



# Effect of carrot puree edible films on quality preservation of fresh-cut carrots

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## Abstract

The effect of edible films based on carrot puree, chitosan, corn starch, gelatin, glycerol and cinnamaldehyde on fresh-cut carrots was studied during storage. Several parameters, such as firmness, colour, weight loss, total carotenoids, total phenols, polyphenol oxidase (PPO) activity and peroxidase (POD) activity in coated carrots were determined at regular intervals and then compared with the uncoated carrots throughout the storage period. Significant and expected changes were observed in all carrot samples that were compared. The coating treatment significantly ( $P < 0.05$ ) delayed the senescence, reduced the deterioration of exterior quality and retained total carotenoids well compared with control ( $P < 0.05$ ). In addition, significant inhibition of PPO activity ( $P < 0.05$ ) and POD activity ( $P < 0.05$ ) as well as reduced accumulation of polyphenols ( $P < 0.05$ ) were observed for all coated samples. All of these favourable responses induced by coating treatment on minimally processed fresh-cut carrots showed beneficial physiological effects, which would give some useful references to the fresh-cut fruit and vegetable processing industry and satisfy people's requirements allowing for extending product shelf life without negatively affecting the sensory quality or acceptability.

## Keywords

carrots • edible films • enzyme activity • food quality • coating treatment

## Introduction

Carrot is one of the most popular vegetables in many countries because of its nutritional value (Wang *et al.* 2011). The predominant carotenoid identified in carrot cultivars is  $\beta$ -carotene (Alasalvar *et al.* 2001). Carrots also contain substantial amounts of vitamin C and phenolic compounds with chlorogenic acid, the most abundant phenolic compound identified in carrot cultivars (Klaiber *et al.* 2005; Kreutzmann, Christensen and Edelenbos 2008). In recent years, due to changes in people's consumption patterns, the demand for natural and fresh-cut products has increased markedly. Fresh-cut carrot products, such as carrot sticks, disks, batons, shredded carrots represent an important part of the fresh-cut vegetable industry (Kenny and O'Beirne 2010). In general, minimal processing of carrots included grading, washing, sorting, peeling, slicing, chopping, packaging and then storage on an industrial scale (Lee *et al.* 2003). However, these operations could damage plant tissue cells causing undesirable biochemical and physiological changes and microbial spoilage to minimally processed vegetables, even decreasing the nutritional quality and thus, shortening the product shelf life (Ahvenainen 1996).

To solve the above problems, research on edible films with functional properties has been on the rapid increase in recent years. Edible films act as a barrier to transport of gas and water vapour in the product, modifying the internal atmosphere,

which delays dehydration and senescence (Baldwin, Nisperos-Carriedo, and Baker 1995) and thus, prolongs the shelf life and potentially the safety of foods (Quintavalla and Vicini 2002). Since carrot mainly consists of water, protein, cellulosic substances and pectin (Bao and Chang 1994), these compounds might provide sufficient properties to form renewable, biodegradable and inexpensive films and packages (Wang *et al.* 2011) and have great benefit on quality preservation of fresh-cut carrots.

However, single carrot film has poor mechanical properties and previous studies (Wang *et al.* 2011) found that adding one kind of biopolymers into carrot films could not solve the problem. Composite edible films can be formulated to combine the advantages of each component (Elsabee and Abdou 2013). Generally speaking, films made from polysaccharides, which provide the supporting matrix for most composite films (Elsabee and Abdou 2013), such as starches, gelatin and cellulose derivatives, are expected to be excellent oxygen barriers due to their tight packing and ordered hydrogen-bonded network structure and have the necessary high tensile strength (Nisperos-Carriedo 1994; Yang and Paulson 2000). However, such films are too brittle for further applications (Jangchud and Chinnan 1999). An effective approach to improving mechanical properties of edible films is to add a plasticiser (a low molecular weight

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non-volatile substance) into the film to reduce biopolymer chain-to-chain interaction resulting in the improvement of film flexibility and stretchability. Glycerol has been found to be the best plasticiser for water soluble polymers (Sánchez-González *et al.* 2011) and contributes to the reduction of material brittleness by the limitation of crosslinking and attenuation of intra- and intermolecular hydrogen bonds (Pereda, Aranguren and Marcovich 2008). Based on this finding, glycerol has been used as plasticiser to enhance flexibility and improve the mechanical properties of edible films.

