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Rapid protocol to evaluate the photoprotective effect of film-forming formulations on mangoes

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Abstract - Sunburn is responsible for significant losses in post-harvest and has motivated studies to diminish its effect on different types of cultivars. Thus, the present study proposes a rapid protocol to evaluate the photoprotective effect of suppressive formulations. The experiment consisted in placing fruits (mango) covered with different suppressor formulations about 7 centimeters from an incandescent source and measuring the surface temperature variation employing a thermometer and thermal camera. The formulations were based on a filmogenic polysaccharide (galactomannan) and reflective additives (hydroxyapatite, montmorillonite and bentonite clays). The best result was achieved with a coating with bentonite as an additive, which showed a statistical difference in temperature (43.3 ± 2.1 °C) compared with the control (52.1 ± 3.0 °C) and the standard kaolinite coating (39.4 ± 1.7 °C). Thus, it was proven the viability of the protocol as well as of the alternative formulations in to reduce the sunburn effect. This contributes to solution for the establishment of the Tropical Agriculture. **Index Terms**: nanocomposite, galactomannan, sunburn, montmorillonite.

Protocolo rápido para avaliar o efeito fotoprotetor de formulações filmogênicas em mangas

Resumo - A queimadura solar é responsável por uma parcela significativa de perdas pós-colheita e tem motivado estudos para mitigar seu efeito em diversos tipos de cultivares. Desta forma, o presente estudo propõe um protocolo rápido para avaliar o efeito fotoprotetor de formulações supressoras. O experimento consistiu em posicionar frutos (manga) recobertos com diferentes formulações supressoras acerca de 7 centímetros de uma fonte incandescente e medir a variação da temperatura superficial por meio de termômetro e de câmera térmica. As formulações foram baseadas em polissacarídeo filmogênico (galactomanana) e aditivos reflexivos (argilas montmorilonita, bentonita e hidroxiapatita). O melhor resultado foi alcançado com revestimento, tendo a bentonita como aditivo, o qual apresentou diferença estatística de temperatura (43,3 \pm 2,1 °C) em relação ao controle (52,1 \pm 3,0 °C) e ao revestimento padrão caulinita (39,4 \pm 1,7 °C). Assim, ficou comprovada a viabilidade do protocolo bem como de formulações alternativas para diminuir o efeito da queimadura solar. Isto contribui como soluções para o estabelecimento da Agricultura Tropical.

Termos para indexação: nanocompósito, galactomanana, queimadura solar, montmorilonita.

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Introduction

The sunburn effect is responsible for considerable economic loss in many orchids around the world. Mainly in the tropical region, when the air temperature is above 27 °C, allowing fruit surface temperature to reach 41 °C (ALDEBENITO-SANHUEZA et al. 2016). The intense sun exposure can cause physiological changes and compositional variation of the surface pigment (TARTACHNYK et al. 2012). Furthermore, fruits with sunburn symptoms are predisposed to develop postharvest disorders mainly due to the fungal attack (LAL; SAHU 2017).

To mitigate such a problem, studies were done with crops of economic importance, such as apple (ALDEBENITO-SANHUEZA et al. 2016; RACSKO; SCHRADER 2012), pineapple (LOPES et al. 2014), pomegranates (SHARMA et al. 2018) and pears (COLAVITA et al. 2011). For mangoes, despite its economic importance, few works are issuing this problem, except one recommending wool-lined plastic caps (SILIMELA; KORSTEN 2005) and other, more recently, comparing kaolin and Screen Duo® coatings in a field study with Keitt mango (BAIEA et al. 2018). In general, the sunburn prevention is based on suppressing techniques such as shade nets, fruit bagging and particle films, e.g., kaolin clay (RACSKO; SCHRADER 2012). The last one, although efficient (GLENN; PUTERKA 2005), shows, as a major drawback, the difficulty of completely removing the white residues that resemble for the consumer some pesticide residue.

