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RENATO PEREIRA LIMA

NOVAS ABORDAGENS PARA OTIMIZAR A FUNCIONALIDADE DE RECOBRIMENTOS À BASE DE AMIDO PARA MANTER A QUALIDADE E CONSERVAÇÃO PÓS-COLHEITA DE FRUTOS TROPICAIS

NEW APPROACHES TO OPTIMIZING THE FUNCTIONALITY OF STARCH-BASED COATINGS FOR MAINTAINING QUALITY AND POSTHARVEST CONSERVATION OF TROPICAL FRUITS

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Trabalho de Tese apresentada ao Programa de Pós-Graduação em Agronomia da Universidade Federal da Paraíba, como requisito parcial à obtenção do título de **Doutor em Agronomia**.

Orientadora: Profa. Silvanda de Melo Silva, PhD

Coorientador: Prof. Randolph M. Beaudry, PhD

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RESUMO

Esta pesquisa teve como objetivo otimizar a funcionalidade de recobrimentos à base de amido para manter a qualidade e aumentar a conservação pós-colheita de frutos tropicais. Os recobrimentos estudados foram à base de fácula de mandioca (amido). Foram realizadas quatro abordagens experimentais: **I** - Propor um método para estimar a proporção da superfície com recobrimentos à base de amido utilizando processamento digital de imagens de frutos corados com iodo, e otimizar o potencial do recobrimento em aderir a superfície de diferentes frutos; **II** - Desenvolver um método não destrutivo para estimar a difusão de gases através da superfície do fruto recoberto, e estudar o impacto da interação recobrimento-epiderme/resistência às trocas gasosas na composição da atmosfera interna (atm^i) do mamão; **III** - Monitorar as concentrações internas de O_2 e CO_2 em banana recobertas em relação à proporção da superfície recoberta e à composição da atm^i; **IV** - Avaliar os efeitos do recobrimento combinado com solvente eutético profundo (NADES) de ácido ascórbico/cloreto de colina na qualidade pós-colheita de manga. Os principais resultados foram: **I** - A capacidade do recobrimento em aderir a diferentes superfícies de frutos foi otimizada pela adição de surfactante e o aumento da concentração nas dispersões. A adição de Tween 40 na dispersão resfriada (18 ºC) resultou em recobrimentos com alta capacidade de aderência à superfície de diferentes de frutos (tangerina, pimenta, banana e mamão), que pode ser devido ao aumento da adsorção do recobrimento às superfícies dos frutos sem diminuir as interações amilose-amilose. **II** – A quantificação da resistência à difusão de gases através da superfície pode ser útil em definir a atm^i alvo em frutos recobertos, especialmente considerando a interação entre o recobrimento e a casca. Na definição das mudanças na composição da atm^i, a resistência à difusão de gases foi a variável mais apropriada do que a taxa respiratória. **III** - O nível de 3,0% de amido cobriu toda a superfície da banana e resultou em anaerobiose, indicada pela $P^i_{O_2}$ muito baixa, $P^i_{CO_2}$ muito alta e aumento do quociente respiratório. A $P^i_{O_2}$ de 6 kPa em câmaras fixadas na casca foi o indicativo de que uma atm^i adequada foi atingida nas bananas recobertas, cuja taxa respiratória foi minimizada, sem resultar em anaerobiose. Com base nisso, as concentrações de 1,5 e 2,0% de amido podem ser eficazes em retardar a maturação, manter a qualidade e estender a vida útil de banana. A proporção de 75% da superfície recoberta foi estimada como o limite para uma atmosfera modificada segura para bananas. **IV** - A $P^i_{O_2}$ imediatamente abaixo da casca foi bem menor em mangas recobertas com amido+NADES comparadas com as com amido+glicerol e as não recobertas. A atm^i garantida pelo amido+NADES foi eficaz em retardar o amadurecimento da manga e manter a qualidade superior em relação aos demais recobrimentos. Além disso, mangas com amido+NADES preveniu e/ou reduziu os danos induzidos por *ROS*, mantendo maior teor de ácido ascórbico e atividades SOD, POD e APX balanceadas em comparação com as com amido+glicerol e as não recobertas durante o armazenamento.

Palavras-Chave: Atmosfera modificada. Mamão. Banana. Manga. Solvente eutético profundo. Atmosfera interna.

ABSTRACT

This work was aimed at optimizing the functionality of starch-based coatings for maintaining quality and improving postharvest conservation of tropical fruits. The coatings studied were based on cassava flour (starch). Four experimental approaches were carried out: **I** – Propose a method to estimate the proportion of the surface with starch-based coatings, using digital processing of iodine-stained fruit images, and optimize the potential of the coating to adhere to the surface of different fruits; **II** – Develop a non-destructive method to estimate the diffusion of gases through a coated fruit surface, and study the impact of the coating-epidermis/gas exchange resistance on the composition of the coated papaya's internal atmosphere (atm^i); **III** – Monitor the internal O_2 and CO_2 concentrations in coated banana in relation to the proportion of the coated surface and the atm^i composition; **IV** – To evaluate the effects of combined coating with deep eutectic solvent (NADES) of ascorbic acid /choline chloride as a plasticizer on postharvest quality of mango. The main results were: **I** – Coatings ability to adhere on different fruit surfaces were improved by adding surfactant and increasing starch concentration in the dispersions. Adding Tween 40 in a cooled (18 ºC) dispersion resulted in coating with high ability to adhere to the surface of different fruits (mandarin, pepper, banana and papaya), leading to a blockage of the pores, which may be due to the increased adsorption of the coating to the fruit' surfaces without decreasing amylose-amylose interactions. **II** – The quantification of the resistance to the diffusion of gases through the fruit' surface can be useful in defining the target atm^i in coated fruits, especially considering the interaction between the coating and the peel. In defining changes in the composition of the atm^i, the resistance to gas diffusion was the most appropriate variable than the respiratory rate. **III** – A 3.0%-starch coated the entire banana surface and resulted in anaerobiosis, indicated by a too low P^i_{O2} and a burst in P^i_{CO2} and increased respiratory quotient. The P^i_{O2} of 6 kPa in chambers attached on the fruit skin was the indicative of an adequate atm^i was set for starch coated bananas, at which respiratory rate was minimized without development of anaerobiosis. Based on that, 1.5 and 2.0% starch coatings might be effective for delaying ripening, maintaining qualities, and extend shelf life of bananas. A proportion of 75% of coated surface was estimated as the limit for secure modified atmosphere for bananas. **IV** – The P^i_{O2} immediately beneath the coated skin was much lower in mangoes coated with starch + NADES compared to those with starch + glycerol and those not coated. The atm^i ensured by starch + NADES coating was effective in delaying mango ripening and maintaining the superior quality in relation to the other coatings. In addition, mangos with starch + NADES prevented and / or reduced the damage induced by ROS, maintaining a higher content of ascorbic acid and balanced SOD, POD and APX activities compared to those with starch + glycerol and those not coated during storage.

Keywords: Modified atmosphere. Papaya. Banana. Mango. Deep eutectic solvent. Internal atmosphere.

SUMÁRIO

1 GENERAL INTRODUCTION

Food insecurity compromises quality of life, health, and subjective well-being in world-round, leading to the need for sustainable development concerned by reducing food insecurity (FRONGILLO et al., 2017). About one third of all food produced in the planet and about a half of all fruit and vegetables are lost and not consumed (PORAT et al., 2018). Mechanical injury, diseases, physiological disorders, fast ripening, and senescence are the major causes of postharvest losses of fruit and vegetables during postharvest handling and storage, processing, distribution, and consumption (BANTAYEHU; BIZUAYEHU, 2017; PORAT et al., 2018).

Brazil is the third largest fruit producer in the world, from north to south of the country there are more than 2.5 million hectares cultivated (ABRAFRUTAS, 2019). In 2018, Brazil was the second-largest producer of papaya (*Carica papaya* L.) worldwide, with 1.06 million metric tonnes; the fourth-largest producer of banana (*Musa* sp.) worldwide, with 6.75 million metric tonnes; the sixth-largest producer of tangerines/mandarins (*Citrus reticulata*) worldwide, with approximately 1 million metric tonnes; and the seventh-largest producer of mangoes (*Mangifera indica* L.) worldwide, with 1.32 million metric tonnes (FAOSTAT, 2019; IBGE, 2019). Brazil also stands out in terms of orange, pineapple, guava, and grape production. However, in the country, fruit and vegetable losses accounts to approximately 30% and occur in the processing, handling and storage steps. Among fruits, banana, papaya, and mango stand out as those which commonly present higher losses, while for vegetables tomato and bell pepper are noteworthy (SANTOS et al., 2020).

Modified atmosphere (MA) with regard to oxygen (O_2) and carbon dioxide (CO_2) gases has been successfully used for fruit and vegetables quality preservation. The levels of O_2 and CO_2 are key points in controlling ethylene synthesis and action (XU; ZHANG, 2015; BOTTON et al., 2019), which is the crucial regulator of the ripening process of most climacteric fruits (KUBO, 2014; XU; ZHANG, 2015; CHANG et al., 2017). O_2 is used as a co-substrate for the conversion of 1-aminocyclopropane-1 carboxylic acid (ACC) into ethylene by ACC oxidase. Thus, ethylene production is O_2 dependent and low O_2 levels can reduce ethylene synthesis and action (KUBO, 2014; BOTTON et al., 2019). Low O_2 also reduces the produce sensitivity to ethylene (AMARANTE; BANKS, 2010). CO_2 can act as an inhibitor of ACC oxidase activity, depending on exposure time and CO_2 level (CHANG et al., 2017). The reduction in O_2 and the increase in

CO_2 when it exceeds a certain limit induces anaerobic respiration, resulting in physiological damage and quality depreciation (KUBO, 2014).

Coating is a MA technology applied directly to the product surface in addition to or as a replacement for natural protective waxy, and provides a thin layer barrier to moisture, O_2 and CO_2 exchange (Fig. 1A; PORAT et al., 2018). Its main goals are to inhibit ethylene production and/or action, which, in turn, delay respiration rate, delay ripening, prolonging shelf life, and reducing postharvest losses (Fig. 2B; AMARANTE; BANKS, 2010).

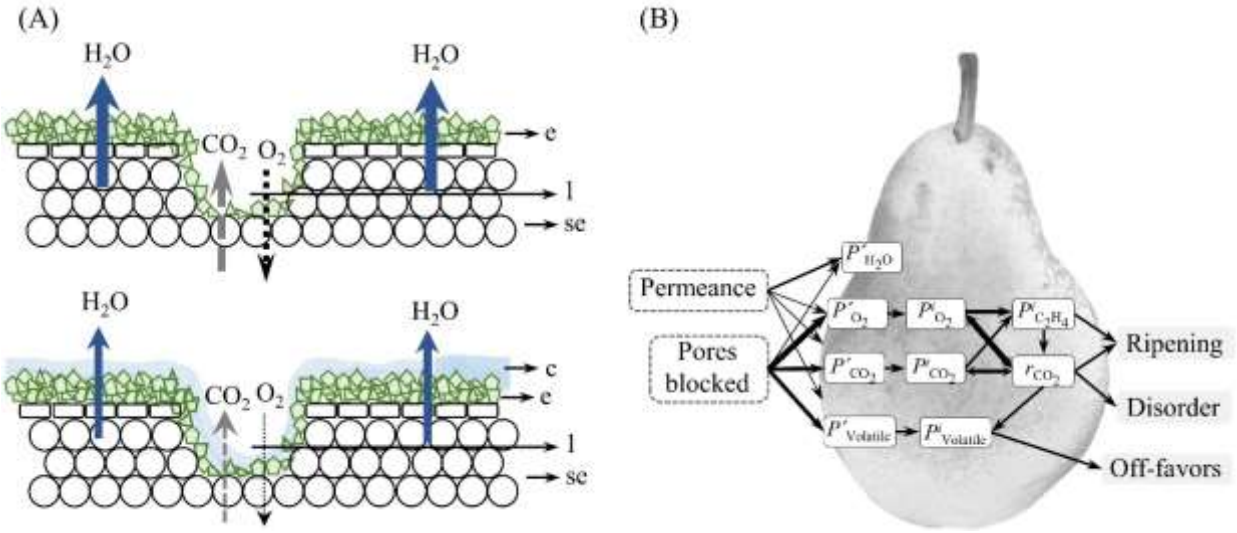

Fig. 1. Hypothetical model for the differences in skin permeance to O_2, CO_2 and water vapor (H_2O) in uncoated and coated fruit surface with lignified cells in the skin (A), and schematic diagram of the effects of coatings on gas exchange and physiology of coated commodities (B). Arrow thickness is proportional to the differences in permeance values and the magnitude of physical, compositional, or physiological effect. e = epidermis; 1 = lenticel; se = sub-epidermis; w = coating layer; subscript "volatile" represents anaerobic volatiles that accompany fermentation; P' = permeance to gas j; P^i_j =internal partial pressure of gas j; r_{CO_2} = rate of total respiratory CO_2 production. Adapted from Amarante and Banks (2010).

Edible coatings are among the most promising technologies for the preservation of fruit and vegetables due to the low-cost, availability of raw materials and performance in extending the shelf life of coated produce (PARVEN et al., 2020). In order to reduce fruit and vegetable losses and increasing the supplies through better preservation techniques, several studies have reported the efficiency of various biodegradable coatings formulated from different polymers,

such as alginates (MANTILLA et al., 2013; AZARAKHSH et al., 2014), carrageenans (SKURTYS et al. 2014; HAMZAH et al., 2013), cellulose derivatives (OMS-OLIU et al., 2010; GHANBARZADEH et al., 2011), chitosan (HONG et al., 2012; ARNON et al., 2014), gums (SKURTYS et al. 2014; KHALIQ et al., 2016), pectin (OMS-OLIU et al., 2010), and starches (CASTRICINI et al., 2012; DE AQUINO et al., 2015), to prolong shelf life of coated produce. These biopolymers are potential for the formation of coatings because of the solubility in water and unique colloidal properties, which include the gelling properties or thickener, and emulsion stabilizer (SKURTYS et al. 2014), besides are low-costy and widely available sources (REIS et al., 2015). Coatings are usually colorless, odorless and tasteless, and Generally Recognized as Safe (GRAS).

Coated fresh fruit and vegetables can last much longer after harvest than uncoated products. However, there are some risks when using surface coatings to generate modified atmosphere benefits, such as a very low permeance of skin to O_2 which can excessively decrease internal O_2 partial pressure and result in anaerobiosis, fermentation, and development of off-flavours (BANKS et al., 1997; AMARANTE; BANKS, 2010; MAQBOOL et al., 2011). Too thick and low-permeability coatings lead to fermentation and result in loss of quality and off-flavor development (PARK, 1999; HAGENMAIER, 2005).

In order to propose a safe biodegradable coating for a given product, it is necessary to set a control over the internal composition of the gases ensured by the coating (PARK, 1999). The gas permeance in a coated product depends on the proportion of pores blocked on its surface (BANKS et al., 1997; AMARANTE; BANKS, 2010). Thus, the character of coating must be evaluated to set a target internal atmosphere and enable a better description of a coating system. There is a high variability of data that arises from fruit-to-fruit within a single coating treatment and it hampers in selecting suitable coating, that provides a standardized control of the fruit quality changes (BANKS et al., 1997; HAGENMAIER, 2005). Uniform distribution of coating dispersion on the product surface is necessary (LIN; ZHAO, 2007). Accurately determining coated surface area is a needed tool precise evaluation of the altering gas exchange in the coated fruits (PEREZ; BEAUDRY, 1998).

The core of a fruit normally experiences significantly lower O_2 levels than those at the surface for any given external O_2 level in MA storage, due to resistance to the diffusion of gasses through the skin and flesh of the fruit (RAJAPAKSE et al., 1990). Additionally, in comparison

with controlled atmosphere storage, coating results in large differences of the partial pressure between internal and external atmospheres of the fruit (BANKS et al., 1993).

Commercial applications of biodegradable coatings in a broad range of fruit and vegetables are still very restricted due to still limited studies on the properties of the coatings. Those includes matrices uniformity, safety, proper gasses diffusion, appropriate coating formulation with better moisture-barrier property, complete surface adhesion on the produce, desirable sensory quality, and feasibility of scale-up to an industrial setting (LIN; ZHAO, 2007). As MA system, the effects of coatings on the produce internal levels of O_2 and also CO_2, and these gases concentration on the its physiology must be careful evaluated for selection of new coatings. However, much of the work in this area has been largely empirical, describing the effects of changes in quality that occur as a result of the application of a particular coating treatment (Table 1; AMARANTE et al., 2001; VARGAS et al., 2008), without describing the impact of internal atmosphere in fruit's metabolism. In other words, the relationship between a coated surface area and the internal atmosphere composition of coated produces has not been deeply reported yet, as summered in the Table 1. Most of the studies do not take into account the interaction between the coating and a fruit surface and its subsequent influence on coating properties (VARGAS et al., 2008). Also, little is known about the relationship between the extent of changes in the internal atmosphere of coated produces and the specific physiological ripening processes, such as respiration, softening and color changes, as well as fermentation during storage (AMARANTE et al., 2001).

Table 1. Recent studies on the effect of different coatings (in vivo tests) to preserve fruits and vegetable qualities pointing out those who assessed the effects of coatings on the number of blocked pores (BP) and internal atmosphere (atm^i) composition of coated products to explain changes in the physiological processes of ripening.

Commodity	Coating material	RR	atm^i	BP	References
Apple	Arabinoxylan and β-glucan stearic acid ester	CO_2 released	ND	ND	Ali et al., 2019
	chitosan combined with ethanolic extract of liquorice	ND	ND	ND	Madanipour et al., 2019
Banana	Cellulose nanomaterials, sucrose ester fatty acid, chitosan, and oleic acid	CO_2 released O_2 absorbed	ND	ND	Deng et al., 2017
	Rice starch and sucrose fatty acid esters	CO_2 released	ND	ND	Thakur et al., 2019
Bell pepper	Chitosan and Byrsonima crassifolia	CO_2 released	ND	ND	González-

	extract (L.) Kunth				Saucedo et al., 2019
Blueberry fruits	Sodium alginate and pectin	ND	ND	ND	Mannozzi et a., 2017
Citrus fruit	Carboxymethyl cellulose and chitosan	ND	CO2 levels	ND	Arnon et al., 2014
	Polysaccharides	ND	CO2 levels	ND	Arnon et al., 2015
Cucumber	Starch and glucose	ND	ND	ND	Patel et al., 2019
Fig fruit	Chitosan and thymol essential oil	CO_2 released	ND	ND	Saki et al., 2019
Goji fruit	Sodium alginate, Konjae glucomannan, Starch and lotus leaf extract	ND	ND	ND	Fan et al., 2019
Grapes	Chitosan and poly-ε- lysine	ND	ND	ND	Chen et al., 2019a
	Chitosan, polyvinyl alcohol and salicylic acid	ND	ND	ND	Lo'ay et al., 2019
Guava	Chitosan and alginate with pomegranate peel extract	CO_2 released	ND	ND	Nair et al., 2018
	Galactomannan-carnauba wax	CO_2 released	ND	ND	Germano et al., 2019
Indian jujube	Carnauba wax and glycerol monolaurate	CO_2 released	ND	ND	Chen et al., 2019b
Kiwifruit	Lacquer wax	CO_2 released	ND	ND	Hu et al., 2019
Kumquat fruit	Chitosan and essential oils	ND	ND	ND	Hosseini et al., 2019
Loquat	Chitosan	ND	ND	ND	Petriccione et al., 2015a
Mango	Gum arabic and calcium chloride	CO_2 released	ND	ND	Khaliq et al., 2016
	Chitosan	CO_2 released	ND	ND	Jongsri et al., 2016
	Chitosan and spermidine	ND	ND	ND	Jongsri et al. 2017
	chitosan and spermidine	ND	ND	ND	Zahedi et al., 2019
Orange	Pea starch and guar gum	CO_2 released	ND	ND	Saberi et al., 2018
	Salicylic acid and Aloe vera gel	ND	ND	ND	Rasouli et al., 2019
	Cinnamaldehyde and chitosan	ND	ND	ND	Gao et al., 2018
	Polyethylene, carnauba and shellac	ND	O2 and CO2 levels	ND	Ferdous Chowdhury et al., 2016
Papaya	Aloe vera	ND	ND	ND	Mendy et al., 2019
Pears	Soy protein isolate, hydroxypropyl methylcellulose, olive oil and potassium sorbate	ND	ND	ND	Dave et al., 2017
	Carboxymethylcellulose, candelilla wax and potassium sorbate	CO_2 released O_2 absorbed	ND	ND	Kowalczyk et al., 2017
	Semperfresh™	CO_2 released	ND	ND	Zhi et al., 2018
Plums	Rice starch and sucrose fatty acid esters	CO_2 released	ND	ND	Thakur et al., 2018
	Ascorbic acid and chitosan	CO_2 released	ND	ND	Liu et al., 2014

	Chitosan	CO_2 released	ND	ND	Kumar et al., 2017
Pomegranate	Carboxymethyl cellulose, Chitosan, oxalic and malic acids	ND	ND	ND	Ehteshami, et al., 2019
	Chitosan	CO_2 released	ND	ND	Varasteh et al. 2017
Potato	Cactus Opuntia dillenii polysaccharide	CO_2 released	ND	ND	Wu, 2019
Sapota	Aloe vera and Fagonia cretica plant extract	ND	ND	ND	Khaliq et al., 2019
	Pullulan with n-octenyl succinic anhydride and palm oil	CO_2 released O_2 absorbed	ND	ND	Shah et al., 2016
Strawberries	Alginate ad Limonene liposomes	CO_2 released	ND	ND	Dhital et al., 2018
	Chitosan and carboxymethyl cellulose	ND	ND	ND	Yan et al., 2019
	Aloe vera and ascorbic acid	ND	ND	ND	Sogvar et al., 2016
Sweet Cherry	Chitosan	CO_2 released	ND	ND	Petriccione et al., 2015b
Tangerine	Chitosan and montmorillonite	ND	CO2 levels	ND	Xu et al. 2018
Tomato	Ethanol extracts of Flourensia cernua, alginate and chitosan	CO_2 released O_2 absorbed	ND	ND	Salas-Méndez et al. 2019
	Mango kernel starch	ND	ND	ND	Nawab et al., 2017

The research was carried out through different combinations of six general terms (coating, fruit and vegetables, internal atmosphere, respiration rate, coated surface, blocked pores) in three academic research databases (Periódicos Capes, Google Scholar, and PubMed). RR = respiration rate; ND = Not Determined.

