmness was measured on two pared sides of each fruit using a Push Pull dynamometer (Model FD 101). To measure respiration rate, CO₂ output was recorded by titration and respiration rate was calculated as mg CO₂ kg⁻¹ h⁻¹ (Schirra, 1992).

Preparation of PFK Extract

Preparation of PFK extract was performed as described by (Gyulakhmedov *et al.*, 2006). Sections were cut from five fruits and peeled. Middle samples were taken from 2-mm-thick parenchymal tissue. The samples were minced in an ice-cooled porcelain mortar and homogenized in a medium containing 25 mM Tris-HCl pH 8.5, 5 mM EDTA, 2.5 mM MgCl₂, 10 mM 2-mercaptoethanol, 10 mM Cis, 2% polyvinyl pyrrolidone (PVP-40), and 0.5% BSA. The ratio between the tissue and the extraction buffer was 1: 3 v/v. The homogenate was filtered into an ice-cooled beaker through a double layer of nylon. The filtrate was centrifuged at 10000g for 5 min. The sediment was discarded, and the precipitate was used as a crude PFK preparation.

Assay of PFK Enzyme

Quantification of PFK enzyme was analyzed by using commercial quantitative immunoassay kits for PFK (Usen Life Science & Technology Company, PFK ELISA kit; R&D systems, Japan). Values of PFK are assigned in (ng/mL).

Statistical Analysis

Complete randomized design was used. New Least Significant Difference (new LSD) at 5% level was calculated to compare between

treatments mean. Statistical analysis was determined using Statgraphics plus 5.1 statistical package software (Statpoint Technologies, Inc., United States) and Microsoft Excel.

Results

Overall accessibility ratings of fruits stored in CA were lower than those of fruits stored in the air.

Effect of (CAS) on PFK Activity

There were significant differences in the activity of PFK as compared with control under (CAS) after 5 weeks of storage which continued until the end of storage, Fig.1.

New LSD at 0.05 = 1.83

Fig. 1. Phosphofructokinase (PFK) @13°C after storage under the controlled atmosphere.

A: (3% O₂ + 6%CO₂+91% N₂), B: (6% O₂ + 6%CO₂+88% N₂), C: (6% O₂ + 9%CO₂+85% N₂) and D: control (air)

Effects of (CAS) on Ripening and Respiration Rates

Fruits exposed to CA used in this work remained greener and more firm than those in the air, (Fig. 2). Flesh firmness of fruit kept in air was significant decreased very rapidly from 1960.0 (g/cm²) to 1074.0 (g/cm²) after 4 weeks of storage, and to 289.17 (g/cm²) after 6 weeks (*New LSD at 0.05 = 74.87*). The flesh firmness of fruits which were kept under (CAS) also decreased, but it was in the air higher than them.

New LSD at 0.05 = 74.87

Fig. 2. Flesh firmness of mangoes @13°C after storage under the controlled atmosphere.

A: (3% O₂ + 6%CO₂+91% N₂), B: (6% O₂ + 6%CO₂+88% N₂), C: (6% O₂ + 9%CO₂+85% N₂) and D: control (air)

The storage of fruits under the CA resulted in a significant reduction in the respiration rate and in curtailment of the climacteric respiratory rise that accompanies ripening of mango fruit, Fig. 3.

New LSD at 0.05 = 0.65

Fig. 3. Respiration rate of mangoes after storage under the controlled atmosphere.

A: (3% O₂ + 6%CO₂+91% N₂), B: (6% O₂ + 6%CO₂+88% N₂), C: (6% O₂ + 9%CO₂+85% N₂) and D: control (air)

Effects of (CAS) on Total Soluble Solids Content (TSS), Titratable Acidity (TA) and pH

No significant changes in TSS; (New LSD at 0.05 = 0.53), TA; (New LSD at 0.05 = 0.07) and pH; (New LSD at 0.05 = 0.06) between fruits kept in the air and those kept under CA conditions were observed, Fig. 4.

