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Browning characteristics of fresh-cut 'Tsugaru' apples as affected by pre-slicing storage atmospheres

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ABSTRACT

The change in browning characteristics of the slices processed from 'Tsugaru' apples stored at 0 °C for 5 months under controlled atmosphere (CA, 1 kPa O_2 + 1 kPa CO_2, 3 kPa O_2 + 3 kPa CO_2) or air has been investigated for 5 days at 20 °C. Respiration and ethylene production of the slices from apples stored in CA were retarded. Electrolyte leakage and browning index were lower in the slices from apples stored under CA than air. Vitamin C and phenolic contents in the slices from apples stored under air were maintained at higher level compared to the slices from apples stored under CA. Polyphenol oxidase activity in the slices was not affected by pre-slicing storage atmospheres. Therefore, the atmospheres of pre-slicing storage affected browning development in fresh-cut products of 'Tsugaru' apples and browning was found to be correlated with the levels of electrolyte leakage and phenolic compounds.

1. Introduction

'Tsugaru', the major apple variety among a precocious species grown in South Korea, is consumed mainly for short-term as a fresh fruit because good storage and processing conditions are not available (Chung, Lee, Kim, Lee, Jeong & Choi, 2000). The long-term storage and processing of fresh apples are an important concern after harvest. The technology of controlled atmosphere (CA) storage at low temperature can be used to prolong the storage life of 'Tsugaru' apples (Chung et al., 2000). However, little is known about the processing quality, including the effect of storage on fresh-cut quality.

The industry of fresh-cut fruits and vegetables is continuously growing due to consumers demand for fresh, convenient and healthy foods (Tomas-Barberan, Allende, & Gil, 2006). Fresh-cut operations have been defined as those procedures, such as washing, sorting, trimming, peeling, slicing, or chopping, that do not affect the fresh-like quality of the fruit or vegetable (Cantwell, 1992). Shelf-life of fresh-cut produces is greatly shortened as compared with the intact fruits and vegetables because fresh-cut means the tissues are wounded (Cantwell, 1992). Usually, wounding of plant tissues induces elevated respiration and ethylene production, enzymatic browning, membrane lipid degradation, production of secondary metabolites, and water loss (Watada, Abe, & Yamauchi, 1990). Among these negative consequences of wounding in fresh-cut products, enzymatic browning is considered one of the main limitations on shelf-life of fresh-cut products (Brecht, 1995). The browning of fresh-cut produces occurs via the reaction of polyphenol oxidase (PPO) with phenolic compounds and is facilitated by the diffusion of O_2 into the cut tissue (Gil, Gorny, & Kader, 1998). PPO catalyses the hydroxylation of monophenols to diphenols and oxidation of diphenols to diquinones followed by non-enzymatic formation of melanines (Tomas-Barberan & Espin, 2001). In case of some apple varieties, there is no clear relationship between the extent of enzymatic browning and PPO activity (Rocha & Morais, 2002), between the extent of enzymatic browning and phenolic contents (Coseteng & Lee, 1987), and between PPO activity and phenolic contents (Podsedek, Wilska-Jeszka, & Anders, 2000).

In previous studies on the inhibition of enzymatic browning in fresh-cut apple processing, anti-browning agents treatment (Son, Moon, & Lee, 2001), edible coating (Perez-Gago, Serra, & del Rio, 2006), mild heat treatment (Kim, Smith, & Lee, 1993), high CO_2 treatment (Gunes, Watkins, & Hotchkiss, 2001), 100 kPa O_2 pre-treatment (Lu & Toivonen, 2000), and modified atmosphere packaging flushed with N_2 (Chung, Moon, & Choi, 1999) have been reported to be effective in various apple varieties. In general, fresh-cut products are processed from stored whole apples. The conditions of anti-browning treatments for fresh-cut apples have been mostly recommended as a constant level without the consideration of conditions of pre-slicing storage. These recommended conditions may depend upon the conditions of pre-slicing storage and will be changed as more research is completed. However,

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the fresh-cut response of 'Tsugaru' apples in relation to the storage conditions has not been demonstrated.

The objective of this study is to evaluate the change in characteristics related with the browning of fresh-cut 'Tsugaru' apples as affected by different pre-slicing storage atmospheres.