The coating process involves dipping the produce in the coating solution. The wettability and adhesion of the liquid-solid surface determines the efficiency of a coating that may block pores by adhering on the produce surfaces (SAPPER; CHIRALT, 2018; SORADECH et al., 2017; SENTURK PARREIDT et al., 2018). Adding surfactants, such as those in the Tween series, to coating solutions reduces cohesion forces and surface tension, and increases wettability, which results in improving the compatibility between the coating dispersion and the fruit skin surface (CARNEIRO DA CUNHA et al., 2009; SENTURK PARREIDT et al., 2018).

Starch is one of the most explored natural polymers for obtaining biodegradable coating due to its low cost, easy availability, and biodegradability (RODRÍGUEZ et al., 2006; EDHIREJ et al., 2016). Cassava (*Manihot esculenta* Crantz) is the cheapest material for starch production in Brazil, costing approximately US$ 0.85/kg (CHIUMARELLI et al., 2011). Nevertheless, starch-based film and coatings are brittleness with poor mechanical properties. Thus, the incorporation of a plasticizer is required to reduce intermolecular forces and increase the mobility of polymer chains (EDHIREJ et al., 2016).

In this sense, the natural deep eutectic solvents (NADESs) are mixtures of two or more components having a melting temperature much lower than the melting temperature of the individual components (ZDANOWICZ et al., 2016). They are easy to prepare, ecofriendly, and chemically and thermally stable (GÓMEZ et al., 2019). Especially on starch-based films, NADES forms stronger interactions with starch than glycerol, thus leading to better mechanical properties and inhibited tendency to starch recrystallization (ZDANOWICZ et al., 2019). However, little is known about the plasticization effect of NADES on coating formulation and no reports of the application of NADES-based coating on fruit and vegetables have been found.

This work was aimed at optimizing the functionality of starch-based coatings for maintaining quality and improving postharvest conservation of tropical fruits. Approaches were made in order to reach a target internal atmosphere in starch-based coated produces, while developing methodologies to optimize the preparation and formulation of coating dispersion and, better understand the relationship between coated surface area and O_2 and CO_2 internal partial pressure. The independent variables include surfactant and coating dispersion temperature at which surfactant is added, plasticizer, polymer concentration, and species of fruit and vegetables.

Based on the above, this research had the following specific objectives:

- To develop a method for the estimation of surface coverage for starch-based coatings using digital image processing of iodine-stained fruit surfaces;
- To improve the capability of starch-based coating to covering surface of different fruit;
- To quantitatively assess the impact of coating levels on covered surface of different fruit and infer the impact on internal O_2 and CO_2 levels;
- To developed a nondestructive method for estimating gas diffusion through a coated fruit surface;
- To quantitatively assess the permeability of coatings as a result of its interaction with the fruit surface;
- To study the impact of coating-skin interaction/resistance to gas exchange on the internal atmosphere composition of starch-based coated papaya;
- To assess the internal gas concentrations in banana fruit coated with starch-based dispersions as related to the proportion of coated surface and the internal atmosphere composition;

- To evaluate the effects of starch-based coating with ascorbic acid/choline chloride deep eutectic solvent as a plasticizer on postharvest quality of mango fruit.

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2 ARTIGO I

STARCH-BASED COATING MODIFICATION TO SELECTIVELY BLOCK SURFACE PORES OF BULKY FRUITS AND ACHIEVE TARGET INTERNAL ATMOSPHERES

Highlights

A method for estimating the proportion of starch-coated fruit surface was developed.

Image segmentation of stained starch-coated fruits was used to assess coated area.

Decreasing temperature of adding surfactant enhanced fruits coated surface area.

Internal levels of O_2 decreased and CO_2 increased at higher starch concentrations.

Journal: Food Control

Abstract

Surface coating of fruit reduces gas exchange and modifies the internal atmosphere composition by blocking a proportion the pores on fruit skin. Modification of the internal atmosphere has potential to slow ripening and improve storability. Using a method for estimating surface coverage on freshly coated produce, the effects of starch content, surfactant, and coating solution temperature at which surfactant was added on the proportion of coated surface area were evaluated for banana, mandarin oranges, papaya, and bell peppers. The internal levels of O_2 and CO_2 were also assessed. The coating surface was visualized by staining starch granules with an iodine solution. The projected area of coated surface was quantified by digital image processing, using color pixels information for coated/uncoated regions segmentation. Addition of the surfactant Tween 40 at a 60 °C coating solution, resulted in a weak interaction with the skin of fruits known to have high cutin and wax content (mandarins and peppers), but had good adhesion on the banana surface. Adding Tween 40 to a cooled coating solution (18 ºC) enhanced the coating capability to block pores by adhering to different fruit skins, which may due to the improvement of the adsorption of the coating solution on the fruit surfaces without decreasing the amylose-amylose interactions. Increasing starch concentration in the coating also increased the proportion of coated surface, indicating improved starch polymer chain association. As coating deposition increased on banana and papaya fruit surfaces, the internal O_2 levels decreased and CO_2 levels increased. The results herein describe how application variables (starch content, surfactant, and coating solution temperature at which surfactant was added) affect the percentage of surface coated in a species-specific manner. Iodine staining of starch-based coatings is a suitable method to quantitatively assess the impact of the coating on blocking pores of different fruits and thereby understand its impact on the internal gas concentrations.

Keywords: digital image processing, image segmentation, gas exchange, modified atmosphere,

1 Introduction

Surface coatings provide a thin layer barrier to moisture, O_2 and CO_2 exchange and can increase skin resistance to water and gas diffusion in fruit. Increasing resistance causes a reduction in the internal partial pressure of O_2, which is one of the main benefits in applying coatings on fruit surface (Amarante, Banks & Ganesh, 2001; Amarante & Banks, 2010). Coatings can reduce weight loss and respiration rate, delay ripening, maintain quality and storability and extend shelf life (Saberi et al., 2018; Germano et al., 2019). The mechanism through which a coating affects resistance to gas diffusion is by covering pores and cuticle on the fruit surface such that the pore-coating and cuticle-coating resistances are added in parallel (Banks, Cutting & Nicholson 1993). Coating effects on gas exchange will depend upon the numbers of pores blocked by the coating, but will not depend on the O_2 permeance of the coating (Banks et al., 1993; Banks, Cutting & Nicholson, 1997; Cisneros-Zevallos & Krochta, 2005). Therefore, the coating character of covering pores must be fully evaluated to enable the generation of safe and effective modified internal atmospheres.

The effectiveness of coating to cover and block pores by adhering onto the fruit surfaces relies on the wettability and hydrophobicity of its formulations as well as the surface tension with fruit skin (Deng, Jung, Simonsen, Zhao, 2017). Adding surfactants, such as those in the Tween series, to coating solutions reduces cohesion forces, reduces surface tension, and increases wettability, which results in improved the compatibility between the coating solution and the fruit skin surface (Carneiro-da-Cunha et al., 2009; Senturk Parreidt, Schott, Schmid & Müller, 2018). Differences in the wax layer and epidermis uniformity between different fruit can affect the surface tension (Casariego et al. 2008; Senturk Parreidt et al., 2018) and thereby influence the adhesion potential of the coating to the fruit surface. Therefore, the optimization of surface coatings must take into account differences in the nature of the fruit skin and its waxes, the coating properties of the solution and its interaction with the fruit surface, and the influence of the final coating on gas diffusive resistance (Amarante et al., 2001; Basiak, Linke, Debeaufort, Lenart & Geyer, 2019; Vargas, Pastor, Chiralt, McClements & Gonzalez-Martinez, 2008). The internal O_2 in coated plant organs is decreased due to its consumption by fruit respiratory metabolism while the surface coatings limits the O_2 uptake (Amarante & Banks, 2010).

Amylopectin and amylose are the major molecular components of cassava starch. An iodine solution will stain amylose black and can be used visualize the presence of starch (Zhu, 2015).

Iodine staining permits determination of the presence and uniformity of starch-based coatings on fruit surfaces (Oliveira, Cruz & Alves, 2016). Accurately determining coated surface area is a precise means of altering gas exchange in the coated fruits (Perez & Beaudry, 1998). However, it is still necessary to develop a method for quantifying coating efficacy that is objective and rapid and will also permit estimation of the degree of pore blockage.

Machine-vision technology enables extraction of patterns or other information from a digital image that often is not possible by human sight. Characterization of fruit quality by digital image processing can recognize defects, damage, and maturity of the fruit by using color, size, and shape, via differing intensities of pixels in the image (Momin et al., 2017; Arunachalam, Kshatriya & Meena, 2018; Soradech, Nunthanid, Limmatvapirat & Luangtana-anan, 2017). The segmentation of an image into regions that differ in the intensity of constituent pixels enables the identification of specific areas of interest in the fruit relative to its total area (Arunachalam et al., 2018). Machine vision should be an effective method for evaluating starch-based coating when stained with an iodine solution (Oliveira et al., 2016).

The present research had the following two major objectives: (a) to develop a method for the estimation of surface coverage for starch-based coatings using digital image processing of iodine-stained fruit surfaces, and (b) to improve the capability of starch-based coating to covering surface of different fruit.

2 Materials and Methods

2.1 Fruit material and coating chemicals

Mandarin (*Citrus reticulata*), sweet peppers (*Capsicum annuum* L.) and banana (*Musa sp.*, AAA group, Cavendish subgroup, cv. Valery) fruits were purchased at the commercial maturity from a market in East Lansing, Michigan, United States. Papaya (*Carica papaya*, cv. Havaí) fruit were obtained from Central Estadual de Abastecimento S/A (CEASA) in Campina Grande, PB – Brazil. Cassava (*Manihot esculenta* Crantz) starch was extracted from roots purchased at the street market in Areia, Paraíba, Brazil and air-dried to 15% water content. Tween 40® and glycerol were purchased from Sigma Aldrich (St. Louis, MO, US).

2.2 *Preparation and application of coatings*

All coating solutions used in this study were prepared as follows: cassava starch solutions (at 1.5%, 2%, 2.5%, and 3%; w/v) were prepared by heating the starch-water mixture to 70 °C using

a hot plate magnetic stirrer and left at 70 °C for 5 min to obtain complete gelatinization. Coating solutions were cooled to 60 °C and glycerol (1%; w/v) was added as a plasticizer under constant stirring. Tween 40 (0.05%; w/v) was added as surfactant also during cooling phase, at a 60 °C or 18 °C. For coating, fruit were dipped for 1 min in the coating solutions at 20±2 °C. Following coating, all fruit were air-dried at room conditions (20 °C, ~50% RH), then placed in polystyrene trays and stored at room conditions.

2.3 Increasing blocked pores by coating properties on different fruit surfaces

Two experimental approaches were carried out in this study: first, the goal was to improve the capability of starch-based coating of covering surface of different fruit (mandarins, peppers, and bananas), which have different characteristics of epidermis (Wang et al., 2014; Parsons et al., 2013; Li et al., 2019). The major purpose of the second experimental approach was to ensure different levels of the proportions of coated surface in papaya and banana fruits, allowing to infer the impact of covered surface on internal O_2 and CO_2 levels. The two experimental approaches are described below:

1) Cassava starch solution (3% w/v) and glycerol 1% (w/v) were used and Tween 40 0.05% (w/v) added to increase wettability and adhesion properties of the coating (Carneiro-da-Cunha et al., 2009; Senturk Parreidt et al., 2018). To evaluate the impact of Tween 40, coating solutions without Tween 40 and with Tween 40 were applied to mandarin fruits (14 fruits), bananas (12 fruits) and peppers (12 fruits). Tween 40 was added when the coating solution was in the cooling phase, at 60 °C or 18 °C.

2) Coating solutions were prepared from different concentrations of cassava starch (1.5%, 2%, 2.5%, and 3%, w/v) and tested for their potential to covering banana and papaya fruit surfaces. 1% glycerol and 0.05% Tween 40 (added to the coating solution at 18 °C) were added. For each coating solution, 12 banana and 8 papaya fruits were used. To examine the effect of coated surface on gas exchange, internal O_2 and CO_2 levels were determined.

In both experimental approaches, a fruit was considered as an experimental unit and each experiment was replicated at least twice. In the first experiment, a total of 42 mandarin fruit (14 fruit x 3 replications), 36 banana fruit (12 fruit x 3 replications), and 24 pepper fruit (12 fruit x 2 replications) were evaluated, while in the second experiment 36 banana fruit (12 fruit x 3 replications) and 16 papaya fruit (8 fruit x 2 replications) were tested. For each replication, new coating solutions were prepared.

2.4 Determination of the coated surface area in a projected area

The coated surface area was estimated from projected area of fruits by the image processing technique as adapted from Arunachalam et al. (2018). Coated fruit were dipped in an iodine solution (0.25% of KI and 1% of I_2 in water) to stain the starch granules, 48 h after the coating application, so the coating presence could be made visible by the black coloration (Fig. 1A). The images were captured from opposite sides of each coated fruit using a Coolpix p500 camera with 12.1megapixel resolution. For control of lighting, fruit were placed inside a square box especially constructed from diffuse white paper with four 12 W lamps arranged distally (10 cm) on the outer sides. Image processing was performed using the ImageJ® 1.52a program (Bethesda, MD, USA). To develop the image segmentation based on the luminance, green↔red spectrum, and blue↔yellow spectrum (Lab) color space. For selecting the coated area of fruits, each image was divided into its Lab channel, which are grayscale monochrome, see Fig. 1B. The histogram of the entire image (both of the three grayscale images) was analyzed and an threshold level was set using the 'auto-threshold' setting to create the binary image (Fig. 1C). For binarization, if the value of a pixel is greater than or equal to the threshold value then set 1 (white), and if it is less than the threshold value set 0 (black) (Arunachalam et al., 2018).

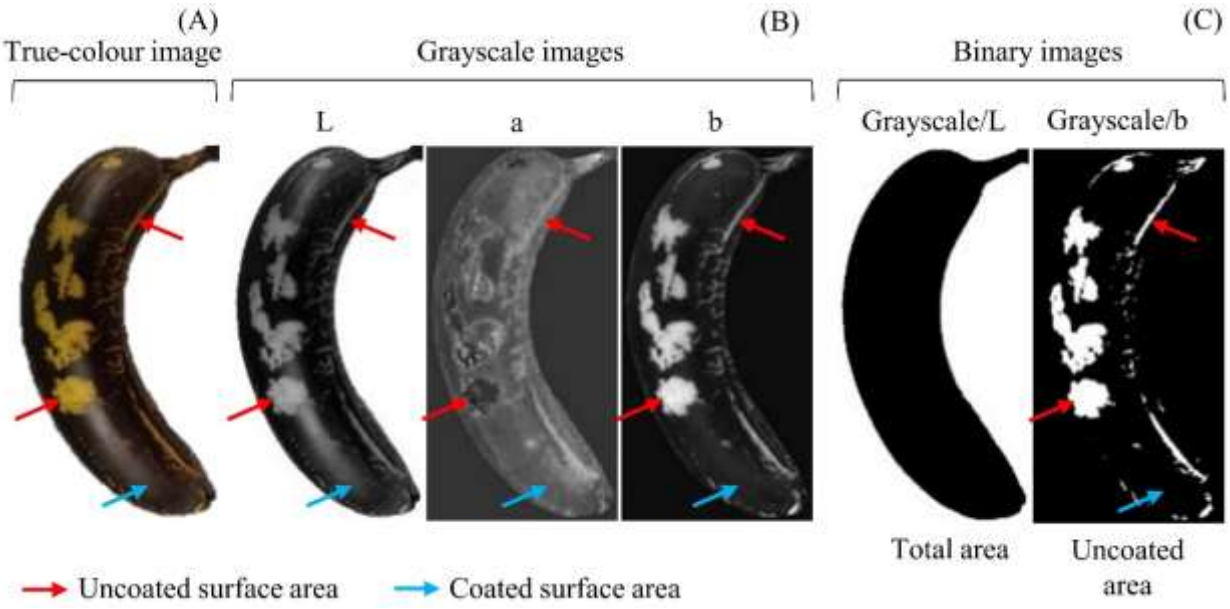

Fig. 1. Steps of the digital image processing of starch-coated fruit aiming to estimate the projected coated-surface area by computer vision. Blue arrows = coated, red arrows = uncoated areas, L, a, and b = Luminance, Green↔Red and Blue↔Yellow.

From the binary images, the projected area of uncoated surface was obtained and also the projected area of the fruits, in pixels. The coated surface area were expressed as a percentage, relative to the projected area of total fruit surface, as follows:

$$CSA\ (\%) = (TA - USA/TA) * 100$$

where CSA is the coated surface area, TA is the projected area of total fruit surface, and USA is the projected area of uncoated surface.

2.5 Levels of O_2 and CO_2 in chambers adhered over fruit surface

The reduced O_2 levels achieved with the coating was determined from 2-mL chambers adhered to the fruit surface as described by Rajapakse, Banks, Hewett and Cleland (1990). Cylinders of 16-mm long and 14-mm diameter were sealed with a rubber septum (6-mm thick). Silicone grease was applied at the septum-glass interface to prevent leaks. Two chambers were attached to the fruit skin at an equatorial position, on opposite sides of each of 6 fruits in each treatment using silicone sealant (*Dow Corning*® PV-804 Neutral Sealant). After sealing the chambers, fruit were left for 36 h before applying the coatings. A 0.1-mL gas sample was taken from each chamber at 0, 1, 3, 6, 9, and 12 d after coating the fruit. Gas samples were injected in oxygen analyzer PA-10a (Sable Systems International, Las Vegas, USA) operated in a flow-through mode with N_2 as the carrier gas and a flow rate of 100 mL min^{-1}. O_2 concentration was calculated relative to the certified gas standard noted previously.

2.6 Statistical analysis

Experimental data were subjected to analysis of variance (ANOVA) using software R 4.00. Means of treatments were separated using Tukey's test ($P \leq 0.05$). Data were presented as mean ± standard deviation means.

3 Results and Discussion

3.1 Determination of the proportion of coated surface in a projected area

The coated surface area was measured using an image segmentation approach. Fig. 2 corresponds to a 3D surface plot from stained starch-coated pepper fruit. The luminance of the image is interpreted as height (z-axis) for the plot, which ranges from 0 to 250 (Ferreira and Rasband, 2012). From this specific color range, different regions of neighboring/connected pixels on the image could be identified (Fig. 2B and C), the projected area of coated and uncoated fruit

surface are separated (Fig. 2C). Thus, captured color images of coated fruit stained by iodine solution (Fig. 1A; Fig. 2; Fig. 3A) indicated that the uncoated surface areas are easily distinguishable from the coated ones using image color segmentation.

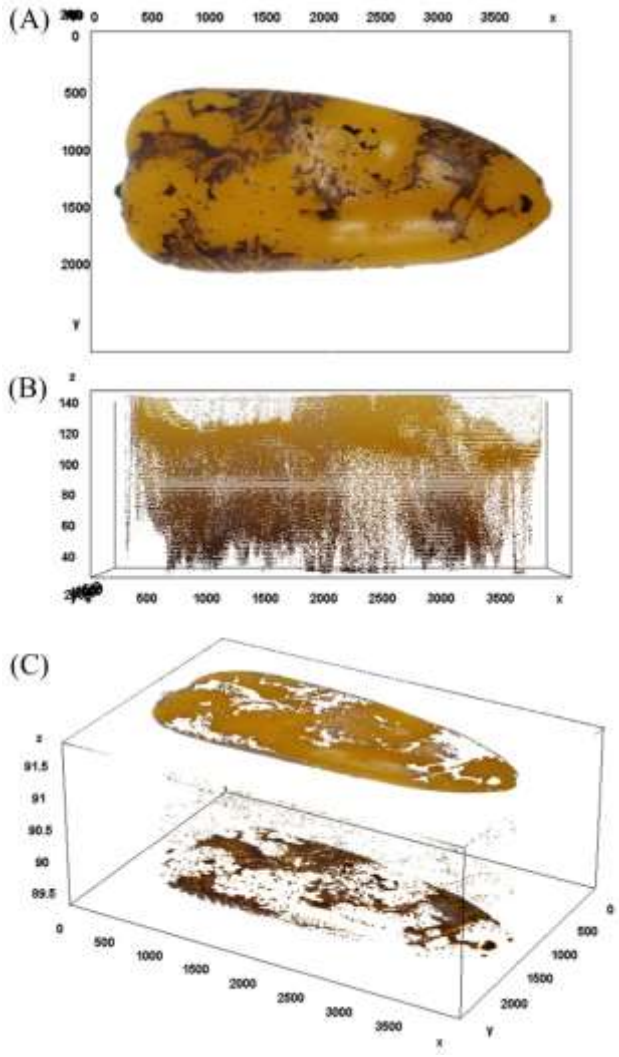

Fig. 2. 3D surface plot from coated pepper fruit shown from the top (A), from the front (B) and, showing separated the coated and uncoated regions (C) through the pixels drawn as small dots.

A single grayscale threshold was used to determine if an image pixel belongs to the uncoated or the coated region. For coated banana these two regions were more distinguishable in *b* scale monochromatic images (Fig. 1A). In the resulting binary image, the uncoated region boundary was drawn and the number of pixels counted. Herein, the coated surface area was expressed as a percentage, relative to the projected area of total fruit surface.

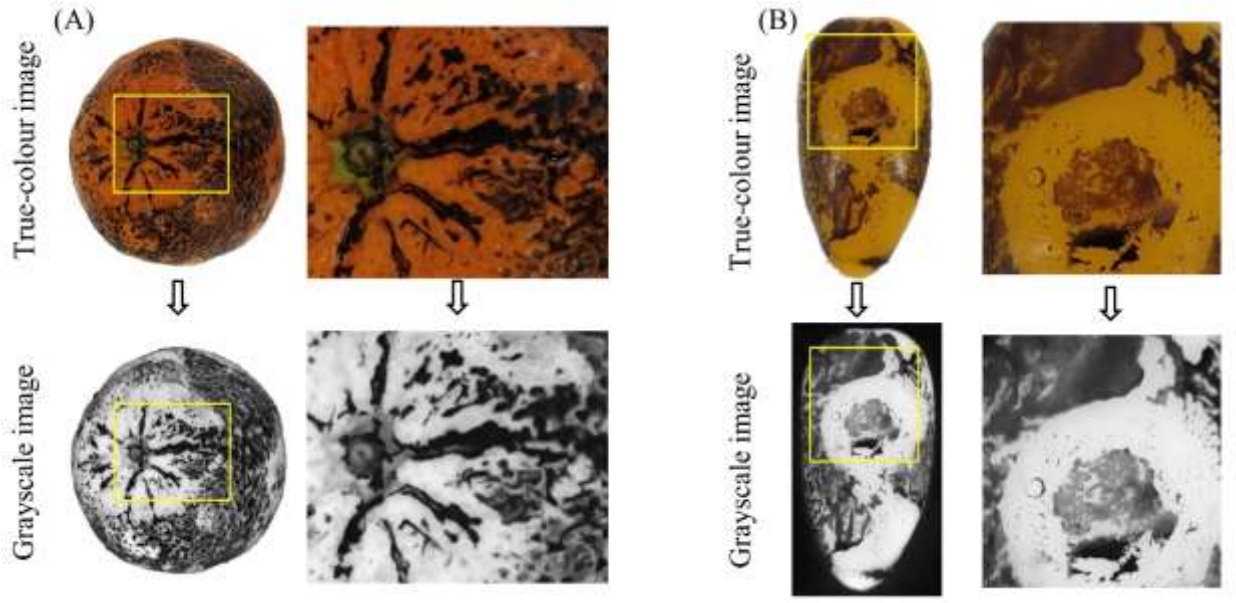

Fig. 3. True-color image from coated mandarin (A) and pepper fruits (B) and their monochromatic images (Lab) used for the coated surface area measurement.

