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Gas Transmission Rates of Mango (*Mangifera indica* L. 'Carabao') Peel as Affected by Chitosan-Nanosilica Composite Fruit Coating

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ABSTRACT

Direct measurement of gas transmission rates of coated commodity under controlled environmental conditions is needed in formulating coatings to extend shelf life of fruits. The gas transmission rate of coated commodities, along with its respiration rate can be used to predict the level of modification of internal atmosphere and the quality of the product during storage. Oxygen (O_2) and carbon dioxide (CO_2) transmission rates of mango (Mangifera indica L. cv Carabao) peel coated with different formulations of chitosan-nanosilica at three storage temperatures were estimated using Exponential Decay Method. The coating was prepared by dissolving chitosan (CS) in aqueous solution of glacial acetic acid (0.25% v/v) and blending with nanosilica (NS) dispersion. The resulting films have microcracks and agglomeration of NS throughout the surface. 0.75% CS at 25°C had the highest gas transmission rate (1.136 $mLO_2\ cm^{-2}\ h^{-1}$ and 3.243 $mLCO_2\ cm^{-2}\ h^{-1}$) whereas 1% CS+ 0.03% NS at 15°C had the lowest gas transmission rate (0.406 $mLO_2\ cm^{-2}\ h^{-1}$ and 1.586 $mLCO_2\ cm^{-2}\ h^{-1}$). At constant temperature, increasing chitosan concentration decreases O_2 and CO_2 transmission rates by almost 44% and 41%, respectively, whereas the incorporation of NS decreases the O_2 transmission rate by 10% to 20% and CO_2 transmission rate by 5% to 14%. Gas transmission rates were highest at 25°C and lowest at 15°C.

Keywords: Chitosan, Fruit Coatings, Nanosilica, Oxygen and Carbon Dioxide Transmission Rate

INTRODUCTION

One of the most employed postharvest treatment/ MAP for fruits and vegetables is fruit coating (Aramante *et al*., 2001). It is a form of modified atmosphere packaging (MAP) that involves the modification of atmospheric condition inside the produce, usually by O_2 reduction or CO_2 elevation to prevent or minimize deterioration of commodity. Some of the benefits of fruit coatings include delayed ripening of fruits and vegetables, retarded softening of tissues, reduced rates of respiration and ethylene production, and delayed various compositional changes related to ripening (Ben-Yehoshua *et al*., 1993). Fruit coating causes a depletion of O_2 and a buildup of CO_2 within the fruit by restricting the gas exchange between the fruit and the surrounding atmosphere, thus causing an effect like MAP (Aramante *et al*., 2001). During MAP, O_2 passes through the film slower than CO_2 since it diffuses 2-6 times slower than CO_2. This results to low O_2 level and relatively low CO_2 in a MAP at steady state (Zagory, 1997).

To optimize the benefits of modified atmosphere packaging, knowledge on the respiration rate of the commodity, storage temperature and gas transmission rate of the film or coating must be understood. Respiration rate of fruits depends on the concentration of gases in the surroundings and the ability of the barrier-like peel to restrict the movement of gases between the fruits and the surrounding. Gas transmission rate, on the other hand, is the amount of gas that passes through a film per unit area over time. There are various models that have been developed to determine barrier properties of packaging materials, coatings and even fruit peels (Ben-Yehoshua *et al*., 1993). The model like Exponential Decay Method follows the Fick's law to predict the barrier characteristics of films (Moyls *et al*. 1992). This law states that the flux of gas which diffuses through a barrier is dependent on the fruit thickness, effective area of diffusion and its resistance, and the difference in concentration of gases between the two sides of the barrier.

Fruit coatings must have balanced gas permeability properties to allow a normal exchange of O_2 and CO_2, limited water vapor permeability to inhibit the escape of moisture and antimicrobial activity, and good adhesion to fruit surface (Arnon-Rips and Poverenov, 2018). Several authors have published values of water and gas diffusion characteristics of coating films (Hagenmaier and Shaw, 1991; Hong and Krochta, 2006) but limited information on gas diffusion characteristics of coated commodities. Gas transmission rates of coating films and coated commodities cannot be compared because the measurement of barrier properties of coating films were carried out using different techniques in a range of environmental conditions of temperature, RH and gas partial pressures that are very different during storage of coated products (Aramante *et al*., 2001; Hong and Krotcha, 2006). Hence, for optimization of surface coating for fruits and vegetables, direct measurement of gas transmission rates of coated commodity under controlled environmental conditions should be employed.

