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Starch to Sugar Conversion in "Tsugaru" Apples under Ethylene and 1-Methylcyclopropene Treatments

M. Thammawong[1]*, and O. Arakawa[2]

ABSTRACT

The effects of ethylene and 1-methylcyclopropene (1-MCP) on sugar accumulation during storage of *Malus domestica* Borkh. *cv.* "Tsugaru" apples were evaluated. Fruit was harvested and treated with ethylene and 1-MCP at both immature and mature stages. The loss of starch content in immature "Tsugaru" fruit was observed in ethylene-treated fruit at days 7-10, and the total sugar content of all tissue zones only changed slightly following storage. The highest sugar content was of fructose, and the lowest was of sorbitol. The difference in sugar content between ethylene-treated and untreated fruit was observed only after four days of treatment. Other ripening aspects of the immature fruit, such as respiration rate and ethylene production, were not affected by ethylene and 1-MCP. In the mature fruit, the sugar content changed slightly between days 4-7 and then dropped on day 10. Ethylene treatment resulted in an increase in starch hydrolysis and also affected the ripening characteristics of the fruit. However, exogenous ethylene did not seem to induce sugar accumulation in mature "Tsugaru" fruit during storage. Therefore, this study indicates that the accumulation of sugars in the detached 'Tsugaru' fruit during storage seems to correlate differently with the ripening properties and starch hydrolysis, depending on fruit maturity at harvest and its storage duration.

Keywords: 1-methylcyclopropene, Apple, Ethylene, Starch, Sugar, "Tsugaru".

INTRODUCTION

Fruit sweetness is one of the major characteristics of fruit quality and market value, reflecting the concentrations of sucrose, glucose, fructose, and sorbitol in the fruit flesh. In the early stages of maturation, starch accumulated in the fruit is progressively hydrolyzed in order to increase sweetness, thus affecting fruit taste during ripening (Brookfield *et al.,* 1997; Lau, 1988; Magein and Leurquin, 2000; Warrington *et al.*, 1999). These changes in starch and sugar content during the ripening process can greatly affect the sweetness of the fruit since the apple is generally harvested at the mature stage and allowed to ripen up until consumption. In addition, once the fruit has been harvested, the sweetness quality of the detached fruit seems to be influenced mainly by starch accumulated in the fruit cells and cellular sugar levels at the time of harvest. However, little is known about the effects of ripening and starch loss on the characteristics of sugar accumulation during storage of apple fruit.

Ethylene has been suggested for stimulating the conversion of starch to sugar (Kader, 1985; Watkins, 2003), and 1-MCP has been recommended as a valuable tool for post-harvest handling (Fan *et al.,* 1999; Pre-Aymard *et al.,* 2003). Both ethylene and 1-MCP have been previously shown to affect

[1] National Food Research Institute, National Agriculture and Food Research Organization, 2-1-12 Kannondai, Tsukuba, Ibaraki, 305-8642, Japan.
* Corresponding author; e-mail: manasika@affrc.go.jp
[2] Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyocho, Hirosaki, Aomori, 036-8561, Japan.

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starch degradation and physiological properties of fruit during storage depending on cultivars and their harvesting dates (Thammawong and Arakawa, 2007). Nevertheless, the effects of ethylene and 1-MCP on fruit sweetness and the individual sugar content of the detached fruit flesh during ripening has not been well studied. A study on changes in the sugar content of detached apple fruit may provide better understanding of starch degradation, and also increase knowledge that can be helpful to further improve fruit quality and commercial use.

In this study, the changes of sugar concentrations in different flesh zones of fruit treated with either ethylene or 1-MCP were investigated. "Tsugaru" fruit harvested at two different developmental stages was used in the study since it has been shown to respond well to ethylene and 1-MCP treatment. This fruit also shows a nearly simultaneous increase in ripening characteristics with hydrolysis of accumulated starch.

MATERIALS AND METHODS

Apple Fruit

"Tsugaru" fruits were obtained from the experimental orchard at the Faculty of Agriculture and Life Science, Hirosaki University. The fruits were harvested at two maturing stages in the year 2006 on August 8 (immature stage; 80 days after full bloom (DAFB)), and September 7 (mature stage; 110 DAFB). The fruits of each harvesting time were divided into three groups for ethylene treatment, 1-MCP treatment, and a control group.