Computer vision technology has made huge progess in the inspection of fruit and vegetable quality. The images segmentation is a technique of digital image processing used to divide the image into parts and extract patterns (Arunachalam et al., 2018). This approach has been used to estimate volume, mass, and maturity, as well as defects and damage of different fruits (Jana, Parekh & Sarkar, 2020; Momin et al., 2017; Arunachalam et al., 2018). Herein, the surface of coated fruits with starch-based solution was analyzed by digital image processing and the regions of the fruit where the coating adhered were segmented from those where coatings did not adhere. This approach provides a good estimation of the how much of the fruit surface is coated, which in turn is the main factor limiting gas exchange in coated fruits (Amarante & Banks, 2010; Amarante et al., 2001).

3.2 Effects of adding surfactant on the coating solution at different temperatures

Starch-based coating at 3% without adding surfactant showed high potential of coating up the banana surfaces (90%). On the other hand, the same coating concentration coated only 42% of the mandarin and 26% of the pepper surfaces (Fig. 4). Mandarins and pepper fruits are naturally coated by a relatively thick layer of both wax and cutin. The total wax concentration in mandarin (cv. Satsuma) was reported to be 3.8±0.2 μg cm^{-2} (Wang et al., 2014). However, pepper have shown an even thicker wax layer among different accessions, ranging from 0.968 μg cm^{-2} to

13.77 μg cm^{-2} with an average wax load of 5.30 μg cm^{-2} (Parsons et al., 2013). Fruit skins with higher cutin and wax content have lower surface free energy and polarity, providing low interaction with some coating solutions (Soradech et al., 2017), and hence, resulting in the lower percentage of coated surface area by applying a starch-based coating, if the surfactant was not added.

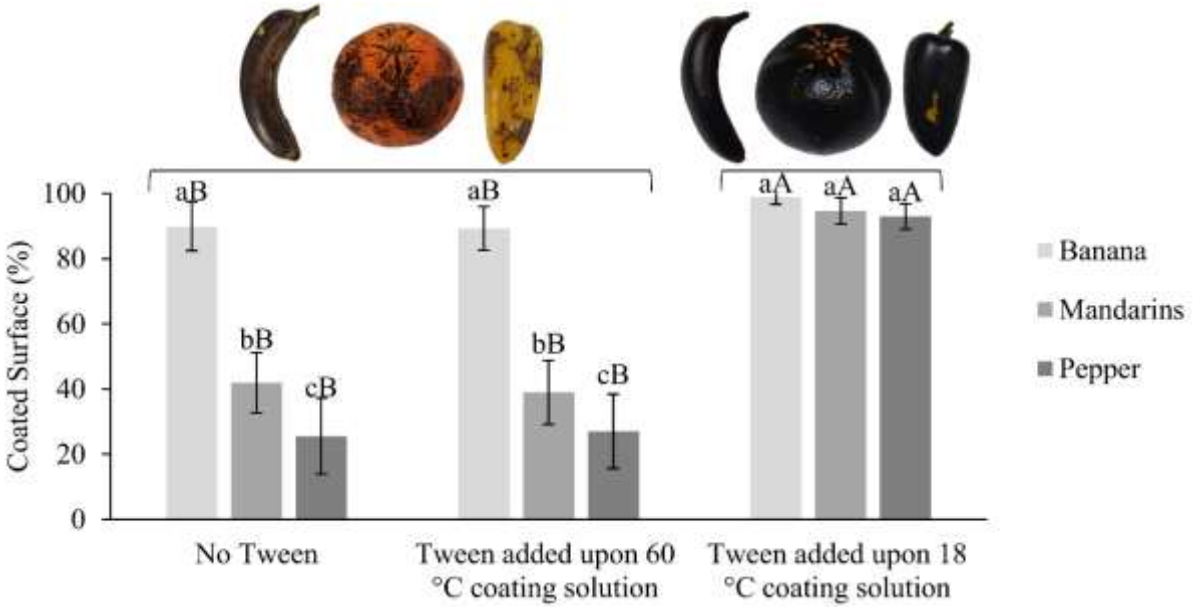

Fig. 4. Coated surface area of banana, mandarins and pepper fruits by starch-based coating without surfactant (No Tween 40) and with surfactant added to the coating solution at different temperature (Tween 40 added to 60 °C coating solution and Tween 40 added to 18 °C coating solution). Vertical bars represent Standard Deviation. Same lowercase letters among fruits within the same coating and uppercase between coatings within the same fruit do not differ according to Tukey's test ($P \leq 0.05$).

Adding Tween 40 to a coating solution at 60 °C did not improve the proportion of coated surface as compared to that without Tween 40 (Fig. 4). Under these conditions, the coated surface for banana, mandarin, and pepper fruits were 89%, 39%, and 27%, respectively. Starch-based films with surfactants, produced by blow extrusion, showed aggregates surrounded by micro-cracks and had lower structural integrity than those without surfactants (Brandelero, Yamashita & Grossmann, 2010). These authors suggested that the poor mechanical properties of films with surfactant in the extrusion may be related to the increase in the free volume between the adjacent chains of starch. A similar result was also reported by Rodríguez, Oses, Ziani and Mate (2006)

when studying the effects of plasticizers and surfactants on physical properties of starch-based edible film. According to these authors, the film solution was kept hot (70 ºC) up to the point of surfactant incorporation and the film samples were dried in a climatic chamber at 60 ºC for approximately 24 h. They also stated that surfactants can form aggregates between the starch chains, which leads to weak interactions between the surfactant and starch chains. This behavior could cause a phase separation during drying that results in brittle film formation (Rodríguez et al., 2006).

Fruit species affected the percent surface coverage of the coating (Fig. 4). For each fruit species, there was a high variability observed for the coated area values without and with Tween 40 added to a coating solution at 60 °C. This behavior supports previous reports that variability hampers the selection of a suitable coating (Hagenmaier, 2005; Banks et al., 1997). Since the final gas permeance of a coated product is heavily dependent upon the numbers of pores blocked on the product surface (Banks et al., 1997; Amarante et al., 2001), the high variability in the percentage of coated surface area within a single coating treatment is likely to lead to a high range of internal O_2 and CO_2 levels. Furthermore, coating operations in a factory scale demand a uniform distribution of coating solution on the product surface and efficient drying to obtain a feasible coating system (Lin & Zhao, 2007), that provides a standardized control of the fruit quality changes (Perez & Beaudry, 1998).

The starch coating covered a high percentage of the fruit surface when Tween 40 was added to a coating solution at 18 ºC (Fig. 4). Under these conditions, the proportion of coated surface was 99% in banana, 95% in mandarins and 93% in peppers. During the preparation of starch-based film solutions, the high-temperature breaks down the intermolecular bonds of starch granules, leading to absorbing water and disordering of the organization of the starch chains (Zhu, 2015). The solubilized amylose chains allow Tween 40 to access more hydrogen bonding sites and form aggregates between starch chains, which lead to the formation of less compacted films (Brandelero et al., 2010). However, when the gelatinized starch solution is subjected to lower temperatures, the disordered chains undergo re-ordering and re-association through inter- and intrachain interactions and hydrogen bonding, resulting in a compacter starch matrix (Rompothi, Pradipasena, Tananuwong, Somwangthanaroj & Janjarasskul, 2017; Zhu, 2015). Therefore, when Tween 40 is added to a coating solution at lower temperature it acts on reducing the surface tension between the coating solution and the fruit skin surface, increasing the

wettability (Carneiro-da-Cunha et al., 2009; Senturk Parreidt et al., 2018) without decreasing the amylose-amylose interactions and disturbing the film structure.

Furthermore, very low dispersion of the percentage of coated surface values was achieved when the starch coating solution was cooled up to 18 ºC before adding Tween 40, indicating uniformity of the number of blocked pores from fruit-to-fruit as well as among different fruits within the same coating treatment.

3.3 Effect of starch concentration on proportion of coated surface

Increasing starch concentration from 1.5 to 3% in the coating solutions improved its adhesion onto banana and papaya surfaces, which is taken to mean that the blockage of pores was increased by increased starch content in the coating (Fig. 5A, B). When the starch concentration was 1.5%, the coated surface area was 28.6 ± 14.5 and 42.0 ± 18.2% in bananas and papaya, respectively. In turn, when the coating's starch concentration was 3%, the percentage of the coated surface area was 99.5±1.1 and 99.1±1.8% in bananas and papaya, respectively. These results are supported by the findings of Maqbool, Ali, Alderson, Zahid and Siddiqui (2011), who found that increasing gum arabic (5, 10, 15, and 20%) within chitosan (1.0%) based solution increased coating deposition on the banana surface. The samples were photographed under a scanning electron microscope and showed that higher concentrations of arabic gum (15 and 20%) plus chitosan coatings completely covered the cuticles and blocked the pores on the fruit surface. Probably, herein the high starch concentration increased the starch polymer chain association, resulting in more compact films (Rompothi et al., 2017) and hence increasing the proportion of coated surface.

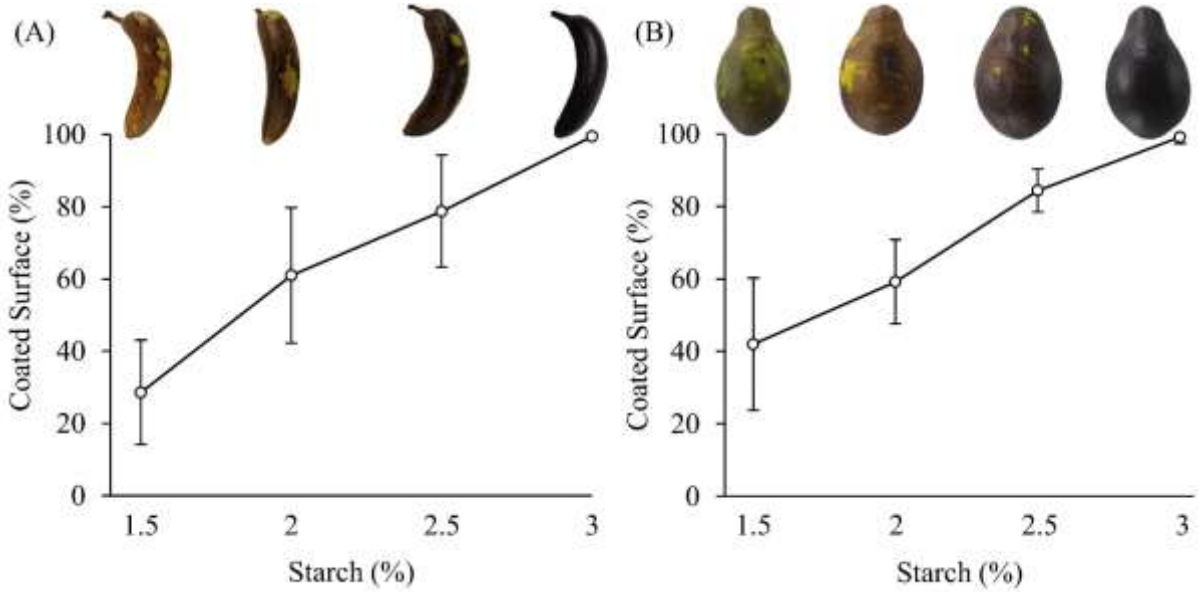

Fig. 5. Coated surface area of banana (A) and papaya (B) fruits by starch-based coatings at different concentrations of starch. Vertical bars represent Standard Deviation.

It is worth mentioning that at the 3% the starch concentration yielded very low variability in the coated surface area, although this indicates uniformity of coating, highlights the probable high number of blocked pores from fruit-to-fruit, within the same coating condition.

3.4 Relationship of coated surface and internal gas concentration

The respiratory gaseous concentrations in sealed chambers attached to the fruit surface are close to equilibrium with the fruit's internal atmosphere (Banks & Nicholson, 2000). Herein, for the first time is shown that as the starch concentration in the coating solution increased, the O_2 partial pressure in the chambers attached to the fruit surface decreased (Fig. 6A, B). Probably, increasing starch concentration in the coating solutions promoted high diffusive resistance to the gas, which led to a high gradient between internal and external atmospheres (Banks et al., 1993). The decline in O_2 level with increasing starch content was greater in banana (81%) than in papaya (64%). A 3% starch solution yielded 1.9±0.7 and 5.8±2.3 kPa of O_2, respectively, for banana and papaya, two days after the coating application. The higher O_2 gradient between internal and external atmospheres in coated banana compared with coated papaya may be related to their different respiration rates, which requires different minimum oxygen transfer rates

through the coatings to avoid anaerobiosis and unwanted metabolic changes (Sapper & Chiralt, 2018).

Herein, a reduced O_2 level is desirable for inhibiting ethylene action, which in turn, delays ripening and prevents losses of quality during storage of fruits (Solomos & Kanellis, 1997). However, when internal O_2 levels are at a low level, anaerobic respiration takes place in the fruit respiratory metabolism leading to the formation of acetaldehyde and ethanol (Saltveit, 2019; Vanoli et al., 2015). In this process, the oxidation of glucose is incomplete - glycolysis only captures about 20% of the energy for a glucose molecule and the accumulation of ethanol is potentially toxic to the cell (Saltveit, 2019), which also modifies the typical fruit aroma composition, causing a negative impact on fruit quality (Saberi et al., 2018; Arnon, Granit, Porat & Poverenov, 2015).

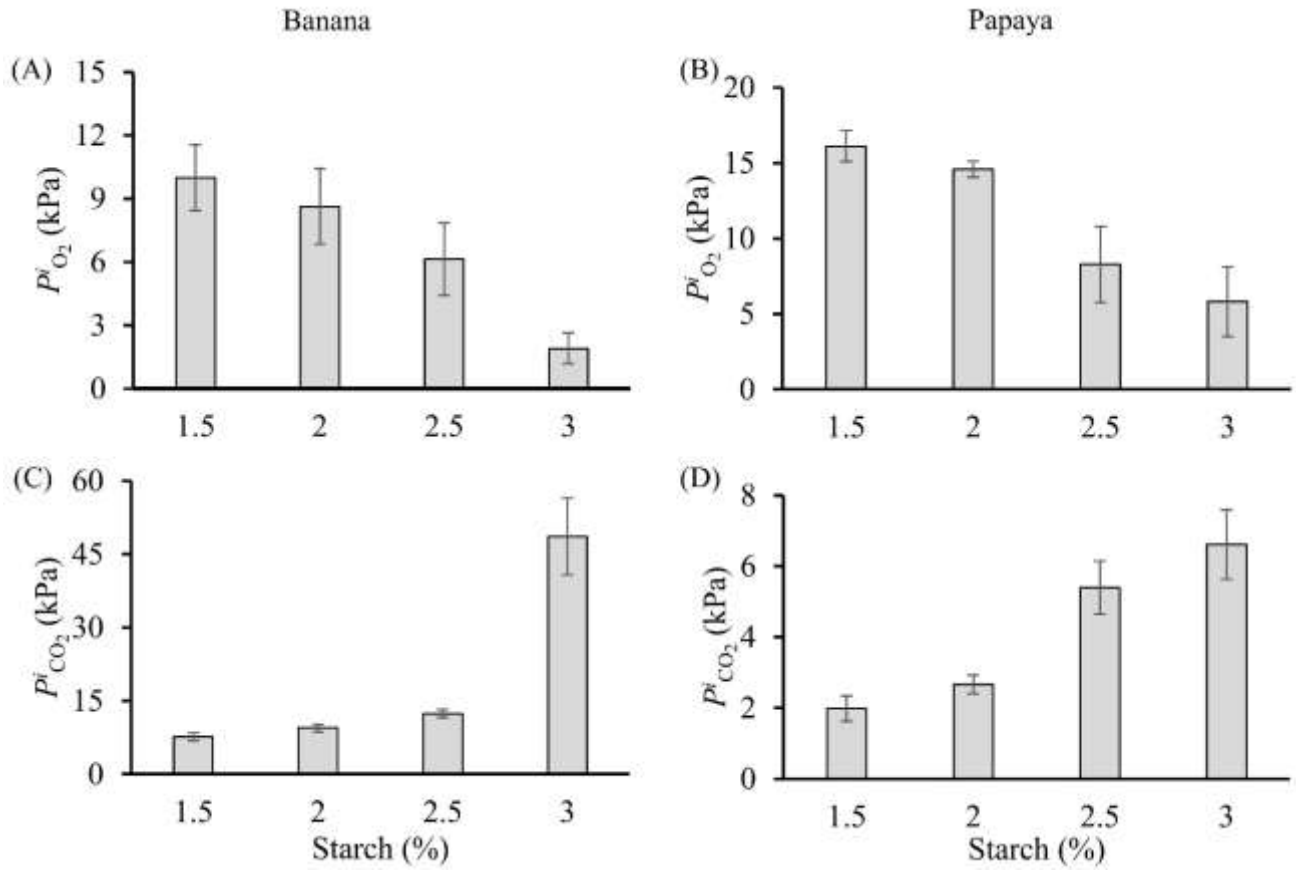

Fig. 6. Internal O_2 and CO_2 levels in coated banana (A and C, respectively) and papaya (B and D, respectively) fruits, under different levels of starch coatings. Vertical bars represent Standard Deviation.

The fruit coated with 3% starch solution had the highest internal CO_2 partial pressures, which were 48.6±7.8 and 6.6±1.0 kPa for banana and papaya fruit, respectively (Fig. 6C, D). Like O_2, the gradient for CO_2 is increased by the relatively lower permeance of the fruit skin, leading to a higher internal CO_2 concentration (Hagenmaier, 2005). Generally, increasing CO_2 concentrations up to 5% within the atmosphere surrounding the fruits reduces the biological action of ethylene and, therefore, delays ripening (Irtiza, Wani, Khan, Murtaza & Wani, 2019; Saltveit, 2019). However, excessive CO_2 concentrations may damage internal tissues and hence reduce fruit quality (Amarante & Banks, 2010).

Taken together, increasing coating deposition on the fruit surface increases the resistance to gas diffusion of the coated fruits, creating an internal atmosphere within reduced O_2 and increased CO_2 levels. In this sense, a uniform continuous coating all over the banana and papaya surfaces were obtained with a 3% starch-based coating solution (Fig. 5A, B), probably blocking most of the pores and lenticels on fruit skins (Maqbool et al., 2011). As a result, banana fruit were unable to ripen properly even after 7 days storage at room conditions, skin color did not change (from unpublished data) and fruit were off-flavored, suggesting that an excessively low O_2 level (1.9±0.7 kPa) and an excessively high CO_2 level (48.6±7.8 kPa) developed within 3.0% starch coated fruit leading to fermentation (Fig. 6A, C). Similar results were observed in banana fruit coated with Arabic gum (15 and 20%; w/v) plus chitosan (1%; w/v) and having pores completely blocked by the coating (Maqbool et al., 2011), once fruit were unable to ripen properly even after 33 days storage and exhibited increased decay. However, for papaya fruit (Fig. 6A, C), the internal gaseous concentrations of O_2 (5.8±2.3 kPa) and CO_2 (6.6±1.0 kPa) obtained with the 3%-starch were reported to be useful for prolonging the storage life of this fruit (Ali, Muhammad, Sijam & Siddiqui, 2011).

The pattern of the proportion of coated surface assessed by digital image processing was in agreement with data shown by a confocal scanning microscope (Amarante et al., 2001) and a scanning electron microscope (Maqbool et al., 2011). Furthermore, the relationship between internal gas concentrations and the proportion of coated surface proved the usefulness of the color segmentation for estimating the percentage of coated surface area of coated fruit by image segmentation and, therefore, this simple procedure may allow the optimization of coating technology. Further studies, therefore, should focus on targeting the optimum internal O_2 and

CO_2 concentrations of a range of coated fruits, which has to take into account the physiological features of the coated commodity and the efficient control of the number of blocked pores.

4 Conclusion

This study was the first to employ stained starch granules coloring to segment digital images captured from starch-based coated fruits as a method for estimating the proportion of coated surface. It was verified that coatings ability to adhere on different fruit surfaces were improved by adding surfactant and increasing starch concentration in the coating solutions. It was demonstrated for the first time that adding Tween 40 in a warmed coating solution, just after starch gelatinization, leads to weak interaction of the coating solution with the skin of fruits known to have high cutin and wax content (mandarins and peppers) and caused low proportion of coated surface area. On the other hand, adding Tween in cooled coating solution improved the coating ability to block pores by adhering to different fruit skins (mandarin, pepper, banana, and papaya), which may be due to the improvement of the adsorption of the coating solution on the fruit surfaces without decreasing the amylose-amylose interactions.

Based on that, it was possible to quantitatively assess the impact of coating levels on the coated surface of different fruit and infer the impact on internal O_2 and CO_2 levels.

Acknowledgements

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3 ARTIGO II

GASES EXCHANGE AND FRUIT'S INTERNAL ATMOSPHERE BUILD UP IN STARCH-BASED COATED PAPAYA FRUIT

Highlights

A method to measure gas diffusion through a coated fruit surface was developed.

The approach helps on setting a target internal atmosphere in coated fruits.

Coatings increase O_2 and CO_2 gradients between the surface and fruit`s core.

Coatings increase fruit's pulp resistance to gas diffusion.

