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Full Length Research Paper

Physicochemical changes in fresh-cut Honeydew melon fruit during storage

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The purpose of this study was to investigate certain physicochemical changes in fresh-cut Honeydew melon fruit during storage. Flesh colour, soluble solid content, firmness and cell wall hydrolases, such as pectinesterase, polygalacturonase, β-galactosidase and galactanase activities were determined. Flesh colour and soluble solid content remained constant whilst firmness decreased significantly throughout storage. The loss of firmness was associated with the increases in polygalacturonase and galactanase activities. A high β-galactosidase activity was detected; however, a significant change in the enzyme activity was not found. Pectinesterase activity declined throughout storage. These results indicated that firmness is a key factor affecting quality of minimally processed Honeydew melon fruit and associates with the increase in polygalacturonase and galactanase activities.

Key words: Minimally processed, Honeydew melon, physicochemical changes, cell wall hydrolases.

INTRODUCTION

Recently, consumers have been more concerned about their health problem from food consumption (Watada and Qi, 1999). Consumers demand convenient and fresh-like food which makes the minimally processed fruit and vegetable product have a role in the world food market. However, minimally processed fresh fruit and vegetables are more perishable than intact fruit (Huxsoll and Bolin, 1989; King and Bolin, 1989). Changes of texture, flavour and appearance are the limiting factors of minimally processed products quality which have been reported in melon fruits (Portela and Cantwell, 1998; Lamikanra et al., 2003; Ergun et al., 2007).

Melon is the 4th important fruit in the world fresh fruit market (Aguayo et al., 2004). Melon is a kind of fruit having a big market share in minimally processed products. In United States, the most common melon used for minimal process is cantaloupe (Aguayo et al., 2004). The quality of minimally processed cantaloupe changes rapidly during storage (Lamikanra et al., 2000). Changes in texture and flavour are the main factors limiting the quality of minimally processed cantaloupe melon (Lamikanra et al., 2003; Aguayo et al., 2004). However, Portela and Cantwell (2001) had reported that there were no changes of aroma, off-odor and total sugar in minimally processed cantaloupe melon but the texture significantly declined during storage. Furthermore, the rapid decrease in texture during storage was also reported in other minimally processed melon fruits such as Honeydew (Portela and Cantwell, 1998), Amarillo (Aguayo et al., 2003) and Galia (Ergun et al., 2007). Ranwala et al. (1992) suggested that the softening of intact muskmelon fruit during ripening resulted from the degradation of pectin polymers by the action of –galactosidase. Furthermore, the presence of β-galactosidase/galactanase activities have also been

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reported in intact melon fruits during ripening (Rose et al., 1998). The objective was to examine physicochemical changes and cell wall hydrolases in minimally processed Honeydew melon fruits during storage at 4±1°C.

MATERIALS AND METHODS

Plant material

Honeydew melon fruits (*Cucumis melo* var. *inodorous*) were obtained from a local food processor, Orchard House Foods, Corby. The fruits were screened for uniformity, such as being free from any mechanical damage and diseases and the same stage of maturity, and were washed with tap water containing 200 µll^{-1} sodium hypochlorite solution, and then air dried.

Sample preparation

All materials, knife and cutting board were washed with tap water and disinfected using 70% ethanol and then air dried. The fruits were cut into half with a sharp knife and each half was cut at the exposed end into 4 equal pieces. The seeds, cavity tissues and peel were then removed. The fruits were cut into cubes approximately 1.5×1.5×1.5 cm^3 size. The cubes were kept in plastic containers wrapped with punctured wrapping film, (10 holes were made using a needle) and then kept at 4±1°C for 5 days. Physicochemical quality attributes, namely flesh colour, firmness, soluble solid content (SSC) and cell wall hydrolases such as pectinesterase, polygalacturonase, galactosidase and galactanase were determined.

Flesh colour

Colour of melon flesh was measured using a Minolta CR-200 reflectance colorimeter (Japan). The chroma meter was calibrated against a white tile. The melon flesh were measured for L* (lightness), a*[green (-) to red (+)], b* [blue (-) to yellow (+)].

Total soluble solid

Juice extracted from 5 cubes of the minimally processed cantaloupe from each experiment was used to assay soluble solid content and was evaluated using a hand refractometer (Bellingkam and Stanley Ltd, UK).

