International Food Research Journal 26(2): 547-556 (April 2019)

Journal homepage: *http://www.ifrj.upm.edu.my*

Enzymatic activity of alginate coated and pulsed light treated fresh-cut cantaloupes (*Cucumis melo* L. var. *reticulatus* cv. *Glamour*) during chilled storage

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Article history

Received: 6 May, 2018
Received in revised form: 2 September, 2018
Accepted: 11 November, 2018

Keywords

Repetitive pulsed light
Alginate coating
Fresh-cut cantaloupes
Phenolic enzyme
Textural enzyme

Abstract

The objective of the present work was to investigate the effects of alginate coating combined with repetitive pulsed light (RPL) treatment on the enzymatic activity of fresh-cut cantaloupes during chilled storage. Fresh-cut cantaloupes were coated with alginate (1.86%, w/v) followed by RPL treatment (0.9 J/cm^2 at every 48 h up to 26 d) during storage. Untreated samples and samples with alginate or RPL alone were used as controls. Enzymatic activities [polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), peroxidase (POD), pectinesterase (PE), polygalacturonase (PG), pectate lyase (PEL), β-galactosidase (β-Gal), α-arabinofuranosidase (α-AF) and cellulase] of fresh-cut cantaloupes were evaluated every 4 d during storage. Neither alginate coating nor RPL treatment has any effect on the activities of PE and PEL of fresh-cut cantaloupes during storage. The individual treatment or in combination did not increase the PAL, PPO, PG and cellulase activities of fresh-cut cantaloupes throughout the storage. Alginate coating with or without RPL was effective in maintaining low POD activity of fresh-cut cantaloupes. In addition, treatments using alginate coating or alginate coating+RPL were effective in reducing the β-Gal and α-AF activities of fresh-cut cantaloupes during storage. Overall, alginate without or with RPL was effective in reducing changes of enzymatic activities in fresh-cut cantaloupes during storage.

Introduction

Fresh-cut fruit industry has received great interest as it produces fresh-cut fruits that are fresh, convenient and nutritious (Fan *et al*., 2009). However, fresh-cut fruits have a shorter shelf life as compared to whole fruits because the rate of physiological and biochemical changes are enhanced thereby resulting in high respiration rate and quality deterioration (González-Aguilar *et al*., 2004). Cantaloupes (*Cucumis melo* L. var. *Reticulatus* cv. *Glamour*) are popular mainly due to its sweet pulp and pleasant aroma (Villanueva *et al*., 2004). Firmness and colour are among the important factors that determine consumers' acceptance of fresh-cut cantaloupes (Martiñon *et al*., 2014). Cantaloupes are low in energy and are a rich source of nutrients particularly provitamin A (carotenoids) and vitamin C (Laur and Tian, 2011). From 2011 to 2015, the production of cantaloupes in Malaysia has increased from 310 to 5,117 MT according to Malaysian Department of Agriculture, which indicates the increase in its demand. The common quality losses in fresh-cut cantaloupes are juice leakage, softening and translucency (Cantwell and Suslow, 2004).

The loss of fruit quality is due to the stimulation of enzymatic activities as a result of tissue disruption, which leads to mixing of enzymes and substrates that are sequestered within vacuoles (Lamikanra and Watson, 2001). The activities of peroxidase (POD) and polyphenol oxidase (PPO) enzymes mainly affect the flavour and colour of fruits and vegetables (Lamikanra and Watson, 2001). Phenylalanine ammonia lyase (PAL) catalyses the determining step in the biosynthesis of phenylpropanoid-derived secondary products such as phenolic compounds,

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which are substrates for PPO and POD (Sun *et al*., 2009). Lamikanra and Watson (2000) suggested that POD activity in cut cantaloupe could be due to the tissue response to enhanced oxidative stress. The POD may cause browning by oxidising hydrogen donors in the presence of H_2O_2 (Richard-Forget and Guillard, 1997). Textural changes of a fruit can be caused by cell wall degrading enzymes and reduction in turgor as a result of water loss (Beaulieu and Gorny, 2002). The postharvest solubilisation and depolymerisation of pectins and hemicelluloses are mainly caused by enzymatic activities, which result in cell wall loosening and degradation (Ergun *et al*., 2011). Among the enzymes that involve in the hydrolysis of cell wall polymers are pectinesterase (PE), polygalacturonase (PG), pectate lyase (PEL), β-galactosidase (β-Gal), α-arabinofuranosidase (α-AF) and cellulase (Deng *et al*., 2005).