Journal: Postharvest Biology and Technology

Abstract

Coating increases fruit skin resistance to gas diffusion. Thus, a proper O_2 and CO_2 exchanges in coated fruit are essential for setting a target internal atmosphere. In this study, a nondestructive method for estimating the gasses diffusion through a coated fruit surface was developed. The objective was to quantitatively assess the permeability of coatings as it interacts with the fruit surface and, thereby, evaluate the impact of the coated skin resistance to gas exchange on the internal atmosphere composition of starch-based coated papaya. Fruits were dipped in starch-based coating dispersions at 1.5, 2, 2.5, or 3% (w/v) and stored at 21±2 °C for five days. A flow-through system was adapted to sealed chambers (head space) attached to the skin of the coated fruit, and the difference in the CO_2 concentration of the inlet-flow to outlet-flow gave the rate of CO_2 released by the coated fruit. The diffusive transmission rate of CO_2 was estimated at the steady-state rate of gas transfer using the Fick's equation based on the first law of diffusion. The permeability to gasses diffusion was more appropriated than fruit respiration rate to indicate the changes in internal atmosphere composition. The relationship between the coated skin resistance to gas exchange and the O_2 and CO_2 internal partial pressure in the tissues immediately beneath the skin and also in the core of fruit were described for starch coated papaya. Besides increasing skin resistance to gas exchange, coatings can also increase flesh resistance to gas diffusion by reducing pressure gradients in the flesh tissues. The approaches described in here will be very useful to compare the effect of coatings on skin resistance to gas diffusion and on setting a target internal atmosphere in a coated fruit, especially taking into account the interaction between the coating and the fruit skin.

Keywords: coating permeability, *Carica papaya* L., gas diffusion, internal partial pressure.

1 Introduction

Papaya (*Carica papaya* L.) is one of the most important and popular tropical fruit due to its unique flavor and high content of antioxidants (such as β-carotene, lycopene, and ascorbic acid), rich nutrient content, and the associated economical potential (Parven et al., 2020; Maringgal et al., 2020). However, due to its perishable nature, it is a fruit with fast ripening, short shelf life, and high susceptibility to quantity and quality postharvest losses (Vilaplana et al., 2020; Parven et al., 2020). Surface coating has been shown to delay ripening and maintain quality of coated papaya during storage and also to prolong its storage life (Ali et al., 2011; Mendy et al., 2019; Maringgal et al., 2020).

Biodegradable coatings are among the currently most explored technologies for quality preservation of fruit and vegetables due to their low cost, availability of raw materials and potential for extending the shelf life of coated produce (Parven et al., 2020). However, the design of a thin layer barrier to moisture and gas exchange adhered to the fruit surface, must assure a safe modified internal atmosphere composition avoiding anaerobiosis (Tabassum and Khan, 2020). In this sense, taking into account fruit respiration, the proportion of blocked pores in the fruit surface, and coating permeability to O_2 and CO_2 determine the internal atmosphere that will be built up in a given coated produce (Banks et al., 1993; Park, 1999).

Naturally, skin resistance to gas diffusion results in O_2 and CO_2 gradients between the external atmosphere and the atmosphere just beneath the fruit skin. In turn, fruit pulp resistance to gas diffusion results in O_2 and CO_2 gradients between tissues immediately beneath the skin and those at the fruit core (Rajapakse et al., 1999). Coating can change the fruit skin resistance to gas exchange by blocking a greater or lesser proportion of the pores in the fruit surface, however the pore-coating and cuticle-coating resistances in a coated fruit are added in parallel (Banks et al., 1993). Too thick and low-permeability coatings can result in very low permeance of skin to O_2, which can excessively depress internal O_2 partial pressure and result in anaerobiosis, fermentation, development of off-flavors, and, thus, loss of product quality (Park, 1999; Hagenmaier, 2002). Furthermore, thicker-barrier coatings result in high variation in ripening, thus, in produce quality (Hagenmaier, 2005).

It is not easy to measure the gas permeation rates of a given coating followed its application in a fruit surface (Park, 1999). Therefore, as a step of developing a new coating, a basic procedure must be to characterize its properties as it is casted and then peeled off from a plate,

such as a film (Vargas et al., 2008; Salas-Méndez et al., 2019). However, this approach does not take into account the interaction between the coating and the fruit surface and its subsequent influence on the coating and fruit properties (Vargas et al., 2008).

Fick's law has been used to quantify the diffusion transmission rate of gases from the internal and external atmospheres of bulky fruits through the skin and also through flesh (Banks et al., 1993; Park, 1999; Amarante et al., 2001). The flux of a gas based on Fick's law is dependent on the concentration gradient and diffusivities of bulky organs (Park, 1999). The O_2 and CO_2 partial pressures in the tissues immediately beneath the skin can be assessed by measuring its concentrations in sealed chambers attached to the fruit surface (Perez & Beaudry, 1998; Rajapakse et al., 1999; Banks & Nicholson, 2000). In turn, the O_2 and CO_2 partial pressures in the core of the fruit can be determined by direct gas sampling from the central core cavity by using a syringe fitted with a needle (Rajapakse et al., 1999). Although, these analytical approaches can be helpful to study gaseous diffusion in uncoated and coated fruits (Vargas et al., 2008), they are still not much used for the evaluation of the coating permeability and efficiency, and the latter is limited to fruits that have a cavity.

In this study, a nondestructive method for estimating gas diffusion through a coated fruit surface was developed. The objective was to quantitatively assess the permeability of coatings as a result of its interaction with the fruit surface and, thereby, study the impact of coating-skin interaction/resistance to gas exchange on the internal atmosphere composition of starch-based coated papaya.

2 Materials and Methods

2.1 Fruit material and coating chemicals

Papaya (*Carica papaya*, cv. Havaí) fruit were obtained from Central Estadual de Abastecimento S/A (CEASA) in Campina Grande, PB – Brazil. Cassava (*Manihot esculenta* Crantz) starch was extracted from roots purchased at the street market of Areia, Paraíba, Brazil and dried at 55 ^{o}C to 15% water content. Tween 40 were purchased from Sigma Aldrich (São Paulo, SP, Brazil). Glycerol was purchased from Dinâmica Química Contemporânea LTDA (São Paulo, SP, Brazil).

2.2 *Preparation and application of coatings*

Cassava starch solutions (at 1.5%, 2%, 2.5%, and 3%; w/v) were prepared by heating the starch-water mixture to 70 °C using a hot plate magnetic stirrer and left at 70 °C for 5 min to obtain complete gelatinization. Coating solutions were cooled to 60 °C and glycerol (1%; w/v) was added as a plasticizer under constant stirring. Tween 40 (0.05%; w/v) was added as surfactant also during cooling phase, at 18 °C. For coating, fruit were dipped for 1 min in the coating solutions at 20±2 °C. Following coating, all fruit were air-dried at room conditions (20 °C, ~60% RH), then placed in polystyrene trays and stored at room conditions. A fruit was considered as an experimental unit and 16 papaya fruit were tested for each coating solution. The experiment was replicated twice. For each replication, new coating solutions were prepared.

2.3 Determination of the coated surface area in a projected area

The coated surface area was estimated from projected area of fruits by the image processing technique as adapted from Arunachalam et al. (2018). Coated fruit were dipped in an iodine solution (0.25% of KI and 1% of I_2 in water) to stain the starch granules, 48 h after the coating application, so the coating presence was shown by the black coloration. The images were captured from opposite sides of each coated fruit using a Coolpix p500 camera with 12.1 megapixel resolution. For control of lighting, fruit were placed inside a square box especially designed from diffuse white paper, which had four 12 W lamps arranged distally (10 cm) on the outer sides. Image processing was performed using the ImageJ® 1.52a program (Bethesda, MD, USA). To develop the image segmentation based on the luminance, green↔red spectrum, and blue↔yellow spectrum (Lab) color space. For selecting the fruit's coated area, each image was divided into its Lab channel, which are grayscale monochrome. The histogram of the entire image was analyzed and an threshold level was set using the 'auto-threshold' setting to create the binary image. For binarization, in case of the value of a pixel be greater than or equal to the threshold value then the value 1 (white) was set, and if it was lower than the threshold value, 0 (black) was set (Arunachalam et al., 2018). From the binary images, the projected area of uncoated surface and also the projected area of the fruits, in pixels were obtained. The coated surface area was expressed as a percentage, relative to the projected area of total fruit surface, as follows:

$$CSA\ (\%) = (TA - USA/TA) * 100$$

where CSA is the coated surface area, TA is the projected area of total fruit surface, and USA is the projected area of uncoated surface.

2.4 Coating relative resistance

The coating resistance to CO_2 diffusion was determined by monitoring the difference in equilibrium rate of CO_2 partial pressure (P_{CO_2}) between the chambers adhered directly to the fruit surface and chambers adapted on the coated-skin surface. The 2-mL volume glass chambers [cylinders of 16-mm long and 14-mm diameter were sealed with a rubber septum (6-mm thick), see Fig. 1], and attached to the fruit skin at an equatorial position, using silicone sealant (*Dow Corning*® PV-804 Neutral Sealant). After sealing the chambers, fruit were left for 36 h before applying the coating. Followed the coating application, fruits were left for 36 h at room conditions (20 °C, ~50% RH) until the coatings were completely dried. Following, another glass chamber was attached on the skin-coated (one quadrant apart from where the first chamber was attached) of the coated fruits and left for more 48 h before analysis. Silicone grease was applied at the septum-glass interface to prevent leaks. For estimating the coating resistance, the chamber system was opened by placing three needles through the septum. Then, through one of the needles chambers were slowly flushed with a 10 mL sample of air, with the aid of a 10 mL syringe, such that the air went into the chamber from one of the needles and gone out through the other two. Immediately after flushing with air, the needles were removed, thus, the chamber system was closed. The CO_2 equilibrium rate in the chamber were monitored.

This analysis was performed for 3% starch coated fruit. For the analysis, 6 fruit were used. The CO_2 steady state time reached in the chambers was monitored in a preliminary experiment. It was observed that 60 min after flushing, the increase in CO_2 concentration in the chambers adhered directly to the fruit surface lost linearity (data not shown). Therefore, for determining coating resistance, a 0.1-mL gas sample was taken from each chamber at 10, 20, 30, 40, 50, and 60 min after flushing, and immediately injected in an infrared gas analyzer (model 225-MK3; Hoddesdon, England) operated in a flow-through mode with N_2 as the carrier gas and a flow rate of 100 mL min^{-1}. CO_2 concentration was calculated relative to the certified gas standard noted earlier. Immediately after sampling, the chamber system was opened again by placing three needles through the septum and left at rest for at least 60 min. Six chambers from three fruits

(adhered either directly to the fruit surface or on the skin-coated surface) were once evaluated every 60 min using a 6 x 6 Latin square experimental design so that each chamber was located at each of six periods (10, 20, 30, 40, 50, and 60 min) of analysis. The Latin square experimental design was replicated twice, resulting in six fruit (12 chambers) evaluated.

2.5 Rate of CO_2 diffusing through the fruit-coated system

For estimating the rate of CO_2 diffusing through the coating, a flow-through system was adapted to the chambers (head space ~ 2-mL) attached to the skin of the coated fruit, as above described: after the coating application, fruits were left for 36 h at room conditions. Following, two glass chambers were attached on the coated fruit skin and left for more 48 h before analysis. The flow-through system with air was set in the chambers using two needles that had latex rubber tube fixed in its upside, while the needle blossom end was fixed in the chamber, through a rubber septum (Fig. 1A). A flow rate of 35 mL min^{-1} of air was flushed into the chamber from one of the needled-tube and gone out through the other one. The flow rate of air was measured at the outlet latex rubber tube (~20 cm long), aiming to confirmed the steady state of the flow-through system. For these analyses, 16 fruits were used per each coating solution.

Gas samples (100 µL) were withdrawn from the inlet and outlet tube and immediately injected in an infrared gas analyzer (model 225-MK3; Hoddesdon, England) operated in a flow-through mode with N_2 as the carrier gas and a flow rate of 100 mL min^{-1}. CO_2 concentration was calculated relative to the certified gas standard noted earlier. The difference in concentration of the inlet-flow rate and outlet-flow rate gave the rate of CO_2 released ($mL.\mu m.min^{-1}.m^{-2}.kPa^{-1}$).

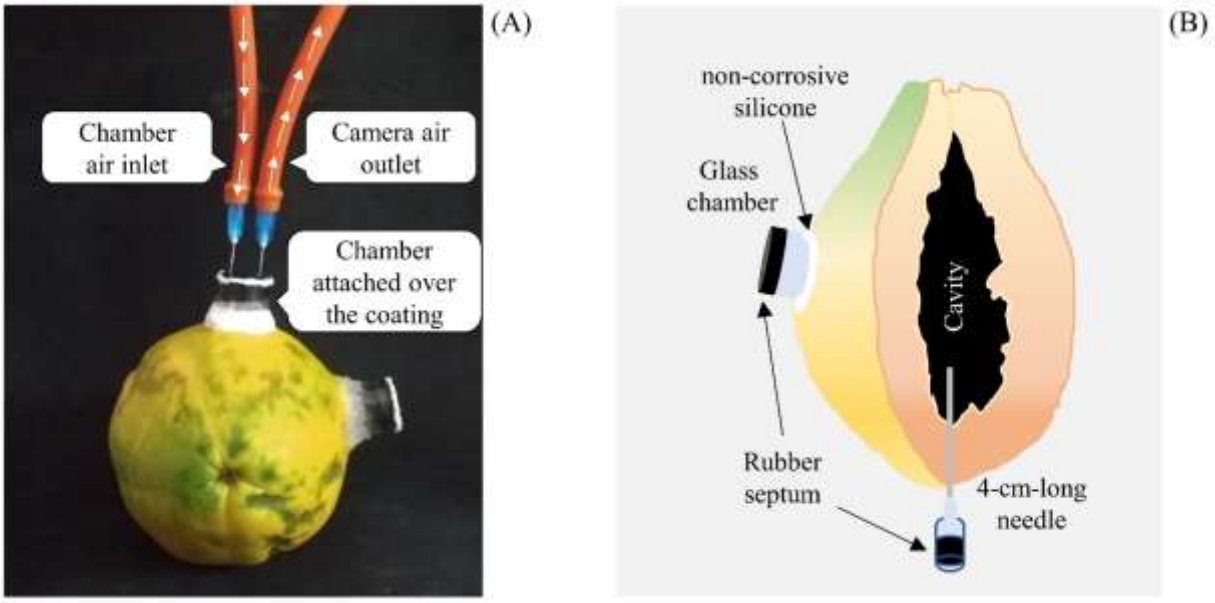

Fig. 1. Flow-through system adapted to the chambers attached on the coating-skin interface of the coated fruit for estimating CO_2 diffusion (A) and, chambers adhered to the fruit surface and needle applied to the core cavity fruit to measure the internal gas concentrations (B) of papaya.

The permeation of CO_2 was estimated from the steady-state rate of gas transfer using the Fick's equation based on the first law of diffusion (Park, 1999), assuming that composition of the internal atmosphere was uniform throughout the fruit, according to the following adapted equation:

$$P = Q.X/(A.\Delta p)$$

where, Q is the rate of gas diffusing through the coating ($mL.min^{-1}$), X is the thickness of the coating (µm), A is the base area of the chamber (m^2), Δp is the partial pressure difference of the gas across the coating (kPa) and *P* is the permeability of CO_2 ($mL.\mu m.min^{-1}.m^{-2}.kPa^{-1}$). For measuring thickness, the chambers were carefully removed from the fruit surface and the coatings were peeled off from the chamber base. Following, the thickness of the removed coating was measured with a digital micrometer. Three random measurements were performed in each piece of coating, from each chamber. The mean values were used to calculate the coating permeability.

2.6 Internal O_2 and CO_2 concentrations

The reduced O_2 levels reached with the coating was determined from the 2-mL chambers adhered to the fruit surface as described by Rajapakse et al. (1990). Cylinders of 16-mm long and

14-mm diameter were sealed with a rubber septum (6-mm thick). Silicone grease was applied at the septum-glass interface to prevent leaks. Two chambers were attached to the fruit skin at an equatorial position, in opposite sides of 12 fruits in each treatment using silicone sealant (*Dow Corning*® PV-804 Neutral Sealant). After sealing the chambers, fruit were left for 36 h before applying the coatings. A 0.1-mL gas sample was taken from each chamber at 1, 2, 3, 4, and 5 d after coating the fruit. Gas samples were injected in oxygen analyzer PA-10a (Sable Systems International, Las Vegas, USA) operated in a flow-through mode with N_2 as the carrier gas and a flow rate of 100 mL min^{-1}. O_2 concentration was calculated relative to the certified gas standard noted previously.

2.7 Respiration rate of the whole fruit

The respiration rate was determined for the whole fruits in a closed system at room condition as described by Saltveit (2019). For each treatment, four jars [1-L Teflon containers (Savillex Corporation, Minnetonka, MN)] containing one fruit each were used for measuring the respiration rate, which were sealed for 60 min. For gases analysis, samples (100 µL) were withdrawn from the outlet port and immediately injected in an infrared gas analyzer (model 225-MK3; Hoddesdon, England) operated in a flow-through mode with N_2 as the carrier gas and a flow rate of 100 mL min^{-1}. CO_2 concentration was calculated relative to the certified gas standard noted earlier. The CO_2 released were expressed as mL kg^{-1} h^{-1}.

2.8 Statistical analysis

Experimental data were subjected to analysis of variance using software R 4.00. Means of treatments were separated using Tukey's range test ($P \leq 0.05$). Data were presented as mean ± standard deviation means. Proportion of coated surface data were analyzed in function of starch concentration by using beta regression technique as recommended for continuous proportions data (Douma & Weedon, 2019).

3 Results and Discussion

3.1 Resistance of coated surface to gas diffusion

The coating deposition on the fruit surface was significantly increased by increasing starch concentration in the coating solution (Fig. 2A). Starting from 1.5 to 3% (w:v) starch, there was an increase of 62% in the proportion of coated surface area. A uniform continuous coating all over

the papaya surface were obtained with a 3% starch-based coating solution, probably blocking most of the pores and lenticels on fruit surface (Perez & Beaudry, 1998; Maqbool et al., 2011). The coated surface resistance to CO_2 diffusion was determined on fruit coated with 3% starch by monitoring the difference in the CO_2 partial pressure (P_{CO_2}) equilibrium rate between chambers adhered directly to the fruit surface (applied before coating application) and chambers on the coating-skin surface (applied after coating application). The coated skin showed a ~2-fold higher resistance to CO_2 diffusion than the uncoated skin surface (Fig. 2B), as indicated by the increased linear rate of P_{CO_2} inside the chambers. The result herein supports previous reports that coating increases skin resistance to gas diffusion (Banks et al., 1993; Perez & Beaudry, 1998).

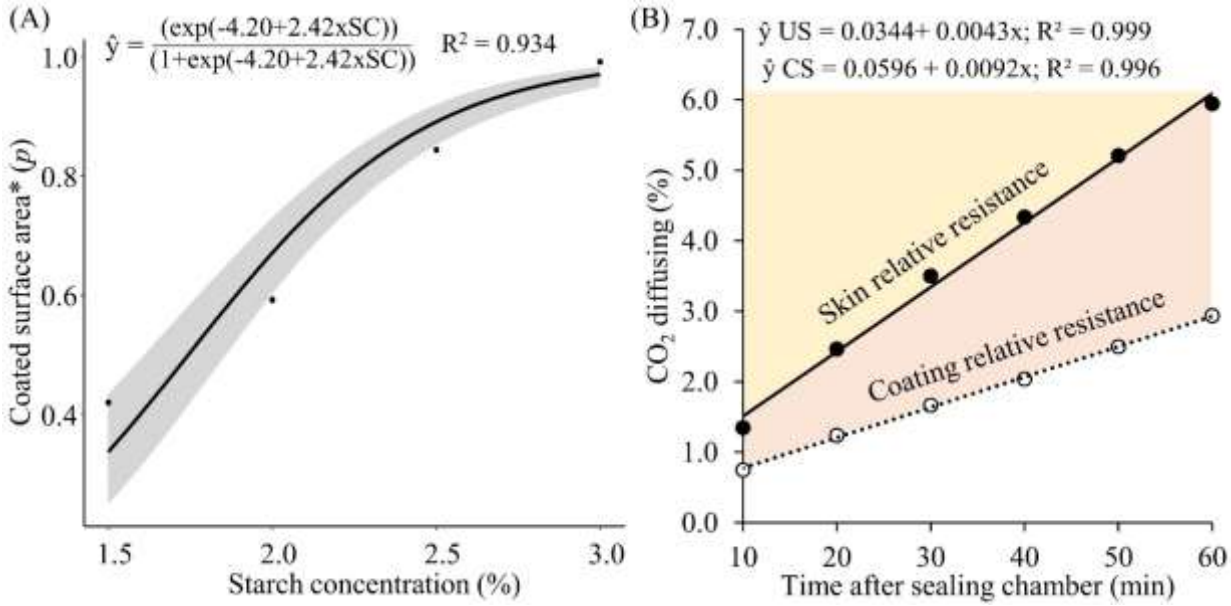

Fig. 2. Proportion of coated surface (A) of papaya fruit treated with starch-based dispersion at different starch concentration, and (B) percentage of CO_2 diffusing through the coated skin surface of a 3% starch-based coated fruit. Shaded zone is the confidence interval. *Area (*p*) is relative to the projected area of fruit. ○ = coated surface (CS) and ● = uncoated surface (US).

A flow-through system was adapted to the sealed chambers attached on the skin of the coated fruit, on the dried coating, and in the head space the difference in CO_2 concentration of the inlet- and outlet-flow gave the rate of CO_2 released through the coated papaya fruit. The CO_2 released at 1.5 and 2% starch-based coated fruit was higher as compared whit 2.5 and 3% starch-based coated fruit (Fig. 3A). The result suggests that the higher starch concentration of the coated fruit

may provide a reduced respiration rate and/or increased resistance to gas diffusion, reducing the rate of CO_2 released.

The permeation of CO_2 was estimated from the steady-state rate of gas transfer using the Fick's equation, which is based in the first law of diffusion (Park, 1999), taking into account the P_{CO_2} in the tissues immediately beneath the skin. Fruit coated with 2% starch-based solution showed highest permeability to CO_2 diffusion (Fig. 3A). At 2.5 and 3% starch-based coated fruit, the permeation to CO_2 diffusion were 53 and 66% lower than that observed for 2% starch-based coated fruit, respectively. The decreasing in permeability of CO_2 diffusion with increasing starch concentration in the coating solution confirms the effect of coatings on the increasing skin resistance to gas diffusion. This effect is probably due to the increase proportion of the blocked pores on the fruit surface (Banks et al., 1993; Amarante et al., 2001), as also indicated by a strong negative correlation (r = -0.78) between the proportion of coated surface and permeability to CO_2 diffusion (Fig. 4B).