Treatments

For the ethylene treatment, pure ethylene gas (4.0 mL, GL Sciences Inc., Japan) was injected into a closed container (40 L) to produce a final concentration of 100 μL L^{-1}. The container was kept at 25ºC for 24 hours. For the 1-MCP treatment, 0.13 g of SmartFresh™ (0.14% A.I., Rohm and Haas Co., Japan) powder was first placed in a flask within a container, and then 25 mL distilled water was added with a syringe through a cap and rubber hose into the flask, producing a final concentration of 2 μL L^{-1} 1-MCP. This concentration is a proper level for apple fruit according to Blankenship and Dole (2003). The fruits were treated with 1-MCP at 25ºC for 24 hours. After the treatments, all fruits were kept at 25ºC in a storage room for ripening.

Measurements

Respiration Rate, Ethylene Production, and Starch Content

The production of CO_2 and C_2H_4 was measured by the methods described in Thammawong and Arakawa (2007). The starch distribution was measured by dipping an apple slice taken from the equatorial region in I_2-KI solution (10 g 25 g^{-1} in 1 L distilled water), and the starch-iodine rating was carried out using the generic starch-iodine index chart for comparison (Watkins, 2003). This method uses a 1 to 8 scale, with 1= All starch and 8= No starch.

Sugar Content

Preparation of samples for HPLC analysis: The sugar content of the samples was determined according to Pharr and Sox (1984) and Wang *et al.* (1999). Briefly, 100 mg of dried sample was extracted three times with 2 mL of 80% (v/v) ethanol at 80ºC for 20 minutes per extraction. The homogenates were centrifuged at 15,000×g for 10 minutes to give ethanol-soluble and ethanol-insoluble fractions. The ethanol-soluble fractions were pooled, evaporated to dryness with a concentrator, redissolved in 2 mL of de-ionized water, and then filtered.

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Preparation of standards for HPLC analysis: Stock solutions (1 mg mL^{-1}) of sucrose, glucose, fructose, and sorbitol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were prepared by dissolving the mixed standards in distilled water (HPLC grade).

HPLC analysis: The mixed standard or sample (20 µL) was measured with a high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan). De-gassed and de-ionized, distilled water at 1 mL min^{-1} at was used as the mobile phase. A refractive index detector (RID-10A; Shimadzu) was used to quantify sugar content following separation with a Shim-pack SCR-101C column (Shimadzu) at 80ºC. The recovery rate was determined by comparison with standard samples of known concentrations of glucose, fructose, sucrose, and sorbitol. The total sugar content was obtained from the sum of these four sugar contents.

Data Analysis

Analysis of Variance (ANOVA) with Completely Randomized Design (CRD) using chemical treatments as a factor was performed using SPSS (SPSS, IL, USA), and Tukey's multiple-range test was used to test significant differences at the 95% confidence level of each variable.

RESULTS

Starch Rating and Iodine Staining Test

In the flesh of an immature "Tsugaru", the starch content values indicated starch hydrolysis in all fruit. Although starch loss of the 1-MCP-treated fruit did not significantly differ from that of the control fruit (Figure 1A), the iodine staining test revealed a rapid loss of starch in fruit treated with ethylene (Figure 1B). The starch loss of the ethylene-treated fruit was found to be