2. Materials and methods

2.1. Materials

Apples (*Malus domestica* Borkh. cv. Tsugaru) were harvested at commercial maturity from a commercial orchard in the Andong region of South Korea and graded according to size and defects. Sound and uniform size apples were stored under CA (1 kPa O_2 + 1 kPa CO_2 and 3 kPa O_2 + 3 kPa CO_2) and normal air for 5 months at 0 °C. CA conditions used in the storage of apples were obtained from previous studies (Chung et al., 2000) for the investigation of storage atmosphere for apples' quality during preservation. Folin-Ciocalteu reagent was purchased from Junsei Chemical Co. (Tokyo, Japan), L-ascorbic acid, chlorogenic acid, and 2,4-dinitrophenylhydrazine were from Sigma–Aldrich Chemical Co. (St. Louis, Mo., USA) and other chemicals used for analyses were high purity grade.

2.2. Preparation of apple slices

Whole apples that had been stored for 5 months under different atmospheres were washed in sodium hypochlorite solution (100 μl l^{-1}, pH 6.5, 5 °C) for 2 min, dried for 10–15 min, then peeled using a sharp stainless steel knife. Individually peeled apples were cored and sliced into eight wedges using a hand corer and slicer. Fifteen 200 g lots of the slices were immediately placed in plastic trays, covered with unsealed individual polyethylene film bags, and kept at 20 °C for up to 5 days. Three replicates from each treatment for analyses were taken at 1 day intervals.

2.3. Analysis of respiration rate and ethylene production

Apples slices (200 g) were put into a 1.4 l glass jar and sealed with a cap. After standing for 1 h at 20 °C, headspace gas (1 ml) was withdrawn using a gas-tight syringe. Carbon dioxide concentration was determined using a gas chromatograph (5890A, Hewlett Packard, CA, USA) equipped with a thermal conductivity detector and a 1.7 m glass column (4.0 mm i.d.) packed with 80/100 mesh Porapak Q (Alletech Associates Inc., IL, USA). Flow of He gas was 40 ml min^{-1}, and oven, injector and detector temperatures were 70, 100, and 140 °C, respectively. Respiration rate was calculated as mg CO_2 kg^{-1} h^{-1}.

Ethylene concentration was determined using a gas chromatograph (5890A, Hewlett Packard, CA, USA) equipped with a flame ionisation detector and the same column for carbon dioxide determination. Gas flows for N_2, H_2 and air were 30, 30 and 300 ml min^{-1}, respectively, and oven, injector and detector temperatures were 80, 100, and 140 °C, respectively. Ethylene production was calculated in μl C_2H_4 kg^{-1} h^{-1}.

2.4. Analysis of electrolyte leakage

Apples tissue discs (2 mm thick, 15 mm diameter) were prepared from the slices using a cork borer. Five discs were rinsed briefly with deionized water and placed in 50 of 0.5 M manitol solution, and incubated for 3 h at 20 °C in a shaking water bath. Conductivity of the surrounding solution was determined with a Conductivity/TDS meter (CDS 5000, Lamotte Co., WA, USA). The tissue was then boiled for 30 min and the total conductivity recorded. Electrolyte leakage was expressed as a percentage of total electrolytes in the tissue.

2.5. Analysis of vitamin C content

Vitamin C content of apple slices was measured according to 2,4-dinitrophenylhydrazine colorimetric methods (Roe & Osterling, 1943), 10 g of samples was added to 50 ml of 5% metaphosphoric acid, then the samples were ground and filtered.

2.6. Analysis of phenolic compounds content

Content of phenolic compounds was determined according to the Folin-Ciocalteu procedure (Singleton & Rossi, 1965). Ten grams of apple slices were homogenised in 90 ml of 50 mM phosphate buffer (pH 7.0). The homogenate was filtered through filter paper (Toyo No. 2). The extract (5 ml) was transferred into a volumetric flask. Folin-Ciocalteu reagent (5 ml) was added, and mixed thoroughly. After 3 min, 5 ml of 10% Na_2CO_3 solution was added and left for 1 h. The absorbance of the resulting solution was measured with a spectrophotometer (UV1601, Shimadzu, Kyoto, Japan) at 760 nm. The concentration of total phenolic compounds was determined by comparison with the absorbance of chlorogenic acid used at different concentrations as standard.

