



Environmentally Friendly and  
Safe Technologies for Quality  
of Fruits and Vegetables

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Authors are responsible for content and accuracy of their papers.

**Proceedings of the International Conference “Environmentally friendly and safe technologies for quality of fruit and vegetables”**, held in Universidade do Algarve, Faro, Portugal, on January 14-16, 2009. This Conference was a joint activity with COST Action 924.

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SECTION 4. ENVIRONMENTALLY FRIENDLY AND SAFE  
METHODS TO CONTROL POSTHARVEST LOSSES

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## 28. EFFECT OF COATING APPLICATION ON THE GAS FLOW AND *P. DIGITATUM* GROWTH IN LEMON CV. 'VERNA'

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### Abstract

All citrus fruits are waxed during postharvest, independent of their final destination for reducing the weight loss. Any physical barrier applied on the surface can have a significant influence on the various metabolic pathways acting on the fruit-environment system. The effect of applying a wax coating on the percentage of blocked stomata and the diffusivity of water vapor was studied in lemon fruits cv. 'Verna', healthy and inoculated with *P. digitatum*. In addition, the growth of *P. digitatum* was modelled with the Gompertz equation modified to three parameters for non-linear regression. The analysis of the percentage of blocked stomata based on the respiratory gas exchange in the healthy coated fruit, suggests that the obstruction is transient. The coating application significantly reduced water vapor diffusivity as a result of the reduction in the gas permeability of fruit peel, being this parameter affected by the mould development in the skin fruit surface. Wax coating significantly delayed the lag phase and reduced the rate of relative growth of *P. digitatum*.

**Keywords:** citric, decay, stomata, water diffusivity, water wax

### Introduction

Citrus fruits are waxed during postharvest manipulation for storage and direct commercialization. The waxing process reduces weight loss, increase fruit resistance during manipulation, and gives an intense shine attractive to the consumers. The interface between the fruit and its environment is very complex due to the diversity of flows of matter and energy across the interface, and the major internal metabolic activity. This complexity can be increased by the application of physical barriers on fruit surface and by atmospheric composition that surrounds it, which can influence the different metabolic pathways involved with it. In the closed system fruit-environment it can be defined the input of O<sub>2</sub>, output of CO<sub>2</sub> and input and output of H<sub>2</sub>O. The flow of CO<sub>2</sub> and O<sub>2</sub> are due to the respiration process, while water flow is mainly caused by dehydration of fruit surface that will depend on environment temperature and relative humidity. The mechanism by which the coating restricts gas exchange depends not only on the properties of coating (composition and thickness) and fruit, but also on its mode of distribution on the fruit surface (Banks *et al.* 1993). For the non-coated fruits lenticels, stomata, scars and injuries are probably the main route of gas exchange. For coated fruits, on the other hand, it is possible that these spaces are filled by coating (Hagenmaier & Baker 1993). The coating also influences in different ways the growth of microorganisms. The wax film in fruit surface forms a barrier to the secondary infections during the large periods of conservation or transport. In many cases, waxed fruits show less percentage of decay and greater shelf-life than non-waxed fruits. The coating also reduces the injuries produced in the fruit surface during manipulation (Ben-Yehoshua *et al.* 1994).

The objective of this work was to evaluate the effect of applying a wax coating on the percentage of blocked stomata and the diffusivity of water vapor in lemon fruits cv. 'Verna' healthy and inoculated with *P. digitatum*; and also modeling the growth of *P. digitatum* with the Gompertz equation modified to three parameters for non-linear regression.

## Material & Methods

### Plant Material

Lemons cv. 'Verna' were used in the experiments. At the laboratory fruits were washed, dried and maintained at 13 °C. A lot of eight fruits were used to perform the analysis.

### Measurements of Gas Exchange

The respiratory gas exchange was evaluated by head space analysis (Carvalho *et al.* 2002) with a micro gas chromatograph (MicroGC HP M200 Model G2890A). Each analysis was performed during 50 min at 5 min intervals. The quantification was made with the HP EZ Chrom Chromatography Data System. After each analysis the air inside the chamber was refreshed, considering 3% of CO<sub>2</sub> as the limit concentration. All the experiments were made in a thermostatic chamber at 13 °C and 86.3% RH (10<sup>-3</sup>M HCl dissolution saturated with KCl). The initial volume of fruit was determine by the water immersion method. With the change in concentration of the respiration gases analyzed in the headspace (d[x<sub>i</sub>]/dt), the diary flux of each gas through the interface (N<sub>i</sub> kmol i kg<sup>-1</sup> h<sup>-1</sup>) was determine by the following equation:

$$N_{i|_{t_1}}^{t_2} = \frac{d[x_i]}{dt} \cdot \frac{P \cdot V_g}{M \cdot R \cdot T} \quad \text{Eq 1}$$