The objective of this study was to determine the O_2 and CO_2 transmission rates of mango peel as affected by composite coatings of chitosan (CS) polymer and nanosilica (NS) filler under different storage temperatures.

MATERIALS AND METHODS

Gas Diffusion Chamber

The gas diffusion chambers were based on Flores *et al*. (2014) as seen in Figure 1a. It was made of 152.4 mm diameter polyvinyl chloride (PVC) pipe, 9.5 mm thick clear acrylic sheet with three 25 mm holes where the samples were fitted, and 1 mm thick galvanized iron sheet. The sample cover was made of nylon rod and metal screws were used to hold the sample cover and the acrylic sheet (Figure 1.b). The average volume of the chambers was 1860.87 cm^3 and the effective gas diffusion area was 7.18 cm^2.

Leakage Testing

A leak test in the chambers was made to ensure that the exchange of gases inside the chamber and the surrounding air will only occur through the peel sample. To lower the O_2 level inside the chamber, pure N_2 gas was flushed for 10 minutes or until O_2 level inside the chamber reached 5%. The gas concentration was measured using PBI Dansensor Checkmate III gas analyzer at time 0, 1 hour, 17 hours, and 24 hours. Leakage was computed using Equation 1; where $\%O_{2i}$ = initial oxygen level in the chamber and $\%O_{2f}$ = final oxygen level in the chamber (Moyls *et al*., 1992).

$$\%\ leakage\ \left(\frac{\%O_2}{hr}\right) = \frac{(\%O_{2i} - \%O_{2f})}{t} x\ 100$$

Equation 1

Preparation of Coating Solutions

High molecular weight CS powder (Sigma-Aldrich, United States) was used as polymer matrix while amorphous NS powder (99.5% purity, 303.89 m^2/g SA, 16.97 Å pore radius) provided by UPLB Nanotechnology Laboratory was used as filler material. Different formulations of CS-NS composite fruit coatings were prepared by dissolving CS powder (0.75% and 1%) in aqueous solution of glacial acetic acid (0.25% v/v) and stirring for two hours using magnetic stirrer under 800 rpm at 60°C to form a viscous solution (Song *et al*., 2016). In a separate container, 0.03% NS-in-fluid dispersion was prepared by stirring the solution for 30 minutes using magnetic stirrer under 800 rpm at 30°C before sonicating for 5 hours using an ultrasonic vibrator to ensure complete dispersion (Ignacio *et al*., 2014). The CS and NS solutions were mixed to achieve the desired formulations of 1% CS, 1% CS+0.03% NS, 0.75% CS, 0.75% CS+0.03% NS. Afterwards, the solution was amended with 0.1% (w/v) Tween 80 and stirred at room temperature for 30 minutes and adjusted to pH 5.6 by adding 1 M NaOH. The surface morphology of the 1% CS and 1% CS+0.03% NS was observed under scanning electron microscopy (SEM).

Preparation of Peel and Coating Samples

Only full green and mature mangoes were used in the experiment. Flotation method in 1% NaCl solution was used to determine the maturity of the fruits. A cylindrical disc corer made of stainless steel was used to obtain the peel samples from the fruit surface with an average thickness of 1.50 mm and diameter of 25 mm. For obtaining the transmission rate of peel with coating, the 1.5 mm peel was dipped in the coating solution for one minute, ensuring that only the outer surface of the peel was coated. The thickness of the coating was determined by measuring individually the mass of coating that adhered to ten 2.5 cm-diameter peel samples, and by ratio and proportion, the equivalent amount of coating for a 90 cm-diameter Petri dish was determined. The computed mass of coating was poured in the Petri dish and allowed to dry evenly in a flat surface. A bubble level was used to ensure the level of the surface. The dried coating was peeled off from four random spots in the petri dish and the thickness was measured using a micrometer (0-1” range 0.00005” resolution, Fowler High Precision, United States). Five replicates were made for each coating formulation.