Pulsed light (PL) treatment is a decontamination technique used to rapidly inactivate microorganisms on food surfaces by utilising short time pulses of an intense broad spectrum (Gómez *et al*., 2012). Other than microbial inactivation, PL could also inactivate enzymes related to quality loss of fruits. Enzymes are one of the main targets for photoinduced modifications as their structures are made up of high quantity of endogenous chromophores (Davies and Truscott, 2011). The effect of PL for PPO inactivation has been demonstrated by Manzocco *et al*. (2013). The inactivation of textural enzymes by using UV irradiation has been reported by Manzocco *et al*. (2009) and Barka *et al*. (2000) on fresh-cut apple and tomato, respectively. However, radiation involving UV also produces reactive oxygen species (ROS) such as singlet oxygen, H_2O_2 and hydroxyl radicals that can affect cell membranes and cell walls (Turtoi, 2013).

Edible coating is another technique used to preserve fresh-cut fruits. The main functions of edible coating are to reduce moisture loss and maintain the texture of fresh-cut fruits. Polysaccharide-based coatings serve as a sacrificial moisture barrier to minimise moisture loss from the fruits to the atmosphere (Dhall, 2013). Furthermore, $CaCl_2$ that is used as the crosslinking agent for the polysaccharide coatings can stabilise membrane system and form Ca-pectates to enhance the rigidity of middle lamella and cell wall (Luna-Guzmán *et al*., 1999). As a result, the accessibility of enzymes to cell walls will be reduced leading to a reduction of fluid loss and increase of firmness.

Koh *et al*. (2017) demonstrated that alginate coating combined with repetitive pulse light (RPL) could improve the quality of fresh-cut cantaloupes by maintaining colour, enhancing firmness and reducing fluid loss, which are the important parameters defining the quality of cantaloupes. However, there is a lack of information on the maintenance of colour and improvement of firmness and fluid loss in fresh-cut cantaloupes by alginate and RPL. Firmness and fluid loss of fruits are interrelated and affected by enzymatic activities that lead to the modification of cellular structure. The evaluation of the enzymatic activity could provide detailed information on the effects of alginate coating and RPL on the changes of colour, firmness and fluid loss in fresh-cut cantaloupes during storage. Therefore, the objective of the present work was to investigate the effects of optimised alginate coating combined with RPL treatment on the enzymatic activity of fresh-cut cantaloupes during storage at 4 ± 1°C.

Materials and methods

Experimental design

The effects of optimised alginate coating in combination with RPL on the enzymatic activity of fresh-cut cantaloupes were investigated. The treatments were divided into four groups: untreated, RPL, alginate coating and alginate coating with RPL. During 36 d storage, the enzymatic activities (PPO, PAL, POD, PE, PG, PEL, β-Gal, α-AF, and cellulase) of fresh-cut cantaloupes were determined at 4-d intervals. Results were then compared with a set of untreated samples and samples treated with either alginate coating or RPL (controls).

Coating solution preparation

The coating formulation used was based on the optimisation carried out previously for combination with RPL treatment on shelf life extension of fresh-cut cantaloupes. Alginate coating (1.86%, w/v) was prepared by dissolving the alginate (1.86%, w/v) in distilled water at 70°C while stirring until the polysaccharide fully dissolved. Glycerol (1.47%, w/v) and sunflower oil (0.025%, w/v) were added to the alginate solution. The mixture was then made up to a final volume of 1 L using distilled water. The coating solutions were homogenised using Diax 900 homogeniser (Heidolph Instruments, Schwabach, Germany) at 24,000 rpm for 5 min and degassed under vacuum.

Sample preparation

Fresh cantaloupes at commercial maturity were harvested from a commercial farm in Klang, Malaysia, and were stored at 4 ± 1°C for 24-48 h. The cantaloupes and cutting utensils were first

washed with running potable water. The fruits were then halved using a sharp knife and the seeds were removed. The fruits were scooped into spheres with a diameter of 3 cm using a melon baller (Koh *et al*., 2016a). The spherical cantaloupes were coated by dipping the samples in the coating solution for 2 min. The excess coating solution was dripped for 1 min, and then the cantaloupes were dipped in 2% (w/v) $CaCl_2$ solution for 2 min to allow gelling of the coatings via cross-linking between calcium ions and alginate coating. The coated cantaloupes were placed on the grid shelf and air-dried for 30 min at ambient temperature (26 ± 1°C). Approximately 150 g coated cantaloupes were packed into a polypropylene bag (dimension of 12.5 cm × 18 cm, thickness of ~40 µm) and the bag was sealed. The O_2 and CO_2 permeability of the bag is 100 cc/m².d.atm and 190 cc/m².d.atm, respectively. One set of cantaloupes with coatings only was prepared. The coated cantaloupes were treated with RPL and were kept at chilled condition prior to analysis. Cut cantaloupes with RPL or alginate alone were used as controls.