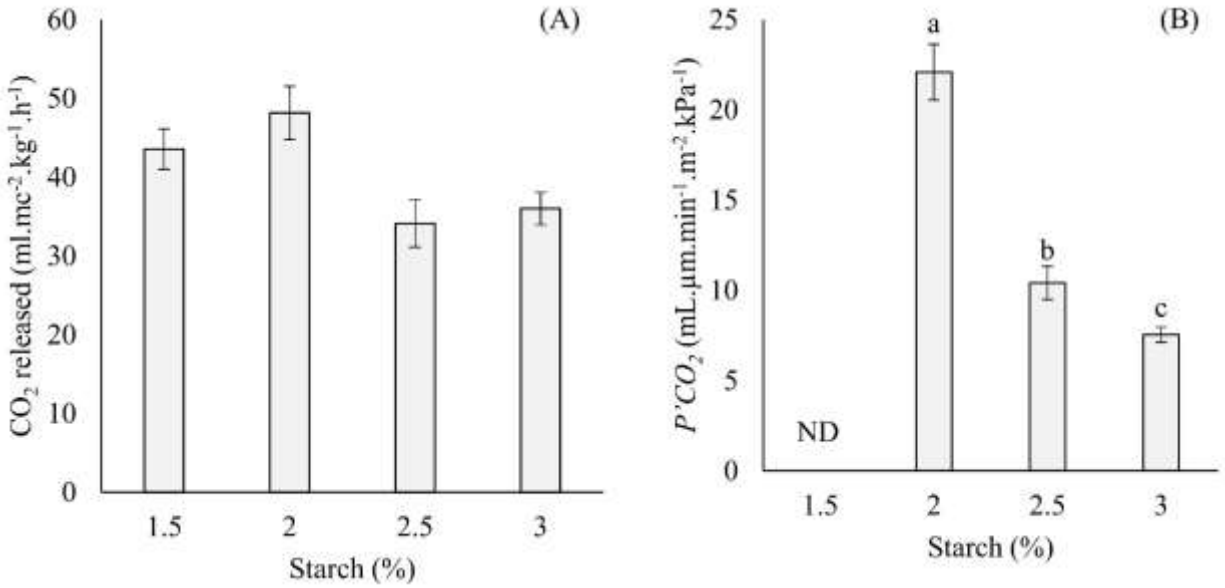

Fig. 3. Amount of CO_2 diffusing through the coated fruit surface (A) and, coated surface permeability to gas diffusion (B) in coated papaya fruit as affected by starch concentration in the coating solution. ND = Not Determined. Different letters indicate statistical difference among treatments according to Tukey's range test ($P \leq 0.05$).

The gas permeability of coatings is usually determined in films obtained by drying the coating solutions on plates, from where they are peeled off and evaluated (Vargas et al., 2008).

Herein, an effective nondestructively method was described for the first time to measure the gas permeability of a coating followed its application to the fruit surface. In fact, this method uses a flow-through system adapted to chambers (head space) attached on the skin of the coated fruit, fitted directly on the dried coating. This approach can be very useful to compare the effect of coatings on fruit surface resistance to gas diffusion, especially taking into account the interaction between the coating and the fruit skin.

A gradual decrease in fruit respiration rate was observed with increasing starch concentration from 1.5% to 3% in the coating solution (Fig. 4.A). No significant difference in respiration rate was observed between uncoated and 1.5% starch-based coated fruit during storage. A slight decrease in respiration rate was observed in fruits of all treatments throughout storage, which was more pronounced in uncoated fruit. Reduced respiration rate is usually associated with a decreased rate of ethylene production and delaying in fruit ripening (Irtiza et al., 2019). In addition, fruit respiration rate has been alternatively used to indicating changes in internal atmosphere composition and gas exchange in coated fruit (Vargas et al., 2008; Maringgal et al., 2020). Herein, a moderate positive correlation (r = 0.42) was found between the respiration rate and the permeability to CO_2 diffusion of coated fruit.

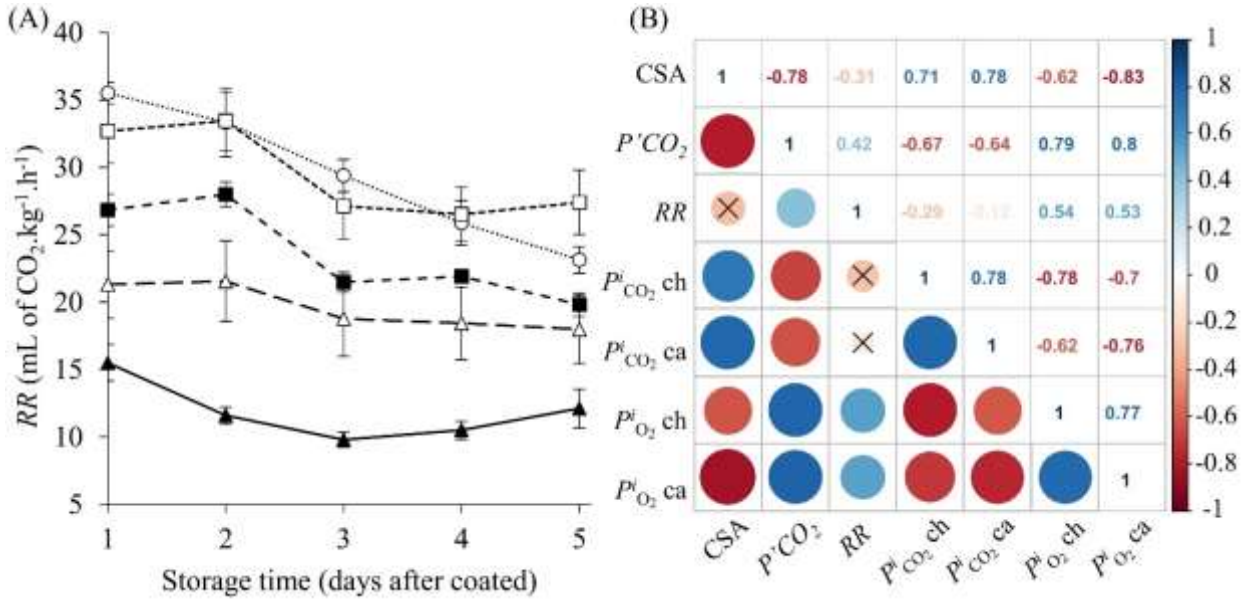

Fig. 3. Respiration rate (RR) of coated papaya fruit as affected by starch concentration in the coating solution (A) and, Pearson correlation between coated surface area (CSA), permeability to gas diffusion ($P'CO_2$), RR and internal atmosphere composition of coated papaya. Legend: ○ =

Uncoated; □ = 1.5% starch; ■ = 2.0% starch; Δ = 2.5% starch; ▲ = 3.0% starch. Internal partial pressures of O_2 ($P^i_{O_2}$) and CO_2 ($P^i_{CO_2}$); ca = cavity; ch = chamber. Vertical bars represent standard error means.

3.2 Internal atmosphere and flesh resistance to gas diffusion

The partial pressure of O_2 and CO_2 inside the chambers attached to fruit surface was assumed to be equal the internal O_2 ($P^i_{O_2}$) and CO_2 ($P^i_{CO_2}$) partial pressures, respectively, immediately beneath the fruit skin (Rajapakse et al., 1990). The $P^i_{O_2}$ in uncoated fruit decreased from 17 kPa to 12.2 kPa, beneath the skin (Fig. 4A), and from 16 kPa to 8.3 kPa at the core of fruit (Fig. 4B) followed 5d storage. The increase in O_2 gradients between the internal and external atmospheres during storage can be associated with senescence process, wherein cell wall and membrane were broke down and cell contents filled some intercellular spaces, resulting in a higher diffusive resistance to the gasses (Kader et al., 1989). In fact, O_2 exchange in the flesh tissue occurs mainly through the intercellular space, the cell wall network and less through the intracellular liquid (Ho et al., 2009; Ho et al., 2011). This phenomenon resulted, therefore, in the highest O_2 gradient cross the flesh in uncoated fruit on the 5^{th} day of storage (Fig. 5A).

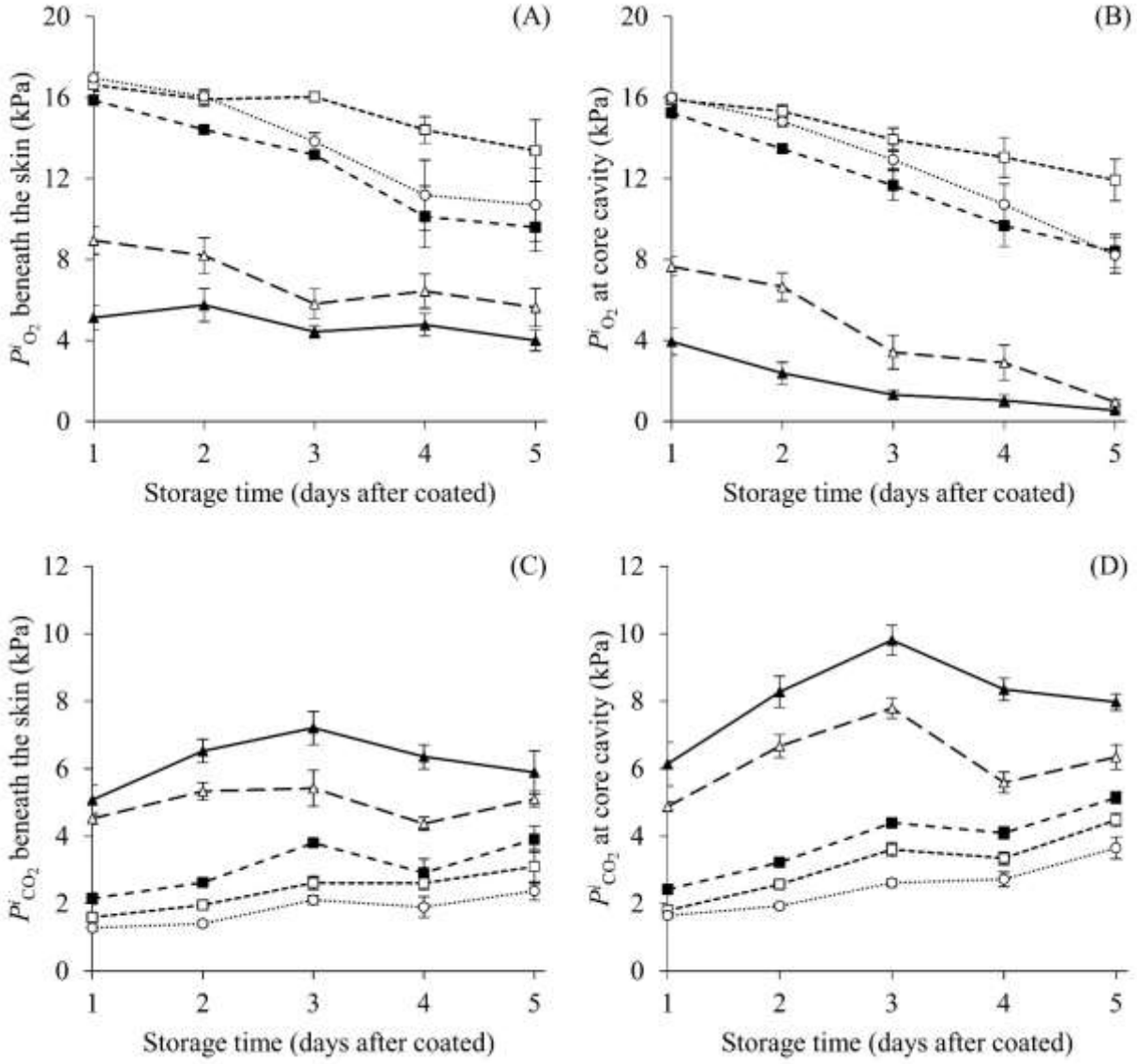

Fig. 4. Changes in O_2 (A) and CO_2 (B) concentrations in chambers adhered on papaya fruit surface and O_2 (C) and CO_2 (D) concentrations in the cavity of papaya fruit as affected by starch-based solution at different starch concentration and kept at 20 °C for 5 days after coating. Legend: ○ = Uncoated; □ = 1.5% starch; ■ = 2.0% starch; Δ = 2.5% starch; ▲ = 3.0% starch. Vertical bars represent standard error means.

From the 3rd day after coating, the 1.5% starch-based coated fruit showed higher $P^i_{O_2}$ than uncoated fruit, beneath the skin and also at the core cavity. The lower O_2 gradient between internal and external atmospheres as compared with uncoated fruit suggests that 1.5% starch-based coating did not exert a sufficient enough barrier to O_2 diffusion to cause a build up in the

internal atmosphere with decreased O_2 levels, instead, fruit skin and flesh had a lower resistance to O_2 exchange than uncoated fruit, which is associated with cell wall and membrane integrity (Kader et al., 1989; Rajapakse et al., 1990; Ho et al., 2009).

The $P^i_{O_2}$ in 2% starch-based coated fruit was not significantly different from the $P^i_{O_2}$ in uncoated fruit throughout storage. In turn, applying 2.5 and 3% starch-based coating the $P^i_{O_2}$ decreased by 47% and 70% as compared to the $P^i_{O_2}$ in uncoated fruit, respectively, beneath the skin at 1st day after coating. Similar behavior was found for the $P^i_{O_2}$ in the core of the fruit, where in 2.5 and 3% starch-based coated fruits the $P^i_{O_2}$ decreased by 52% and 75% as compared to uncoated fruit, respectively, at 1st day after coating. Since surface pores are the main path for O_2 exchange (Amarante et al. 2001), the higher O_2 gradients between internal and external atmosphere developed with increasing starch concentration in the coating that is probably due to the greater proportion of coated surface (Fig. 2A), which probably occluded a greater number of pores on the fruit surface (Banks et al., 1993) that, in turn, increased fruit skin resistance to gas diffusion.

Thus, the $P^i_{CO_2}$ beneath the skin and in the core of fruit increased with increasing starch concentration in the coating solution (Fig. 4C and D). Furthermore, a slight increase in the $P^i_{CO_2}$ beneath the skin and in the core of fruit was observed in fruits of all treatments. These results are similar to those reported by Maringgal et al. (2020) in the core cavity of uncoated or Malaysian stingless bee honey at 1.0% and 1.5% coated papaya and also by Ali et al. (2011) in at 0.5%, 1.0%, 1.5% and 2.0% chitosan coated papaya. Herein, there were no significant differences between the $P^i_{CO_2}$ at 1.5% starch-based coated fruit and the uncoated fruit throughout storage. In turn, 2.5 and 3% starch-based coated fruit had a $P^i_{CO_2}$ ~2 and ~3-fold higher than uncoated fruit, respectively. Similar to O_2, the gradient for CO_2 was increased by the relatively lower permeance of the coated fruit skin (Hagenmaier, 2005; Ali et al., 2011). In fact, both coated surface resistance to CO_2 diffusion (Fig. 3B) and internal CO_2 concentrations (Fig. 4C and D) increased with increasing starch concentration in the coating.

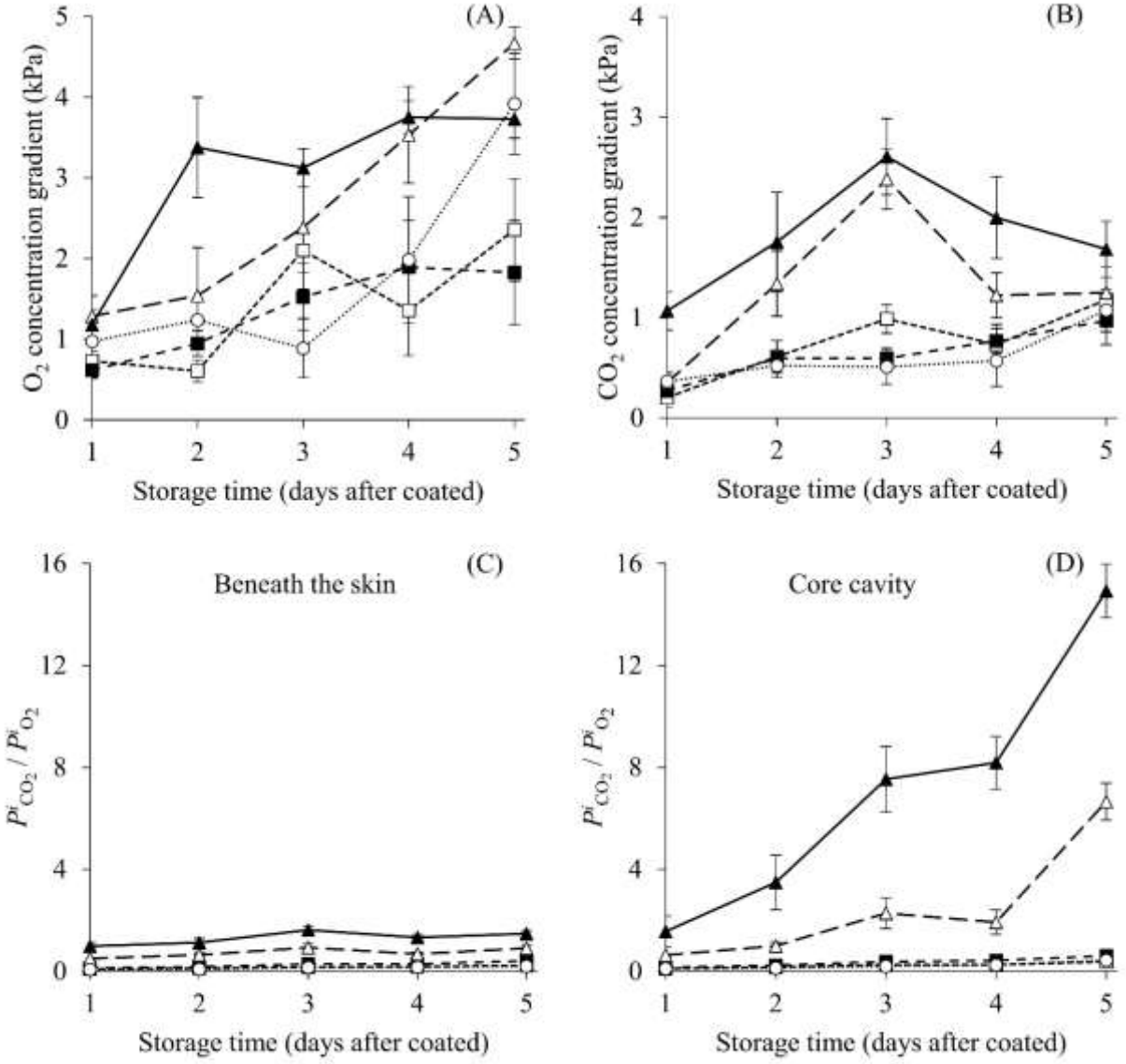

Fig. 5. Gradients of O_2 (A) and CO_2 (B) between internal partial pressures (P^i) in the chambers attached to the coated papaya fruit surface and at core cavity of coated papaya fruit, and $P^i_{CO_2}/P^i_{O_2}$ ratio in the chambers attached to the coated papaya fruit surface (C) and, at core cavity of coated papaya fruit (D). Legend: ○ = Uncoated; □ = 1.5% starch; ■ = 2.0% starch; Δ = 2.5% starch; ▲ = 3.0% starch. Vertical bars represent standard error means.

It is worth to mention that the permeation to CO_2 diffusion showed strong positive correlations (r = 0.79 and r = 0.8) with the $P^i_{O_2}$ beneath the skin and at the core of coated fruit, respectively, and also showed moderate negative correlations (r = -0.67 and r = -0.64) with the $P^i_{CO_2}$ beneath the skin and at the core of the coated fruit, respectively. On the other hand, fruit's

respiration rate showed only a moderate positive correlation (r = 0.54 and r = 0.53) with the $P^{i}_{O_2}$ beneath the skin and at the core of the coated fruit, respectively, and no significant correlation with the $P^{i}_{CO_2}$ was found. These results clearly indicate that the permeation to CO_2 diffusion of coated fruit, assessed as described in this work is more appropriate than using fruit respiration rate to indicating the changes in internal atmosphere composition and gas exchange of coated fruit.

For 2.5 and 3% starch-based coated fruit, the decline in O_2 concentration at the core cavity was more intense that its decline beneath the fruit skin throughout storage. As a result, the O_2 gradient between the surface and core of 3% starch-based coated fruit was the highest from the 2nd day of storage, and 2.5% starch-based coated fruit showed higher O_2 gradient cross the flesh from the 4th day of storage. In turn, the O_2 gradient developed between the surface and core of uncoated and coated with 1.5 and 2% starch-based solution had a slight increase throughout storage excepted by that uncoated fruit that showed higher O_2 gradient at the 5th day of storage (Fig. 5A). Similarly, just a slight increase in the CO_2 gradient was developed between the surface and core of uncoated and coated with 1.5 and 2% starch-based fruits, whereas those with 2.5 and 3% showed a higher CO_2 gradient across the flesh (Fig. 5B). Taken together, these higher gradients in O_2 and CO_2 suggest that higher concentration coatings can also increase flesh resistance to gas diffusion of coated fruit (Perez & Beaudry, 1998), besides increasing skin resistance to gas exchange.

According to Cukrov (2018), gas diffusion in fruit tissues may occur driven by concentration gradients as well as by permeation due to pressure gradients in the tissues. Under normal oxygen conditions, the inflow of O_2 and outflow of CO_2 are boosted as these gases are consumed and released, respectively, by the respiratory process (Hagenmaier, 2005; Cukrov, 2018). Herein, the higher skin resistance to gas exchange in the 2.5 and 3% starch coated fruit (Fig. 3B) decreased O_2 and increased CO_2 levels beneath the skin (Fig. 4A and C). The established $P^{i}_{CO_2}$ and $P^{i}_{O_2}$ inside the coated fruit may have caused a reduced pressure gradient between the tissues immediately bellow the skin and in the core of the fruit, reducing the flesh resistance to gas diffusion (Cukrov, 2018).

Only a slight increase in the ratio between $P^{i}_{CO_2}$ and $P^{i}_{O_2}$ beneath the skin was found with increasing starch concentration in the coating (Fig. 5C). In addition, the $P^{i}_{CO_2}/P^{i}_{O_2}$ beneath the skin was invariable throughout storage, for uncoated and coated fruit. However, in the core of

fruit, there were a sudden increase in the $P^{i}_{CO_2}/P^{i}_{O_2}$ for 3% and also for 2.5% starch coated fruit at the end of storage (Fig. 5D). These results suggest that 2.5% and 3% starch coatings leaded to an anaerobic condition at the core of the fruit, which eventually could lead to physiological disorders (Banks et al., 1993; Ho et al., 2008). It is worth mentioning that at the end of storage 3% starch coated papaya seemed not to evolve in ripening and showed dark depression spots in the epidermis.