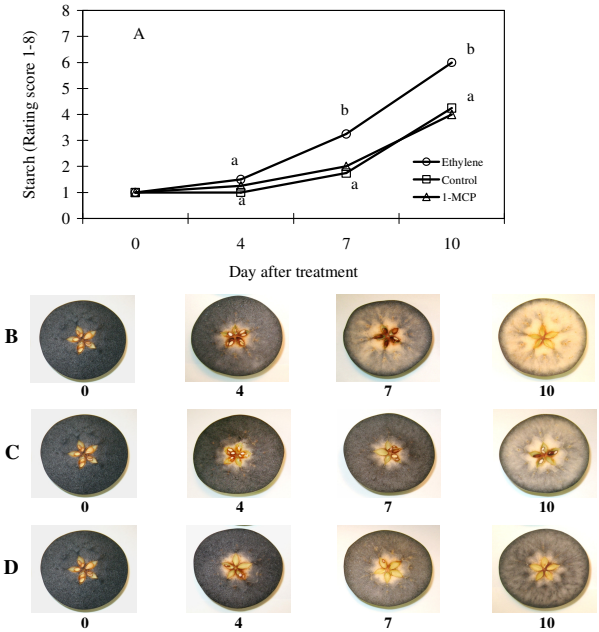

Figure 1. Starch rating scores (A) and iodine staining test of immature 'Tsugaru' (harvested at 80 DAFB); Ethylene treatment (B), Control (C), and 1-MCP treatment (D) during storage at 25ºC. Each value is the mean of four replicates.

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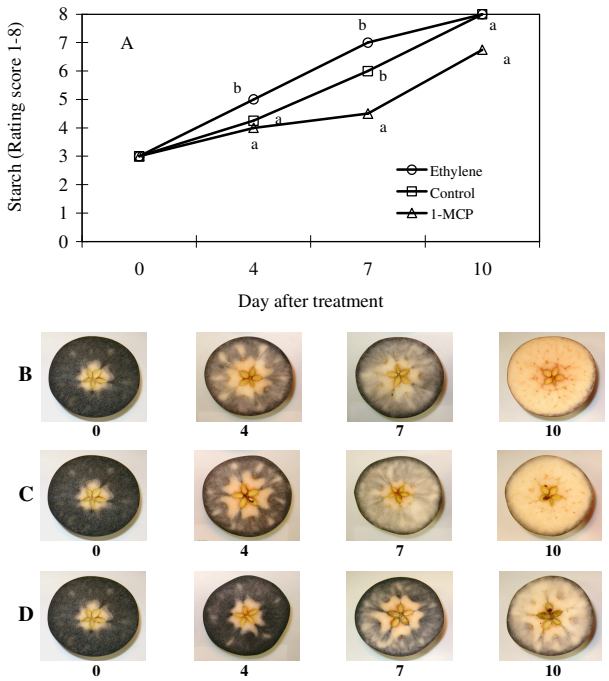

Figure 2. Starch rating scores (A) and iodine staining test of mature 'Tsugaru' (harvested at 110 DAFB); Ethylene treatment (B), Control (C), and 1-MCP treatment (D) during storage at 25ºC. Each value is the mean of four replicates.

significantly greater than both the control and the 1-MCP-treated fruit on day 7 to day 10 after treatment.

For a mature "Tsugaru", ethylene treatment resulted in a greater loss of starch compared to any other treatment. However, by day 10, the starch levels did not differ from the control fruit (Figure 2A). Although there was no statistical difference in the starch content of 1-MCP treated and control fruit on days 4 and 10, the iodine staining test showed a greater amount of starch remaining in the fruit treated with 1-MCP (Figure 2D).

Physiological Aspects

Regardless of the treatment to which the fruit was subjected, the respiration rate of all the immature "Tsugaru" fruit decreased during storage, although the differences between treatments were not clear (data not shown). Unfortunately, ethylene production of the immature fruit was too low to be determined accurately in this study.

In the mature fruit, not only did the respiration rate of 1-MCP-treated fruit decrease slightly following treatment, it was also the lowest rate found in this investigation. The respiration rate of both the ethylene-treated fruit and control fruit increased between days 4-7, and then dropped at day 10 (Figure 3A). Although ethylene production of the ethylene treated fruit did increase between days 4-10, it was not significantly different from the control fruit. Production of ethylene in 1-MCP-treated fruit was observed to be at very low levels and was significantly lower than levels found for ethylene-treated and control conditions (Figure 3B).

Sugar Contents

The differences in total sugar content among flesh zones were small for immature "Tsugaru" fruit. The amount of total sugar in

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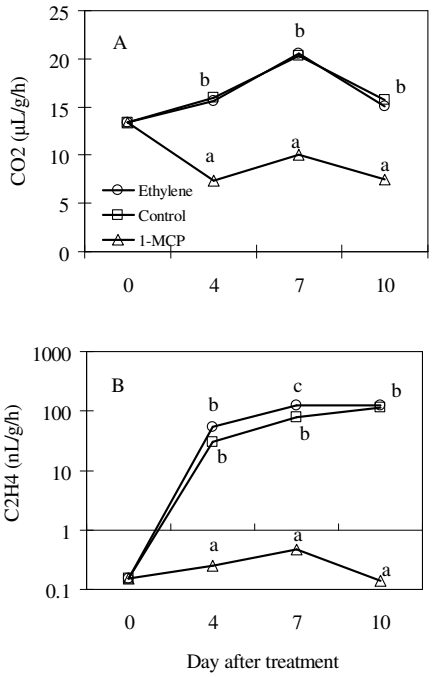