2.7. Analysis of polyphenol oxidase (PPO) activity

PPO activity was assayed with some modifications of the procedure described by Galeazzi, Sagarbieri, and Constantidines (1981). Enzyme extraction was performed under cooling with ice. Ten grams of polyvinyl polypyrrolidone (PVPP) was added to 10 g of apple slices and homogenised in 90 ml of 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000 g for 5 min, and the supernatant was used as a crude enzyme solution. The reaction mixture consisted of 1 ml of the crude enzyme solution, 1 ml of 100 mM phosphate buffer (pH 5.8) and 1 ml of 1% cathecol solution. Enzyme activity was measured by the increase in absorbance at 420 nm. One unit of enzyme activity was defined as the increase in absorbance of 0.001 per min under the conditions of the assay.

2.8. Analysis of surface color

The colour of apple slices was measured with a chromameter (CR-200, Minolta Co., Osaka, Japan), which had been calibrated with a standard white plate ($Y = 92.30$, $x = 0.3162$, $y = 0.3323$). Three readings of X, Y and Z were recorded for each slice, converted into browning indexes according to the following equation (Perez-Gago et al., 2006). Browning index = $(x - 0.31) \times 100/0.172$, where, x is the chromaticity coordinate calculated from the X, Y, Z values according to the following equation $x = X/(X + Y + Z)$.

2.9. Statistical analysis

Experimental data were subjected to analysis of variance using the GLM procedure of the SAS statistical package (Statistical Analysis System, SAS Institute, Cary, NC, USA). Mean separation was determined using Duncan's multiple range test ($P < 0.05$).

3. Results and discussion

3.1. Changes in respiration rate and ethylene production

Respiration is a basic physiological process in all living tissues (Brady, 1987). Higher respiration rates mean a faster overall

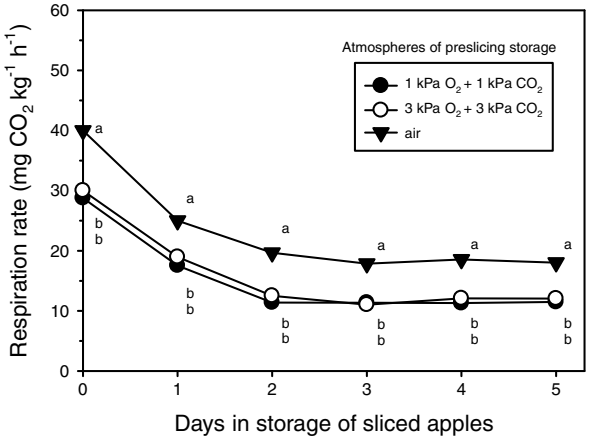

Fig. 1. Change in respiration rate of sliced 'Tsugaru' apples as affected by atmospheres of pre-slicing storage. Whole apples were stored at 0 °C for 5 months, sliced, and the slices were kept at 20 °C for 5 days. Means ($n = 3$) with different letters are significantly different at the 5% level.

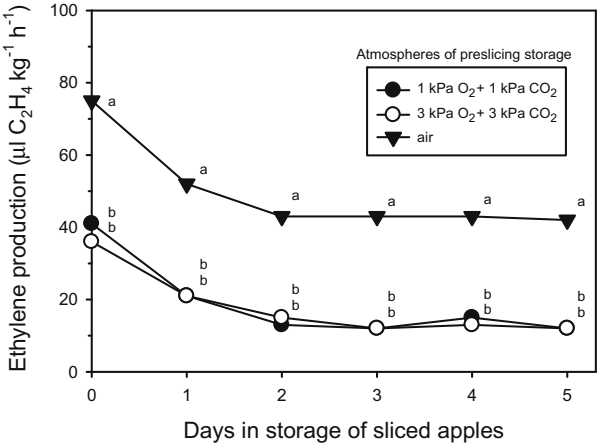

Fig. 2. Change in ethylene production of sliced 'Tsugaru' apples as affected by atmospheres of pre-slicing storage. Whole apples were stored at 0 °C for 5 months, sliced, and the slices were kept at 20 °C for 5 days. Means ($n = 3$) with different letters are significantly different at the 5% level.

