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Interaction between surface coating using cabbage leaf wax extract and temperature on water vapour and gas exchange properties of fresh okra (*Abelmoschus esculentus* (L.) Moench)

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Abstract

Significant shelf-life losses can occur in fresh okra fruit because of weight loss and shrivelling. The effects of surface coating using wax extracted from cabbage leaf (CB-Wax), and storage temperature on water vapour and gaseous exchange properties of fresh okra were investigated. CB-wax was prepared by immersion of outer cabbage leaves in dichloromethane solvent for 15 hr at ambient temperatures and stored in a dried form. The extract was mixed with ethanol for coating the intact okra surface through brushing. CB-Wax coated okra were kept in three different temperatures (10, 25 and 35°C) and effective skin permeance to water vapour (effective SPW), respiration rate, and effective skin permeance to CO_2 (effective SPC) were studied and compared to those of a non-coated control. Values of effective SPW, respiration, and effective SPC of CB-Wax coated okra were significantly lower than those of non-coated fruit. These values showed temperature dependence where could be described by the Arrhenius model; values of error root mean squares were in a range of 0.001-0.422. However, CB-Wax coated okra apparently were less sensitive to temperature changes compared to non-coated okra as determined by activation of energy estimated from the model using a non-linear regression approach. CB-Wax has external benefits in reducing water vapour loss, respiration rate, and CO_2 permeation through the skin of fresh okra.

Introduction

Water loss from fresh okra *(Abelmoschus esculentus)* after harvest causes reductions in its shelf life of fruits and market value because of wilt or a wrinkled appearance and more, importantly, it is due to weight loss (Ben-Yehoshua, 1987; Finger *et al.*, 2008; Dhall, 2013; Shiekh *et al.*, 2013). The market acceptance of weight loss for okra is limited to 4-5% (Finger *et al*., 2008; Nunes, 2015). Like other horticultural products, water loss is the major contributor to this weight loss. Water vapour continuously diffuses from the cuticle of the okra fruit skin to the surrounding environment due to the water vapour pressure gradient, in order to equilibrium condition (Kays, 1991; Magwaza *et al*., 2013). Although plants have natural waxes on their surface to minimize water loss, some losses of these waxes are unavoidable and may result from for example, harvesting and cleaning processes (Srilaong *et al*., 2013). Losses of wax subsequently lead to weight loss, through facilitating water vapour losses from the fruit (Dagostin *et al*., 2015). To compensate for waxes lost from the fruit, surface coating with either natural or synthetic waxes including carnauba wax, shellac wax, rice bran wax, chitosan, alginate and oxidized polyethylene have been commercially applied to a range of fruit to prevent water loss, as well as to increase fruit surface appearance (Cisneres-Zevallos and Krochta, 2002; Ramos *et al*., 2012; Pilon *et al*., 2013; Xin *et al*. 2017).

In this study, wax was extracted from the outer leaves of fresh cabbage as surface coating material (CB-Wax). Outer fresh cabbage leaves are typically discarded as part of normal marketing practices because of their tough hard texture, and unpleasant appearance caused by, for example, bruises, pathogens and holes from insect damage (Thammawong *et al*., 2011; Nugrahedi *et al*., 2016). Cabbage leaf has high amounts of surface wax which can exert a so-called 'lotus leaf effect' in which the surface is considered unwettable or self-cleaning (Koch *et al*., 2006; Zhang *et al*., 2016). Utilisation of the outer cabbage leaves for wax extraction is a value-added approach

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for what is otherwise a waste product. These appear to be no other reports on using wax extracted from cabbage leaves for use on okras or other horticultural products. Wax coating is considered to provide additional barriers to water vapour and to gaseous permeations through the skin of fruit (Kays, 1991; Magwaza *et al*., 2013). Consequently changes to water vapour diffusion and to other gaseous exchange properties of coated okra fruit could be expected when the fruit are treated with cabbage leaf waxes. Storage temperatures of horticultural products can often fluctuate and vary widely. Such changes can have negative consequences to fruit including the promotion of microbial proliferation, water loss, reductions in firmness and colour changes (Celikel *et al.,* 2002; Aloui *et al.,* 2015). The influence of temperature on okra fruit that were either untreated or coated with wax extracted from cabbage leaves (denoted as CB-Wax) was also studied. The aims of this study were to investigate the effectiveness of CB-Wax surface coating as well as the influences of temperature on respiration and skin permeance to water vapour and gaseous exchange in okra fruit.