Apparatus

Pulsed light treatment was carried out using the Steribeam SBS XeMatic-2L-A system (SteriBeam Systems GmbH, Baden-Württemberg, Germany). The machine composed of two xenon lamps; located above and below the sample shelf. The distance between the lamp and the sample shelf was 10 cm. The emitted spectrum's wavelengths ranged from 180 nm to 1100 nm. The total fluence per pulse emitted was 0.3 J/cm².

Pulsed light treatment

The packaged and coated cantaloupes were exposed to RPL treatment as described by Koh *et al*. (2016b). A fluence of 0.9 J/cm² was applied at 48-h intervals during 26 d storage. The total number of treatments was 13 and the total cumulative fluence for RPL treatment was 11.7 J/cm². The RPL treatment was stopped on day 26 even though the storage period was 36 days as the maximum cumulative dose allowed for PL treatment of food is 12 J/cm² according to the FDA. The coated cantaloupes were kept at 4 ± 1°C in a cooler box during the transfer of the samples from the chiller to the treatment chamber.

Storage study

Samples treated with optimised alginate coating and RPL were stored at 4 ± 1°C for 36 d. The storage period was 36 d as the shelf life of the coated fresh-cut cantaloupes treated with RPL could be extended up to 36 d based on microbiological quality. The enzymatic activities were determined at 4-d intervals during the storage. Results were compared with a set of untreated samples and samples treated with either alginate coating or RPL (controls).

Methods of analysis

Phenolic enzyme extraction and assays

Polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL) were extracted according to Sun *et al*. (2009) with slight modifications. All steps were performed at 4°C. Blended cantaloupes (5 g) were extracted with 10 mL 0.1 M sodium phosphate buffer (pH 6.8) containing 0.1% (w/v) PVP and 0.1% (v/v) Triton X-100 for 20 min. The mixture was centrifuged for 15 min at 5,000 g and the supernatant was used for PPO and POD assays. The enzyme solution (0.2 mL) was added to 2.8 mL 10 mM catechol in 0.01 M sodium phosphate buffer (pH 6.8). The absorbance was read after 3 min at 25°C using Thermo Scientific G10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Wisconsin, USA). One unit of enzymatic activity was defined as the amount of the enzyme that causes a change of 0.001 at 400 nm per min.

For POD activity determination, 0.1 mL enzyme solution was added to the mixture containing 2.7 mL 0.1 M sodium phosphate buffer (pH 6.8), 0.1 mL 0.46% (v/v) H_2O_2 and 0.1 mL 4% (w/v) guaiacol. The absorbance was recorded at 470 nm after 3 min at 25°C using Thermo Scientific G10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Wisconsin, USA). One unit of enzymatic activity was defined as the amount of the enzyme that causes a change of 0.01 at 470 nm per min.

For PAL activity determination, blended cantaloupes (5 g) were extracted with 5 mL 0.05 M sodium borate buffer (pH 8.8) containing 5 mM mercaptoethanol and 0.1 g PVP for 20 min. The mixture was centrifuged at 5,000 g for 15 min. The supernatant was used for the PAL assay. Enzyme solution (0.1 mL) was added into the mixture containing 1.9 mL 0.05 M sodium borate buffer (pH 8.8) and 1 mL 20 mM L-phenylalanine. The mixture was incubated for 1 h at 37°C. For the control sample, 1 mL 0.05 M sodium borate (pH 8.8) was used to replace the enzyme extract. Following incubation, the absorbance was read at 290 nm using Thermo Scientific G10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Wisconsin, USA). One unit of enzymatic activity was defined as the amount of the enzyme that causes a change of 0.01 at 290 nm per hour.

Textural enzyme extraction and assays

For pectinesterase (PE), β-galactosidase (β-Gal), polygalacturonase (PG), pectate lyase (PEL) and cellulase, the extractions of the enzymes were carried out as described by Deng *et al*. (2005) with slight modifications. All the steps were performed at 4°C. Blended cantaloupes (10 g) were homogenised for 30 min in 10 mL 0.5 M Tris-HCl (pH 8.0) added with 1 mM CDTA, 5% insoluble PVP (w/v) and 2 M NaCl for PE and β-Gal, and in 10 mL 0.1 M phosphate buffer (pH 7.0) containing 1 mM CDTA, 5% insoluble PVP (w/v) and 0.5 M NaCl for PG, PEL and cellulase. The mixture was centrifuged at 5,000 g for 15 min. The supernatant was used for enzyme assays.