Firmness

The firmness of minimally processed cantaloupe was determined using a TA-XT II texture analyzer, Stable Microsystem England, equipped with P4 probe (2 mm diameter). The probe was driven at a crosshead speed of 5.0 mm s^{-1} to a depth of 5 mm. The maximum force-exerted (expressed as g-force) was used for firmness data.

Electrolyte leakage

Electrolyte leakage determination was modified from the method described by Ergun et al. (2005). Flesh melon cylinders (9 mm in diameter) were taken from equatorial part of a fruit using a cork-borer. The cylinders were cut to produce 5 mm thick discs. Six discs per replication were rinsed with de-ionized water and dried using Whatman filter paper no. 1. The discs were put into 50 ml beaker and then 30 ml of 500 mM mannitol solution was added. The conductivity of the solution was immediately measured using a conductivity meter (EC/TDS waterproof Hanna Instruments, Mauritius). The discs were incubated in the mannitol solution for 5 h and then the conductivity was again measured. The discs and bathing solution were then stored in a freezer (-20°C) for 24 h, thawed, and then boiled for 15 min. After the solution had dropped to room temperature, the total conductivity of the solution was recorded. The result was expressed as a percentage of electrolyte leakage.

Determination of cell wall hydrolases

Acetone insoluble solid (AIS) preparation and protein extraction

Twenty grams of flesh tissue was homogenised using a Polytron Homogenizer in 80 ml of 100% cold acetone and then filtered. The precipitate was washed with 80% cold acetone and then rinsed with 100% cold acetone. The precipitate was collected and dried. Proteins from AIS were extracted following the procedure described by Pressey (1983), using sodium acetate buffer (pH 6). Twenty milliliters of extraction buffer were added to AIS and the pH adjusted to 6. The sample was stirred continuously at 4°C for 3 h. After that, the sample was centrifuged at 1000 x *g* for 15 min. The supernatant was collected. Ammonium sulphate was added at 80% of saturation. It was then incubated at 4°C for at least 1 h. The precipitated proteins were collected by centrifugation at 18,000 g for 25 min at 4°C. The pellet was re-suspended in a small amount of acetate buffer (pH 6) and then dialyzed in the buffer at 4°C overnight.

Determination of protein content

Protein concentrations were determined using the method described by Bradford (1976). A 20-800 ∝l sample was mixed with Bio Rad reagent. The absorbance was measured at 630 nm using a plate reader (Bio-Rad model 550). Standards were included in each assay, using increasing amounts of bovine serum albumin (BSA 1 mg/ml, Pierce, UK).

Pectinesterase

PE activity was determined using the method described by Tucker et al. (1982). The reaction mixture contained 10 ml of substrate solution (pH 9) and 100 ∝l of the crude protein extract. The reaction was started when the pH of the mixture reached 8. The volume of 5 mM NaOH used in the titration was recorded every 30 s for 5 min. The PME activity was calculated as microequivalents per mg protein (µeqH$^+$ mg protein^{-1}).

Polygalacturonase

PG activity was measured as described by Gross (1982) by the release of reducing sugar using 2-cyanoacetamide. A 100 µL of the extract was incubated in 100 L of substrate solution (2.0%