Gas Flushing and Sampling

To lower the O_2 level inside the chamber, pure N_2 gas was flushed for 20 seconds using a nitrogen generator (Domnick Hunter, United Kingdom) attached to a needle that is inserted to the rubber septum of the chamber. While flushing, another needle was inserted to the other rubber septum of the chamber which served as an outlet of the flushed gas. The chamber was settled for 30 seconds to avoid bulging of samples due to high pressure. The procedure was repeated for 10-15 minutes or until the O_2 level dropped to 5%. Following the

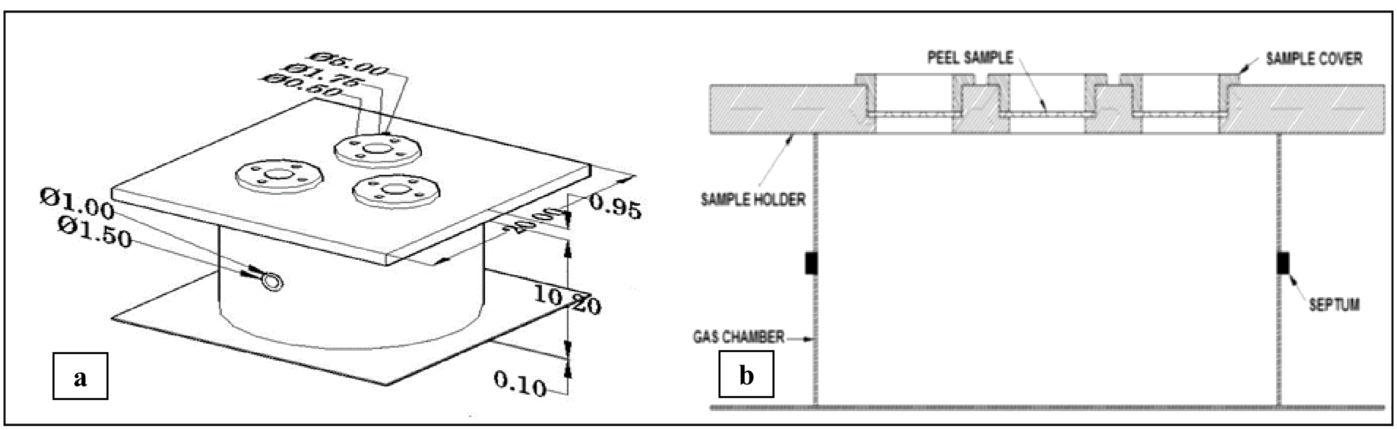

Figure 1. (a & b) Schematic diagram of the gas diffusion chamber showing the dimensions in cm and the parts (Flores et al., 2014).

procedure for N_2 gas flushing, the chamber was then flushed with gas mix until it reaches 5% O_2 and 5% CO_2. The chambers were placed in plastic trays and were partially covered with plastic sheets to ensure still air above the peel samples. The gas concentration inside the gas chamber (under 15˚C, 20˚C and 25˚C storage temperatures) was measured every hour for 12 hours using PBI Dansensor Check Mate III (AMETEK MOCON, Denmark) gas analyzer. The air temperature and relative humidity in the cold room were monitored every using EL-USB-2 RH/TEMP Data Logger (Omega Engineering, United States).

Gas Transmission Rate Computation

The gas transmission rate of films is described by Moyl's Exponential Decay Method, where Fick's law was modified to relate the gas transmission rate with the partial pressures of the gas concentration inside the chamber and the outside condition (Moyls *et al*., 1992). Plotting $\ln \Delta P/\Delta P^o$ versus time using Microsoft Excel, the slope was obtained which represents the exponential decay constant of the partial pressure difference across the peel. The peel transmission rate is given by Equation 2:

$$Trans\left(\frac{mL}{cm^2 h}\right) = -\frac{SV}{A}$$

Equation 2

where, S = slope, A = effective area of the film in cm^2 and where V = volume of test cell or chamber in mL.

Statistical Analysis

The statistical analysis was performed using R (Version 3.5.3) software. The analysis of variance (ANOVA) was used to evaluate the variation among measurements and significant differences were analyzed using the Tukey's Honestly Significant Difference (HSD) Test at $p<0.05$ (Flores *et al*., 2014).

RESULTS AND DISCUSSION

Surface Properties of the Films

The SEM micrographs of the 1% CS and 1% CS+0.03% NS films are shown in Figure 2. It is shown that under 1000x magnification, the 1% CS film have microcracks and discontinuities throughout its surface that could be due to the existence of pores in pure chitosan film (Martínez-Camacho *et al*., 2010). On the other hand, 1% CS+0.03% NS film shows a high degree of agglomeration of the nanoparticles throughout the film due to high surface energy of the nanoparticles (Friedrich *et al*., 2005). The measured diameter of the NS particles ranges from 22 to 78 nm.