For PE assay, 5 mL 1% (w/v) citrus pectin solution added with 0.6% (w/v) NaCl solution (pH 4.5) was used as a substrate and the pH was set at 7.0 by using 0.02 M NaOH prior to the introduction of 1 mL crude enzyme. During the reaction, the reaction mixture was maintained at 35°C and pH 7.0 by using 0.01 M NaOH with continuous stirring. The volume of 0.01 M NaOH added in 10 min was recorded. One unit of PE activity was defined as 1 μmol ester hydrolysed per min per g of fresh weight of cantaloupes.

PG was assayed by using reaction mixture consisted of 0.5 mL sodium acetate (0.2 M, pH 4.5), 0.4 mL 1% (w/v) citrus pectin solution with added 0.6% (w/v) NaCl solution (pH 4.5) and 0.1 mL mixture of crude enzyme and distilled water. Following incubation at 37°C for 1 h, DNS was added into the mixture. The reaction was terminated by heating in a boiling water bath for 5 min. The blank was prepared by heating in the boiling water bath before adding the substrate. Following heating, the reaction mixture was measured at 540 nm using Thermo Scientific G10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Wisconsin, USA). Quantification of the reducing groups was performed by using D-galacturonic acid as the standard. One unit of PG activity was defined as the amount of enzyme that catalyses the production of 1 μmol of reducing groups per min per g of fresh weight of cantaloupes.

PEL activity was determined by adding diluted crude enzyme using distilled water into a mixture containing 0.5 mL 0.36% (w/v) polygalacturonic acid in 0.05 M Tris-HCl buffer (pH 8.5) and 0.3 mL 4 mM $CaCl_2$ with the total reaction mixture of 3.0 mL. The mixture was incubated at 37°C for 30 min followed by heating in a boiling water bath for 2 min to stop the reaction. For blank, the substrate was added after incubation. The absorbance of the mixture was measured at 232 nm using Thermo Scientific G10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Wisconsin, USA). One unit of PEL activity was defined as the amount of enzyme that produces 1 μmol of 4,5-unsaturated product per min based on conditions of the assay.

For β-Gal assay, 0.08 mL crude enzyme was added to a mixture containing 0.4 mL 13 mM π-nitrophenyl-β-galactopyranoside, 0.4 mL 0.1% (w/v) BSA and 0.52 mL 0.1 M sodium citrate (pH 4.1), and incubated for 15 min at 37°C. Then, 2 mL 0.2 M sodium carbonate was added into the mixture to stop the reaction. The absorbance of the mixture was read at 415 nm using Thermo Scientific G10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Wisconsin, USA). One unit of β-Gal activity was defined as the amount of enzyme needed to produce 1 nmol π-nitrophenyl per min at 37°C.

Cellulase activity was assayed by using reaction mixture containing 0.1 mL mixture crude enzyme and distilled water, 0.4 mL 1% (w/v) carboxymethyl cellulose and 0.5 mL 0.1 M sodium acetate buffer (pH 5.0). DNS was added into the mixture after incubation for 1 h at 37°C. The reaction was stopped by heating the mixture in a boiling water bath for 5 min. For blank, the substrate was added after incubation. The absorbance of the mixture was read at 540 nm using Thermo Scientific G10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Wisconsin, USA). Quantification of the reducing groups was performed by using D-glucose as the standard. One unit of cellulase activity was the amount of enzyme that produces 1 μmol of reducing groups per min per g of fresh weight of cantaloupes.

For α-arabinofuranosidase (α-AF) activity, the extraction of the enzyme was performed according to the method described by Gwanpua *et al*. (2014) with slight modifications. Blended cantaloupes (10 g) were homogenised with 10 mL 20 mM borate buffer containing 2 M NaCl, 2% (v/v) Triton X-100, 3 mM $ZnCl_2$ and 2% (w/v) sucrose for 30 min. The mixture was centrifuged at 5,000 g for 15 min. The supernatant was used for the α-AF assay. The reaction mixture comprised of 0.4 mL crude enzyme, 0.5 mL 0.1 M citrate buffer (pH 4.0), 0.1 mL BSA solution and 0.4 mL 3.6 mM π-nitrophenyl-α-L-arabinofuranoside. The reaction mixture was incubated at 37°C for 15 min. After incubation, 2 mL 0.2 M sodium carbonate was added into the mixture to stop the reaction. The absorbance of the mixture was read at 415 nm using Thermo Scientific G10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Wisconsin, USA). One unit of α-AF activity was defined as the amount of enzyme that catalyses the release of 1 μmol π-nitrophenyl per min based on conditions of the assay.