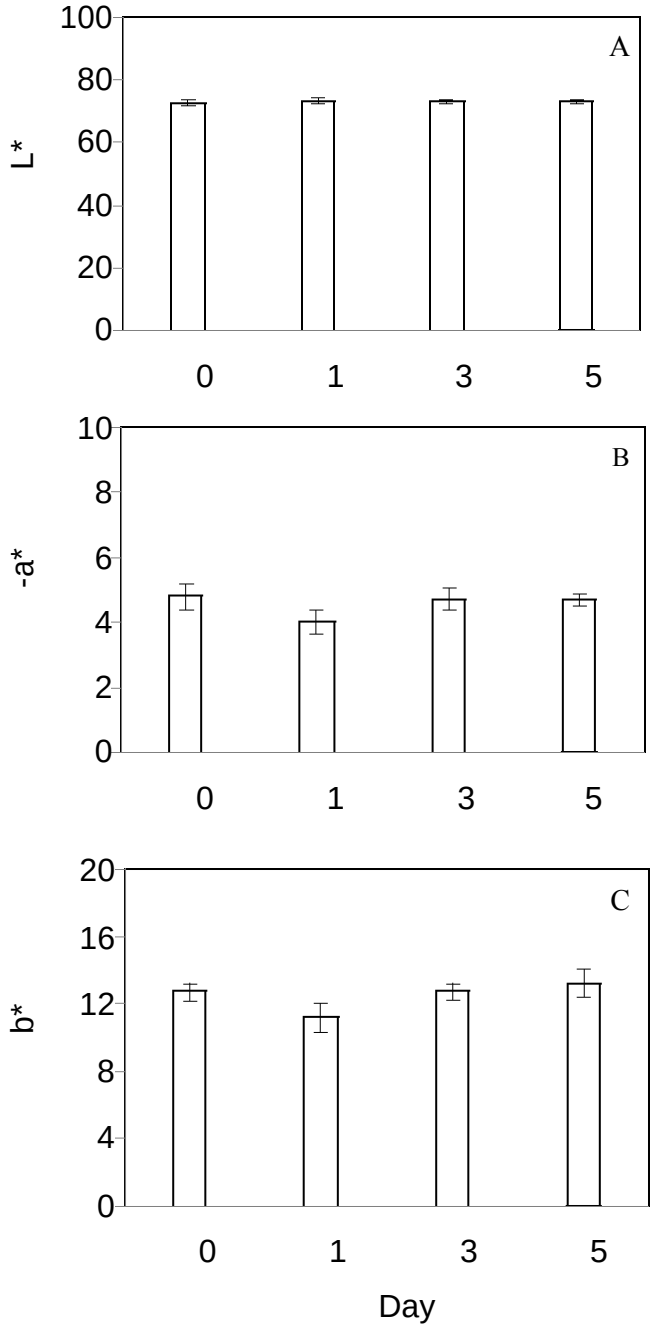

Figure 1. Flesh colour of fresh-cut Honeydew melon stored at 4 ± 1°C for 5 days. Vertical bars present the standard error of means (n = 3). A) Lightness value (L*), B) red to green (a*value) and C) yellow to blue (b*value).

polygalacturonic acid in sodium acetate buffer of pH 4) at 37°C for 20 h. The reaction was stopped by adding 1 ml of 100 mM borate buffer of pH 9.2 and then 200 L of 1% (w/v) 2-cyanoacetamide followed by incubation at 100°C for 10 min. The liberated reducing sugar from the reaction was measured using a UV/visible spectrophotometer at 276 nm. The concentration of galacturonic acid was calculated using a standard curve and the data expressed as nmole mg protein^{-1}.

β-galactosidase

β-Gal activity was assayed as described by Chung et al. (2006) by following the release of ρ-nitrophenol from substrate (ρ-nitrophenyl D-galactopyranoside). The reaction was started when 5 ∝l of the extract mixed with 130 ∝l of the substrate solution and was allowed to proceed at 37°C for 5 min. The reaction was stopped by adding 100 ∝l of 0.4 M Na_2CO_3. The concentration of liberated ρ-nitrophenol was measured using the absorbance at 415 nm. The β-Gal activity was calculated as mmole mg protein^{-1} at 37°C by using a standard curve of ρ-nitrophenol.

Galactanase

Galactanase activity was assayed by following the release of mono-galactose from a galactan substrate. The assay was started by the adding 200 µl of substrate solution (1% galactan) with 400 µl of 0.1 M sodium acetate buffer of pH 4, and 400 µl of extract. The mixture was incubated at 37°C for 1 h after which the reaction was terminated by boiling for 10 min, followed by cooling at room temperature and centrifugation at 13000 g for 3 min. The liberated galactose was measured using a Lactose/D-galactose UV-test KIT (R-Biopharmrhone Ltd, Scotland). The galactanase activity was expressed as D-galactose mg protein^{-1}.

Statistic analysis

The data are present as the mean of triplication and standard deviation bar. Statistic analysis was carried out using a one-way analysis of variance performed in SPSS. The treatment means were compared using a Post Hoc least significant difference test at a significance level p≤0.05.