Materials and Methods

Raw material

Cabbages and okra fruit were purchased from markets in Warin Chamrap, Ubon Ratchathani, Thailand. Both products were transported to the postharvest laboratory, Faculty of Agriculture, Ubon Ratchathani University within 2 hr. Outer cabbage leaves were detached out from the cabbage head and left at room temperature (approximately 35°C) prior to the extraction process. Okra fruit were separated into three groups in accordance to experimental temperatures (10, 25 and 35°C). The fruits were kept in the desired temperatures for 18hr to equilibrate with the storage temperature treatment prior to applying the coating. Preliminary tests showed that the fruit temperature, measured using a thermocouple probe, was equal to the storage room temperature in which the fruit was stored after this equilibration period.

CB-wax extraction and preparation

The CB-Wax extraction procedure used was an immersion method as reported by Bohinc *et al*. (2014) with some modifications. Fresh leaves were cut size into 1 x 3 cm pieces and 150 g of leaf was placed in to a 2 L glass jar. A 300 mL volume of dichloromethane liquid (99% w/v) was added and the jars were left for 15 hr at ambient temperature (35ºC). The extract was then separated from the leaves by filtration. The solvent was evaporated by placing the extract solvent solution in a beaker in water bath (50ºC) and aerating with nitrogen gas (5 mL min^{-1} flow rate) within a fume hood. The dried wax was stored in a desiccator.

Coating of okra surface using CB-wax

To prepare the wax surface coating material, the dried CB-Wax was mixed with ethanol (99%) at a ratio of 0.5: 5 (w/v). Individual okra fruits were coated using a brush dipped in the coating material. The okra surface was brushed thoroughly and left to dry in a ventilated area, i.e. the time period was at least 12 hr. The time period was considered sufficient for gas transport processes achieving their steady-state condition. Non-coated okra fruit were designated as the control (Ctrl). From our studies undertaken (data not shown), there were no significant effects of ethanol as a wax solvent on gaseous exchange properties of okra studied. Values of the properties measured from okra either control or only brushed with ethanol were comparable. The effects of cabbage wax extracted surface coating on the gaseous exchange properties of the okras should be considered results of the wax per se.

Measurement of effective skin permeance to water vapour

Values of effective skin permeance to water vapour (denoted as effective SPW) for both coated and non-coated fruit were estimated according to Fick's first law for steady-state diffusion (Equation 1) following Maguire (1998):

$$P_{okr}^{H_2O} = \frac{r_{okr}^{H_2O}}{\left(p_{\text{int}}^{H_2O} - p_{env}^{H_2O}\right) \times A_{fr}} \qquad \text{(Equation 1)}$$

where $P_{okr}^{H_2O}$ is the effective SPW (mol s^{-1} m^{-2} Pa^{-1}); $r_{okr}^{H_2O}$ is the steady-state rate of water loss from fruit (mol s^{-1}); A_{okr} is the fruit surface area (m^2); $p_{\text{int}}^{H_2O}$ and $p_{env}^{H_2O}$ and are the partial pressure of water vapour inside the fruit and in the atmosphere surrounding the fruit (Pa), respectively. The value of $r_{fr}^{H_2O}$ was estimated by subtracting the rate of carbon loss attributed to respiration processes from the steady-state rate of weight loss (Equation 2).

$$r_{okr}^{H_2O} = r_{okr}^{wt} - r_{okr}^{Closs} \qquad \text{(Equation 2)}$$

where r_{okr}^{wt} is the steady-state weight loss of fresh okra (mol s^{-1}); and r_{okr}^{Closs} is the rate of carbon loss attributed to respiration processes of fresh okra (mol s^{-1}). To quantify r_{okr}^{wt}, fresh okra fruit were weighed periodically using a digital balance until the weight reduction became linear and the value of r_{okr}^{wt} was represented by the slope of the linearized weight change which was estimated using linear regression (Microsoft Excel

2010). The value of r_{okr}^{Closs} was estimated using equation 3.

$$r_{okr}^{Closs} = r_{okr}^{CO_2} M_{okr} \quad \text{(Equation 3)}$$

where $r_{okr}^{CO_2}$ is the respiration rate (mol s^{-1} kg^{-1}); and M_{okr} is the okra mass (kg). The respiration rate of fresh okra was measured through CO_2 generation rate in a closed system following Maguire (1998). Values of $p_{fr}^{H_2O}$ and $p_{env}^{H_2O}$ were calculated using equation 4 and equation 5, respectively.