Considering the time-consuming field experiments to discriminate potential coating to suppress the sunburn effect, this work proposes a fast method to evaluate the coating effectiveness on mangoes (*Mandifera indica* L.). It is based on the radiation incidence of the incandescent electric bulb. Further, considering the good filmingform property of very common polysaccharides such as galactomannans (GLM), combined with the reflexive property of clay minerals, it is proposed some functional composite material suitable for sunburn protection. Clay minerals such as montmorillonite (DORNELAS et al. 2015), hydroxyapatite (TEIXEIRA et al. 2017) and kaolinite (GLENN; PUTERKA 2005) has been studied due to its reflexive property mainly as sunscreens ingredients or suppressor for sunburn prevention.

Materials and methods

Plant Material and Chemicals

The mango (*Mandifera indica* L.) was harvested at the experimental station of the Embrapa Semiarid at physiological maturity, characterized by a light green background (maturation stage 2). After the harvest, the fruits were transported to the Embrapa Semiarid Post-Harvest Physiology Laboratory and submitted to the analysis without previous treatment. The experiment was done in the period of March/2019 to December/2019.

The clay minerals were kaolinite (KLT, positive control), hydroxyapatite (HAP), montmorillonite (MMT) and bentonite (BTT). Bentonite was acquired as a commercial product and used as received. Montmorillonite was isolated from clay soil (EMBRAPA, 2011). Hydroxyapatite was synthesized from Ca(NO₃)₂ and (NH₄)₂HPO₄ solutions in stoichiometric concentrations of 1M, under nitrogen flow and pH 11 (CRUZ et al. 2014).

The filmogenic polysaccharide matrix was galactomannan (GLM) extracted from mesquite gum (*Prosopis juliflora* (Sw.) [D.C.]) with hot water at a proportion of 50 g of powdered seed to 300 mL of water. After the extraction and filtering, the GLM was precipitated with ethanol and freeze-dried (SOUZA FILHO et al. 2013).

Proposed Apparatus for the Rapid Photoprotection Effect Evaluation on Mangoes

The apparatus for sunburn evaluation was constituted by an incandescent lamp (100 W) positioned equally 7.0 cm away from 4 fruits (Figure 1).



Figure 1. Picture of the apparatus used to evaluate the sunburn effect on mangoes.

The surface fruit temperature was taken with a portable infrared laser thermometer (Instrutherm®, TI-550, São Paulo, Brazil) at the time interval of 1, 2 and 4 hours. Also, the infrared picture of the fruit was taken with help of a thermography camera (FLIR ® Systems, Inc., TG165, Oregon, USA).

Evaluation of Different Clay Addition on the Mango Photoprotection

The first study was conducted to determine the effectiveness of two kind of clay additive as photoprotective additive. Based on the literature (LIMA et al. 2010) and preliminary tests, it was prepared two formulations with HAP and MMT at fixed concentration of 0.1% (w/v). The solution matrix consisted of GLM dissolved in deionized water at concentrations of 5.0 mg mL⁻¹. The coating was applied with a paintbrush. For comparison, it was adopted two controls, a negative one, consisted of samples without coating (CTL) and other positive, made of a highly concentrated kaolinite suspension (KLT) in water, generally used in the field for mango protection. The experimental design was completely randomized in a 4x3 factorial design (coating x exposition time), with three replicates each composed of two fruits. The data were submitted to analysis of variance (ANOVA), checking for the F test significance and the averages were compared by the Tukey test at 5% probability (Origin 9.0).

Evaluation of the Galactomannan and Clay Concentrations on the Mango Photoprotection

This second study was conducted to evaluate the best GLM and clay additive concentrations that resulted in active photoprotection effect. Due to the close similarity found in the first experiment, only HAP was chosen for this study. The influence of the GLM concentration was done with three different GLM concentrations at 5.0, 10.0 and 15.0 mg mL⁻¹ in deionized water, having fixed HAP concentration at 0.1% (w/v). The experiment was arranged as described in the first experiment, except the photoprotection effect that was evaluated only after 4 hours of light incidence.

Based on the best photoprotection effect, the 10.0 mg mL⁻¹ GLM concentration was chosen for the determination of the best clay additive concentration. In this way, it was prepared three different proportions at 0.1, 0.5 and 1.0% (w/v) of the clay minerals HAP in the formulations. The experiment was arranged as described above.

Final Test with Bentonite and the best Achieved Condition

Considering the best found results in the previous tests, it was proposed a final test, taking in account a row clay additive that should be cost-effective for large volume preparation. For this, it was prepared a formulation with GLM at 10.0 mg mL⁻¹ and BTT at 0.5% (w/v) in deionized water. The experiment was arranged as described in the first experiment.