4 Conclusion

It was developed a method for quantifying the permeability to gas diffusion through a coated fruit surface. The proposed approach is to our knowledge the most comprehensive method to date for measuring permeability to gas diffusion through a coating followed its application on a fruit surface. Based on that, the measurement of the permeability to gas diffusion was more appropriated than fruit respiration rate to indicate changes in the internal atmosphere composition. Evidence for that was the stronger positive correlation with the O_2 and stronger negative correlation with the CO_2 internal partial pressures relatively to respiration rate during storage of coated papaya. The relationship between the coated skin resistance to gas exchange and the O_2 and CO_2 internal partial pressure in the tissues immediately beneath the skin and also in the core of fruit were described for starch coated papaya. Besides increasing skin resistance to gas exchange, coatings can also increase flesh resistance to gas diffusion by reducing the pressure gradients in the flesh tissues. The approaches described in here will be very useful to compare the effect of coatings on skin resistance to gas diffusion and helpful on setting a target internal atmosphere in a coated fruit, especially taking into account the interaction between the coating and the fruit skin.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data 1

Table. S1. Changes in respiration rate (RR) O_2 and CO_2 concentrations in chambers adhered on papaya fruit surface and in the cavity of papaya fruit as affected by starch-based solution at different starch concentration and kept at 20 °C for 5 days after coating.

	Storage time (days after coating)	Uncoated	Starch concentration (%)			
			1.5	2	2.5	3
RR (mL of $CO_2.kg^{-1}.h^{-1}$)	1	35.51a	32.70a	26.83b	21.31c	15.50d
	2	33.33a	33.44a	27.98b	21.56c	11.59d
	3	29.38a	27.13a	21.48b	18.78b	9.80c
	4	25.87a	26.52a	21.92ab	18.43b	10.50c
	5	23.13a	27.40ab	19.81b	17.99c	12.12d
P_{CO_2} in chamber (kPa)	1	1.29c	1.59bc	2.16b	4.53a	5.07a
	2	1.41d	1.96cd	2.63c	5.33b	6.52a
	3	2.11d	2.61d	3.80c	5.42b	7.21a
	4	2.17d	2.61cd	3.33c	4.37b	6.35a
	5	2.53e	3.30d	4.17c	5.10b	6.29a
P_{O_2} in chamber (kPa)	1	16.97a	16.63a	15.87a	8.93b	5.12c
	2	16.05a	15.92a	14.41a	8.19b	5.75c
	3	13.82b	16.02a	13.17b	5.80c	4.43c
	4	12.77ab	14.40a	11.56b	6.43c	4.77c
	5	12.21b	14.27a	10.23c	5.62d	4.26d
P_{CO_2} in cavity (kPa)	1	1.65c	1.79c	2.43c	4.88b	6.14a
	2	1.93d	2.57cd	3.22c	6.67b	8.28a
	3	2.62d	3.60cd	4.39c	7.80b	9.81a
	4	2.74d	3.34cd	4.10c	5.59b	8.35a
	5	3.61d	4.48cd	5.14c	6.35b	7.97a
P_{O_2} in cavity (kPa)	1	15.99a	15.90a	15.25a	7.65b	3.94c
	2	14.81a	15.31a	13.46a	6.65b	2.38c
	3	12.93a	13.92a	11.65a	3.42b	1.30b
	4	10.79ab	13.04a	9.67b	2.90c	1.02c
	5	8.29b	11.92a	8.41b	0.95c	0.53c

Different letters within the same period indicate statistical difference between treatments according to Tukey's test ($P \leq 0.05$).

4 ARTIGO III

RELATIONSHIP BETWEEN STARCH-BASED COATING AND THE INTERNAL OXYGEN AND CARBON DIOXIDE CONCENTRATIONS IN COATED BANANA

Highlights

The optimum internal O_2 concentration was estimated for starch-coated bananas.

Coating with 3.0%-starch leads to too-low O_2 and too-high CO_2 internal levels.

Anaerobic respiration was induced in fruits with continuously coated surface.

Proportion up to 75% of coated surface area provides safe internal O_2 and CO_2.

1.5 and 2.0% starch coatings were effective in delaying ripening of bananas.

Journal: Postharvest Biology and Technology

Abstract

Banana is a climacteric fruit with a short shelf life. An alternative to maintain quality and storability of this fruit could be the surface coatings. However, if the coating occludes most the cuticle and pores in the fruit surface, the respiratory metabolism becomes anaerobic and fruit quality will be rapidly lost. Therefore, the aim of this work was to assess the internal gas concentrations in banana fruit coated with starch-based dispersions as related to coated surface area and the internal atmosphere composition. Fruits were dipped in starch-based coating dispersions at 1.5, 2.0, 2.5, or 3.0% (w/v) and stored at 21±2 °C for 8 days. Increasing starch concentration in the coating continuously decreased O_2 and increased CO_2 internal concentrations in the coated bananas, which were correlated with the increasing of the proportion of coated surface. Thus, a 3.0%-starch coating covered the entire banana surface. Under this condition, internal O_2 was too-low and there was a burst in CO_2 internal levels, which increased the respiratory quotient, as indicative of anaerobic respiration. At O_2 internal partial pressure of 6 kPa inside the chamber attached onto the fruit skin would be adequate for starch coated bananas, which stood just above the estimated internal lower O_2 limit value and, at which respiration rate was minimized without development of anaerobic respiration. 1.5 and 2.0% starch coatings might be effective for delaying ripening, maintaining qualities, and extend shelf life of bananas without any risk of development of anaerobic respiration. A proportion of 75% of coated surface area was estimated as the threshold for an adequate modified atmosphere by surface coatings on bananas.

Keywords: *Musa* sp., internal atmosphere, anaerobic respiration, ripening

1 Introduction

Banana is one of the most popular and consumed tropical fruit worldwide due to its aroma, taste, and high nutritional value (Jirukkakul and Chanshotikul, 2019; Yuan et al., 2017). However, as a typical climacteric fruit, bananas display a burst in biosynthesis of ethylene and respiration at the onset of ripening, which leads to rapid physiological changes, such as chlorophyll degradation and cell wall degradation, which results in fast ripening and short-shelf life (Gao et al., 2020; Chen et al., 2019). Actually, the energy derived from this rise in respiration is used to supply power for these metabolic processes that accompany ripening (Saltveit, 2019). Therefore, the ripening processes might be delayed and shelf-life extended by reducing ethylene biosynthesis and action and respiratory metabolism in harvested banana fruit (Chen et al., 2019; Deng et al., 2017).

The modified atmosphere generated by coatings technology can delay ethylene production and reduces respiration rate in coated bananas, improves storability and commercial value of the fruit (Thakur et al., 2019). However, if the coating occludes nearly 100% of the pores of the banana surface (Maqbool et al., 2011), even in a fraction of the fruit (Perez and Beaudry, 1998), it will not ripen properly and will lose quality. Notably, the application of some coatings has been shown to result in fermentation and build up of off-flavors in many fruits (Arnon et al., 2014; Perdones et al., 2015; Pérez-Gallardo et al., 2015; Arnon et al., 2015; Maqbool et al., 2016; Kowalczyk et al., 2017; Saberi et al., 2018).

The mechanism through which coatings generally exert their effects involve decreases in gas exchanges, reducing internal oxygen (O_2) and increasing internal carbon dioxide (CO_2) concentrations (Amarante & Banks, 2010). In turn, reduced O_2 and elevated CO_2 atmospheres reduce the rate of ethylene biosynthesis and action as well as respiration rate (Botton et al., 2019). The decreased respiration rate coupled with the selective permeability to the gases by a surface coating results in a larger modification of internal partial pressure of O_2 than of the internal partial pressure of CO_2 (Banks et al., 1997). However, this behavior is observed only when the internal O_2 partial pressure remains above the internal lower O_2 limit, otherwise, the produce starts fermenting and a burst of internal partial pressure of CO_2 increase the respiratory quotient compensating the differential effect of coating on the permeance to the two gases (Amarante & Banks, 2010).

A coating that allows appropriate gas exchange (O_2 and CO_2) will delay fruit ripening and reduce the accumulation of volatile compounds associated to fermentative metabolism (Pérez-Gallardo et al., 2015). In this sense, monitoring the internal gas concentrations and the proportions of occluded pores of the coated fruit are the main features for selecting appropriated coating composition and identifying its appropriate concentrations, seeking to achieve a proper modified atmosphere without excessive risks of fermentation (Banks et al., 1993; Banks et al., 1997).

Cassava (*Manihot esculenta* Crantz) is the cheapest material for starch production in Brazil (Chiumarelli, Ferrari, Sarantópoulos, Hubinger, 2011). Cassava starch is widely consumed and it is an important biopolymer composed of two α-D-glucose based polysaccharides, amylose and amylopectin (Zhu, 2015). Recently, cassava starch has become a source of interest in composing biodegradable coating for fruit and vegetables postharvest conservation due to its low cost, easy availability, and biodegradability (Chiumarelli et al., 2011; Ferreira et al., 2019; Edhirej et al., 2016). However, for our knowledge no information is available on the changes in internal atmosphere composition in response to starch-based coatings applications, especially in banana fruit, which can elucidate the mechanism of gas exchanges and help to manage the effects of coatings in fruit during storage. Therefore, the aim of this work was to assess the internal gas concentrations in banana fruit coated with starch-based dispersions as related to the proportion of coated surface and the internal atmosphere composition.

2 Material and Methods

2.1 Fruit material and coating chemicals

Banana (*Musa sp.*, AAA group, Cavendish subgroup, cv. Valery) fruits were purchased at the commercial maturity from a market in East Lansing, Michigan, United States. The cassava starch (*Manihot esculenta* Crantz) was purchased at the street market in Areia, Paraíba, Brazil. Tween 40® and glycerol were purchased from Sigma Aldrich (St. Louis, MO, US).

2.2 *Preparation of starch solutions and application of coatings*

The coating solutions were prepared as follows: cassava starch solutions (at 1.5, 2, 2.5, and 3; w/v) were prepared by heating the starch-water mixture up to 70 °C using a hot plate magnetic stirrer and left at 70 °C for 5 min to obtain complete gelatinization. Coating dispersions were cooled to 60 °C and glycerol (1%; w/v) was added as a plasticizer under constant stirring. Tween

40 (0.05%; w/v) was added as surfactant also during cooling phase, at 18 °C coating dispersions. For coating, fruit were dipped for 1 min in the coating solutions at 20±2 °C. Following coating, all fruit were air-dried at room conditions (20 °C, ~50% RH), then placed in polystyrene trays and stored at 23±2 °C and 92±1% relative humidity. This experimental approach was repeated three times, which are presented in supplemental files. For each experimental approach, new coating dispersions were prepared.

2.3 Measurement of the proportion of coated surface

For measurement of the proportion of coated surface, twelve banana fruits were used for each coating solution. The proportion of coated surface was evaluated 48 h after the coating application. Coated fruit were dipped in an iodine solution (0.25% of KI and 1% of I2 in water) to stain the starch granules, so the coating presence could be made visible by the black coloration. The proportion of coated surface was quantified by digital image processing as adapted from (Arunachalam et al., 2018), as follows: the images were captured from opposite sides of each coated fruit using a Coolpix p500 camera with 12.1megapixel resolution and 36× zoom. Preliminary tests with lighting control and background color were performed to obtain optimal conditions for capturing images. Image processing was performed using the ImageJ® 1.52a program (Bethesda, MD, USA). To develop the image segmentation based on the luminance, green↔red spectrum, and blue↔yellow spectrum (Lab) color space. For selecting the coated area of fruits, each image was divided into its Lab channel, which are grayscale monochrome. The histogram of the entire image (both of the three grayscale images) was analyzed and an threshold level was set using the 'auto-threshold' setting to create the binary image. For binarization, if the value of a pixel is greater than or equal to the threshold value then set 1 (white), and if it is less than the threshold value set 0 (black) (Arunachalam et al., 2018).

From the binary images, the projected area of uncoated surface was obtained and also the projected area of the fruits, in pixels. The coated surface area were expressed as a percentage, relative to the projected area of total fruit surface, as follows:

$$CSA\ (\%) = (TA - USA/TA) * 100$$

where CSA is the coated surface area, TA is the projected area of total fruit surface, and USA is the projected area of uncoated surface.

2.4 Levels of O_2 and CO_2 into the chambers adhered to fruit surface

The reduced O_2 and increased CO_2 levels achieved by applying the coatings was measured from the interior of the chambers adhered to the fruit surfaces as described by Rajapakse et al. (1990). For each coating solution, twelve banana fruits were used to measure the levels of O_2 and CO_2. Cylinders of 16-mm long and 14-mm diameter were sealed with a rubber septum (6-mm thick). Silicone grease was applied at the septum-glass interface to prevent leaks. Chambers were attached to the fruit skin, at the apex and base of each (Fig. S1) using silicone sealant (*Dow Corning*® PV-804 Neutral Sealant). After sealing the chambers, fruit were left at room condition for 36 h before applying the coatings. A 0.1-mL gas sample was taken from each chamber at 0, ¼, 1, 2, 3, 4, 5, 6, 7, and 8 d following coating. Gas samples were injected in oxygen analyzer PA-10a (Sable Systems International, Las Vegas, USA) operated in a flow-through mode with N_2 as the carrier gas and a flow rate of 100 mL min^{-1}. O_2 concentration was calculated relative to the certified gas standard noted previously.

2.5 Respiration rate

The O_2 uptake and CO_2 production was determined for whole fruits in a closed system at room condition as described by Saltveit (2019). For each treatment, three jars [1-L Teflon containers (Savillex Corporation, Minnetonka, MN)] containing two fruit without chamber attached were used for measuring the respiration rate, which were sealed for 60 min. For gas analysis, samples (100 µL) were withdrawn from the outlet port and immediately injected in an infrared gas analyzer (model 225-MK3; Hoddesdon, England) operated in a flow-through mode with N_2 as the carrier gas and a flow rate of 100 mL min^{-1}. O_2 and CO_2 concentrations were calculated relative to the certified gas standard noted earlier. The O_2 uptake and CO_2 released were expressed as mL kg^{-1} h^{-1}. The respiratory quotient (RQ) was obtained by dividing the CO_2 released per the O_2 uptake.

2.6 Rate of CO_2 diffusing through the fruit-coating system

For estimating the rate of CO_2 diffusing through the coating, a flow-through system was adapted to the chambers (head space ~ 2-mL) attached on the skin of the coated fruit, on the dried coating, as described: after the coating application, fruits were left for 36 h at room conditions (20 °C, ~50% RH) until the coatings were completely dried. Following, two glass chambers were attached on the skin of the coated fruits and left for more 48 h before analysis. The flow-through

system with air was set in the chambers using two needles that had latex rubber tube fixed in its upside, while the needle blossom end was fixed in the chamber, through a rubber septum. A flow rate of 35 mL min^{-1} of air came into the chamber from one of the tube-needle and gone out through the other one (Fig. 4A). The flow rate of air was measured at the outlet latex rubber tube (~20 cm long), aiming to confirmed the steady state of the flow-through system. For this analyses, six fruits were used per each coating solution.

Gas samples (100 µL) were withdrawn from the inlet and outlet tube and immediately injected in an infrared gas analyzer (model 225-MK3; Hoddesdon, England) operated in a flow-through mode with N_2 as the carrier gas and a flow rate of 100 mL min^{-1}. The difference in concentration of the inlet-flow rate and outlet-flow rate gave the rate of CO_2 released. CO_2 concentration was calculated relative to the certified gas standard noted earlier.

The permeation of CO_2 was calculated as recommended by Park (1999) for gases permeability in coatings, according to the following adapted equation:

$$P = Q.X/(A.\Delta p)$$

where, Q is the rate of gas diffusing through the coating (mL min^{-1}), X is the thickness of the coating (µm), A is the area of the coating (m^2), Δp is the partial pressure difference of the gas across the coating (Pa) and *P* is the permeability of CO_2 (mL.µm.min^{-1}.m^{-2}.kPa^{-1}).

For measuring thickness, the chambers were carefully removed from the fruit surface and the coatings were peeled off from the chamber base. Following, the thickness of the removed coating was measured with a digital micrometer. Three random measurements were performed on each piece of coating, from each chamber. The mean values were used to calculate the coating permeability.

2.7 *Weight loss and skin color*

Weight loss was measured by recording the fruit weight during the storage time. Eight fruits per treatment were weighed 1 day after coating applications and after 2, 3, 4, 5, 6 and 7 days of room storage. The percentage of weight loss was calculated based on the initial weight. Skin colour was evaluated with a CR-10 (Konica Minolta Sensing, Inc., Osaka, Japan), using *L** (lightness), *a** (redness and greenness) and *b** (yellowness and blueness) scale (*CIELab* system) equipped with an 8-mm measuring probe.

2.8 Statistical analysis

Experimental data were evaluated by a two-way analysis of variance and the means of coating with significant differences were compared using Tukey's test ($P \leq 0.05$) (Table S1, S2). Data were presented as mean ± standard error. Proportion of coated surface data were analyzed by using beta regression technique as a function of starch concentration as recommended for continuous proportions data (Douma & Weedon, 2019). LOESS (Locally Estimated Scatterplot Smoothing) local regression were applied for predicting the dependence of internal CO_2 on the concentrations of internal O_2, and the dependence of internal O_2 and CO_2 on occluded proportion of coated fruit surface. The subset range of the data were determined by alpha, and the fit by lambda. None robustness value was applied to weighted downward the outliers.

3 Results

3.1 Changes in internal O_2 and CO_2 concentrations and respiration rate in response to coating

The internal partial pressure of O_2 ($P^i_{O_2}$) and CO_2 ($P^i_{CO_2}$) and the respiratory rate of bananas under different starch coating concentrations are shown in Fig. 1. Followed six hours after applying 1.5, 2.0, 2.5, and 3.0% the starch coatings, there was a sharp decrease of O_2 levels, in the order of 56.22, 61.11, 66.77 and 74.02 kPa, respectively, as related to uncoated fruit (Fig. 1A). Banana coated with 3.0% starch reached 1.37 kPa internal O_2 at the 1st day of storage that remained unchanged throughout the storage. In turn, the $P^i_{O_2}$ in fruits coated with 1.5, 2.0 and 2.5% of starch increased in the first 3 days of storage and then declined until the 8th day.

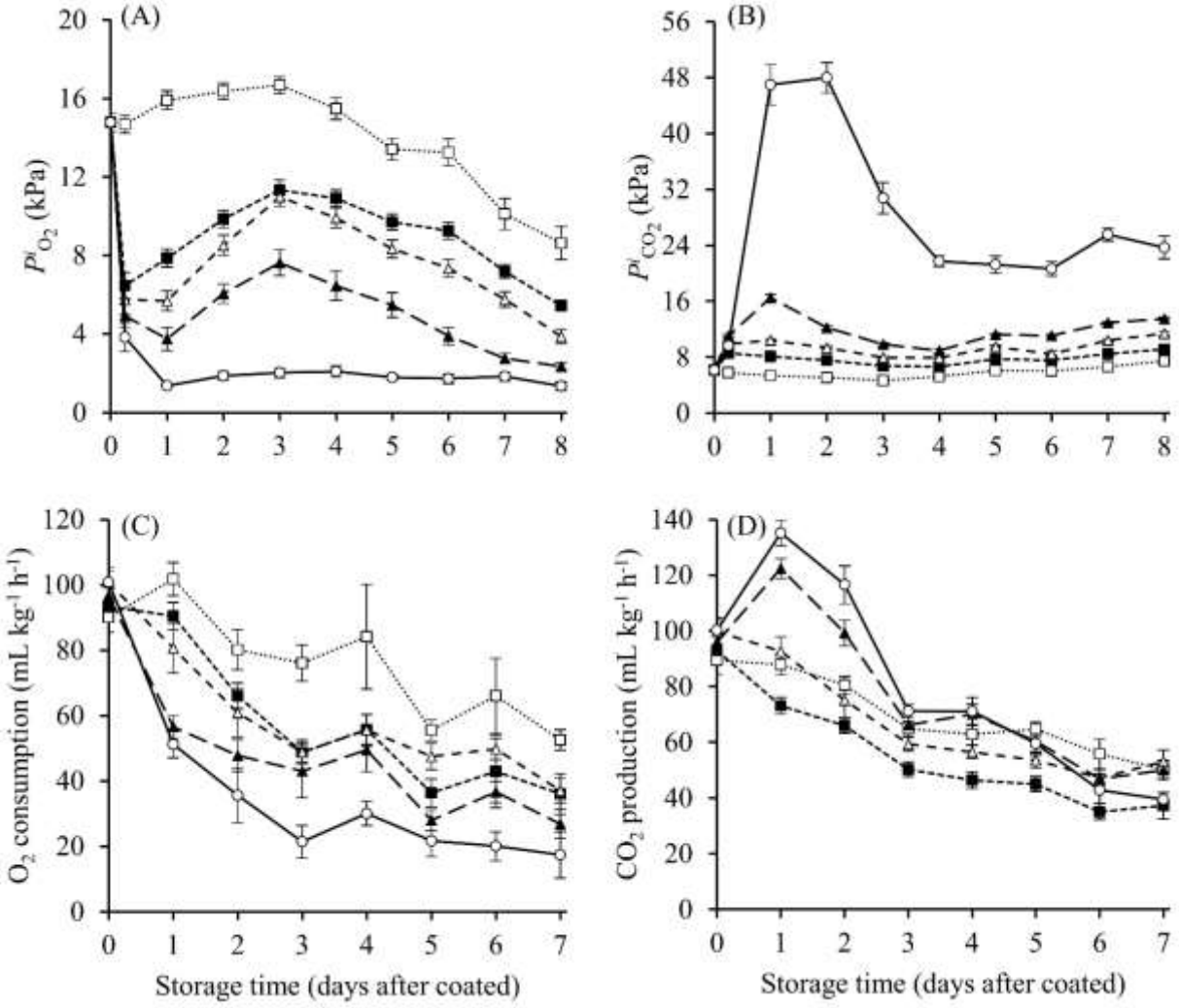

Fig. 1. Changes internal partial pressure of O_2 ($P^i_{O_2}$; A) and CO_2 ($P^i_{CO_2}$; B) in chambers adhered on banana fruit surface, and O_2 uptake (C) and CO_2 released (D) of coated bananas as affected by starch-based solution at different starch concentration and kept at 20 °C for 8 days. □ = Uncoated; ■ = 1.5% starch; Δ= 2.0% starch; ▲ = 2.5% starch; ○ = 3.0% starch. Vertical bars represent standard error means (n = 12).

The $P^i_{CO_2}$ in 3.0% coated fruit shown a peak of 47 kPa in the 1st day storage that remained high with 48 kPa at the 2nd day, then gradually declined to 22 kPa until the 4th day, remaining almost unchanged afterwards (Fig. 1B). For uncoated as well as 1.5, 2.0 and 2.5% coated fruits, the $P^i_{CO_2}$ remained unchanged throughout storage, excepted by the 1st day at which 2.5% coated fruit showed higher $P^i_{CO_2}$ (16.5 kPa). Thereby, from the 1st to 8th day storage, the mean $P^i_{CO_2}$ was 5.81, 7.83, 9.47, 11.92 kPa in uncoated and 1.5, 2.0, and 2.5% coated fruits, respectively.