Leakage Testing

Nine chambers are positive to very minimal leakage after 24 hours, averaging to 1.92%. Since the average value is less than the allowable leakage value of 4%, no correction on the partial pressure in the gas chambers was applied. Also, since the volume of the gas samples extracted from the chamber is only 7 mL from the total volume of 1860.87 mL, there was no correction applied for the change in partial pressure in the chamber when reading (Moyls *et al*., 1992).

Coating thickness

The thickness of the different coating formulations is shown in Table 1. Analysis of variance (ANOVA) shows that coating type significantly affects the thickness of coating. Increasing the concentration of CS means increasing thickness while incorporating NS filler does not affect the thickness of the coating. Higher concentration of CS in acetic acid solution yields to higher viscosity at constant temperature (El -Hafian *et al*., 2010). CS is essentially composed of cellulose which gives a high magnitude of intrinsic viscosity (Hwang and Shin, 2000). Previous study concluded that thickness increases with increasing chitosan concentration that leads to an increased resistance to mass transfer across the films (Casareigo *et al*., 2007).

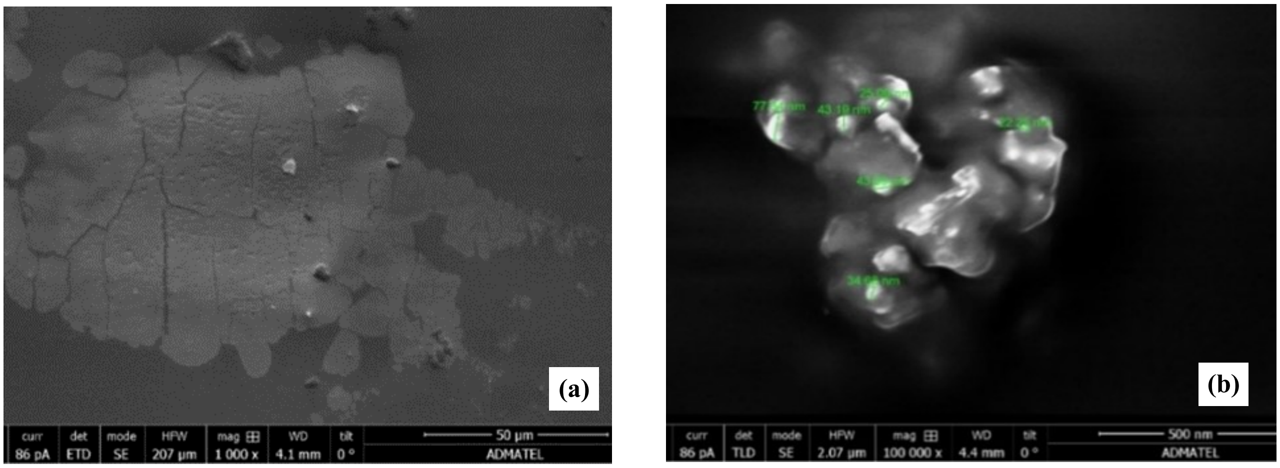

Figure 2. SEM micrographs of (a) surface of 1%CS at 1kx magnification and (b) SEM-EDX analysis of 1%CS+0.03%NS coating at 100kx magnification.

Table 1. Measured average thickness of the coatings

COATING	MEAN ± SE
1% CS+0.03% NS	3.7 ± 0.3 a
1% CS	3.6 ± 0.1 a
0.75% CS+0.03% NS	2.7 ± 0.1 b
0.75%CS	2.4 ± 0.1 b

In a column, means with a common letter are not significantly different at the 5% level by Tukey's HSD.

Table 2. Measured peel transmission rate of mango peel at different temperatures.

TEMPERATURE (°C)	TRANSMISSION RATE ($mL\ cm^{-2}h^{-1}$)		SOURCE
	O_2 Mean ± SE	CO_2 Mean ± SE	
15	1.68 ± 0.12 b	3.29 ± 0.11 a	Experimental
20	2.16 ± 0.10 a	3.80 ± 0.19 a	
25	2.39 ± 0.08 a	3.71 ± 0.11 a	
14	1.11 ± 0.04	3.51 ± 0.18	Flores et al. (2014)
27	1.43 ± 0.02	3.76 ± 0.17	

In a column, means with a common letter are not significantly different at the 5% level by Tukey's HSD.