Figure 3. Effects of ethylene and 1-MCP treatments on the respiration rate of mature 'Tsugaru' apples. Each value is the mean of four replicates. Mean values with the same letter are not significantly different at the 5% level by Tukey's multiple-range test.

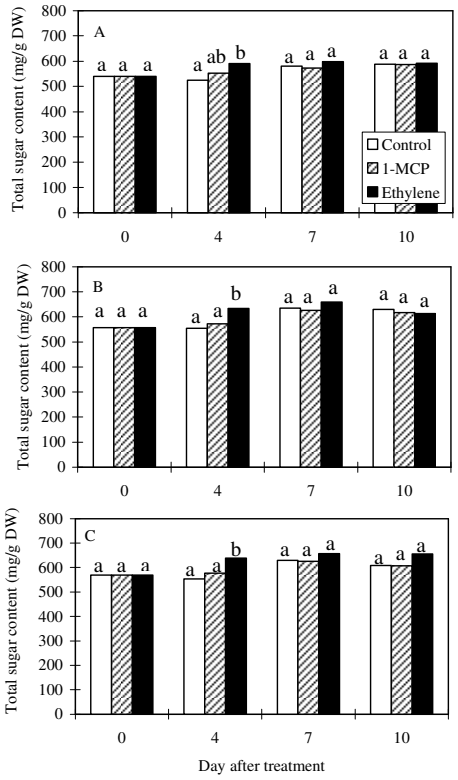

Figure 4. Total sugar content of Inner (A), Middle (B), and Outer (C) tissues of control fruit and fruit treated with 1-MCP and ethylene. Treated fruit was immature 'Tsugaru' apples placed in storage. Each value is the mean of four replicates. Mean values with the same letter are not significantly different at the 5% level by Tukey's multiple-range test.

all zones of the ethylene-treated fruit increased significantly at day four, while there was no observable difference from day zero onwards in both control and 1-MCP (Figure 4) treated fruit. For the levels of individual sugars taken from the middle flesh zone, the sucrose content of the control fruit first decreased slightly on day four, and then greatly increased on days 7 and 10 (Figure 5A). However, although the sucrose content of the ethylene-treated and 1-MCP-treated fruits were initially lower than levels found in the control fruits, the sucrose content on days 7-10 for the treated fruits were not much different than levels for control fruits on day 0. The glucose content of all fruits clearly increased during storage from day 0-10 (Figure 5B). In contrast, fructose content was stable throughout the study and there was no observable difference in the fructose content among treatments (Figure 5C). Sorbitol content was observed to be at a low level in the tissues of all fruits (Figure 5D).

With regards to the mature "Tsugaru" fruits, the total sugar content of the control fruits increased between days 4-7, but decreased on day 10 back to levels similar to day 0 (Figure 6). The total sugar content in fruits treated with either 1-MCP or ethylene increased slightly on day four, but then dropped sharply between days 7-10. For the individual sugars, an increase in sucrose content was generally observed for all fruit between days 4-7, but the increase in sucrose content of 1-MCP-treated fruit was significantly inhibited between days 7-10