Increasingly, consumers demand more natural and safe products. To satisfy these requirements, one of the major challenges for the modern food industry is to reduce conventional chemical additives in food production. In this sense, the alternative use of natural plant products has been receiving more and more attention as many of them have additional functional properties. Among natural antimicrobials, essential oils have been widely studied, presenting a large spectrum of action (Sánchez-González *et al.* 2011). They have also been shown to possess antibacterial properties against a range of pathogenic and spoilage microorganisms (Scollard, Francis and O'Beirne 2013) and are also commonly used as flavouring in the food industry. Cinnamaldehyde had the best antimicrobial effect among four common essential oils, including oregano essential oil, carvacrol, citral and cinnamaldehyde (Wang *et al.* 2010). As a result, cinnamaldehyde was selected to improve edible films. Therefore, in this study, chitosan, corn starch, gelatin, glycerol and cinnamaldehyde were added. These may have a synergistic effect to improve the properties of carrot films (Wang *et al.* 2011).

In the present study, carrot puree films were applied to carrot slices by immersing them in film-forming dispersions containing carrot puree, chitosan, corn starch, gelatin, glycerol and cinnamaldehyde to improve the combination property of carrot films. The aim of this work is to study the effect of the new composite edible film on the overall quality and preservation of fresh-cut carrots.

## Materials and methods

### Materials

Carrots (named 'Early Bolting of Five Inches Ginseng') were purchased from a local supermarket (Changchun, China). Chitosan (degree of deacetylation, 0.958) was obtained from Jinan Haidebei Marine Bioengineering Co., Ltd (Jinan, China). Corn starch (food grade) containing 100% (w/w) starch was purchased from Dalian Linmei Food Co., Ltd (Dalian, China).

Glycerol and acetic acid (both analytically pure) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Bovine gelatin and sodium bicarbonate (both food grade) were obtained from Guangdong Xilong Chemical Co., Ltd (Guangzhou, China). Cinnamaldehyde was purchased from Wuhan Yuancheng Technology Development Co., Ltd (Wuhan, China). Carrot puree, chitosan, corn starch and gelatin were the primary ingredients in all films. Sodium bicarbonate was used in the step of carrot puree production. Glycerol was added as a plasticiser. Cinnamaldehyde was added as an antibacterial agent and antioxidant.

### Preparation of the film-forming dispersions

**Carrot puree production:** Carrots were washed, cut into 10-mm cubes, soaked in 0.1% (w/w) boiling sodium bicarbonate solution for 2 min to prevent browning reaction and then washed in cold water. Next, the carrot cubes were boiled in water for 5 min and then cooled to room temperature. Finally, the A-88 Tissue Pulverizer (Laiheng Lab-Equipments, Beijing, China) was used to turn the carrot cubes into carrot puree with the particle size of carrot puree at about 0.1mm.

**Chitosan solutions:** Chitosan (1% w/w) was dispersed in acetic acid (1% v/v) to prepare a solution. The dispersion was put on a magnetic stirrer for 60 min under continuous stirring to dissolve chitosan completely.

**Corn starch and gelatin solutions:** Corn starch (1% w/w) was prepared by heating aqueous solutions to 72°C and maintained at that temperature for 30 min in a water bath with constant stirring, to accomplish complete starch gelatinisation. Then gelatin (0.5% w/w) was added to the solution. The solution was cooled to room temperature.

**Blend solutions:** The corn starch and gelatin solutions were added to the carrot puree (30%, w/w), and then glycerol (1.5%, v/v) was added. The mixtures were allowed to undergo plasticisation reactions (50°C) for 20 min. In turn, the solution was added to the chitosan solution. Cinnamaldehyde (1%, v/v) was then incorporated into the solutions. These solutions were homogenised for 3 min at 2366 ×g using a FA25 Superfine Homogenizer (Fluko Equipment Shanghai Co., Ltd, Shanghai, China). Each homogenate was degassed under vacuum for 15 min.

The concentration of all constituents in the final film-forming dispersion are listed as follows: carrot puree, 30%, w/w; corn starch, 1%, w/w; gelatin, 0.5%, w/w; glycerol, 1.5%, v/v; chitosan, 1%, w/w; cinnamaldehyde, 1%, v/v.