Gas Transmission Rate of Mango Peel

The average temperature and relative humidity inside the three cold rooms were 15.05±0.17°C and 85.43±1.10% RH, 19.67±0.93°C and 76.18±4.64% RH, and 24.77±0.69°C and 65.49±3.87% RH, respectively. The measured O_2 and CO_2 transmission rate of the mango peel at different storage temperatures are summarized in Table 2. Gas transmission rates are increasing with increasing temperature, except for CO_2 transmission rate at 20°C which is slightly higher than at 25°C. Plotting the trend of gas transmission rates with temperature, it is still evident that temperature is directly proportional with transmission rate value and lowest at 15°C (Figure 3). ANOVA shows that temperature has a significant effect ($p<0.05$) on O_2 and CO_2 transmission rates through the peels. High temperature increases the energy level of permeating molecules, thereby increasing the permeability values (Rogers, 1985 as cited by Gennadios *et al*., 1993). The values of the measured gas transmission rates of mango peel are close to published data of Flores *et al*. (2014). The average O_2 and CO_2 transmission rate of mature mango with peel color index 1 (green) was higher at 27°C than at 14°C.The slight variation in the published data with measured data may be due to the differences in time and place of harvest of fruits during the experiment. In a study on 'Braeburn' apple fruits, more than 50% of the total variation in water vapor permeance was associated with harvest date, 22% was accounted to fruit to fruit differences, and 7% was due to the interaction between harvest date and orchard effects (Maguire *et al*., 2000).

Gas Transmission Rate of Coated Mango Peel

The summary of O_2 and CO_2 transmission rates of mango peel coated with 0.75% CS and 1% CS, with and without 0.03% NS, stored at 15°C, 20°C and 25°C are shown in Tables 3 to 5. It is evident that in all temperature regimes, O_2 and CO_2 transmission rates are higher in peel coated with 0.75% CS than 1% CS. Also, the incorporation of 0.03% NS particles in the CS solution decreased the O_2 transmission rate by 10% to 20% and CO_2 transmission rate by 5% to 14%. ANOVA indicated that CS and NS concentrations have a significant effect ($p<0.05$) on both O_2 and CO_2 transmission rates of the coated peels with the relationship being indirect. This means that increasing the amount of CS or NS decreases the gas transmission rate of the peel.

The presence of CS on the surface of starch-blown films improved its morphological characteristics and reduced water vapor and O_2 barrier properties by reducing the surface hydrophilicity of the film (Dang and Yoksan, 2016). With increasing amount of CS (0.37%- 1.45%), the water vapor permeability and O_2 permeability were significantly reduced by 32% and 40%, respectively. On the other hand, *in situ* addition of 0.6% silica particles in polyvinyl alcohol-CS biodegradable films reduced O_2 and moisture permeability by 25.6% and 10.2%, respectively (Yu *et al*., 2018). Another factor that affects the gas transmission rates in barrier materials such as peel and coatings is thickness (Ray and Okamoto, 2003). The measured thickness of 1% CS was 3.65 µm and 2.55 µm for 0.75% CS. This means that the effective path length for diffusion is longer when peels are coated with higher concentrations of CS. Therefore,

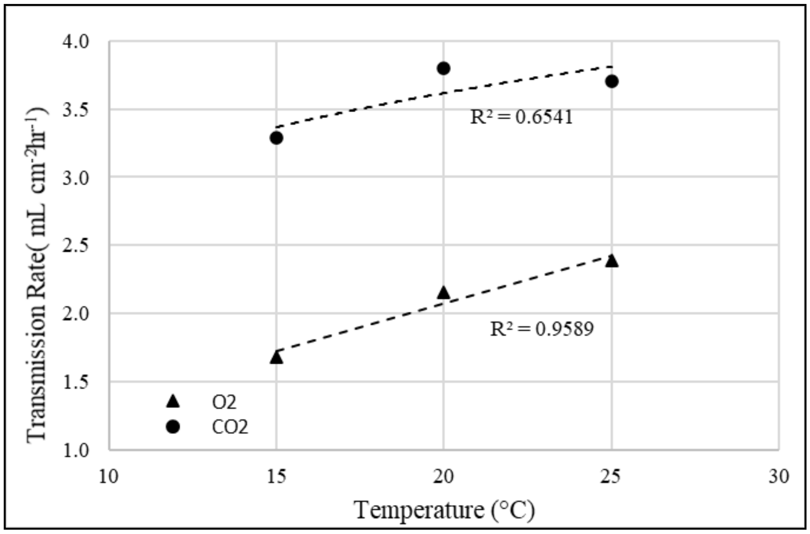

Figure 3. Temperature and gas transmission rate relationships of mango peel.