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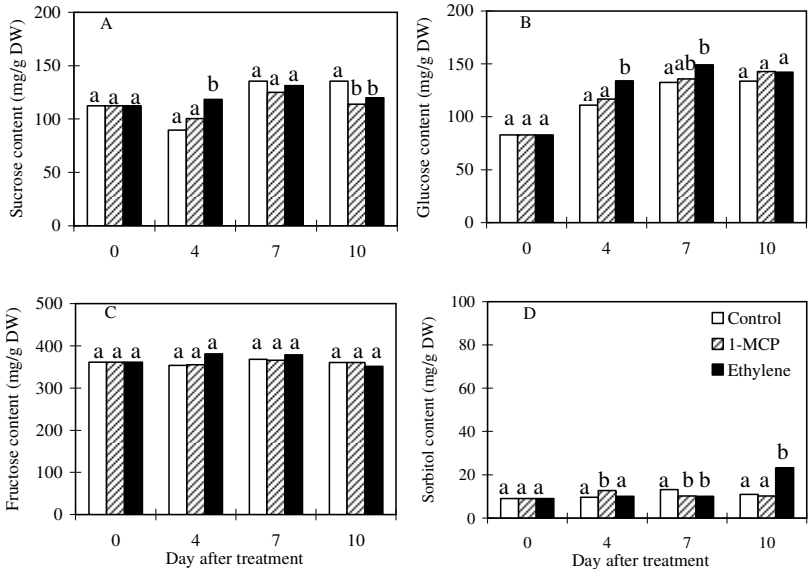

Figure 5. Content of Sucrose (A), Glucose (B), Fructose (C), and Sorbitol (D) of control fruit and fruit treated with 1-MCP and ethylene. Treated fruit was immature 'Tsugaru' apples placed in storage. Each value is the mean of four replicates. Mean values with the same letter are not significantly different at the 5% level by Tukey's multiple-range test.

(Figure 7A). The glucose content increased between days 4-10 for all treatments, and was highest in the inner tissue zone (Figure 7B). Although, ultimately, the glucose content on day 10 was higher than day 0, there was evidence of an inhibitory effect of ethylene and 1-MCP on glucose accumulation on days 7 and 10, respectively. An increase in fructose content was found between days 4-7 for the control fruits, followed by a decrease on day 10 in all tissue zones. In contrast, the fructose content of 1-MCP- and ethylene-treated fruit only changed slightly during storage (Figure 7C). The sorbitol content of all tissues was very low during the investigation period and the differences among treatments were small (Figure 7D).

DISCUSSION AND CONCLUSION

As starch hydrolysis begins to occur during fruit ripening, the levels of sugar accumulation in mature fruit is generally observed to be higher compared with that of the immature fruit. Ethylene has been suggested to play a role in the conversion of starch to sugar (Kader, 1985; Watkins, 2003), and it has been demonstrated that ethylene-induced loss of starch in apples affects the fruit differently depending on the cultivar and stage of harvest (Thammawong and Arakawa, 2007). However, there are few reports that examine both the changing characteristics of sugar content in a particular flesh zone and the rate of starch degradation during storage of detached apple fruit. Moreover, the effects of 1-MCP and ethylene on the process of converting starch to sugar in fruit cells still needs to be clarified.

In immature "Tsugaru" fruit, certain changes in physiological aspects, such as respiration rate and ethylene production, were found to be independent of ethylene treatment. 1-MCP treatment also did not inhibit starch degradation or respiration rate. The total sugar content changed slightly during starch degradation and there was little difference among treatments. Although the results show that the degradation of accumulated starch in the immature fruit

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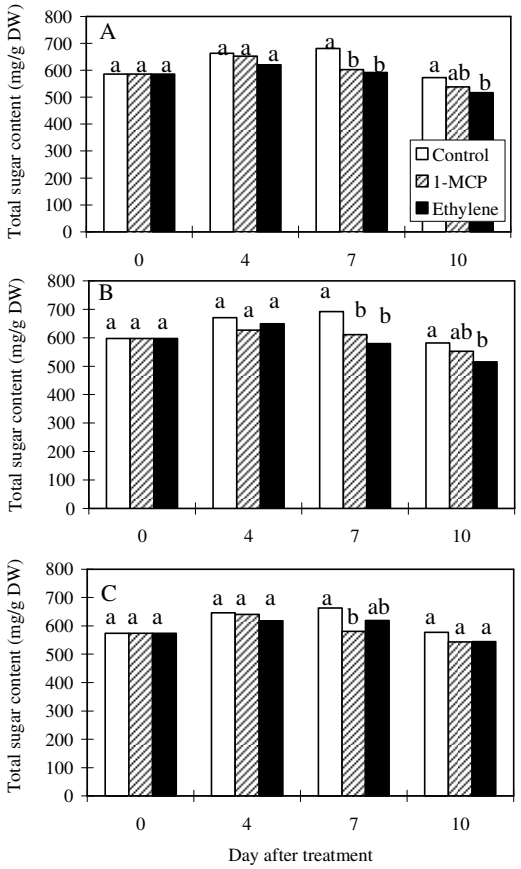