### Coating of carrot sample

Carrots were washed and selected by quality and uniformity. Sliced carrots (2-cm diameter, 1-cm thick), obtained from the central area of the roots by means of a core borer and were dipped in the corresponding coating solution for 5 min. The

coating solutions were allowed to drip off and afterwards the applied coatings were dried with natural convection at room temperature for 2–3 h. Control samples followed the same treatment but were dipped in distilled water. The surface drying time of coated carrot samples was set to eliminate all the solvent content from the adhered coating through their weight control. Samples were packed in 490 ml perforated PET packets (10 samples per packet) and were kept at 5°C in a temperature-controlled chamber for 12 days.

Parameters of firmness, colour, weight loss, total carotenoids, total phenols, polyphenol oxidase (PPO) activity, and peroxidase (POD) activity were determined on production day (Day 0) and every two days throughout the storage period (Days 2, 4, 6, 8, 10 and 12) and were compared with initial values of uncoated carrots. Before the various analyses, the coatings were removed from the stored carrot slices.

#### **Measurement of firmness**

The procedure for firmness measurement of fresh-cut carrots was adapted from the method of Benítez *et al.* (2013) with some modifications. A Texture Analyzer TA.TXPlus (Stable Microsystems, Ltd., UK) was used and a 2-mm diameter stainless steel probe was selected to evaluate the firmness of fresh-cut carrots. Samples were compressed to a depth of 8 mm at a speed of 2 mm/s. Force-distance curves were obtained from the puncture tests and firmness was regarded as the area under the curve expressed in N/mm.

#### **Measurement of colour**

Colour measurement was performed by using a spectrophotometer CM-3600d (Minolta Co., Tokyo, Japan) according to the method of Vargas *et al.* (2009) with some modifications.  $L^*$ ,  $a^*$  and  $b^*$  values from the CIELAB colour space were determined using D65 illuminant and 10° observer. From these values, whiteness index (WI) was obtained. In order to enable colour change of the coated samples to be observed better, slices of 1 cm<sup>3</sup> were obtained from the carrot's parenchyma tissue. The  $L^*a^*b^*$  data was then expressed as the WI according to the equation:  $WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$  (Bolin and Huxsoll 1991).

#### **Measurement of weight loss**

Weight loss was measured by the weighing method (Hong *et al.* 2012). The same carrots were weighed from the beginning to the end of the experiment every 2 days during storage. The results were expressed as the percentage loss of the initial total weight (Velickova *et al.* 2013).

#### **Measurement of total carotenoids**

Total carotenoids were measured by spectrophotometry according to the method of Jagannath (2006) with some

modifications. Total carotenoids were extracted by homogeneously mixing 5 g of fresh-cut carrot samples with 40 ml acetone and 60 ml hexane; 0.1 g of magnesium carbonate was added during the extraction. The homogenate was filtered through a Whatman #4 filter paper (Kenny and O'Beirne 2010), washed twice with the mixture of 25 ml acetone and 25 ml hexane and the filtrates were combined. The supernatant of filtrate was transferred to a 100 ml volumetric flask, 9 ml acetone added and diluted to 100 ml with hexane. The blank was a mixture of 9 ml hexane and 1 ml acetone and a spectrometer was used to measure optical density (OD) at 450 nm. Total carotenoids were calculated according to the equation:

$$TC = \frac{OD \times 10^5}{250 \times W}$$

(where, TC: total carotenoids; W: weight of carrot samples). The results were expressed as mg/kg.

#### **Measurement of total phenols**

Total phenols were determined according to the method of Pirie and Mullins (1976) with some modifications. Carrot samples (5 g) were homogenised with 25 ml methanol containing hydrochloric acid (1%, v/v). The homogenates were centrifuged at 12,000 ×g for 30 min at 4 °C and the supernatant was collected, after which the absorbance at 280 nm was measured. The results were expressed as OD<sub>280</sub> per gram.

#### **Measurement of PPO activity**

PPO activity was measured using a spectrophotometer according to the method of Galeazzi, Sgarbieri and Constantinides (1981) with slight modification. The reaction mixture contained 0.5 ml crude extract and 3 ml substrate solution (0.5 mol/l catechol in 0.2 mol/l phosphate buffer). Buffer solution (0.5 ml) was selected as a substitute for crude enzyme extract and served as a blank control. The absorbance was measured at 398 nm for 30 s. One unit of PPO activity was defined as the amount of crude extract enzyme that caused a change of 0.1 unit of absorbance per min per ml. PPO activity was expressed as enzyme units per gram (U/g).