Table 3. Transmission rate of peel with different formulations of coating at 15°C storage temperatures.

CS CONCENTRATION	NS CONCENTRATION	GAS TRANSMISSION RATE ($mL\ cm^{-2}h^{-1}$) O_2	CO_2
0.75%	0%	0.814 a	3.006 a
	0.03%	0.656 b	2.845 a
1%	0%	0.450 c	1.681 b
	0.03%	0.406 c	1.586 b

In a column, means with a common letter are not significantly different at the 5% level by Tukey's HSD.

Table 4. Transmission rate of peel with different formulations of coating at 20°C storage temperatures.

CS CONCENTRATION	NS CONCENTRATION	GAS TRANSMISSION RATE ($mL\ cm^{-2}h^{-1}$) O_2	CO_2
0.75%	0%	0.935 a	3.106 a
	0.03%	0.833 a	2.905 a
1%	0%	0.528 b	1.915 b
	0.03%	0.449 b	1.672 b

In a column, means with a common letter are not significantly different at the 5% level by Tukey's HSD.

Table 5. Transmission rate of peel with different formulations of coating at 25°C storage temperatures.

CS CONCENTRATION	NS CONCENTRATION	GAS TRANSMISSION RATE ($mL\ cm^{-2}h^{-1}$) O_2	CO_2
0.75%	0%	1.136 a	3.243 a
	0.03%	0.989 a	3.043 a
1%	0%	0.595 b	2.024 b
	0.03%	0.534 b	1.741 b

In a column, means with a common letter are not significantly different at the 5% level by Tukey's HSD.

the measured O_2 and CO_2 permeabilities decrease with increasing chitosan concentration (Casariego *et al*., 2007).

It is evident from Figure 4 and Figure 5 that the gas transmission rate is directly proportional with temperature as supported by the high value of R^2 which ranges from 0.95-0.99. Storage at low temperatures slows down the gas transmission rate through the coated peel. Previous studies similarly reported significant increase in O_2 permeabilities of edible films from corn zein, wheat gluten, and wheat gluten/soy protein isolate at higher temperatures (Gennadios *et al*., 1993). High temperature enhances the motion of the polymer segments and increases the energy level of permeating molecules, thereby increasing the permeability values (Rogers, 1985 as cited by Gennadios *et al*., 1993).

Based on Figure 6 and Figure 7, the O_2 transmission rate of coated peel is reduced to over half of the value of the uncoated peel. Coatings exert their effect on the peel permeance to gases by blocking the lenticels and improving the coverage of cracks in the cuticle (Aramante *et al*., 2001). Polysaccharide and carnauba wax coatings exhibited lower permeance to O_2 and CO_2 than uncoated mango fruit (Baldwin *et al*., 1999). The improvement in gas barrier properties of CS coatings with NS fillers in films is due to the presence of the ordered dispersed silicate layers with large aspect ratios in the polymer matrix. This forces the gas travelling through the film to follow a tortuous or maze-like path through the polymer matrix surrounding the NS particles thereby increasing the effective path length for diffusion (Ray and Okamoto, 2003). Figure 8 represents a schematic description to explain the way in which inorganic nanomaterials like NS interact in an edible coating. NS fillers reinforce the network of the base film by creating hydrogen bonds between the NS and chitosan polymer matrix (Zambrano-Zaragoza *et al*., 2018).