$$p_{fr}^{H_2O} = 611 \cdot \exp\left(17.27 \cdot \left(\frac{T_{fr}}{T_{fr} + 237.3}\right)\right) \times a_w \quad \text{(Equation 4)}$$

$$p_{env}^{H_2O} = 611 \cdot \exp\left(17.27\left(\frac{T_{wb}}{T_{wb} + 237.3}\right)\right) - \gamma(T_{db} - T_{wb}) \quad \text{(Equation 5)}$$

where T_{fr} fruit temperature (ºC); a_w water activity [defined as 0.995 following Maguire (1998)]; T_{wb} , T_{db} wet and dry bulb temperature (ºC), respectively; γ constant, defined as 67Pa C^{-1} following Maguire (1998). The okra surface area was measured using a leaf area measuring meter (Area meter Li-3100, Nebraska, USA). To measure the surface area, individual okra were wrapped with opaque tape which was later cut and peeled off from the okra using a sharp knife. The tape was spread out and flattened on a transparent sheet inserted into the leaf area meter. The surface area was estimated from the opaque section laid on the sheet.

Measurement of effective skin permeance to CO_2

Effective skin permeance to CO_2 (denoted as effective SPC) was estimated according to Fick's first law for steady-state diffusion following Banks (1983). To quantify CO_2 concentration inside the fruit, the techniques by Banks (1983) were utilized. Cannulae (14 gauge stainless steel needles, cut down to 2-cm length) were inserted through the fruit wall into the cavity of the fruit. The connection between the cannula and the skin was sealed to be gas-tight using an epoxy adhesive. Once the cannulae were attached, fruit were separated into three groups (8 fruits per individual temperature; n = 8) and then equilibrated for 18hr in three different temperatures (10, 25 and 35ºC). Steady-state internal CO_2 ($p_{okr,int}^{CO_2}$) at each storage temperature thereafter were determined by sampling 10 mL from the fruit cavities through the cannulae. Values for effective SPC were then calculated using equation 6 (Cheng, 1999).

$$P_{okr}^{CO_2} = \frac{r_{okr}^{CO_2} M_{okr}}{\left(p_{int}^{CO_2} - p_{env}^{CO_2}\right) A_{okr}} \quad \text{(Equation 6)}$$

where $P_{okr}^{CO_2}$ is an effective SPC (mol s^{-1} m^{-2} Pa^{-1}); and $p_{int}^{CO_2}$, $p_{env}^{CO_2}$ are CO_2 concentration in the fruit interior and in the environment (Pa), respectively. It should be noted that the value of was equal to 0.03 kPa which is the CO_2 pressure in the ambient atmosphere.

Data analysis

The experimental design and statistical analysis was in accordance with a factorial in Complete Randomized Design (CRD) with two replicates of which the coating (CB-Wax and non-coated) and storage temperatures (10, 25 and 35ºC) were main effects. Data for effective SPW, respiration rate and SPC were analysed and expressed according to units proposed by Bank *et al*. (1995). Temperature dependences of the data were analysed using Arrhenius's law (Equation 7). The reference temperature for Arrhenius's law was in all cases fixed at 15ºC (288.15K).

$$k = k_{ref} \exp\left(\frac{Ea}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \quad \text{(Equation 7)}$$

where R is gas constant (8.314J mol^{-1} K^{-1}); energy of activation (J mol^{-1}) expresses the dependence of given rate (either effective skin permeance or respiration rate represented by k) on temperature (T, K), k_{ref} effective skin permeance or respiration rate at arbitrarily chosen reference T_{ref} .

Nonlinear regressions were conducted for fitting data from equation 7 to the empirical results. Values of Arrhenius coefficients were identified through fitting by minimizing the sum of squared residuals in Microsoft Excel®. Statistical analyses were conducted to evaluate the differences between the predicted *(pred)* (i.e. Equation 7) and experimental *(epmt)* results, through calculations of root mean square of error following Yantarasri *et al.* (1995) (Equation 8).

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left((epmt)^i - (pred)^i\right)^2} \quad \text{(Equation 8)}$$

where *RMSE* is root mean square of error, *n* is number of data points, and *epmt* and *pred* are experimental and equation 7's predicted data, respectively.