RESULTS AND DISCUSSION

Flesh colour

During storage for 5 days, no significant differences in L*, a* and b*values fresh-cut Honeydew melon were not found. L*, a* and b*values during storage were about 73.9, -4.6 and 12.5, consequently (Figure 1). It shows that flesh colour did not the key factor limiting quality of fresh-cut Honeydew melon fruit during storage. Portela and Cantwell (2001) and Ergun et al. (2007) suggested that a decreases in L* and chroma values of minimally processed muskmelon fruit was related to the development of translucent or water-soaking symptom. However, the result in this study shows no development of translucent and water-soaking symptom on the fresh-cut Honeydew melon fruit over storage, similar to the results reported by Gil et al. (2006) and Machado et al. (2008).

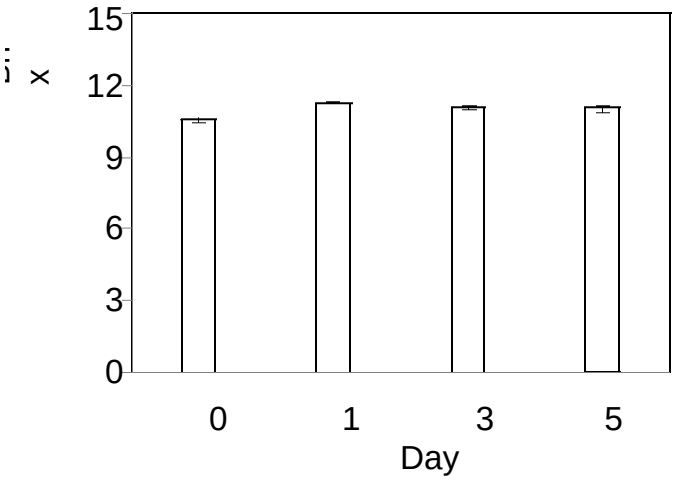

Figure 2. Soluble solid content (°Brix) of fresh-cut Honeydew melon stored at 4±1°C for 5 days. Vertical bars present the standard error of means (n = 3).

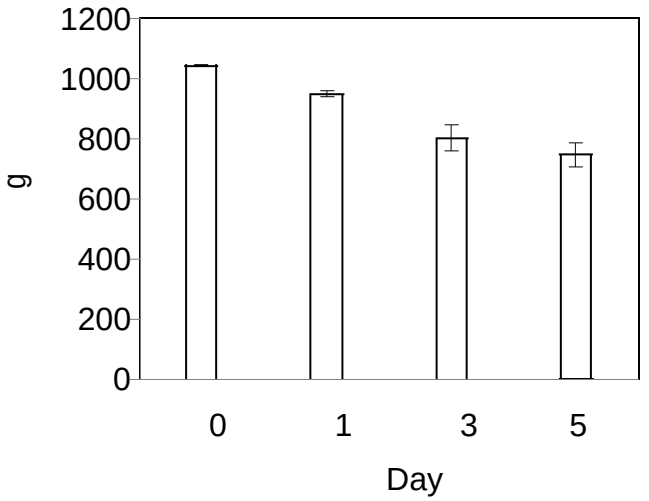

Figure 3. Firmness of fresh-cut Honeydew melon stored at 4±1°C for 5 days. Vertical bars present the standard error of means (n = 3).

Soluble solid content

Soluble solid content of fresh-cut Honeydew melon fruit remained constant and was about 10.9 °Brix over storage (Figure 2). In melon fruit, soluble solid content is a main factor used to determine the quality in commercial. The minimum requirement in TSS of melon fruit should be higher than or equal 10 °Brix (United Nation Economic Commission for Europe, 2006). The results in this study show that there were no significant changes in soluble

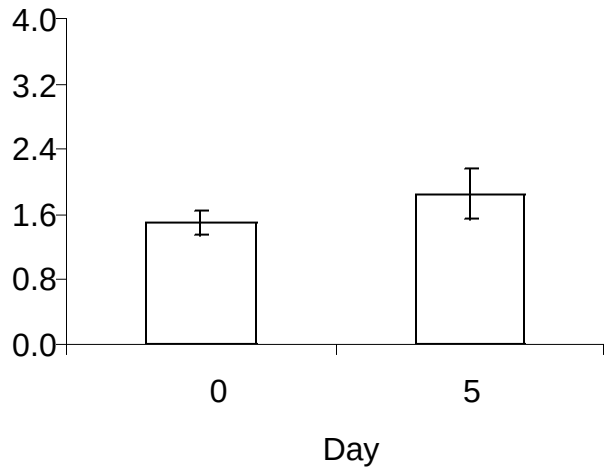

Figure 4. Electrolyte leakage (%) of fresh-cut Honeydew melon stored at 4±1°C for 5 days. Vertical bars present the standard error of means (n = 3).

solid over storage. Similarly, no changes in soluble solid content have been reported for minimally processed Honeydew melon (Portela and Cantwell, 1998) and Cantaloupe melon (Portela and Cantwell, 2001; Gil et al., 2006).