metabolism and deterioration. It is known that wounding fruit tissue induces elevated respiration (Watada et al., 1990). This is probably due to an increase in the surface area exposed to the atmosphere after slicing that allows oxygen to diffuse into the interior cells more rapidly and results in increased metabolic activity of injured cells. Changes in the respiration rate of the slices produced from 'Tsugaru' apples that had been stored at 0 °C for 5 months under 1 kPa O_2 + 1 kPa CO_2, 3 kPa O_2 + 3 kPa CO_2, or normal air prior to slicing are shown in Fig. 1. Initial respiration rate of sliced apples processed from whole apples stored in CA or air were approximately 30 and 40 mg CO_2 kg^{-1} h^{-1}, respectively. This difference of respiration rate between CA and air stored apples may be derived from lowered O_2 and elevated CO_2 (Chung & Choi, 1999). Respiration rates of all slices decreased until the second day of storage, then remained stable. There was no difference in decreased respiration rate among the slices prepared from whole apples stored in the two controlled atmosphere treatments. Higher initial level of the slices from whole apples stored in air was maintained over 5 days of storage. These results show that the pattern of changes in respiration rate of apple slices was not affected by the pre-slicing storage atmosphere used in this study. However, the respiration difference of whole apples induced by the storage atmospheres has persisted for the post-slice storage.

Wounding of plant tissues induces elevated ethylene production rates. Wound ethylene can accelerate ripening and senescence on fruits and vegetables (Brecht, 1995), and can also induce synthesis of some enzymes involved in browning reactions or substrate biosynthesis (Rolle & Chism, 1987). Changes in ethylene productions of the slices processed from 'Tsugaru' apples stored for 5 months under different CA and normal air are presented in Fig. 2. Initial ethylene production of the slices from apples stored under air was about 2-fold higher than the slices from apples stored under CA. This may be due to the inhibition of ethylene biosynthesis in whole apples by lowered O_2 and elevated CO_2 (Chung et al., 2000). Ethylene production of all apple slices tended to decreased until 2 days of storage and then remained stable. However, ethylene production during storage was significantly higher in the slices from whole apples stored under air than in the slices from whole apples stored under CA. There was no significant difference in ethylene production between 1 kPa O_2 + 1 kPa CO_2 and 3 kPa O_2 + 3 kPa CO_2. These results show that pre-slicing CA storage was effective in suppressing subsequent ethylene production in fresh-cut slices.

3.2. Changes in electrolyte leakage

Electrolyte leakage is generally considered an indirect measure of cell membrane damage suffered from adverse circumstance and senescence of tissue (Jiang, Shina, Nakamura, & Nakahara, 2001). Changes in electrolyte leakage of the apple slices in relation to the atmosphere of pre-slicing storage are presented in Fig. 3. The initial electrolyte leakage was higher in the apple slices prepared from apples stored under normal air than under CA. In general, electrolyte leakage of the slices was increased with the increase of storage periods. However, there was no difference in the increment of electrolyte leakage among the atmospheres of pre-slicing storage. These results show that the electrolyte leakage in the sliced apples was accelerated by air storage of whole apples. These results may be explained by the loss of membrane integrity associated with browning of the slices. In case of 'Spartan' apple slices, Lu and Toivonen (2000) reported that the low leakage rate is associated with the low degree of cut surface browning of slices. On fresh-cut potatoes, Cantos, Tudela, Gil, and Espin (2002) found that browning is not rate limited by either the enzymes associated with browning or polyphenol substrate concentration, but membrane stability is potentially a major factor of controlling the browning

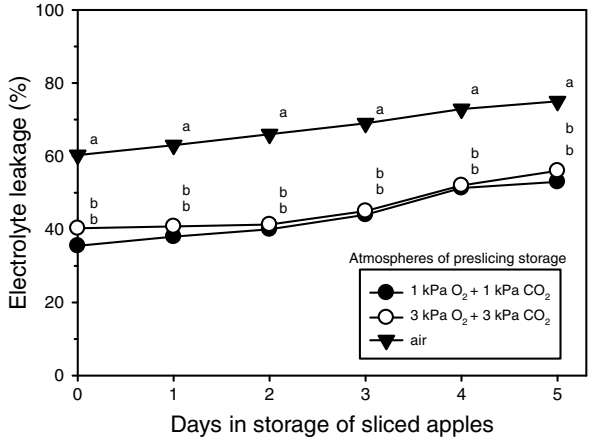

Fig. 3. Change in electrolyte leakage of sliced 'Tsugaru' apples as affected by atmospheres of pre-slicing storage. Whole apples were stored at 0 °C for 5 months, sliced, and the slices were kept at 20 °C for 5 days. Means ($n = 3$) with different letters are significantly different at the 5% level.