Statistical analysis

The experiments were performed in triplicates. The data were analysed using Minitab version 16.0 statistical package (Minitab Inc., Pennsylvania, USA) based on analysis of variance, and expressed as mean value ± standard deviation. The confidence level for statistical significance was set at a probability value of 0.05. Tukey's test was used to determine the significant difference of the data.

Results and discussion

Phenolic enzymes

Figure 1(a) presents the effects of alginate coating and RPL treatment on PPO activity in fresh-cut cantaloupes. PPO is an enzyme that oxidises phenolic compounds thereby producing quinones, which is then converted to brown pigments known as melanins (Charles *et al*., 2013). Generally, the PPO activity of cantaloupes decreased on day 4 for all treatments as compared to the fresh samples (day 0). It is known that O_2 is required for PPO activity to occur (Iyengar and McEvily, 1992). Previously, Koh *et al*. (2017) found that the packages of fresh-cut cantaloupes produced low O_2 concentration, which could explain the reduction of PPO activity in the samples. Thereafter, the PPO activity remained constant throughout the storage for samples treated with either RPL or alginate and alginate+RPL without significant difference ($p > 0.05$). This finding indicates that alginate and RPL treatment did not influence the PPO activity of fresh-cut cantaloupes. These results could be due to the lack of PPO and/or oxidisable phenols in fresh-cut cantaloupe as reported by Boynton *et al*. (2005). For untreated samples, significant decreased ($p \leq 0.05$) in PPO activity on day 28 could possibly be due to high CO_2 concentration in the package. CO_2 could inhibit PPO activity due to conformational changes in the secondary and tertiary structure stimulated by the gas (Queiroz *et al*., 2008).

The effects of alginate coating and RPL treatment on PAL activity of fresh-cut cantaloupes are presented in Figure 1(b). In the response of cell acclimatisation against stress in plants, PAL activity increases leading to the production of phenolic compounds (Murugesan *et al*., 2012). Untreated samples showed stable PAL activity at the beginning of storage. On day 24, the PAL activity in untreated samples started to increase and appeared to be higher than the other treatments. This increase in PAL activity at the end of storage could be contributed by the abiotic stress caused by extremely high CO_2 concentration (~45-80%) in the package as determined in the present work. The