Figure 6. Total sugar content of Inner (A), Middle (B), and Outer (C) tissues of control fruit and fruit treated with 1-MCP and ethylene. Treated fruit was mature 'Tsugaru' apples placed in storage. Each value is the mean of four replicates. Mean values with the same letter are not significantly different at the 5% level by Tukey's multiple-range test.

leads to the production of glucose during storage, and that the glucose content of the ethylene-treated fruit was higher than other treatments after harvesting, there was no increase in respiration and ethylene production observed. These results supported earlier conclusions that immature fruit does not respond to exogenous ethylene used for inducing fruit ripening, and that starch hydrolysis leading to sugar accumulation is not related to the ripening or ethylene treatment (Thammawong and Arakawa, 2007).

A significant inhibitory effect of 1-MCP on starch hydrolysis and physiological aspects of the mature "Tsugaru" was also observed in this study. As 1-MCP has been shown to increase post-harvest life and maintain fruit quality (Fan *et al.,* 1999; Pre-Aymard *et al.,* 2003), previous studies had suggested that 1-MCP treatment inhibits the ripening of apples by preventing or delaying the increase in ethylene production associated with the climacteric ripening stage (Dauny and Joyce, 2002; Defilippi *et al.,* 2004; Fan *et al.,* 1999; Moran and McManus, 2005; Rupasinghe *et al.,* 2000). On the other hand, although it has been reported that 1-MCP has an effect on the soluble solid concentrations (SSC) in apples, SSC in fruit treated with 1-MCP can be lower, higher or equal to those in untreated fruit (Bai *et al.,* 2002; Dauny and Joyce, 2002; DeEll *et al.,* 2002; Fan *et al.,* 1999; Moran and McManus, 2005; Saftner *et al.,* 2003; Watkins *et al.,* 2000). In addition, it is particularly interesting to note that despite the simultaneous increase in respiration rate and ethylene production with the starch loss found in ethylene-treated fruit, this did not induce the accumulation of sugar components.

From the results of this study, we see that starch hydrolysis was occurring during storage. However, the difference in total sugar content between ethylene-treated and untreated fruit was observed after only four days of storage (Figure 4). In addition, there was an initial increase in total sugars between day 4 and day 7 for the mature fruit; however it slightly dropped on day 10 compared with day 7. From a postharvest point of view, since respiration is a central process that mediates the release of energy through the breakdown of carbon compounds and the formation of carbon skeletons, and living cells of harvested fruit respire continuously, it is necessary to maintain this synthetic reaction after harvest. In mature fruit, growth decreases and the fruit proceeds towards the ripening and senescence stages after harvest (Kays, 1991). Consequently, the sugars from starch hydrolysis are not only important for determining fruit flavor, but may also be

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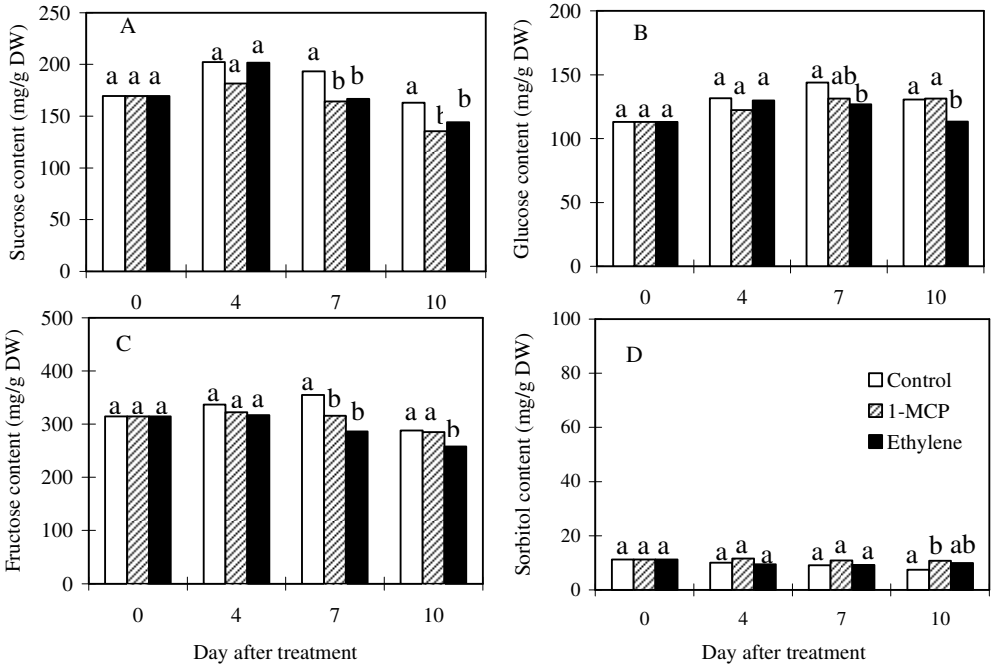