#### **Measurement of POD activity**

POD activity was measured according to the method of Bi *et al.* (2011) with slight modification. Crude extract (0.5 ml) was transferred to a colorimetric tube containing 2 ml guaiacol solution (0.05% in 0.2 mol/l phosphate buffer, pH 6.5) and water bath heated at 30°C for 5 min. Then 1 ml of hydrogen peroxide (0.08% in 0.2 ml phosphate buffer, pH 6.5) was added to the solution. POD activity was measured

at 460 nm for 120 s and 0.5 ml buffer solution was as a blank control. One unit of POD activity was defined as the amount of crude extract enzyme that caused a change of 0.1 unit of absorbance per min per ml under the usual assay conditions. POD activity was expressed as enzyme U/g.

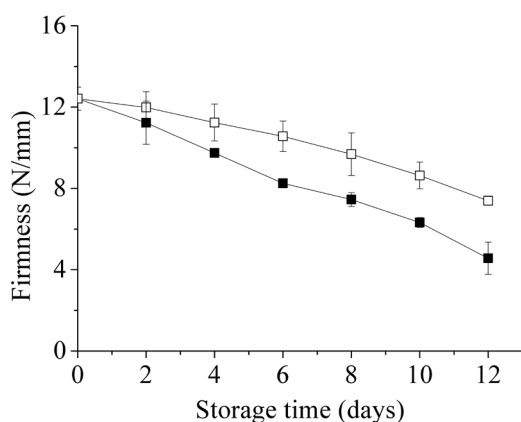
### Statistical analysis

Significant differences were evaluated by analysis of variance and Duncan mean comparison test ( $p < 0.05$ ) with SPSS version 17.0. All experiments were carried out in duplicate and each sample was measured in triplicate every 2 days.

## Results and Discussion

### Firmness

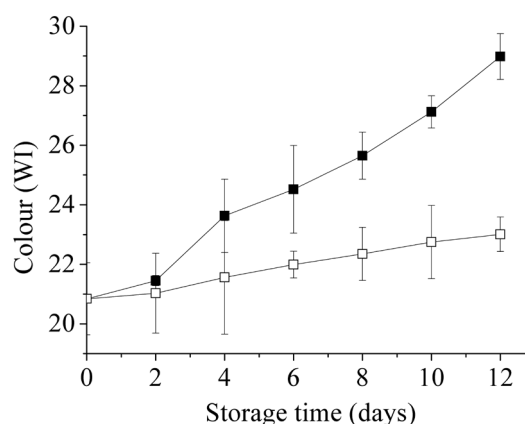
Changes of firmness for coated and uncoated fresh-cut carrots during storage are shown in Figure 1. Overall firmness tended to decrease for both coated and uncoated carrots. However, the rate of firmness reduction in uncoated carrots was less compared with coated samples, which was significant ( $P < 0.01$ ). These changes can be attributed mainly to tissue senescence and cell-wall breakdown as well as sample water loss (Vargas *et al.* 2009). Other studies have already demonstrated that carrot edible films with cinnamaldehyde had good moisture barrier performance, which, in turn, reduced gas exchange and water loss from fresh-cut samples (Brasil *et al.* 2012). Thus, the water loss rate was probably slower in coated carrots, which prevented the decrease of firmness.



**Figure 1.** Changes of firmness in coated (□) and uncoated (■) fresh-cut carrots during storage. Each value is the mean of two duplicates (each duplicate with triplicate measurements) and error bars represent standard errors of means.

### Colour

Changes in colour for coated and uncoated fresh-cut carrots during storage are shown in Figure 2. It was clear that the overall tendency of WI increased for both coated and uncoated carrots. The WI value of uncoated carrots increased significantly ( $P < 0.05$ ) faster than that of coated carrots. Colour is an important quality parameter of fresh-cut carrots. Good colour usually indicates good exterior quality and improved consumer acceptability. In general, the colour of uncoated carrots has low WI. However, cutting operations often induce undesirable changes in colour and result in enzymatic browning (Rojas-Grau, Soliva-Fortuny and Martín-Belloso 2009). Moreover, these changes can be related to surface dehydration and carotenoid oxidation (Chervin and Boisseau 1994). Thus, in practical terms, the changes in the WI value probably indicate quality deterioration of fresh-cut carrots, such as surface dehydration, carotenoid damage and nutrition loss in carrots, which will decrease the product shelf life. In this study, the results for WI indicate that the coating treatment decreased the influence of carotenoid oxidation and enzymatic browning. These findings were similar to the data on chitosan coating treatment of longan (Vangnai *et al.* 2006) and pumpkin (Ponce *et al.* 2008). In that study, chitosan in the films reduced the enzyme activity of PPO delaying the discoloration of carrots. In addition, previous studies have already demonstrated that carrot edible film with cinnamaldehyde had good moisture barrier, oxygen barrier and antioxidant properties (Wang *et al.* 2013). Coated fresh-cut carrots had less contact with oxygen than the uncoated, which probably prevented surface dehydration of fresh-cut carrots and to some carotenoid oxidation degree and reduced the occurrence of the white blush.