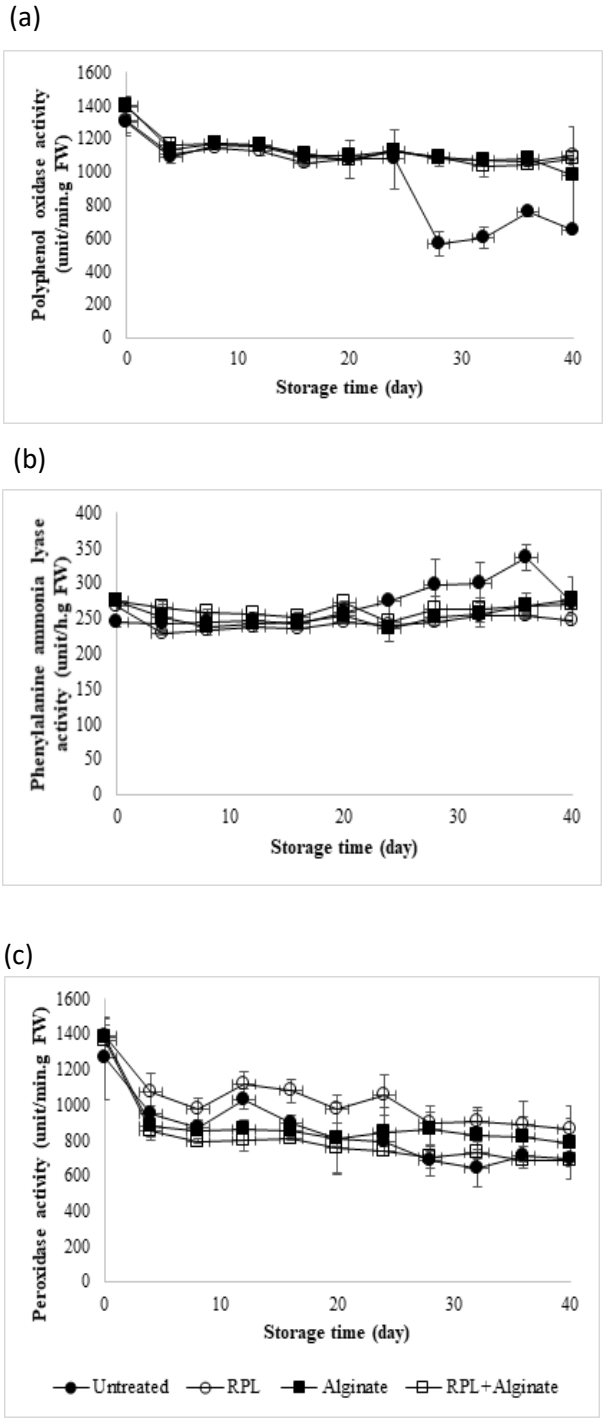

Figure 1. Effects of alginate coating and repetitive pulsed light (RPL) application on phenolic enzymes: (a) polyphenol oxidase, (b) phenylalanine ammonia lyase and (c) peroxidase of fresh-cut cantaloupes.

modification in package headspace atmosphere of fresh-cut cantaloupes during storage was reported to induce abiotic stress that resulted in an increase of PAL activity (Oms-Oliu *et al*., 2008). During storage, the PAL activity in samples treated with RPL or alginate coating alone was not significantly different ($p > 0.05$). For alginate coating+RPL treatment, the PAL activity in cantaloupes was higher as compared

to other treatments from the beginning of storage until day 20. This could be due to the synergistic effect of alginate coating and RPL treatment that generated abiotic stress on the samples. After day 24, the outpaced PAL activity in untreated samples as compared to the other treatments suggested that the abiotic stress caused by extremely high CO_2 concentration as mentioned earlier was greater than that resulted from alginate coating+RPL treatment. Generally, the PAL activity in fresh-cut cantaloupes did not change drastically regardless of the treatments.

Figure 1(c) shows the effects of alginate coating and RPL treatment on the POD activity of fresh-cut cantaloupes. Generally, stored fresh-cut cantaloupes had lower POD activity as compared to the fresh samples (day 0) regardless of treatments. This could be due to the low O_2 level developed in the fruit packages. It is known that O_2 is required for the catalytic activity of POD (Mousavizadeh and Sedaghathoor, 2011). Based on Figure 1(c), the POD activity in untreated samples gradually decreased during storage. As the storage time increased, the elevated CO_2 level (from ~30% to ~80%) in the packages was discovered and this could pose a negative effect on the POD activity in untreated samples. The CO_2 accumulation causes acidification of medium and disrupts enzyme protein structure (Duong and Balaban, 2014). On the other hand, samples treated with RPL alone had higher POD activity as compared to the other samples during storage. This could be due to oxidative stress contributed by RPL. It is known that exposure of plants to UV causes the production of ROS such as singlet oxygen, H_2O_2 and hydroxyl radicals (Turtoi, 2013). POD in RPL-treated cantaloupes could react with these species and yielded higher POD activity. When the alginate coating is combined with RPL, the POD activity in cantaloupes was lower and remained constant during the storage. These activities were not significantly different ($p > 0.05$) as compared to the samples with alginate coating alone. This could be contributed by a coating of the fruit that modified the surrounding atmosphere, i.e. low O_2 and moderate CO_2 levels in the packages and thus minimised the negative effect on the POD.

Generally, the insignificant changes of phenolic enzymatic activities found in the present work explained the retention of colour and total phenolic content of fresh-cut cantaloupes during storage. The maintenance of POD and PAL activities in samples with coating or RPL alone and alginate+RPL indicated the presence of consistent abiotic stress in these samples during storage as compared to untreated samples.

Textural enzymes

Figures 2(a)-(f) show the textural enzymatic activities in fresh-cut cantaloupes during storage as affected by alginate coating and RPL treatments. The mechanism of enzymatic textural changes involves de-esterification of pectin by PE action followed by pectin depolymerisation due to the activities of PG and PEL (Alandes *et al*., 2006). On the other hand, cellulase degrades cellulose and hemicellulose present in walls of dicotyledons (Deng *et al*., 2005). In the present work, the PE activity gradually increased throughout the storage in all fresh-cut cantaloupes regardless of the treatments (Figure 2(a)). It is common that PE activity increases during ripening of fruits leading to pectin degradation (Yaman and Bayoındurlı, 2002). However, the PE activity was not significantly different ($p > 0.05$) for all the treatments during the storage. This suggests that neither RPL treatment nor alginate coating had an inhibition effect on PE activity in fresh-cut cantaloupes.