### Fruit Inoculation

The inoculation of *P. digitatum* was made using the strain 2594 provided by the Spanish Type Culture Collection of the University of Valencia. A spore suspension was prepared and adjusted to 1×10<sup>6</sup> spore mL<sup>-1</sup> using a hemacytometer. Four wounds were made in the fruit equator zone and each wound was inoculated with 20 μL of suspension. The lesion growth was monitored measuring the concentric lesion diameter formed around each wound with a caliper and expressed as mean diameter in cm.

### Coating Treatment

The fruits were coated with a commercial water-wax emulsion (Water Wax® - U.E.), supplied by Fomesa: shellac, polyethylene and other components with a wax and coadjutants composition of 18% (w/w). This procedure was carried out in a pilot scale equipment (Ortolá *et al.* 1999) for 30 s at 25 °C with a turn velocity of 10 rpm for rollers and 50 rpm for brushes. Then fruits were dried in a laboratory dryer (Fito *et al.* 1997) at 25 °C for 2 min with an air velocity of 1.4 m s<sup>-1</sup>.

### Statistical Analysis

Statistical procedures were performed using a commercial statistical software (Statgraphics plus 4.1, Manugistics, Inc., Rockville, MD, USA.). All data were subjected to analysis of variance, and means were compared using LSD test at  $P \leq 0.05$ .

## Results

### Percentage of Blocked Stomata

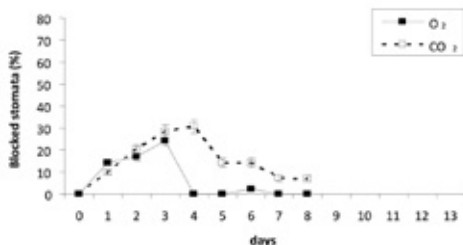
As O<sub>2</sub> and CO<sub>2</sub> flows in a gas phase mainly through the stomata to the outside of fruit, a relationship was search between the percentage of blocked stomata and coating in healthy lemon fruits. The molar flow of O<sub>2</sub> and CO<sub>2</sub> in the system was calculated by the equation 1. The flow of O<sub>2</sub> and CO<sub>2</sub> that go through the stomata could be expressed in a flow rate (Q, m<sup>3</sup> m<sup>-2</sup> s<sup>-1</sup>) according to the following equation (data not shown):

$$Q = \frac{\partial x_i}{\partial t} \cdot \frac{V_E}{S_g} \quad \text{Eq 2}$$

The opening section of the stomata will vary according to the external (surface evaporation) and internal (CO<sub>2</sub> concentration in the interior of the apoplastic way) conditions. At the same time, when the stomata are blocked by coating, the opening section is highly reduced. In this way, an adimensional parameter for stomata blocking caused by coating ( $F = Q_e / Q_{ne}$ ) was defined; being  $Q_e$  the gas flow rate in coated fruits and  $Q_{ne}$  the gas flow rate in non-coated fruits. An open stomata will have an  $F$  equal to 1 and a stomata totally blocked will have an  $F$  equal to 0. Knowing the O<sub>2</sub> and CO<sub>2</sub> flow rate of coated and non-coated fruit, the percentage of stomata blocked by the coat was calculated according the following equation:

$$\% \text{ stomata blocked} = [1 - (Q_e / Q_{ne})] * 100 \quad \text{Eq 3}$$

Figure 1 shows that the coating of lemon cv. 'Verna' originated an immediately obstruction of the stomata in the first day, with a 10-15% of blocked stomata. Nevertheless, this obstruction was transient.



**Fig 1.** Effect of coating with Water Wax® on the percentage of blocked stomata in lemons cv. 'Verna' at 13 °C and 86.3% RH.