**Figure 2.** Changes of the WI of coated (□) and uncoated (■) fresh-cut carrots during storage. Each value is the mean of two duplicates (each duplicate with triplicate measurements) and error bars represent standard errors of means. WI: whiteness index.

### Weight loss

Changes in weight loss of coated and uncoated fresh-cut carrots during storage are shown in Figure 3. Weight loss increased for both coated and uncoated carrots and although, the differences were statistically significant ( $P < 0.05$ ), the rate of loss was not substantial. The maximum weight loss of the uncoated carrots was no more than 2% and that of the coated carrots was 1%. The weight loss of uncoated carrots increased significantly faster ( $P < 0.01$ ) than that of coated carrots. The weight loss is an index of a vegetable dehydration process due to transpiration and it involves water transfer from the cell to the ambient atmosphere, thus becoming a way to evaluate coating treatment efficiency on preservation quality of fresh-cut carrots (Pérez-Gago, González-Aguilar and Olivas 2010). In this study, the new composite carrot edible film added with cinnamaldehyde formed a protective barrier on the surface of carrots to decrease moisture loss. Thus, the coating treatment partially inhibited weight loss of fresh-cut carrots, retaining freshness.

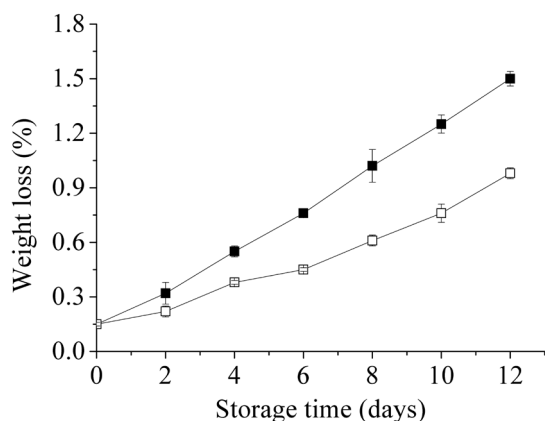
### Total carotenoids

Changes of total carotenoids in coated and uncoated fresh-cut carrots during storage are shown in Figure 4. The total carotenoids in uncoated carrots decreased considerably during storage, from an initial 1103 to 433 mg/kg after 12 days. However, the total carotenoids in coated fresh-cut carrots decreased significantly less ( $P < 0.05$ ) showing a decrease from an initial 1103 to 891 mg/kg after 12 days. This result is of importance as from the nutritional standpoint, carrots are one of the major sources of carotenoids in the human diet (Alegria *et al.* 2012). Previous studies have already demonstrated that carrot edible film with cinnamaldehyde was a good moisture

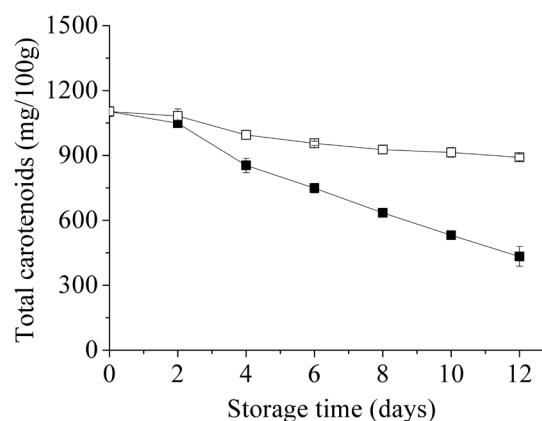
barrier, oxygen barrier and had antioxidant properties (Wang *et al.* 2013). Although, various post-harvest steps, including food-processing operations, had a great influence on the stability of phytochemicals, such as carotenoids in fruits and vegetables (Amorim-Carrilho *et al.* 2014), fresh-cut carrots with coating treatment had less contact with oxygen than the uncoated, which prevented the decrease of total carotenoids to some extent.