Results and discussion

Influence of Different Clay on the Mango Photoprotection

Initial test made with formulation at 5.0 mg mL⁻¹ of GLM and 0.1% of clay showed that the formulations based on hydroxyapatite and montmorillonite have an intermediary photoprotector effect, when compared with the control and the positive reference, kaolinite (Figure 2). The efficiency of kaolinite to reduce the fruit surface temperature is due to its thickness. However, thick film or high concentrated kaolinite solution can cause undesirable physiological disorder and reduce the fruit quality (GLENN; PUTERKA 2005). According, particle film has a great influence on the net gas exchange, radiation reflection and stomata blockage. In this way, thin film can be advantageous to control such physiological parameters and be imperceptible to naked eyes (Figure 3).

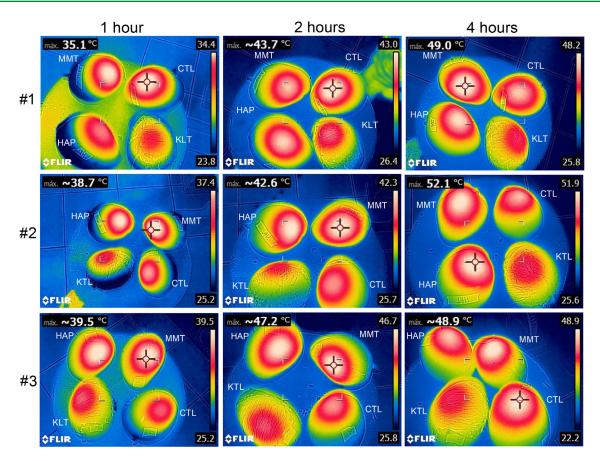


Figure 2. Thermography pictures results (triplicate) of mangoes coated with photoprotective film based on galactomannan at 5.0 mg mL⁻¹ and additive clays (CTL: control, KLT: kaolinite, HAP: hydroxyapatite and MMT: montmorillonite) at 0.1%, submitted to different periods of incandescent radiation.



Figure 3. The general aspect of mangos coated with formulations based on kaolinite (KLT), hydroxyapatite (HAP), montmorillonite (MMT) and non-coated control (CTL), after 4 hours under incandescent radiation.

The temperature analysis also confirms such behavior (Table 1). The kaolinite coating showed the lowest temperature variation. On the other hand, the coating based on montmorillonite and hydroxyapatite showed no significant difference when compared with the uncoated control. Such result was indicative that it was necessary to adjust both polysaccharide GLM and clay concentrations to improve the film effectiveness to blockage the radiation.

Time	CTL	KLT	MMT	HAP
1	34.3±0.5ª	31.0±2.0ª	38.7 ± 0.4^{b}	33.4±2.0ª
2	41.6±1.8°	40.0±0.8°	43.6±1.9°	40.0±0.6°
4	48.7 ± 4.9^{d}	46.3±3.5 ^d	51.0±3.5 ^d	48.3±1.3 ^d

Table 1. The temperature (°C) of the mango surface variation in function of time irradiation (hour) for coatings based on kaolinite (KLT), hydroxyapatite (HAP), montmorillonite (MMT) and non-coated control (CTL). The clay concentration was 0.1% (w/v) the galactomannan was 5.0 mg mL^{-1}).

Different letters in a row indicate that the means difference is significant at the 0.05 level Tukey test.

Influence of the Galactomannan and Clay Concentrations on the Mango Photoprotection

Comparing the effect of the GLM matrix concentration (Figure 4a), it is seen that the most appropriate concentration was 10.0 mg mL⁻¹. This sample resulted in the lowest temperature increasing (51.2 °C) in comparison with the control (59.0 °C) and with the other samples concentrations at 5.0 (55.7 °C) and 15.0 mg mL⁻¹ (60.2 °C), after 4 hours of exposition to incandescent radiation. For soluble polysaccharides like GLM, the solution viscosity is directly proportional to the solution concentration (LIMA et al. 2010). In its turn, the viscosity

solution will define the effectiveness of the coating on the fruit surface after formulation application. This is an empiric value, highly dependent on the kind of polymeric matrix, fruit surface hydrophilic/hydrophobic character and other physical chemistry parameters (BRITTO; ASSIS 2012; BRITTO; ASSIS 2010). For GLM sample at 5.0 mg mL⁻¹, the viscosity was low resulting in the poor coating over the fruit surface. On the other hand, for GLM at 15.0 mg mL⁻¹, the viscosity was high, forming a thick film after drying but with low adherence to the fruit surface. In this way, such concentrations were not feasible for mango coating.