Results and Discussion

Effective skin permeance to water vapour (effective SPW)

The effective SPW values of okra coated with CB-Wax were significantly lower than for non-coated okra (approximately 100-fold) (Figure 1). The reductions were attributed to the CB-Wax layer coated on the okra, which decreased the rate of water vapour transfer from the okra to the immediate environment, due to the differences between the partial pressure of water vapour inside and outside

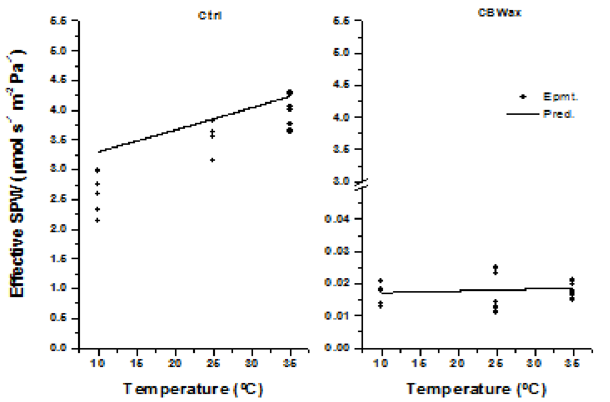

Figure 1. Effects of CB-Wax coating and storage temperature on effective SPW of fresh okra fruit (n = 8). Ctrl (control) represents non-coated okra. Epmt. and Pred. represented experimental and Eq. 7's predicted data, respectively.

the fruit (Magwaza *et al.*, 2013). Hence CB-Wax significantly reduced the loss of water vapour from the fruit and, consequently, minimized the loss in fresh fruit weight. CB-Wax has, therefore, potential as method for effective water vapour and weight loss control. Applications of surface coating materials including wax and others on weight loss reductions among fruit and vegetable have been extensively reported. The coating materials increase resistances to water vapour diffusion out from the commodities (Dagostin *et al.*, 2015). In recent study, Xin *et al.* (2017) reported that chitosan coating materials could reduce weight losses of Chinese cherries through minimising losses of water vapour between the cherries and the surrounding environment. Okra coated with CB-wax had a greener colour and had only minor wrinkle marks on the fruit surface. In contrast, non-coated fruit had a noticeably wrinkled appearance and became slightly yellow in appearance (data not shown).

The effective SPW of both CB-Wax coated and non-coated okra increased with increased temperature (Figure 1). Changes of the effective SPW in relation to temperature were adequately described by Arrhenius's relationship (Equation 7) (Table 1). The value of was lower than 2 which, according to Yang and Chinnan (1998), indicated that the model or equation can reasonably described the experimental data. Variations observed amongst the effective SPW data especially observed in non-coated okra could mainly be attributed to natural characteristics wax coated on fruit surface (Figure 1). Unlike plastic film, the wax layer naturally coated on fruit surface is not even which could be results of, for examples, cracks and losses of wax at some areas on the surface (Rimbai *et al.*, 2012). Such irregularities

Table 1. Parameter estimates resulting from non-linear regression analysis of data of the effects of storage temperature differences on effective SPW, respiration rate, and effective SPC (Ctrl represents non-coated okra; CB-Wax represents coated okra)

Water vapour and gas exchange properties	Parameters	Ctrl	CB-Wax
Effective SPW	$P^{H_2O}_{okr,ref}$	3.484	0.017
	$Ea_{P^{H_2O}_{okr}}$	7.206	2.337
	$RMSE$	0.422	0.003
Respiration rate	$r^{CO_2}_{okr,ref}$	0.023	0.002
	$Ea_{r^{CO_2}_{okr}}$	32.138	20.000
	$RMSE$	0.014	0.001
Effective SPC	$P^{CO_2}_{okr,ref}$	0.230	0.019
	$Ea_{P^{CO_2}_{okr}}$	49.807	14.367
	$RMSE$	0.325	0.006

of wax could provide additional passages for gaseous and water vapour exchanges between fruit and immediate environment. Since individual okra fruit could have different wax irregularities, the effective SPW data of non-coated okra accordingly showed high variations. In contrast, the variations become lesser amongst CB-Wax coated okra (Figure 1). These could be attributed to the fact that CB-Wax additionally applied on the fruit surface filled in some cracks or replaced empty spaces caused by losses of wax.