As shown in Figures 2(b) and (c), there was a similar trend for PG and cellulase activities in fresh-cut cantaloupes during storage. All the fresh-cut cantaloupes showed an increase of PG and cellulase activities at the beginning of storage (day 4). As storage time increased, the PG and cellulase activities of all samples remained constant until the end of storage except for the untreated samples which had significantly lower ($p \leq 0.05$) PG activity on day 40 and cellulase activity on day 28 as compared to the other samples. The constant PG and cellulase activities in all the samples could be due to the development of higher CO_2 and lower O_2 levels than the atmosphere in the packages. It was reported that a controlled atmosphere with low O_2 and high CO_2 levels inhibit PG at the transcription level (Zhou *et al*., 2000). In addition, the high CO_2 level might prevent the conversion of ACC to ethylene by acting as a competitive inhibitor, leading to reduced ethylene production, which decreases cellulase and PG synthesis and activities (Deng *et al*., 2007). Furthermore, O_2 is required for the action of ACC synthetase to synthesis ACC (1-aminocyclopropane-1-carboxylic acid), which is the co-substrate of the ethylene-forming enzyme (EFE) that transforms ACC into ethylene (Ramos-Villarroel *et al*., 2011). As such, low O_2 level will result in lower ethylene production. In the present work, the extremely high CO_2 level (84.36%) developed in the untreated samples was discovered as storage time increased. This observation explained the decrease in PG and cellulase activities at the end of storage. The inactivation of PG and cellulase by low O_2 and high CO_2 levels was also reported by Deng *et al*. (2007) in