Firmness and electrolyte leakage

Firmness of fresh-cut Honeydew melon fruit decreased significantly throughout storage ($p \leq 0.05$). Firmness reduced from 1043 g on day 0 to 747 g on day 5 (Figure 3). This shows that the reduction of firmness was an important factor affecting quality of fresh-cut Honeydew melon fruit during storage. The fresh-cut melon at day 0 and day 5 was selected to measure tissue electrolyte leakage (Figure 4). The electrolyte of the fresh-cut fruit increased slightly from 1.53% at day 0 to 1.82% at day 5 and there was no significant difference.

Softening is universally known as a predominant cause limiting the transportation and shelf-life of fruit. Several previous works of minimally processed melon fruit have reported that the loss of firmness is one of the main factor limiting the quality and shelf-life of cantaloupe melon (Lamikanra et al., 2003; Aguayo et al., 2004), Honeydew melon (Portela and Cantwell, 1998), Amarillo melon (Aguayo et al., 2003) and Galia melon (Ergun et al., 2007). The results in this study show a continuous decrease in firmness of the fresh-cut Honeydew melon which was similar to the previous report. Ergun et al. (2007) reported that the loss of firmness in fresh-cut Galia melon associated with an increase in electrolyte leakage of the tissue. However, a slight increase in

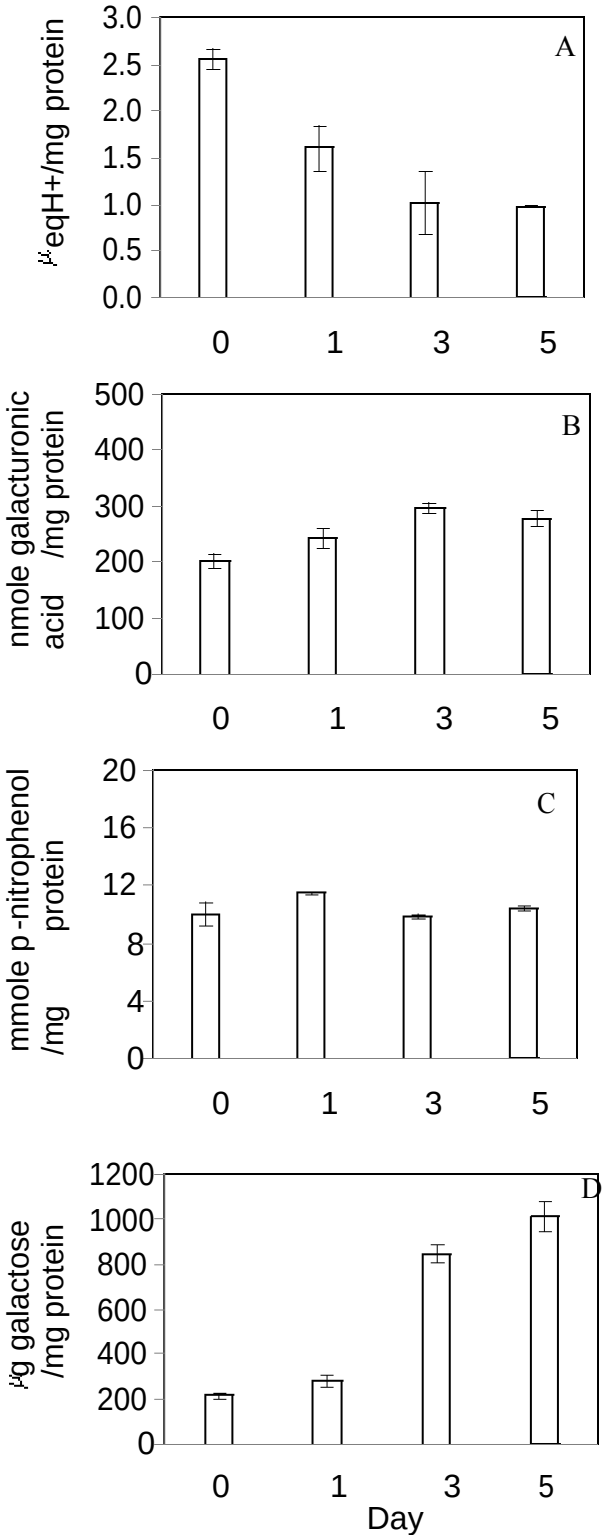

Figure 5. Cell wall hydrolases of fresh-cut Honeydew melon stored at 4±2°C for 5 days. Vertical bars present the standard error of means (n = 3). A) pectinesterase, B) polygalacturonase, C) β- galactosidase, and D) galactanase.

electrolyte leakage was shown in this study. This could suggest that the increase in electrolyte leakage might be associated with the loss of firmness but it is not the key factor affecting the softening of the fresh-cut Honeydew melon fruit.

Cell wall hydrolase activities

It is widely known that softening of fruit is associated with cell wall hydrolase activities. Figure 5 shows cell wall hydrolase activities in fresh-cut Honeydew melon fruit during storage. Pectinesterase activity decreased continuously throughout storage. A small amount of polygalacturonase activity was detected and it increased over storage. A high amount of β-galactosidase activity was found in fresh-cut Honeydew melon fruit; however, it slightly changed during storage. β-galactanase activity increased markedly from days 1 to 3 and 5, respectively.