New LSD at 0.05 = 0.53

Fig. 4. Total soluble solid contents of mangoes @13°C after storage under the controlled atmosphere.

A: (3% O₂ + 6%CO₂+91% N₂), B: (6% O₂ + 6%CO₂+88% N₂), C: (6% O₂ + 9%CO₂+85% N₂) and D: control (air)

Effects of (CAS) on Water Loss

Fruits under the CA had a significant less water loss than fruits which were kept in the air (*New LSD at 0.05 = 1.19*), Fig.5. After 8 weeks of storage under the CA conditions mangoes lost 2.35% to 2.86% of their weight, while in the air fruits lost 9.18% of their weight.

New LSD at 0.05 = 1.19

Fig. 5. Water loss of mangoes after storage under the controlled atmosphere.

A: (3% O₂ + 6%CO₂+91% N₂), B: (6% O₂ + 6%CO₂+88% N₂), C: (6% O₂ + 9%CO₂+85% N₂) and D: control (air)

Discussion

The storage of fruits under the CA at 13°C retarded ripening and did not affect the quality attributes of Keitt mango. (CAS) reduced water loss but it did not decrease the vitamin C content (data not shown). Fruits did not exhibit external or internal injury, no off-flavors and had an acceptable sensory quality.

In order to avoid the risk of chilling injury to the fruit, it would be preferable to hold mature-green mangoes or mangoes at the breaker stage in a controlled atmosphere chamber with 4% O₂ (with the balance of the atmosphere being N₂) and a temperature of 15°C than in a normal air environment at 10°C when attempting to delay ripening (Kader and Mitcham, 2008).

Ripening and senescence rates in many climacteric fruits like mangoes, can be affected by control of the availability of O₂ and CO₂ to the fruit during respiration and that these two compounds can have a significant inhibitory effect on ability of ethylene to initiate ripening (Ben-Yehoshua *et al.*, 2005).

Storage of fruits and vegetables in 5-20% CO₂ cause changes in the activities of specific enzymes of the respiratory metabolism and may have an uncoupling effect on oxidation phosphorylation (Frenkel and Patterson, 1977).

The activity of PFK reduced under (CAS). This reduction by elevated CO₂ could result from an inhibition of PFK synthesis or by inactivation of preexisting PFK. Regulation of glycolysis by PFK depends to a great extent on pH (Turner and Turner, 1980). Theoretical calculations and experimental data indicate that CO₂ concentrations >5% will generally lower intracellular pH (Bown, 1985). High CO₂ concentrations may allow certain intermediates and cofactors to accumulate to a point where secondary reactions can take place. CO₂ can also have a direct effect on metabolic activities distinct from that mediated by changes in pH, where some proteins and complexes are known to dissociate and can be fractionated in the presence of CO₂ (Mitz, 1979).

Rapid and substantial increases in the levels of F-1,6-P₂ and reduction in the levels of F-6-P have been shown to accompany the rise in respiration that occurs during ripening of climacteric fruit (Nair and

Darak, 1981). In all these cases the changes were attributed to an activation of PFK, which enhanced glycolysis and consequently respiration. By analogy, the reduction in PFK activity that we observed in our study, suggest that PFK is a controlling point in the glycolytic pathway and are affected by (CAS). There was a reduction of PFK and PFP activities, and accumulation of F-1,6-P₂ and F-6-P in "Bartlett" bears stored in 10% CO₂ for up to 4 days at 20 °C (Kerbel *et al.*, 1988).