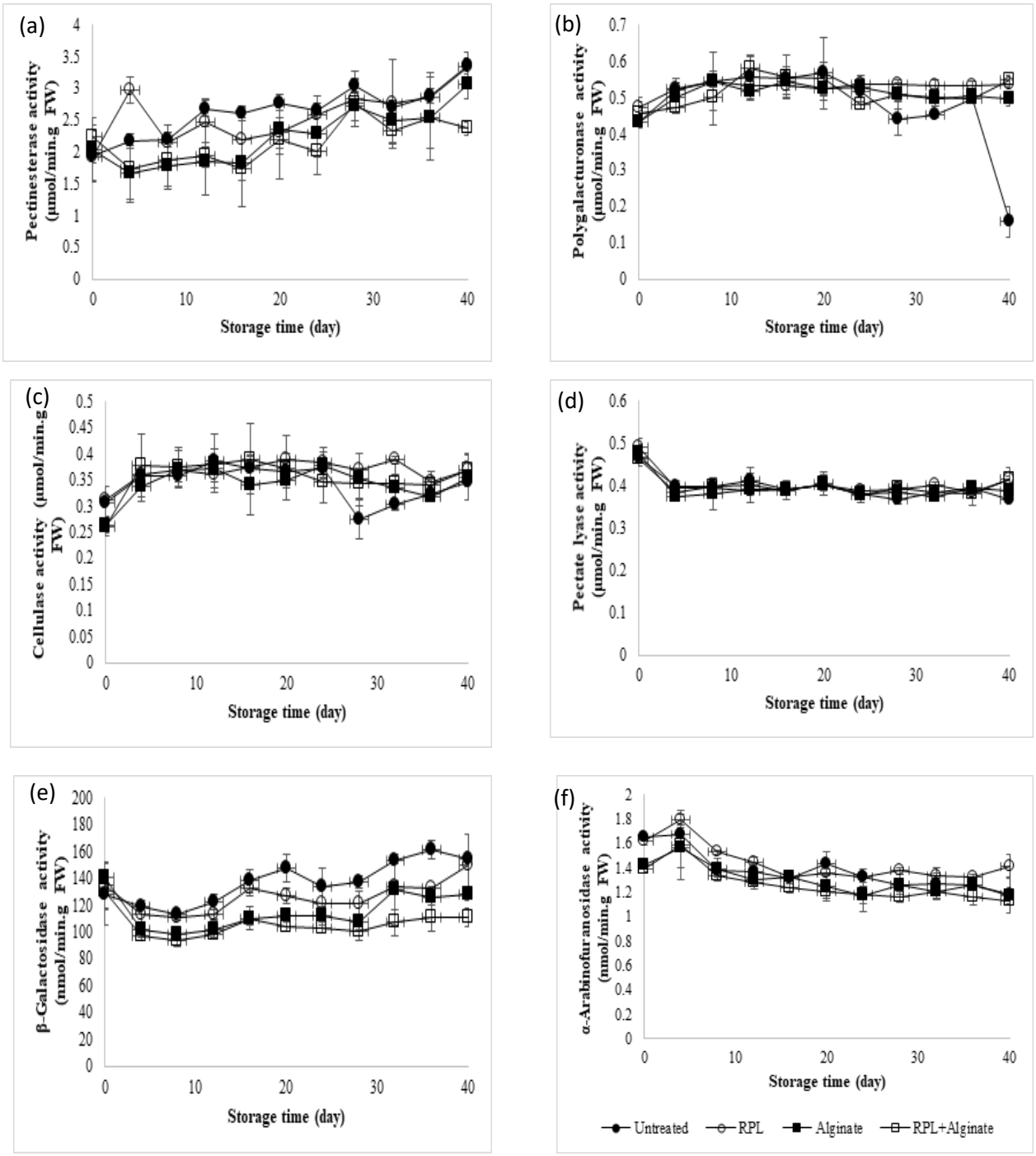

Figure 2. Effects of alginate coating and repetitive pulsed light (RPL) application on textural enzymes: (a) pectinesterase, (b) polygalacturonase, (c) cellulase, (d) pectate lyase, (e) β-galactosidase and (f) α-arabinofuranosidase of fresh-cut cantaloupes.

grapes. Based on Figure 2(d), the PEL activity was initially reduced at the beginning of storage followed by insignificant changes ($p > 0.05$) throughout the storage for all samples. The reduced and constant PEL activity could also be due to the low O_2 and high CO_2 levels in the packages of fresh-cut cantaloupes. PEL activity in fruits was suggested to be regulated by ethylene (Palusa *et al*., 2007).

β-Gal and α-AF enzymes are responsible to break the non-reducing terminal galactosyl (Barnavon *et al*., 2000) and α-L-arabinofuranosyl (Sozzi *et al*., 2002) residues, respectively in pectic and hemicellulosic polysaccharides. It is known that the NS loss of the polysaccharides by these enzymes would cause polysaccharide depolymerisation (Dos-Santos *et al*., 2011). During storage of fresh-cut cantaloupes, untreated samples showed the highest increase of β-Gal activity followed by RPL-treated samples

without coating while coated samples without and with RPL maintained the lowest and insignificant different ($p > 0.05$) β-Gal activity throughout storage (Figure 2(e)). For α-AF, untreated samples and samples treated with RPL alone had higher enzymatic activity followed by samples treated with coating alone during storage (Figure 2(f)). The α-AF activity in alginate+RPL samples remained the lowest throughout the storage. The lower β-Gal and α-AF activities in the coated samples could be contributed by the use of calcium as a crosslinking agent in alginate coating. Ortiz *et al*. (2011b) suggested that calcium could reduce β-Gal and α-AF activities by regulating the release of the enzyme as a result of the protective effect of calcium on cell membranes. Calcium ions are known to strengthen the structure of cellular membranes by binding to phospholipids and stabilising lipid bilayers (Hepler, 2005). In addition, the crosslinking of cell wall polysaccharides in the presence of calcium reduces the entrance of enzymes into cell wall matrix, which prevents polysaccharide hydrolysis (Maruvada and McFeeters, 2009). The reduction of β-Gal and α-AF activities due to the effect of calcium was also reported by Ortiz *et al*. (2011a) in apples. Generally, RPL did not have an important effect on β-Gal and α-AF activities as there was insignificant difference ($p > 0.05$) of the enzymatic activities for uncoated samples without and with RPL or coated samples without and with RPL during storage.

Overall, the textural enzymatic activities were constant and not affected by the treatments except for β-Gal and α-AF. These results indicate that textural changes of fresh-cut cantaloupes in the present work were more likely to be affected by β-Gal and α-AF. Since coated samples without and with RPL maintained lower β-Gal and α-AF activities, these explained the higher firmness (2.19-2.75 N) and lower fluid loss (0-2.33%) of these samples as compared to uncoated samples (1.43-2.14 N for firmness and 1.81-19.62% for fluid loss) as determined during storage.

Conclusion

In conclusion, alginate coating combined with RPL treatment was effective to minimise changes in enzymatic activities such as PE, PEL, PAL, PPO, PG and cellulase in fresh-cut cantaloupes. Meanwhile, alginate coating was effective to reduce the activities of the POD, β-Gal and α-AF in fresh-cut cantaloupes regardless of combination with RPL. Overall, alginate coating imposed greater positive effect for reducing enzymatic activities in fresh-cut cantaloupes during storage.

Acknowledgement

The authors are grateful to Universiti Putra Malaysia for financially supporting the present work through Project GP-IPS/2015/9469100.

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