### Total phenols

Changes of total phenols in coated and uncoated fresh-cut carrots during storage are shown in Figure 5. The results show that total phenols increased in coated and uncoated fresh-cut carrots. However, the rate of accumulation of total phenols in uncoated carrots was significantly higher than that of coated carrots ( $P < 0.05$ ). Accumulation of phenols during the storage of minimally processed carrots has been observed by some researchers (Howard and Griffin 1993; Klaiber *et al.* 2005). A greater accumulation of total phenols is induced by the wound in minimally processed carrots (Kenny and O'Beirne 2010). Moreover, the accumulation of total phenols in wounded carrots is dependent upon wounding intensity (Heredia and Cisneros-Zevallos 2009). Phenols have a wound repair effect on carrots. In a previous study, an increase in total phenols demonstrated that the respiration of fresh-cut carrots was enhanced. After being wounded, the respiration rate and phenylalanine ammonia lyase (PAL) activity of fresh-cut carrots were enhanced with wound induction and the contact area between carrots and oxygen increased, which promoted the rapid synthesis of phenols indicating that the increase in PAL activity activated the biosynthetic pathway of phenolic compounds after wounding



**Figure 3.** Changes in weight loss of coated (□) and uncoated (■) fresh-cut carrots during storage. Each value is the mean of two duplicates (each duplicate with triplicate measurements) and error bars represent standard errors of means.



**Figure 4.** Changes in total carotenoids in coated (□) and uncoated (■) fresh-cut carrots during storage. Each value is the mean of two duplicates (each duplicate with triplicate measurements) and error bars represent standard errors of means.



(Du *et al.* 2012). Although, wound induction also existed in coated carrots, the good oxygen barrier performance of carrot edible film weakened the contact between carrots and oxygen, respiration rate and increase of PAL activity, which probably inhibited the synthesis of phenols.

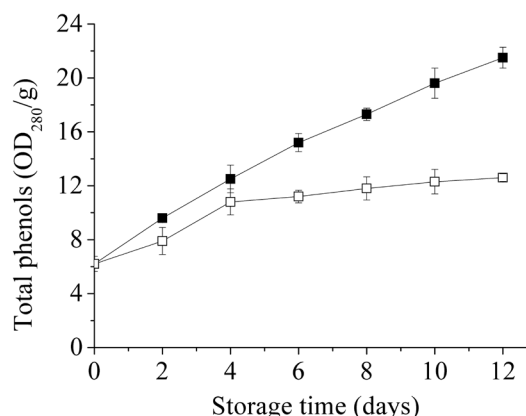
#### PPO activity

Changes of PPO activity in coated and uncoated fresh-cut carrots during storage are shown in Figure 6. The results show that the PPO activity increased for both coated and uncoated carrots. PPO activity is considered to be a measure of the degree of oxidation reaction since PPO acts as a catalyst in a series of oxidation reactions (Jiang 2013). In addition, PPO is the main enzyme responsible for enzymatic browning in vegetable tissues (Falguera *et al.* 2011) and it oxidises catechol to quinones, and then further polymerises to the formation and accumulation of melanin, which is the brown or black pigment in plant tissues (Toivonen and Brummell 2008). PPO is bound with the membrane system, not showing activity in the natural state in unprocessed carrots. When carrots are subjected to the incised wound, the membrane system is impaired, thus leading to PPO activation and enhancement of its activity.

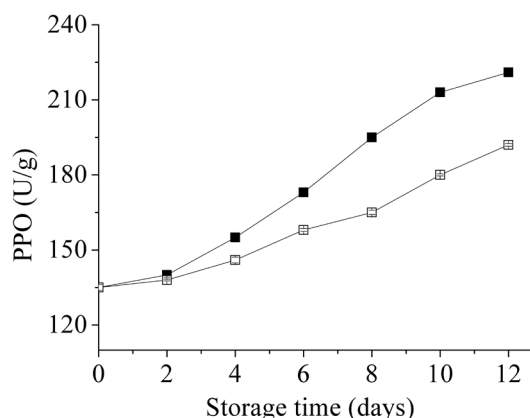
The results also show that changes of PPO activity were small in both coated and uncoated fresh-cut carrots during early storage. In fact, it required an accumulative process for the incised wound to enhance PPO activity. The enhancement of PPO activity accelerated from day 2, with PPO activity in uncoated carrots enhancing significantly faster ( $P < 0.05$ ) than coated carrots. The new composite edible film strongly decreased the contact between the internal part of carrot tissues and external oxygen due to its good oxygen barrier property, which probably weakened the oxidation of catechol by PPO. Thus, it seems that the coating treatment could inhibit PPO activity of fresh-cut carrots decreasing the enzymatic browning and maintaining a good colour of the carrots.