The storage life of mangoes can be extended by holding the fruit in an environment with 3 to 5% O₂ and 5 to 10% CO₂, at 7 to 9 °C and 90% relative humidity atmosphere. They note that mango cultivars respond differently to CA storage, and that ripening delays provided may be minor (several days) and not economically advantageous in all situations (Paull and Chen 2004). The best CA for long-term storage of "Keitt" mango at 13 °C is (5% O₂ +5% CO₂+ 90% N₂), although it did not result in any significant advantage (Hatton and Reeder 1965). There were no significant benefits during storage of "Alfonso" mangos in atmosphere containing (2% O₂ +8% CO₂), or (5% O₂ +10% CO₂) (Spalding, 1977). However, "Haden" mangos have been stored for 6 weeks in 2% O₂ with 1% or 5% CO₂. Based on studies with Florida mango cultivars, the optimal range of O₂ is 3 to 5% and CO₂ is 5 to 10% in modified or controlled atmospheres (Bender *et al.*, 2000, 2000a, 2000b, Kim *et al.*, 2007 and Yahia, 2006). Mangos tolerate short exposures to insecticidal atmospheres with very low O₂ and elevated CO₂ (Yahia and Vasquez-Moreno, 1993). However, exposure of mature-green mangos to O₂ levels below 2% and/or CO₂ levels above 10% for longer than a few days may induce skin discoloration, grayish or pale flesh color, uneven ripening, and off-flavor development due to fermentative metabolism (accumulation of acetaldehyde and ethanol) (Kader 2008).

Conclusion

The atmosphere used in this work resulted in modification of the activity of PFK. In addition, the storage of fruits under the CA retarded ripening and did not affect the quality attributes of Keitt mango. These results indicate a potential for the application of CA for post-harvest ripening control of mango cv. Keitt. The optimal CA for long-term storage of "Keitt" mango at 13 °C is (3% O₂ +6% CO₂+ 91% N₂); under

these conditions the storage life of mango can be extended up to 10 weeks.

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أثر التخزين تحت الجو المتحكم فيه على النشاط الإنزيمي لإنزيم الفسفوفركتوكينيز (*Mangifera Indica L.*) في ثمار فاكهة المانجو صنف كيت

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المستخلص. يتم تنظيم تركيز كل من الأكسجين، وثاني أكسيد الكربون والنيتروجين، درجة الحرارة والرطوبة بواسطة الجو المتحكم فيه خلال فترة التخزين. ويمكن استخدام الجو المتحكم فيه كعلاج إضافي لحفظ الفواكه الطازجة وذلك ضمن نطاقاتها المثلى من الرطوبة النسبية ودرجات الحرارة وذلك لزيادة فترة بقائها بعد الحصاد. يعتبر الجليكوليسيس أساس كل من التنفس اللاهوائي والتنفس الهوائي، ويحدث في جميع الكائنات الحية تقريباً ويتم تنظيمها بواسطة ثلاثة إنزيمات تحفز تفاعلات غير عكسية هي: الهكسوكينيز، الفوسفوفركتو كينيز والبيروفيت كينيز. الهدف من هذا العمل دراسة تأثير تخزين المانجو تحت ظروف الجو المتحكم فيه على نشاط إنزيم الفوسفوفركتو كينيز، كذلك على جودة الثمار. وقد أدت ظروف الجو المتحكم فيه والمستخدمة في هذه الدراسة إلى انخفاض معنوي في النشاط الإنزيمي للفوسفوفركتو كينيز بالمقارنة مع الكنترول وزيادة معنوية في فترة الحفظ تحت هذه الظروف. إضافة

إلى ذلك، كان هناك انخفاض في إنتاج ثاني أكسيد الكربون. وتشير هذه النتائج إلى وجود إمكانية لتطبيق ظروف الجو المتحكم فيه للتحكم في عملية نضج المانجو بعد الحصاد. الجو المتحكم فيه الأمثل للتخزين الطويل الأجل للمانجو صنف كيت عند ١٣ درجة مئوية هو (٣٪ أكسجين + ٦٪ ثاني أكسيد الكربون + ٩١٪ نيتروجين)، ويمكن في ظل هذه الظروف أن تمتد فترة التخزين للمانجو لتصل إلى ١٠ أسابيع.

الكلمات الدالة: التخزين تحت الجو المتحكم فيه، المانجو، التنفس اللاهوائي، التنفس الهوائي، جودة الثمار، الفسفوفركتوليفيناز.