Uncoated fruit showed the highest O_2 consumption throughout storage, while those coated consumed lower O_2 quantity in the same period (Fig. 1C). Additionally, higher starch concentrations in the coating favored lower O_2 consumption, therefore, the 3.0% coated fruit showed the lowest O_2 consumption.

The 1.5% coated fruits showed the lowest CO_2 production during storage. In turn, CO_2 production by bananas coated with 2.5 and 3.0% starch presented a peak at the 1st day storage, decreasing continuously during storage (Fig. 1D). Therefore, on at the 1st and 2nd days the CO_2 production for 2.5 and 3.0% starch coated were higher them for fruits of other coatings.

3.2 Changes in internal CO_2 concentration with variation in internal O_2 concentration

By plotting individual values of $P^i_{CO_2}$ against $P^i_{O_2}$ (Fig. 2A) it was seen that at lower O_2 concentrations (<4 kPa) there was a burst of internal partial pressure of CO_2 which increased sharply the Respiratory Quotient (RQ) (Table 1). This increased CO_2 concentration started to be archived at 2.5% starch coating, however it was under 3% starch coating that $P^i_{CO_2}$ rose above 20 kPa, reaching 62 kPa. The dependence of the $P^i_{CO_2}$ on the internal $P^i_{O_2}$ was modeled and reveled a suitable internal lower O_2 limit (LOL^i) for starch-coated bananas ranging from 5.33 to 5.05 kPa (points of down and up inflection), which was correlated with 10.07 kPa of CO_2 (Fig. 2B).

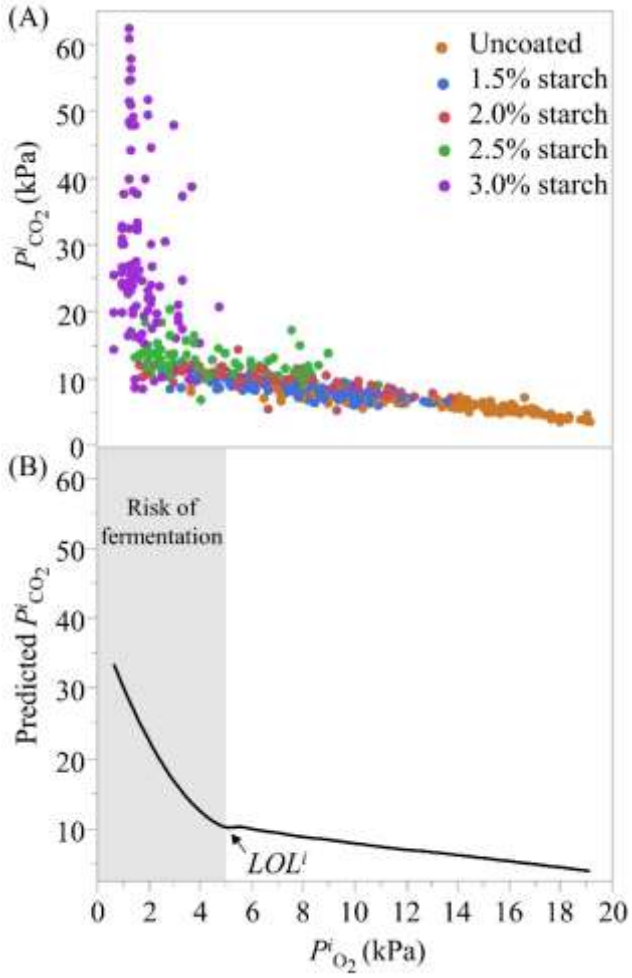

Fig. 2. (A) Plot of individual values for internal partial pressure of CO_2 ($P^i_{CO_2}$, kPa) against internal O_2 ($P^i_{O_2}$, kPa) and (B) predicted internal CO_2 as affected by internal O_2 of banana fruit coated with starch-based solution at different starch concentration and kept at 20 °C for 7 days. Predicted CO_2 was obtained by a Local Regression with R-Square = 0.5971, Local Fit (lambda) = Quadratic, Weight Function = Epanechnikov and Smoothness (alpha) = 0.6. LOL^i = Lower O_2 Limit.

The RQ was approximately 1 for uncoated fruit, as well as for 1.5 and 2.0% starch coated fruit. However, when 2.5 and 3.0% starch coatings were applied the RQ increased to ~2 and ~3, respectively.

Table 1. Respiratory quotient (RQ) of coated banana fruit as affected by starch concentration in coating solution and storage time at 20 °C.

Storage time (days after coated)	Uncoated	Starch concentration in coating solution (%)			
		1.5	2.0	2.5	3.0
0	0.994	0.994	0.991	0.992	0.994
1	0.864	0.809	1.149	2.165	2.639
2	1.006	0.999	1.235	2.078	3.280
3	0.852	1.032	1.215	1.538	3.337
4	0.747	0.832	1.017	1.417	2.365
5	1.167	1.239	1.128	2.161	2.750
6	0.845	0.817	0.949	1.280	2.138
7	0.957	1.035	1.433	1.863	2.274

3.3 Coated surface area

The effect of increasing starch concentration for coating fruit surface, which probably will occlude proportionally the pores of the banana surface (Perez and Beaudry, 1998; Maqbool et al., 2011), is represented in Fig. 3. Increasing starch concentration in the coatings continuously increased the proportions of coated surface, reaching a probability of 0.96 with 3.0% starch concentration.

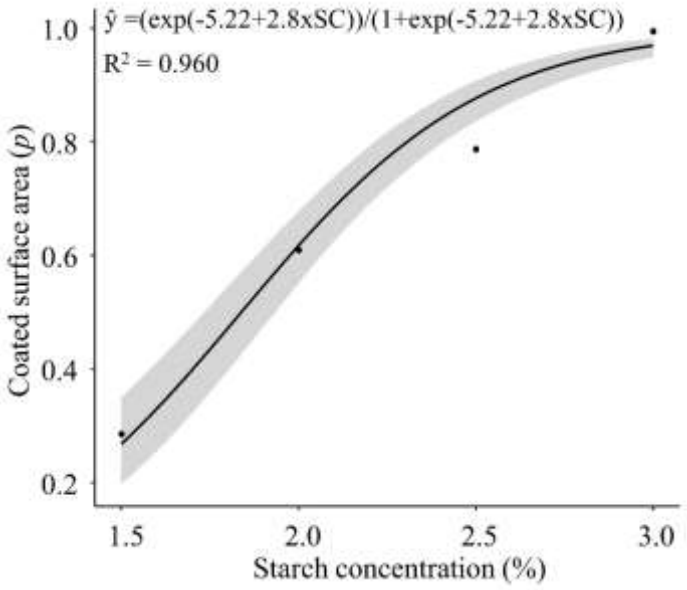

Fig. 3. Coated surface area of banana fruits treated with starch-based solution at different starch concentration. Shaded zone is the confidence interval (95%).

3.4 Dependence of internal O_2 and CO_2 on proportion of coated fruit surface area

Considering that at the 2nd day after coating the fruit metabolism has been adapted to the modified atmosphere provided by the coating, since the steady state was reached, the data ranging from the 2nd to the 4th days were selected to study the relationship between the coated surface area and the internal gasses concentrations. The plots of the individual concentrations for internal O_2 and CO_2, and also RQ against coated surface area of banana fruit are shown in Fig. 4. There was a continuous decline of O_2 concentration with increasing percentage of coated surface area (Fig. 4A). In turn, as the coated surface area was increased toward 100% there was a burst in CO_2 concentration (Fig. 4B) and also in the RQ (Fig. 4C) of the fruits, which was associated with O_2 concentrations below 4 kPa.

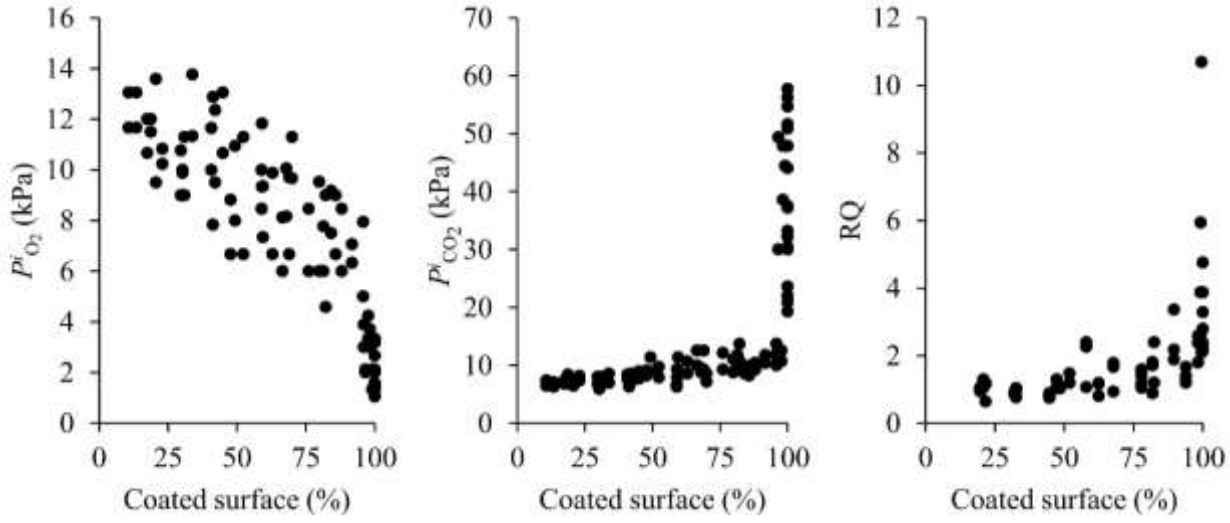

Fig. 4. Plot of individual values for internal O_2 (A) and CO_2 (B), and RQ (C) against coated surface area of banana fruit. Points are data from days 2, 3 and 4 of storage.

The effects of increasing the percentage of coated surface area on gasses internal concentrations were modeled by LOESS regression (Fig. 5). It can be seen that the decrease in O_2 concentration was more pronounced in fruits whose the percentage of coated surface areas was higher than 85%. Actually, for a percentage of 91% of coated surface area, the O_2 concentration was estimated at 5.3 kPa, which was assumed to be the LOL^i for starch coated bananas, based on the down and up inflection of the internal O_2, followed by CO_2 accumulation and increased RQ (Fig. 2A, B and Table 1). In turn, CO_2 concentrations presented a slightly increased as the percentage of fruit-coated surface area increased up to 75%. However, from that level there was a down inflection of estimated CO_2, which quickly increased, again, as the percentage of coated surface areas was higher than 85%.

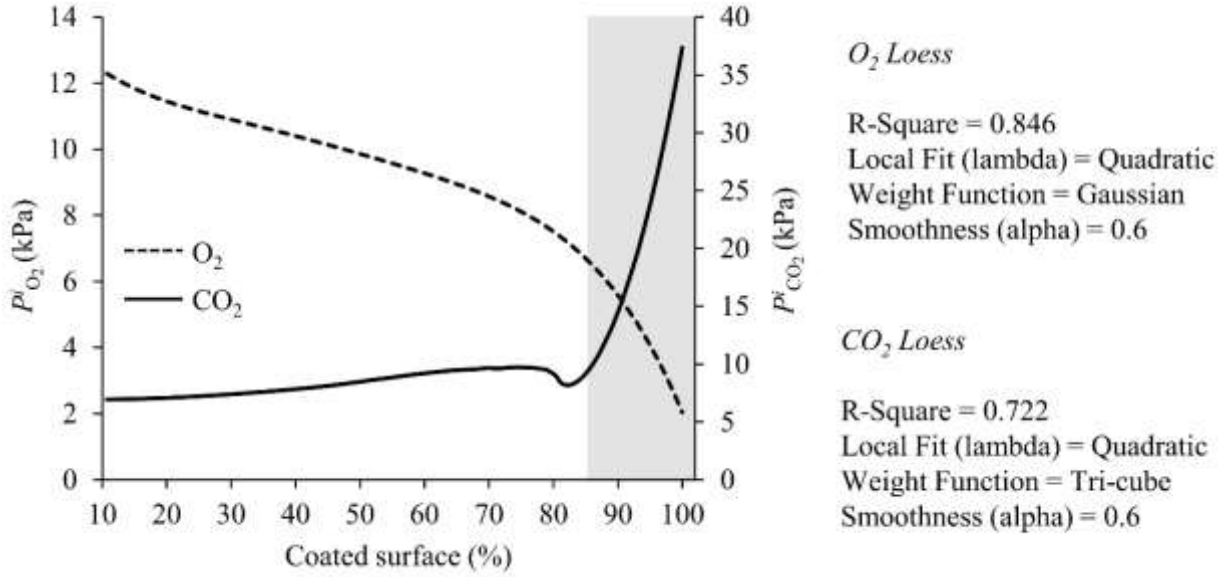

Fig. 5. Predicted dependence of internal O_2 and CO_2 on proportion of coated banana fruit surface area by starch-based coating. Shaded zone indicates high probability of fermentation.

3.5 Rate of CO_2 diffusion through the coating-fruit system

The rate of CO_2 released by the fruit through the barrier provided by the coating applied was measured in a flow-through system in which air was provided throughout the chambers attached on the surface of coated fruit (Fig. 6A). By monitoring the atmosphere in the chamber, it was noticed that at the beginning the flow coming out, there was an increase in CO_2 levels (data not shown). However, followed 15 min the steady state was reached, thus the rate of CO_2 exiting the chamber was closely even to the rate of CO_2 released by the coated fruit. The rate of CO_2 diffusing through the 2.5% starch coated fruit system was two times higher than the 3.0% starch coated fruit sistem (Fig. 6B), which showed higher internal CO_2 concentration (Fig. 1B).

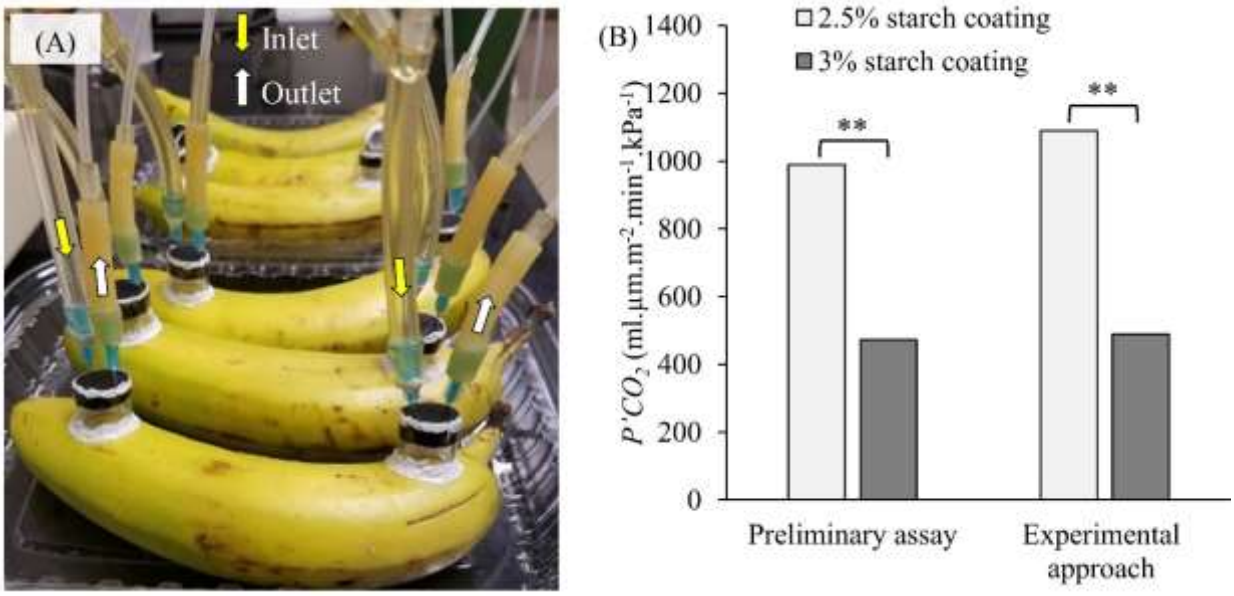

Fig. 6. Flow-through system adapted to the chambers attached on the coating-skin of the coated fruit for estimating CO_2 diffusion (A) and, amount of CO_2 diffusing through the fruit-coating system (B) expressed as permeability.

3.6 Changes in weight loss and colour

Weight loss in coated and uncoated fruits progressively increased during storage (Fig. 7A). However, this increasing trend was more pronounced in the control and 1.5%-starch coated fruit. From the 6th day onward, bananas coated with 2.5% starch showed significantly lower weight loss than the control fruits and 1.5% starch coated fruit. Additionally, weight loss of 3.0% starch coated fruit was significantly lower than the control fruit and 1.5%-starch coated fruit at the end of storage.

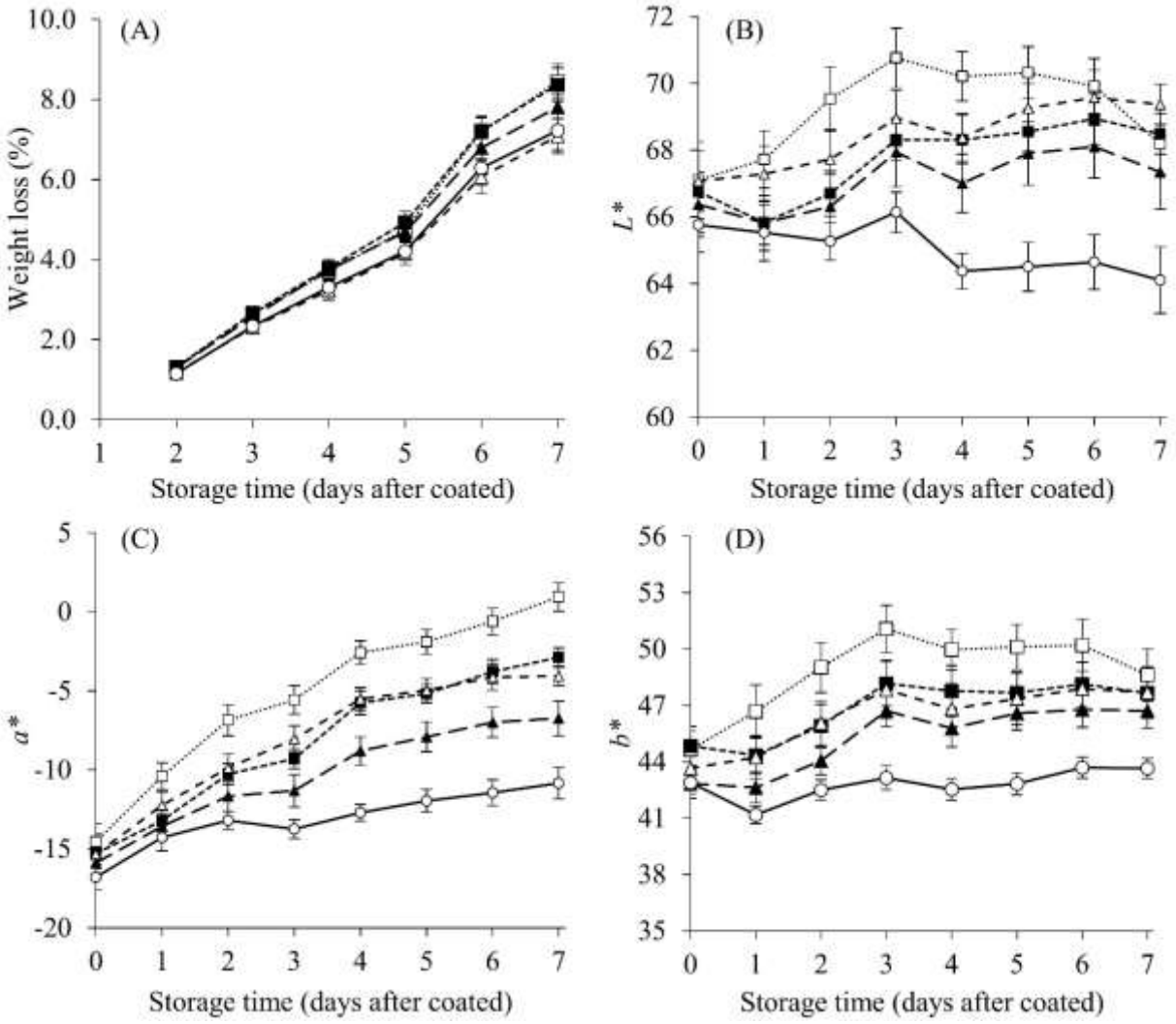

Fig. 7. Changes in weight loss and *L**, *a** and *b** parameters of *CIELab* colour of banana fruit coated with starch-based solution at different starch concentration and kept at 20 °C for 7 days. □ = Uncoated; ■ = 1.5% starch; Δ= 2.0% starch; ▲ = 2.5% starch; ○ = 3.0% starch. Vertical bars represent standard error means (n = 12).

Uncoated fruit lost the green color by to the 4th day of storage, as indicated by the increasing in *L** (Fig. 7B), *a** (Fig. 7C) and *b** (Fig. 7D) values. However, these color changes were delayed in fruits coated with 1.5, 2.0 and 2.5% starch, and did not happen on 3.0%-starch coated fruit. In fact, 3.0%-starch coated fruit presented a decrease in *L**, a small increase in *a** and no changes in *b** values throughout storage.

4 Discussion

The effectiveness of a coating to extend fruit shelf life without inducing fermentation and off-flavor development depends on its ability to allow proper gas exchange, and thereby, provide a safe internal O_2 and CO_2 concentrations build up into the coated produce. The use of chambers attached on the fruit skin has been reported as a satisfactory method for characterizing internal atmosphere composition of fruit and coated fruits (Banks & Nicholson, 2000; Amarante et al., 2001). Herein, the effects of increasing cassava starch concentrations in the coating solutions on the internal atmosphere of coated bananas were studied and correlated with the percentage of the fruit's coated surface. The sharp decline in the internal O_2 (Fig. 1A; Fig. S2A) and increase internal CO_2 (Fig. 1B; Fig. S2B) concentrations observed in the first hours after the coatings application indicate that the fruits were stressed by the probably sudden occlusion of the pores and lenticels by the viscous coating, while the fruit metabolism was running in a regular basis, but little O_2 levels had been uptake. After the coating solutions had been dried on fruit surface, the transfer rate of O_2 through the coating was probably increased, in a manner that increased the internal concentration O_2. Furthermore, the initial low O_2 probably caused a decline in the metabolic rate of fruit (Amarante & Banks, 2010; Jirukkakul and Chanshotikul, 2019), therefore, in its respirations rate (Fig. 1D), thus establishing a modified atmosphere.