Figure 7. Content of Sucrose (A), Glucose (B), Fructose (C), and Sorbitol (D) of control, and fruit treated with 1-MCP and ethylene. Treated fruit was mature 'Tsugaru' apples placed in storage. Each value is the mean of four replicates. Mean values with the same letter are not significantly different at the 5% level by Tukey's multiple-range test.

greatly utilized in respiration and cell maintenance. Kays (1991) also suggested that during the rapid growth of plant products, hexose sugars often only proceed partially through the respiratory system pathway to yield carbon skeletons, and not carbon dioxide. In addition, a high rate of cell elongation and expansion has been observed in immature fruit (Ryugo, 1988). All of these suggest that the sugar products from the degradation of accumulated starch in immature fruit might be used primarily as substrates for respiration in order to produce energy and carbon skeletons that can be then used in other continuous synthetic reactions of fruit cells after harvest.

Overall, the physiological characteristics of the ripening process in detached apples showed different responses to ethylene and 1-MCP treatment depending on the maturation stage at harvest. Neither treatment induced the accumulation of sugars resulting from starch degradation in "Tsugaru" fruit held in storage. Future studies need to be undertaken, taking into account other variables such as genetic variation, in order to understand the individual mechanism of starch to sugar conversion in these apples.

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تبدیل نشاسته به قند در سیب تسوگارو (Tesugaru) در اثر تیمار با اتیلن و ۱-متیل سیکلو پروپن

م. تاماوونگ و ا. آراکاوا

چکیده

اثر اتیلن و ۱-متیل سیکلو پروپن (1-MCP) بر تولید قند در سیب رقم تسوگارو(*Malus domestica* Borkh) ارزیابی شد. میوه سیب پس از برداشت در حالت رسیده و نارس، تحت تیمار اتیلن و ۱- متیل سیکلو پروپن قرار داده شد. کاهش مقدار نشاسته در سیب نارس تحت تیمار اتیلن در روزهای ۷ تا ۱۰ انبارداری مشاهده گردید. مقدار قند کل بافت قسمتهای مختلف سیب کمی تغییر در دوره انبارداری داشت. بیشترین قند موجود فروکتوز و کمترین قند سوربیتول بود. اختلاف مقدار قند در سیبهای تیمار شده با اتیلن و تیمار نشده بعد از ۴ روز مشاهده شد. بقیه خصوصیات رسیدن میوه‌های نارس مثل سرعت تنفس و تولید اتیلن تحت تأثیر اتیلن و ۱- متیل سیکلو پروپن قرار نگرفت. در میوه‌های رسیده مقدار قند در روزهای ۴ تا ۷ کمی افزایش یافت و سپس در روز دهم کاهش پیدا کرد. تیمار اتیلن موجب افزایش تجزیه نشاسته و خصوصیات رسیدن میوه شد ولی به نظر نمی‌رسد که اتیلن خارجی موجب افزایش قند در سیب رسیده تسوگارو در زمان انبارداری شده باشد. بنابراین، این مطالعه مشخص کرد که تولید قند در سیب تسوگارو مورد آزمایش رابطه متفاوتی با خصوصیات رسیدن و تجزیه نشاسته دارد و این تفاوت بستگی به درجه رسیدگی میوه در زمان برداشت و زمان انبارداری دارد.

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