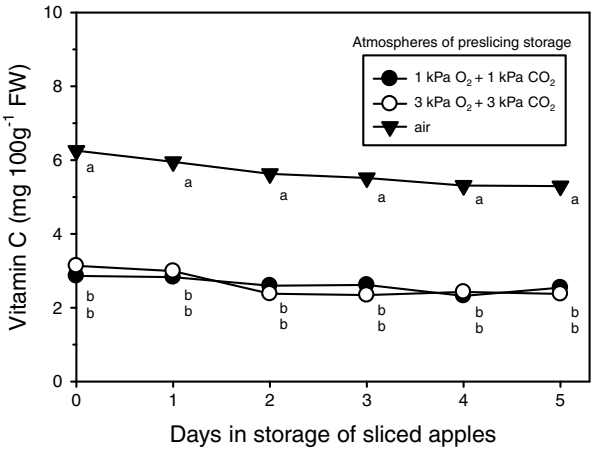

Fig. 4. Change in vitamin C content of sliced 'Tsugaru' apples as affected by atmospheres of pre-slicing storage. Whole apples were stored at 0 °C for 5 months, sliced, and the slices were kept at 20 °C for 5 days. Means ($n = 3$) with different letters are significantly different at the 5% level.

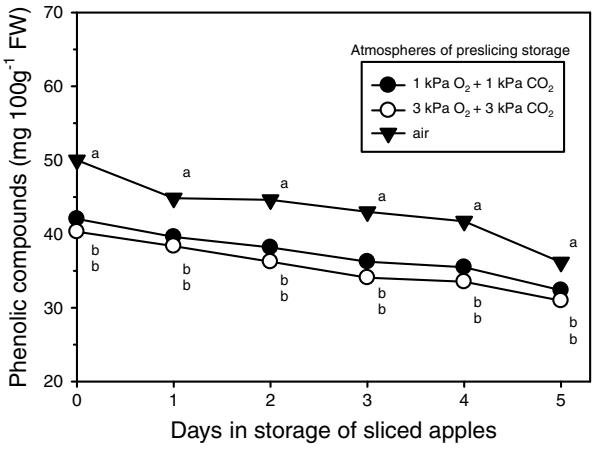

Fig. 5. Change in phenolic compounds content of sliced 'Tsugaru' apples as affected by atmospheres of pre-slicing storage. Whole apples were stored at 0 °C for 5 months, sliced, and the slices were kept at 20 °C for 5 days. Means ($n = 3$) with different letters are significantly different at the 5% level.

rate. In addition, Abreu, Beirao-da-Costa, Goncalves, Beirao-da-Costa and Moldao-Martins (2003) reported that pre-slicing treatment of 'Rocha' pears at temperatures higher than 45 °C enhanced cut surface discolouration due to increased tissue damage.

3.3. Changes in vitamin C contents

Vitamin C is one of the most important nutritional attributes in fruits and vegetables and, has many biological activities in the human body (Lee & Kader, 2000). It is widely used as an antioxidant for preventing enzymatic browning in fresh-cut processing (Son et al., 2001). Changes in vitamin C contents of sliced apples in relation to atmospheres of pre-slicing storage are shown in Fig. 4. The initial level of vitamin C was lower in the slices processed from apples stored under CA than under air. This suggested that the loss of vitamin C in 'Tsugaru' apples was accelerated by low O_2 atmospheres similar to previous reports on some pears (Veltman, Kho, van Schaik, Sanders, & Oosterhaven, 2000). On the other hand, vitamin C was lost more quickly in air than in low O_2 concentration in some vegetables (Platenius & Brown, 1944). Vitamin C contents of sliced apples decreased up to 5 days of storage, but higher levels were maintained in the slices processed from whole apples stored under air in comparison with the slices from apples stored under CA. There was no significant difference in vitamin C levels between the two pre-slicing CA storage atmospheres. These results show that CA had detrimental effects on maintaining vitamin C of whole and sliced 'Tsugaru' apples.

3.4. Changes in phenolic compounds contents

With phenolic compounds, the substrate in enzymatic browning reactions (Tomas-Barberan & Espin, 2001) have many biological and functional activities for fruit quality and human health (Scalbert & Williamson, 2000). The major phenolics found in apples are chlorogenic acid, catechin and epicatechin, these compounds are closely related with enzymatic browning (Murata, Tsurutani, Tomita, & Honma, 1995; Son et al., 2001). Changes in the phenolic compounds of sliced apples processed from apples stored for 5 months under CA and air are shown in Fig. 5. Initial levels of phenolic compounds in the sliced apples processed from apples stored in CA and air were approximately 41 and 50 mg 100 g^{-1} FW, respectively. Phenolic compounds of all slices tended to decrease during storage. Phenolic compounds of the slices from apples stored under air were higher when compared to the slices from apples stored under 1 kPa O_2 + 1 kPa CO_2 or 3 kPa O_2 + 3 kPa CO_2. The changes of phenolic compounds in the slices were not significantly different with the two different storage atmospheres. It is known that ascorbic acid and phenolic compounds contents have a positive correlation due to the reducing action of ascorbic acid that prevents a high level of phenolic compound degradation (Cocci, Rocculi, Romani, & Rosa, 2006).