### Water Vapor Diffusivity

As the total mass loss in the closed system is a result of the weight loss experimented by fruit during storage, a molar balance could be thought. Like this, the total molar flow ( $N_T$ , kmol m<sup>-2</sup> s<sup>-1</sup>) diffused in the closed system during the storage period was defined according to the equation reported by Fito (2002). The molar flows ( $N_i$ , kmol m<sup>-2</sup> s<sup>-1</sup>) of O<sub>2</sub> and CO<sub>2</sub> were calculated by the equation 1 (data not shown). The molar flow of water that goes through fruit skin was calculated by the following equation:

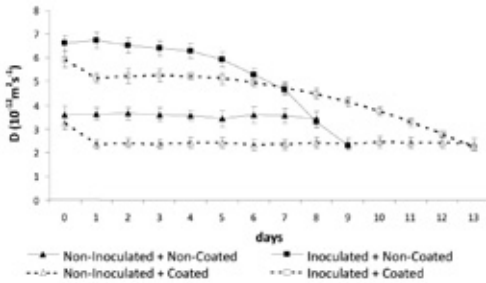
$$\frac{dN_{H_2O}}{dt} = \frac{\frac{dM_T}{dt} - 32 \frac{dN_{O_2}}{dt} - 44 \frac{dN_{CO_2}}{dt}}{18} \quad \text{Eq 4}$$

The total mass flow ( $\frac{dM_T}{dt}$ , kg m<sup>-2</sup> s<sup>-1</sup>) was determined by fruit weight loss, and the molar flow of water ( $\frac{dN_{H_2O}}{dt}$ , kmol m<sup>-2</sup> s<sup>-1</sup>) was calculated with the equation 4 (data not shown). The first law of Fick establish that the gas flow  $N_i$  diffused through a barrier is determined by the diffusivity of this gas  $D$  (m<sup>2</sup> s<sup>-1</sup>), and the concentration gradient through the barrier  $\partial C / \partial x$  (kmol m<sup>-3</sup> m<sup>-1</sup>). The system fruit-environment is stationary, so the concentration gradient is lineal (Banks 1985), and the water vapor that flow through fruit skin ( $N_{H_2O}$ , kmol m<sup>-2</sup> s<sup>-1</sup>) can be calculated by the equation:

$$N_{H_2O} = -c \cdot D_{H_2O} \cdot \left( \frac{x_{H_2O}^E - x_{H_2O}^F}{L} \right) \quad \text{Eq 5}$$

The water molar fraction of the gas phase ( $x_{H_2O}^i$ , kmol<sub>water</sub> / kmol<sub>Total</sub>) present in head space (E) and in fruit skin (F) was calculated in function of the water activity ( $a_w$ , adimensional) of salt and the water activity of

fruit skin (0.97, Cháfer 2000), according the Raoult Law (data not shown). In this way, with the molar flow of  $O_2$ ,  $CO_2$  and water vapor diffused in the system (data not shown) the water vapor diffusivity for the different treatments was calculated. Figure 2 shows that the water vapor diffusivity is reduced after coating (1<sup>st</sup> day) in both fruits (inoculated and non-inoculated) remaining constant after it. Nevertheless, while in non-inoculated fruits the diffusivity remains constant with time in inoculated fruits a significant reduction with time was observe.



**Fig 2.** Effect of coating with Water Wax® on water vapor diffusivity in lemon cv. 'Verna' non-inoculated and inoculated with *P. digitatum* ( $1 \times 10^6$  spore  $mL^{-1}$ ) at 13 °C and 86.3% RH.

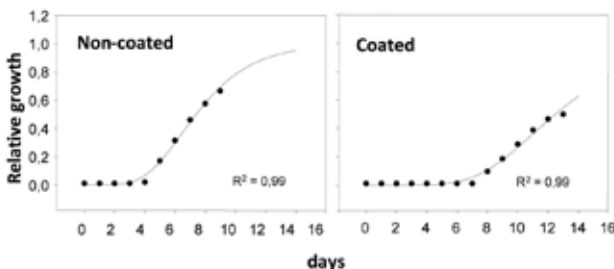
### Modeling the Growth of *P. digitatum*

The relative growth values (lesion diameter/fruit diameter) of *P. digitatum* in fruits inoculated with and without coating, were adjusted to a sigmoid line with the Gompertz equation for three parameters by non-linear regression (Sigmaplot version 8.0):

$$y = a \cdot e^{-e^{-\left(\frac{x-x_0}{b}\right)}}$$

Eq 6

The asymptote (a) was considered 1 as it is the maximum growth of the mould,  $x_0$  is the mean point of the logarithmic phase (days), being directly related with the lag phase; and  $1/b$  is the constant that define the logarithmic phase (relative growth velocity of the mould). In Fig 3, the growth of *P. digitatum* in both fruits (coated and non-coated) was compared.



**Fig 3.** Predicted values (—) versus observed values (●) of the relative growth of *P. digitatum* ( $1 \times 10^6$  spore  $mL^{-1}$ ) in lemon cv. 'Verna' coated with Water Wax® at 13 °C and 86.3% RH. Predicted values at 99% confidence level (t-Test).