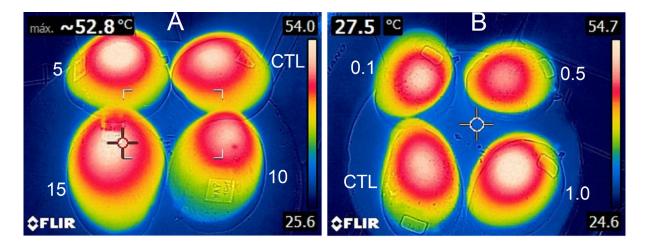


Figure 4. Thermography pictures of mangoes coated with photoprotective films based on galactomannan and hydroxyapatite at a) different galactomannan concentrations of 5.0, 10.0 and 15.0 mg mL⁻¹, at fixed hydroxyapatite concentration of 0.1% (w/v) and b) different hydroxyapatite concentrations of 0.1, 0.5 and 1.0%, at fixed galactomannan concentration of 10 mg mL⁻¹, submitted to 4 hours of incandescent radiation. 'CTL' stands for negative non-coated control.

Equally, the clay additive concentration also influenced photoprotector effectiveness (Figure 4b). Similarly, the intermediary concentration at 0.5% resulted in a better active coating on the mango surface. For the lowest HAP clay concentration (0.1%), the amount

added was not sufficient to form a uniformly distributed film, while for the highest concentration (1.0%), the film distribution had not homogeny with low adherence.

Bentonite as Active Photoprotective Coating on Mangoes

The BTT formulation showed a good suppression ability as photoprotective film (Figure 5). Also, the temperature variation showed that BTT based formulation formed an insulated layer that reduces effectively the surface temperature in comparison with the control (Table 2), mainly after 4 hours of incandescent irradiation. Bionanocomposites have been fully explored due to their good mechanical, optical and barrier properties (VILARINHO et al. 2019), but its insulator and reflexive properties have few works (SUN et al. 2019). However, according to the present results, such montmorillonitebased composite has the potential of application in these areas.

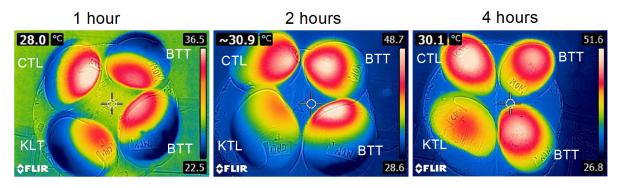


Figure 5. Thermography pictures of mangoes coated with photoprotective film based on galactomannan (5.0 mg mL^{-1}) and additive clays bentonite, BTT, (0.5% w/v), submitted to different periods of incandescent radiation. 'CTL' stands for the negative control and the 'KLT' for the positive kaolinite control.

Table 2. The temperature (°C) of the mango surface variation in function of time irradiation (hour) for coatings based on galactomannan (10 mg mL⁻¹) and bentonite, BTT, (0.5%) compared with the control (CTL) and kaolinite (KLT).

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Time	CTL	KLT	BTT
1	42.0±4.6ª	32.3±1.1ª	33.5±4.1ª
2	40.2 ± 3.0^{a}	34.3 ± 1.0^{b}	37.3±0.6ª
4	52.1±3.0ª	39.4±1.7°	43.3±2.1 ^b

Different letters in a row indicate that the means difference is significant at the 0.05 level Tukey test.

Conclusion

With the help of a simple apparatus, it was possible to evaluate the intensity of the damage caused by the incandescent irradiation similar to the sunburn effect. However, sun irradiation has other components, e.g., UV, that must be evaluated in a field experiment. The formulation based on galactomannan and bentonite was efficient to reduce the sunburn effect and can be used as an alternative to kaolinite which has the drawback of the hard work to complete cleanness from the fruit surface after post-harvesting.

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