3.5. Changes in polyphenol oxidase activity

PPO participates in browning by oxidising the phenolic compounds into quinines which subsequently form brown colour pigments (Tomas-Barberan & Espin, 2001). The level of PPO activity may be considered as an index for prediction of susceptibility to browning. Changes in PPO activity of the sliced apples as affected by the storage atmospheres of whole apples were investigated (data not shown). No difference in PPO activity was observed at initiation of storage. During the 5 days of storage, PPO activity did not change from the initial activity, 40 to 50 unit, and was unaffected by varying the atmospheres of pre-slicing storage. Otherwise, Rocha and Morais (2002) reported that PPO activity in 'Jonagored' apple slices increased with time in storage.

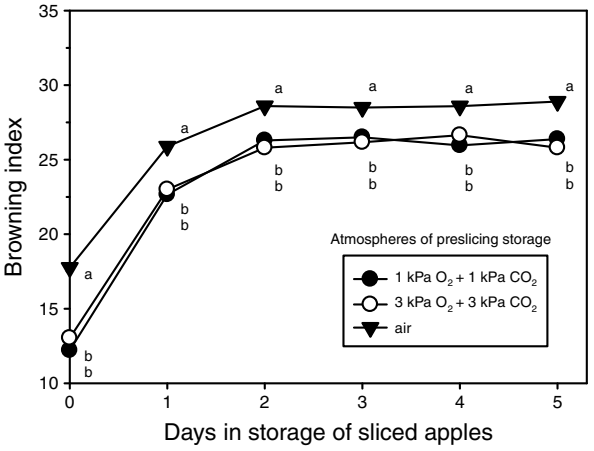

Fig. 6. Change in browning index of sliced 'Tsugaru' apples as affected by atmospheres of pre-slicing storage. Whole apples were stored at 0 °C for 5 months, sliced, and the slices were kept at 20 °C for 5 days. Means ($n = 3$) with different letters are significantly different at the 5% level.

3.6. Changes in browning index

Changes in browning index of the slices processed from whole apples stored for 5 months under 1 kPa O_2 + 1 kPa CO_2, 3 kPa O_2 + 3 kPa CO_2, and air are shown in Fig. 6. Initial browning index was significantly higher in slices from apples stored under air than under two CA conditions. Browning index increased up to 2 days of storage, and did not change thereafter. Initial differences in the browning index between the slices from air and the slices from CA were maintained for 5 days of storage. These results show that CA containing 1 to 3 kPa O_2 and CO_2 inhibited the development of flesh browning in whole apples stored for long-term. However, the atmospheres of pre-slicing storage used in this study did not affect the browning of sliced apples. Coseteng and Lee (1987) reported that 'Classic Delicious', 'Cortland', 'McIntosh' and 'RI Greening' apples showed a good correlation between degree of browning and enzyme activity, while 'Empire', 'Golden Delicious' and 'Rome' apples displayed a good correlation between degree of browning and total phenolic content. Rocha and Morais (2002) indicated that browning of 'Jonagored' apple slices during storage is moderately correlated with PPO activity, but no correlation was found between the browning index and the total phenolic content. From the results of this study, browning in 'Tsugaru' apples was found to be correlated with the level of electrolyte leakage and the contents of phenolic compounds.

4. Conclusion

The initial respiration rate, ethylene production, electrolyte leakage, vitamin C, phenolic compounds, and browning index of the slices processed from whole apples stored in normal air were higher in comparison with the slices from CA stored apples, and these differences persisted during storage of the slices. However, the trend in the changes of the above properties during storage of the apple slices was not affected by the atmosphere of pre-slicing storage. CA was found to be a suitable method of storage for fresh-cut produces of 'Tsugaru' apples. Therefore, this study shows that the pre-slicing storage conditions should be considered an important factor for optimising fresh-cut procedures. Further research is required in order to obtain information about commercial fresh-cut processing in relation to pre-slicing storage.

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