The estimates for activation energy ($Ea_{P^{H_2O}_{okr}}$) of CB-Wax coated okra was approximately 3-fold lower than that of control fruit (Table 1). The experimental results indicated that non-coated okra were more sensitive to temperature changes, compared to the CB-Wax coated okra. The values of $Ea_{P^{H_2O}_{okr}}$ for both CB-Wax coated okra and the non-coated controls were much lower than those of plastic films: for example, 61.9 kJ mol^{-1} polyvinylidene chloride (PVDC), 42.2–65.3 kJ mol^{-1} polypropylene (PP) (Roger *et al.*, 1982), 33.4–61.7 kJ mol^{-1} polyethylene (PE) (Fang *et al.*, 2012), 29 kJ mol^{-1} bees wax film (Donhowe and Fennema, 1993), 14.56 kJ mol^{-1} hydroxypropyl cellulose (HPC), and 16.43 kJ mol^{-1} methyl cellulose (MC) (Bertuzzi *et al.*, 2007). Consequently the rates of water vapour loss from okra are relatively less sensitive to temperature changes compared to those of water vapour permeations through such plastic films.

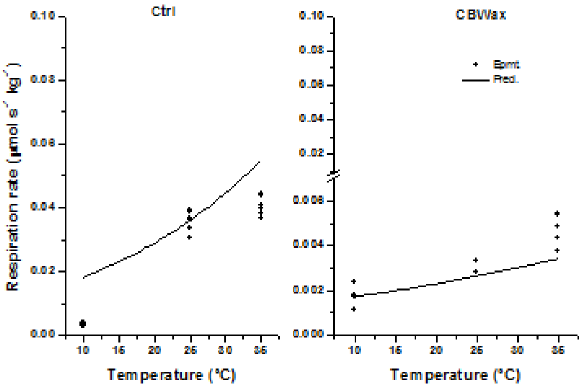

Figure 2. Effects of CB-Wax coating and storage temperature on respiration rate of fresh okra (n= 8). Ctrl represents non-coated okra (control). Epmt. and Pred. represented experimental and predicted data, respectively.

Respiration rates

Values for respiration rate of okra coated with CB-Wax were approximately 10-fold lower than those of non-coated okra (Figure 2). The wax layer is likely to have been an additional barrier that limits oxygen transfer from the outside of the okra fruit to the fruit interior, causing a lower respiration rate as the rate is dependent on oxygen concentration (Cisneros-Zevallos and Krochta, 2002; Arnon *et al.*, 2014). Del Nobile *et al.* (2009) similarly reported that cactus pear coated with agar and alginate coating materials had lower rates of oxygen exchanges between the cactus pear and the environment due to the coating layers. Khorram *et al.* (2017) recently reported that orange fruit coated with shellac, gelatin and persian gum coating materials had lower rates of oxygen exchanges between the orange fruit and the environment due to the coating layers. Experimental findings in the present work and those reported in the literature including the recent review made by Patel *et al.* (2016) confirm effects of wax coating materials on restricting gas exchanges as well as water vapour transport properties of fruit and vegetable. Values of respiration rates of non-coated okra measured at 35ºC were apparently lower than those measured at 25ºC in the control fruit. These lower values could be attributed to effects of high temperature during storage (i.e. 35ºC) stimulating senescence of okra which subsequently led to lower respiration rates. Effects of high storage temperatures on stimulating respiration and senescence of fruit and vegetables have been well documented (Wills *et al*. 1989; Samira *et al*. 2013). In contrast to results obtained from non-coated okra, the respiration rates of CB-Wax coated okra measured at 35ºC were higher than those measured at 25ºC and these could be results of the wax coated lowered the respiration and related metabolic activities, causing the okra having slower senescence rate.

Changes in respiration rate in relation to temperature were adequately described by the Arrhenius relationship (Equation 7) with values of being lower than 2 (Table 1). The estimates for activation energy ($Ea_{r_{CO_2}}$) of okra coated with CB-Wax were lower than there for non-coated okra (Table 1). The experimental result indicated that non-coated okra is more sensitive to temperature change compared with the CB-Wax coated okra. It could be noticeable that the Arrhenius model overestimated respiration rates of non-coated okra measured at 10ºC (Figure 2). The low respiration rate could be results of chilling injury. Whilst there is limited information on effects of the chilling injury on respiration rate of okra, those for example, on mango were reported i.e. the chilling injury suppressed respiration rate and delayed fruit colour development (Nair and Singh, 2009; Cantre *et al.*, 2014; Cantre *et al*., 2017). Although optimal storage temperature of okra is in a range of 7-10ºC, different cultivars and production environment among others would be factors causing the okra became differ in susceptibility to the chilling injury (Cantwell and Suslow, 2001; Huang *et al.*, 2012). Given such information, the low respiration rates measured at 10ºC of non-coated okra would be considered disorders attributed to the chilling injury. In contrast, Arrhenius model sufficiently predicted the respiration rates measured at 10ºC of CB-Wax coated fruit suggested that symptoms of chilling injury occurred to the coated okra were lessened compared to those of the non-coated fruit. There is much evidence to show that surface coating wax and other materials such as chitosan could lower chilling injury incidences among horticultural products including grapefruit, oranges, pineapples, cucumbers (Patel *et al*., 2016), and pomegranate (Meighani *et al*., 2015). Future studies would be conducted to investigate for understanding effects of CB-Wax on chilling injury of fresh okra.