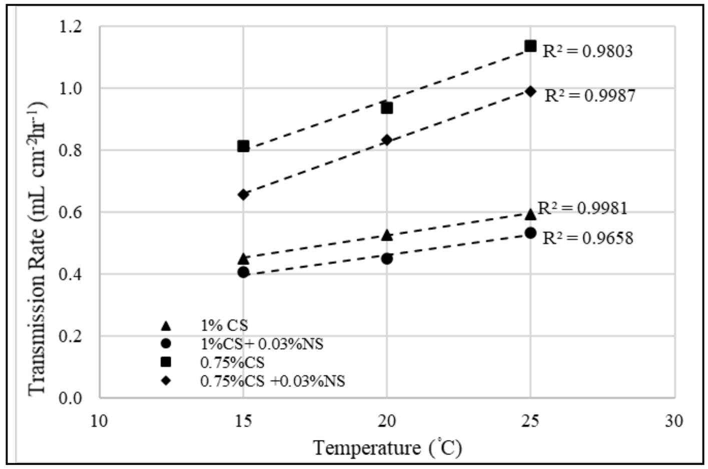

Figure 4. Effect of temperature on O_2 transmission rate of mango peel coated with CS and NS composite.

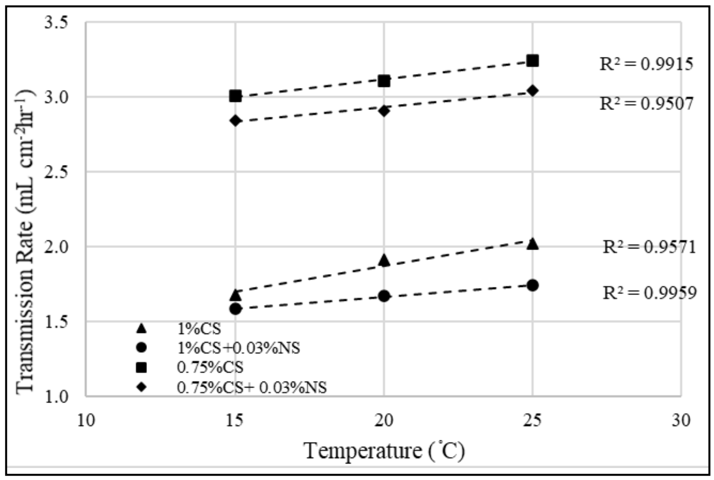

Figure 5. Effect of temperature on CO_2 transmission rate of mango peel coated with CS and NS composite.

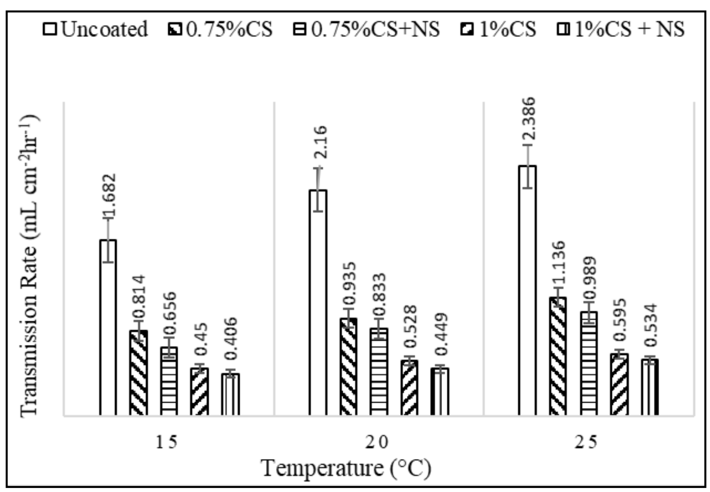

Figure 6. O_2 transmission rate of coated and uncoated mango peel at different storage temperatures. Bars represent the mean ± SD of six replicate samples.

CONCLUSION

Different formulations of CS-NS coating were prepared via solution blending and characterized by SEM. The produced composite films were transparent, smooth and continuous. The films exhibited microcracks and agglomeration of NS particles throughout its surface as observed under SEM.

The gas transmission rates of mango peels coated with the CS-NS composite fruit coating (0.75% CS, 0.75% CS+0.03% NS, 1% CS and 1% CS+0.03% NS) were measured at three storage temperatures (15°C, 20°C and 25°C) using Exponential Decay Method. Results showed that 0.75% CS at 25°C had the highest gas transmission rate (1.136 mLO_2 $cm^{-2}h^{-1}$ and 3.243 $mLCO_2$ $cm^{-2}h^{-1}$) while 1% CS+0.03% NS at 15°C had the lowest gas transmission rate (0.406 mL O_2 $cm^{-2}h^{-1}$ and 1.586 $mLCO_2$ $cm^{-2}h^{-1}$). At 15°C, the gas transmission rate of 0.75% CS+0.03% NS (0.656 mLO_2 $cm^{-2}h^{-1}$ and 2.845 $mLCO_2$ $cm^{-2}h^{-1}$) is significantly higher than 1% CS+0.03% NS. The incorporation of 0.03% NS particles decreased the O_2 transmission rate by 10% to 20% and CO_2 transmission rate by 5% to 14%. Gas transmission rates were highest at 25°C, followed by 20°C and lowest at 15°C.