#### POD activity

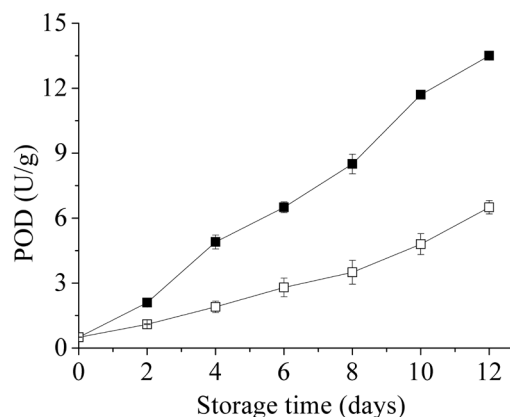
Changes of POD activity in coated and uncoated fresh-cut carrots during storage are shown in Figure 7. POD activity increased in both coated and uncoated carrots during the storage. POD activity in uncoated carrots increased significantly faster ( $P < 0.05$ ) than coated carrots. It is known that POD activity is correlated with various deteriorative reactions, which influence colour, flavour, texture and nutritional properties in processed vegetables (Alegria *et al.* 2010). POD is an enzyme-based  $H_2O_2$  as electron receptor catalysing  $H_2O_2$  to oxidise phenols and amine compounds to form coloured compounds. POD activity in plants increased in response to various biotic and abiotic stresses (Kwak *et al.* 1996), including wounding, physiological stress and the readily available phenolic substrates (Alegria *et al.* 2012). The incised wound probably accelerated the cleavage of histocyte



**Figure 5.** Changes in total phenols in coated (□) and uncoated (■) fresh-cut carrots during storage. Each value is the mean of two duplicates (each duplicate with triplicate measurements) and error bars represent standard errors of means. OD: optical density.



**Figure 6.** Changes in PPO activity in coated (□) and uncoated (■) fresh-cut carrots during storage. Each value is the mean of two duplicates (each duplicate with triplicate measurements) and error bars represent standard errors of means. PPO: polyphenol oxidase.



**Figure 7.** Changes in POD activity in coated (□) and uncoated (■) fresh-cut carrots during storage. Each value is the mean of two duplicates (each duplicate with triplicate measurements) and error bars represent standard errors of means. POD: peroxidase.

cells of carrots, promoted the formation of reactive oxygen species and H<sub>2</sub>O<sub>2</sub> and then led to the enhancement of POD activity. POD is considered as an enzyme that eliminates radicals, which can promote browning. The results indicated that the coating treatment may form a protective barrier on the surface of carrots and reduce the supply of oxygen for enzymatic oxidation of phenolics (Zhang and Peter 1997), thus inhibiting POD activity delaying the ageing of fresh-cut carrots.

## Conclusion

The new composite edible films based on carrot puree, containing chitosan, corn starch, gelatin, glycerol and cinnamaldehyde better retained the important phytochemicals, including total carotenoids, limited the increase of total phenols and inhibited key enzyme activities, such as PPO activity and POD activity, compared with the control. The

composite edible films also provided a good moisture barrier, oxygen barrier and had antioxidant properties, which promoted maintenance of a good exterior quality of fresh-cut carrots and extended the product shelf life. Therefore, it could be concluded that the new composite film, with good comprehensive properties, has extensive application prospects in quality preservation of minimally processed vegetables and fruits. Furthermore, other new composite edible films are expected to be researched and developed, increasing the quality of fresh-cut fruits and vegetables.

## Acknowledgements

Financial support from the high level talent fund of Henan University of Technology Science and Technology (No. 2012BS024) is gratefully acknowledged. The authors also wish to thank foreign teacher Gatenet Solomon for language revision.

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