The $Ea_{r_{CO_2}}$ values of both CB-Wax coated and non-coated okra were lower than those for bell pepper (39.869 kJ mol^{-1}) (Chen *et al*., 2000), hot chillies (69.813 kJ mol^{-1}) (Utto, 2001), avocados (99.80 kJ mol^{-1}) (Maftoonazad and Ramaswamy, 2008) and white kidney bean (31.9-63.4 kJ mol^{-1}) (Bauweraerts *et al.*, 2013), indicating the okra appears to be less sensitive to temperature than other crops referenced. The CB-Wax further reduced okra's sensitivity to temperature differences. The reduced in sensitivity could provide benefit in future supply chain management of fresh okra where temperature fluctuations occur during cool chain transportation and storage.

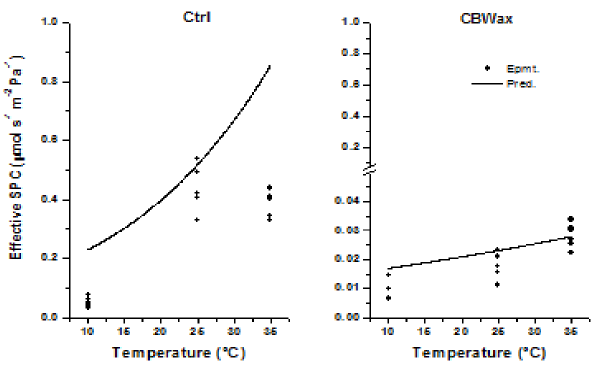

Figure 3. Effects of CB-Wax coating and storage temperature on effective SPC of fresh okra (n= 8). Ctrl represents non-coated okra (control). Epmt. and Pred. represented experimental and predicted data, respectively.

Effective skin permeances to CO_2

The values for effective SPC of okra coated with CB-Wax were approximately 10-fold lower than those of non-coated okra. These lower values were attributed to the coated layer which minimizes gaseous exchanges between okra and the environment (Banks, 1984; Arnon *et al*., 2014). As mentioned above the wax layer can reduce the rate of oxygen transfer into the fruit causing a reduction in respiration rate (Khorram *et al*., 2017). This lowers the concentration of CO_2 which is a key product of respiration. The lower CO_2 concentration accumulated in the internal regions of the fruit in turn lowers effective SPC because of the decreased CO_2 concentration gradient between the internal and the immediate external environment, subsequently reducing CO_2 transfer rates.

Like the effective SPW, values of effective SPC of both coated and non-coated okra increased with increasing temperature (Figure 3). Amongst the control okra, the SPW values measured at 35ºC were lower than those measured at 25ºC. The similar trend was observed in the respiration values (Figure 2) and these could be mainly attributed to senescence of okra which was stimulated when they were kept at 35ºC. The Arrhenius relationship described adequately the experimental data supported by the values of RMSE which were lower than 2 (Table 1). The activation energy of non-coated okra ($Ea_{P^{eff}_{CO_2}}$) was approximately 2-fold high than that of CB-Wax coated okra (Table 1). The experimental data suggested that non-coated okra were more sensitive to temperature changes compared to CB-Wax coated fruit. It can be noticed that the Arrhenius model overestimated values of the effective SPW of non-coated okra measured at 10ºC. Similar prediction of the Arrhenius model was observed in Figure 2. The overestimations among the effective SPW data could be results of effects of the chilling injury on respiration rates of non-coated fruit at 10ºC as discussed previously in the respiration rate.

Conclusion

The experimental results showed that both CB-Wax coating and storage temperatures had significant effects on water vapour and CO_2 gas exchange properties of fresh okra. Applications of CB-Wax as a surface coating material reduced effective skin permeances to both water vapour and CO_2, as well as respiration rates. The CB-Wax developed in this study shows potential benefits for incorporation in future postharvest cool chain management for reducing sensitivity of fresh okra to temperature fluctuations.

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