CS and NS concentrations and temperature have significant effect on O_2 and CO_2 transmission rate of the mango peel and coating solutions. Increasing CS and NS concentrations significantly reduced the gas transmission rates of the coating while increasing the temperature significantly decreased the gas transmission rates of the coating.

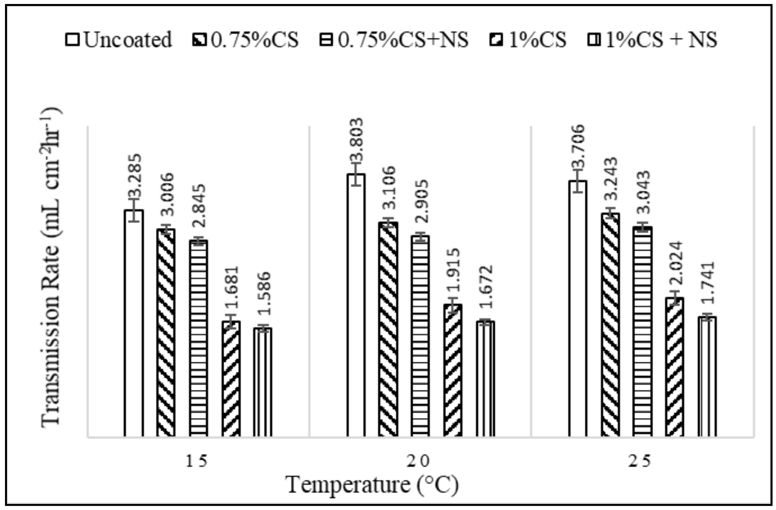

Figure 7. CO_2 transmission rate of coated and uncoated mango peel at different storage temperatures. Bars represent the mean ± SD of six replicate samples.

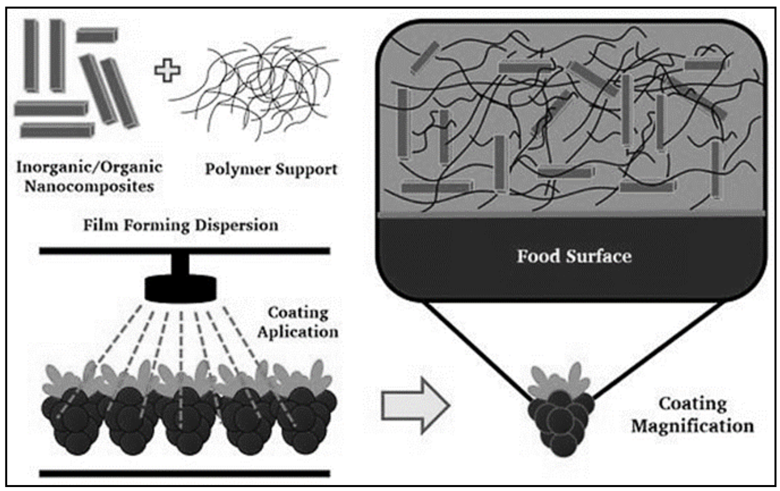

Figure 8. Inorganic nanocomposites incorporated in edible coating (Zambrano-Zaragoza et al., 2018).

RECOMMENDATIONS

The preparation of the nanocomposite coating is very critical as nanoparticles are highly agglomerated especially when applied directly in a bulk composite. It is recommended that other preparation techniques like in situ polymerization be tried and other solvents such as formic and lactic acid be used to improve the dispersion of the nanoparticles in the polymer matrix.

Gas transmission rates through barrier materials such as peel and coatings are greatly affected by relative humidity. To have an accurate measurement, relative humidity should be set constant in the three storage rooms.

The gas transmission rates of the peel coated with CS-NS can be further used in determining the compatibility of a formulated CS-NS composite as coating for mango fruits to extend postharvest life.

The required gas transmission rate of mango fruit can be determined under steady state conditions, where the O_2 diffusing into the packaging film is equal to the O_2 consumed by the commodity during respiration.

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