The coating kept the sigmoid form of relative growth; nevertheless the lag phase was notably extended. The coating also reduced significantly the growth velocity of *P. digitatum* in fruit (0.40 for non-coated fruits and 0.26 for coated fruits); increasing at the same time the  $x_0$  (6.5 for non-coated fruits and 11.1 for coated fruits).

## Discussion

According to Ben-Yehoshua *et al.* (1985) the commercial wax is much more efficient than the natural wax obstructing the stomata because flows into the pore as a fluid. The results of this work, also suggest an obstruction of stomata; however this obstruction was transient. This phenomenon could be due to the releasing of wax plates from the pores of stomata in time as a consequence of the action of CO<sub>2</sub> flow and fruit handling. The coating significantly reduced water vapor diffusivity in the non-inoculated fruits during storage ( $2.9 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$  for coated fruit and  $3.6 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$  for non-coated fruit). In coated fruits higher values in inoculated fruits with respect to non-inoculated was also observed, as a consequence of higher weight loss registered in those fruits. Knoche *et al.* (2001), observed a reduction in the conductance of stomata to water vapor in the cuticle membrane of cherry fruit, attributed to the obstruction of stomata by wax, which would reduce the cross-section area available for the dissemination of water vapor. The fruit inoculated and coating take four days more to reach the same value of water diffusivity reach by the same fruit non-coated on day 9 ( $2.3 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$ ), suggesting that coating reduce the velocity of fruit deterioration. The coating significantly delays the lag phase and reduced the rate of relative growth of *P. digitatum*. According to McGuire & Hagenmaier (2001), the effect of wax on mould growth is mainly due to two factors: the natural physical barrier that forms the coat on the fruit surface and the composition of coat that can have a fungistatic effect.

## Nomenclature Used

P: total pressure of the chamber (atm)	V <sub>g</sub> : volume of the head space (L)
Va: air volume of the chamber (L)	S <sub>f</sub> : surface of the fruit (m <sup>2</sup> )
M: fruit mass (kg)	L: thickness of the fruit skin (m)
R: ideal gas constant (atm L kmol <sup>-1</sup> K <sup>-1</sup> )	c: molar density of the gas phase (kmol <sub>r</sub> m <sup>-3</sup> )
T: temperature (K)	

## References

- Banks NH. 1985. Estimating skin resistance to gas diffusion in apples and potatoes. *J Expl Bot* 36:1842-50
- Banks NH, Dadzie BK, Cleland DJ. 1993. Reducing gas exchange of fruits with surface coatings. *Postharvest Biol Technol* 3:269-84
- Ben-Yehoshua S, Fishman S, Fang D, Rodov V. 1993. New developments in modified atmosphere packaging and surface coatings for fruits, pp:250-60. In: *Postharvest handling of tropical fruits*. Champ BR, Highley E, Johnson GI (eds) ACIAR, Canberra, Australia
- Ben-Yehoshua S, Burg SP, Young R. 1985. Resistance of citrus fruit to mass transport of water vapor and other gases. *Plant Physiol* 79:1048-53
- Carvalho CP, Ortolá MD, Fito P. 2002. Desarrollo de un nuevo sistema analítico de respiración de frutos frescos con renovaciones de aire. *II Congreso Español de Ingeniería de Alimentos - CESIA*, Lleida, España
- Cháfer M. 2000. *Deshidratación osmótica de corteza de naranja (Valencia Late) y mandarina*. PhD Thesis. Universidad Politécnica de Valencia, España
- Fito P, Asensio S, Chiralt A. 1997. *Sistema inteligente de secado superficial de frutos autorregulable*. Patente, nº solicitud: P9701259
- Fito PJ. 2002. *Desarrollo de un nuevo sistema de aplicación de recubrimientos en cítricos*. PhD Thesis. Universidad Politécnica de Valencia. 221pp
- Hagenmaier RD, Baker RA. 1993. Reduction in gas exchange of citrus fruit wax coatings. *J Agri Food Chem* 41:283-7
- Knoche M, Peschel S, Hinz M. 2001. Studies on water transport through the sweet cherry fruit surface: II. Conductance of the cuticle in relation to fruit development. *Planta* 213:927-36
- McGuire RG, Hagenmaier RD. 2001. Shellac formulations to reduce epiphytic survival of coliform bacteria on citrus fruit postharvest. *J Food Protec* 64:1756-60
- Ortolá MD, Fito P, Fito PJ, Asensio S. 1999. *Procedimientos de la aplicación de recubrimientos sobre frutas y hortalizas y dispositivo para su puesta en práctica*. Patente, nº solicitud: P 9901778