The reduction of fruit firmness is widely recognized that it is accompanied by the action of cell wall hydrolases (Brummell, 2006). During ripening, the extensive depolymerisation of cell wall substances have been attributed to the action of cell wall modifying enzymes, such as polygalacturonase (EC 3.2.1.15), pectinesterase (EC 3.1.1.11), β-galactosidase EC 3.2.1.23) (Lester and Dunlap, 1985; Tucker, 1993) and galactanase (EC 3.2.1.89) (Lazan et al., 2004). The results show that all of those cell wall modifying enzymes were detected in fresh-cut Honeydew melon fruit during storage. Increase in polygalacturonase and galactanase activities might associate with the reduction of firmness. Lester and Dunlap (1985) reported that polygalacturonase activity was not detected in muskmelon fruit during ripening. In this study, a small amount of polygalacturonase was found and it also increased over storage. Hadfield et al. (1998) suggested that even though PG activity in muskmelon fruit was small it was enough to degrade pectic polymers at the later stage of ripening. The role of pectinesterase activity in melon fruit softening is still unclear because it decreased over storage. Similarly, Supapvanich (2009) reported that pectinesterase activity of intact and fresh-cut Cantaloupe melon fruits decreased during storage. A high level of β-galactosidase activity in minimally processed Honeydew melon fruit during storage was detected in this study. It might have some effect on the fruit softening. In muskmelon fruit, β-galactosidase was reported as an important enzyme regulating the fruit softening (Ranwala et al., 1992). It is generally accepted that β-galactosidase has an ability to catalyse (1→4)-β -D-galactosidic cross-linkage in the side chains of pectic polymers, resulting in breakdown of the large polymers (Ranwala et al., 1992; Ross et al., 1993; Carey et al., 1995). It is accepted that galactanase is an enzyme in galactosidase group having an ability to

remove the cell wall galactose during fruit ripening (Carey et al., 1995). The data in this study show a marked increase in galactanase activity of muskmelon fruit during storage. Supapvanich (2009) reported that a high level of galactanase activity was found in β-galactosidase isoform II which related to the loss of firmness in muskmelon fruit. Similarly, Ali, et al. (1995) has discovered galactosidase of mango fruit having galactanase activity and the increase in this enzyme was parallel with increase in the fruit softening.

Conclusions

Flesh colour and soluble solid content were not the main factors limiting the quality of minimally processed Honeydew melon fruit. The marked reduction of firmness during storage was an important factor on the fruit. An increase in electrolyte leakage might relate to the loss of firmness but it is not the main factor of the fresh-cut fruit softening. The marked increases in polygalaturonase and galactanase activities were parallel with the loss of firmness. A high level of galactosidase was found. These suggest that increase in cell wall hydrolases, especially polygalacturonase, β-galactosidase and galactanase played a key role in the softening of fresh-cut Honeydew melon fruit during storage.

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