The O_2 consumption was much higher in uncoated fruit, which can be related to intense metabolic activity during ripening (Saltveit, 2019; Gao et al., 2020). In turn, O_2 consumption decreased gradually with increasing starch concentration in the coating solution (Fig. 1C), thus 3.0% starch coated fruit showed the lowest O_2 consumption. The results ratify the increased occlusion of the pores as the starch concentration increased in the coating solution, which, in turn, enhanced the resistance to gasses diffusion (Banks et al., 1997; Amarante & Banks, 2010). Furthermore, a decrease in O_2 consumption was shown throughout the storage for fruit of all coating treatments, which is expected after the fruit's climacteric be reached (Perez and Beaudry, 1998).

Increasing starch concentration in the coating also increased internal CO_2 levels. For fruits coated with 1.5 and 2.0% starch, this increase was proportional to the decrease in O_2 levels; nevertheless, a broad range in CO_2 increases have been demonstrated in fruits 2.5% starch coated. Togheter, the burst in $P^{i}_{CO_2}$, and the increased RQ in 2.5 and 3.0% coated fruit indicate the onset of anaerobic respiration (Amarante & Banks, 2010; Saltveit, 2019), which herein in

starch coated banana was associated with $P^{i}_{O_2}$ below 4 kPa. The internal lower O_2 limit (LOL^{i}) for starch coated bananas was assessed by plotting individual values of internal CO_2 against internal O_2 concentrations. An O_2 concentration of 6 kPa in the chambers attached on the fruit skin would be proper for starch coated bananas still develop aerobic respiration, thus stood just above the estimated LOL^{i} value (Fig 2B) and, at which respiration rate was minimized and bellow that the anaerobic respiration was set. As the data were plotted with coatings at increasing starch concentrations (Fig 2A; Fig. S3A), it was confirmed that 2.5 and 3.0% starch coatings are associated with some degree of risk of anerobic respiration. In the preliminary experiment, a similar behavior was shown. The results also prove that the gaseous concentrations assessed into the chambers attached on the fruit skin are adequate for describing the build up of the modified atmosphere as a result of the surface coating.

Coating solution with 1.5% starch favored the lowest CO_2 releasing by the coated bananas. The result indicates that the 1.5% starch coating reduced respiration rate without triggering any stress associated with a higher internal CO_2 production (Pérez-Gallardo et al., 2015). On the other hand, the highest CO_2 released by fruits coated with 2.5 and 3.0% starch was correlated with high internal CO_2 production in the 1st day after coating application. Similar behavior was reported for blackberries coated with 2% (w/v) modified tapioca starch added with either 0.5 or 1.0 % (w/v) beeswax microparticles, whose coated fruits showed higher respiration rate and also ethylene production than the uncoated (Pérez-Gallardo et al., 2015). According to those authors, that increased respiration rate indicates that fruits were stressed by the applied coatings.

The increased proportion of coated surface with increasing starch concentration in the coating (Fig. 3) would be attributed to the improved association among the starch polymer chains, resulting in more compact film (Rompothi et al., 2017), forming a continuous coating on the fruit surface. Similar behavior was reported by Maqbool et al. (2011), in which increasing Arabic gum (5, 10, 15, and 20%) concentrations combined with chitosan (1.0%) solution increased coating deposition on the banana surface. In this sense, the number of pores that were probably occluded by the coating on the fruit surface was the main factor modifying the internal atmosphere of coated fruit (Banks et al., 1993).

The O_2 and CO_2 concentrations in the chambers attached on bananas skin were modeled as a function of the coated surface area (Fig. 5). The finds herein are supported by previous studies in which increasing coating concentration in pear fruit (*Pyrus communis* L.) cv. ‘Duyenne du

Comice', increased occlusion of pores, reducing the internal partial pressures of O_2 (Amarante et al., 2001). Furthermore, these results are similar to those predicted for a model fruit by Banks et al., 1993. As suggested for a model fruit (Banks et al., 1993) as also shown herein in coated bananas, at lower percentages of coated fruit surface area, most of the gas exchanges occur throughout the unclogged pores and, thus, those pores which have become blocked by the coating contribute very little to total gas exchange. Thus, the initial increases in the coated surface (ranging from 12 to 75% of the coated surface area) resulted in approximately equal changes in the rates of O_2 and CO_2 permeance. However, the decrease in O_2 was much more pronounced than the increase of CO_2 concentration, which can be attributed to the particularly lower O_2-permeability of starch coatings (Basiak et al., 2019), and higher permeability of CO_2 through the probably blocked pores. In this sense, the decrease in CO_2 concentration as the coated surface ranged from 75 to 80% and was associated with a greater proportion of total CO_2 transmission moving throughout the cuticular route and through the possibly blocked pores, both of which are more permeable to CO_2 than to O_2 diffusion (Banks et al., 1993). Furthermore, the lower O_2 concentration severely limited ethylene production and action and, thus, CO_2 production. On the other hand, as the CO_2 permeability thoughout the 3.0% starch coated fruit significantly decreased, there was a burst in internal CO_2, and the internal O_2 concentrations dropped below the internal anaerobic compensation point (Fig. 5), thus the RQ increased dramatically (Table 1), indicating that fruit begun to ferment.

It is well known that a high variability of data arises from fruit-to-fruit within a single coating treatment (Hagenmaier, 2005; Banks et al., 1997). Herein, not all fruits coated with 3.0% and also 2.5% starch presented a too-low internal O_2 and/or a too-high CO_2 internal concentration. Furthermore, fruits coated with 1.5 or 2.0% starch showed a broad range of internal O_2 concentrations among them. In this sense, in order to reduce the risk of a few fruit turning anaerobic, the starch concentration of the coating needs to be designed as much lower than the optimum for the majority of the fruit (Amarante & Banks, 2010). Therefore, 1.5 and 2.0% starch coatings might be effective for delaying ripening and extend shelf life of bananas without any risk of development of anaerobic respiration.

Banks et al. (1993) showed that the final gas permeance of a coated product is heavily dependent upon the numbers of pores blocked on its surface, thereby, it might be hypothesized that the high range of $P^i_{O_2}$ and $P^i_{CO_2}$ is triggered by higher variability in the proportion of the

coated surface within a single coating treatment. In its turn, Perez and Beaudry (1998) demonstrated that using fractional coatings causes a large O_2 gradient along the fraction of paraffin-coated bananas, which causes uneven ripening. However, for our knowledge no information is available on controlling coated surface area and/or blocked pores of bulky fruits coated with solutions that may cover 100% of their surface but only block a fraction of the pores, such as starch-based coating.

Therefore, a mean to obtain standardized changes in the quality of a starch-based coated fruit could be to control the proportions of the coated surface. From this study onward, a preliminary experiment has shown that it is possible to make large (Fig. S4A) or small (Fig. S4B) holes in the surface coatings by applying droplets of alcohol gel and sodium bicarbonate, respectively, just before coating application. These holes in the coating can be helpful in controlling the number of unclogged pores on the surface of a coated fruit. Further research is necessary for setting controlled hole size and distribution along the coating-fruit system, and study the effectiveness of this approach.

The rate of color development in the skin of uncoated bananas might be attributed to the degradation of chlorophyll during a fast ripening and senescence processes of the fruits (Thakur et al., 2019). On the other hand, the delayed changes in color of 1.5, 2.0 and 2.5% coated fruit might be attributed to the decreased O_2 (Fig. 1A) and increased CO_2 (Fig. 1B) internal concentrations, which have slowed down the ripening (Maqbool et al., 2011; Thakur et al., 2019). In its turn, the slight changes in color (a^* and b^* values) of 3.0% starch coated fruit, as well as the opposite change showed for L^* parameter during the storage, indicate that these fruits were unable to ripe properly. Instead, they developed dark brown color, which might be due to a too-low O_2 internal level and a too-high CO_2 internal levels that uncoupled ripening.

5 Conclusions

It was demonstrated for the first time the relationship between the coated surface area and the internal O_2 and CO_2 concentrations in starch-based coated bananas. The proportion of coated surface was increased by increasing starch concentration from 1.5 to 3.0% in the coating solution. A 3.0%-starch coating covered the entire banana surface and led to fruit anaerobic respiration, indicated by a too-low internal O_2 and a burst in internal CO_2 levels, with increased respiratory quotient.

The lower internal O_2 limit was assessed by plotting individual values of internal CO_2 against internal O_2 concentrations. The O_2 concentration of 6 kPa into chambers attached on the fruit skin would be an indicative that an adequate internal atmosphere was set for starch coated bananas, at which respiration rate was minimized without development of anaerobic respiration. Based on that, 1.5 and 2.0% starch coatings might be effective for delaying ripening, maintaining qualities, and extend shelf life of bananas without any risk of developing anaerobic respiration.

A proportion of 75% of coated surface was estimated as the limit for an adequate modified atmosphere by starch coatings for bananas. Further research, however, is necessary for optimizing variability allowed for the proportion of coated surface and quality changes that arises from fruit-to-fruit within a single surface coating.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data 1

Fig. S1. Chambers adhered over banana fruit surface for estimating internal gas concentrations.

Supplementary data 2

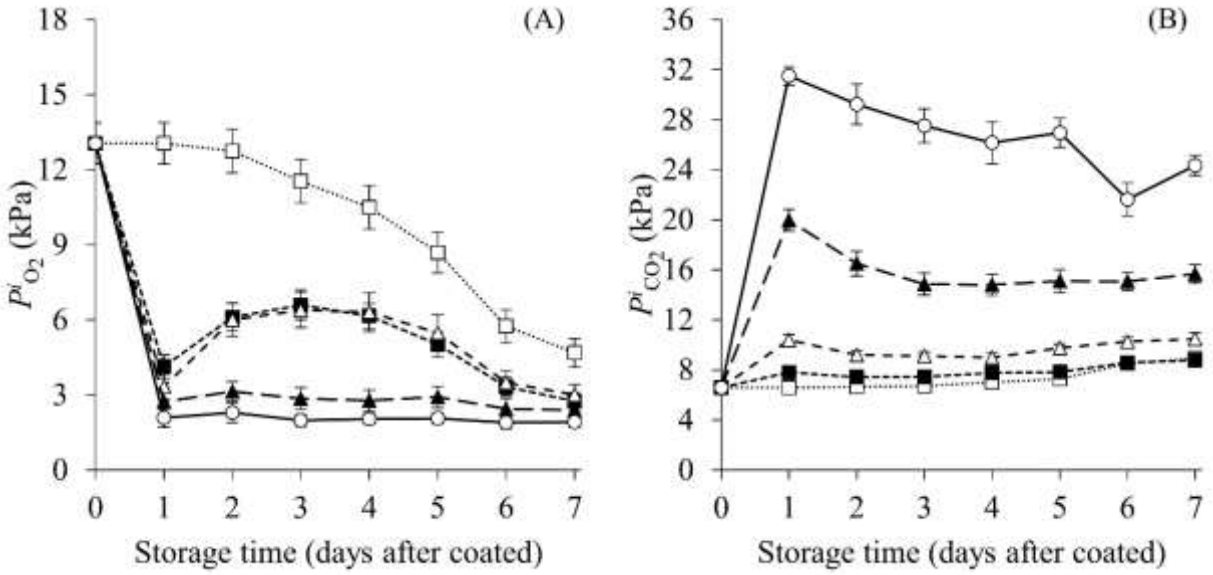

Fig. S2. Changes in O_2 (A) and CO_2 (B) concentrations in chambers adhered over banana fruit surface and respiration rate of coated bananas as affected by starch-based solution at different starch concentration and kept at 20 °C for 8 days. □ = Uncoated; ■ = 1.5% starch; Δ= 2.0% starch; ▲ = 2.5% starch; ○ = 3.0% starch. Vertical bars represent standard error means (n = 12).

Supplementary data 3

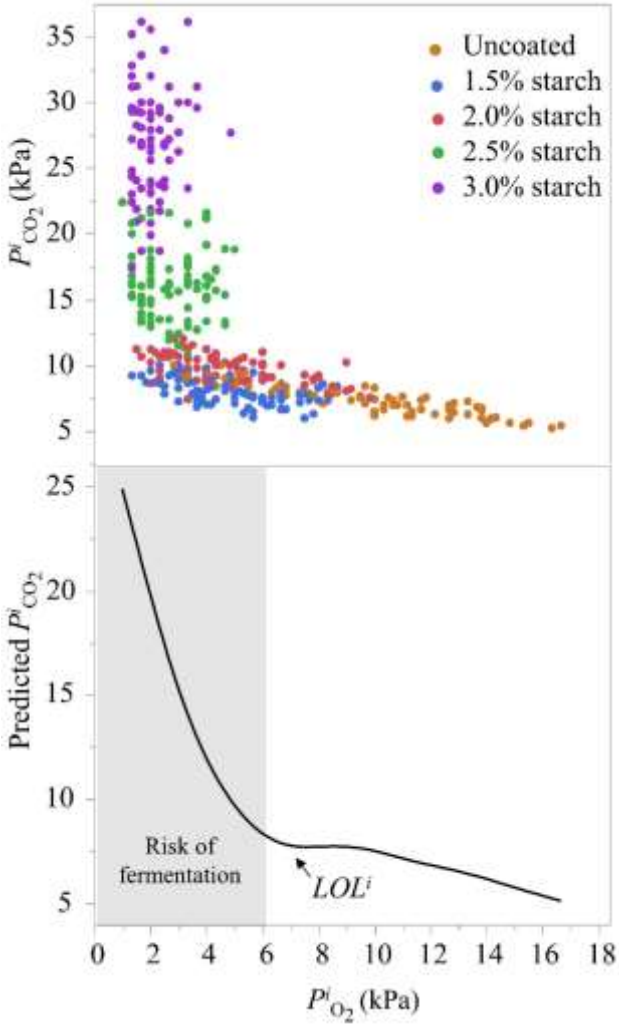

Fig. S3. (A) Plot of individual values for internal carbon dioxide (CO_2, %) against internal oxygen (O_2, %) and (B) predicted internal CO_2 as affected by internal O_2 of banana fruit coated with starch-based solution at different starch concentration and kept at 20 °C for 7 days. Predicted CO_2 was obtained by a Local Regression with R-Square = 0.5971, Local Fit (lambda) = Quadratic, Weight Function = Epanechnikov and Smoothness (alpha) = 0.6. LOLi = Lower O2 Limit.

Supplementary data 4

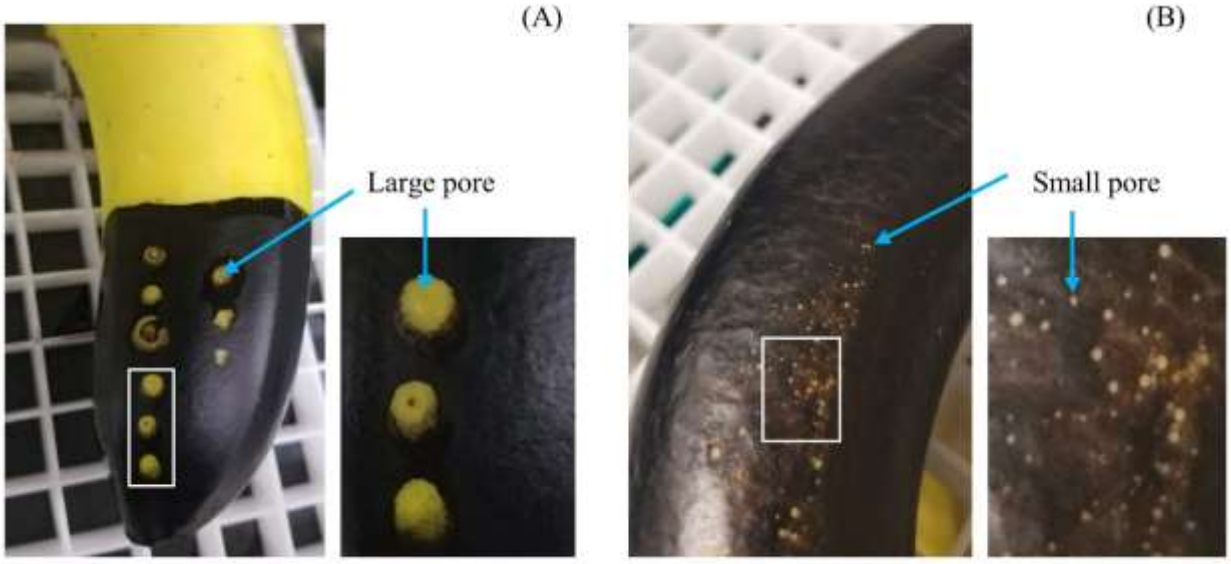

Fig. S4.

5 ARTIGO IV

IMPACTS OF STARCH-BASED COATING ADDED WITH ASCORBIC ACID/CHOLINE CHLORIDE NATURAL DEEP EUTECTIC SOLVENT ON POSTHARVEST QUALITY OF MANGO FRUIT

Highlights

Starch plus NADES coating set an O_2 target level of 2.91 kPa for coated mango.

Starch plus NADES maintained the vitamin C content during mango storage.

NADES improved the coating efficiency of reducing the oxidative damage of mango.

Ripening was delayed and storability enhanced in starch plus NADES coated mango.

Journal: Food Chemistry

Abstract

Natural deep eutectic solvents (NADES), as well as ascorbic acid, has been shown to be better plasticizer then glycerol in biodegradable films. An ascorbic acid/choline chloride-based NADES was prepared and compared to glycerol as plasticizers in starch-based coating regarding their effects on postharvest quality of mango fruit. The concentrations of O_2 immediately beneath the coated skin were significantly lower for starch plus NADES coating as compared to starch plus glycerol coating and uncoated fruits. The internal atmosphere ensured by starch plus NADES coating was effective in delaying mango ripening and maintaining higher qualities as related to both uncoated and starch plus glycerol coated fruits. Furthermore, starch plus NADES coating maintained the balance right to prevent and/or reduce the ROS-induced damage by maintaining a higher ascorbic acid content, and balanced SOD, POD, and APX activities in mango as compared to starch plus glycerol coating and uncoated fruit throughout storage time.

Keywords: *Mangifera indica* L., NADES, internal oxygen, ascorbate peroxidase, ripening

1 Introdução

Mango (*Mangifera indica* L.) is an important and widely consumed fruit due to the delicious flavor, pleasant aroma, and rich source of nutrients and phytochemicals such as ascorbic acid, carotenoids and phenolics (Ntsoane, Zude-Sasse, Mahajan & Sivakumar, 2019; Lawson, Lycett, Ali & Chin, 2019). Mango fruit shelf life is limited due to high respiration rate, ethylene production and very rapid ripening (Liu, Wang & Young, 2014; Ntsoane et al., 2019), as well as susceptibility to diseases during postharvest (Liu, Fu, Guo & Xu, 2018a; Diskin, Sharir, Feygenberg, Maurer & Alkan, 2019). During storage and the steps of transport, distribution and exposure in the market, quality compromising of mangoes includes a significant decrease in fruit firmness and ascorbic acid contents, high weight loss (Liu et al., 2014; Yasunaga, Fukuda, Nagle & Spreer, 2018), bruising damage and chilling injury, archiving about 20% to 30% quantitative loses (Yasunaga et al., 2018). Additionally, chilling injury limits the application of cold treatment to mango fruit, especially on 'Keitt' that is among the most marketed mango cultivars (Sivankalyani, Sela, Feygenberg, Zemach, Maurer & Alkan, 2016).

Modified atmosphere, with regard to reduced O_2 levels and/or increased CO_2 levels, have been shown to extend the shelf life of mangoes (Liu et al., 2018a; Ntsoane et al., 2019). Modified atmosphere by coating provides a thin layer barrier to moisture, O_2, and CO_2 exchanges (Lima et al., 2010), and proves to be a feasible technology to reduce respiration rate and weight loss, that provides a delay in ripening, and prolong shelf life of mango fruit (Khaliq, Mohamed, Ali, Ding & Ghazali, 2015; Jongsri, Wangsomboondee, Rojsitthisak & Seraypheap, 2016; Liu et al., 2014). For example, coated mangoes with a solution of *Adenanthera pavonina* galactomannan (0.5%), collagen (1.5%) and glycerol (1.5%) showed a 28% less O_2 consumption and 11% less CO_2 production when compared with uncoated mangoes (Lima et al., 2010). Furthermore, mangos treated by either bentonite or bentonite/potassium sorbate coatings and stored at room conditions exhibited reduced decay, delayed ripening, decreased water loss, maintained high ascorbic acid levels, and preserved titratable acidity when compared with uncoated fruit (Liu et al., 2014).

A plenty of natural biopolymers and lipids have become a source of interest in biodegradable coating applications for fruit and vegetables (Hassan, Chatha, Hussain, Zia & Akhtar, 2018; Dhumal & Sarkar, 2018). Among them, starch is one of the most used natural polymers due to its low cost, easy availability, and biodegradability (Rodríguez, Osés, Ziani & Mate, 2006; Edhirej Sapuan, Jawaid, & Zahari, 2016). Nevertheless, starch-based materials are brittleness with poor

mechanical properties. Thus, the incorporation of a plasticizer is required to reduce intermolecular forces and increase the mobility of polymer chains (Edhirej et al., 2016). Glycerol is widely used as conventional plasticizer for films and coatings (Shafie, Samsudin, Yusof & Gan, 2018; Rodríguez et al., 2006), although with low efficiency in maintaining the integrity of starch-based matrices on fruit surfaces during medium-term storage due to recrystallization (Zdanowicz et al., 2019). However, Natural Deep Eutectic Solvents (NADESs) are eco-friendly plasticizers and have been dawned as excellent candidates for film and coatings applications (Zdanowicz & Johansson, 2016; Almeida, Magalhães, Souza & Gonçalves, 2018; Galvis-Sánchez, Castro, Biernacki, Gonçalves & Souza, 2018; Shafie et al., 2018; Gómez, Biswas, Tadini, Furtado, Alves & Cheng, 2019; Gouveia, Biernacki, Castro, Gonçalves & Souza, 2019; Zdanowicz Staciwa, Jędrzejewski & Spychaj, 2019), since it enhances interactions between CC and -OH of starch chains, providing better mechanical properties then glycerol (Zdanowicz et al., 2019).

NADES consist in the mixture of two or more components which have a melting temperature much lower than that of the individual components (Zdanowicz et al., 2016). These are easy to prepare, ecofriendly, and chemically and thermally stable (Gómez et al., 2019). Choline chloride (ChCl) is commonly used to prepare NADES plasticizers with a wide variety of organic acids as hydrogen bond donors (Galvis-Sánchez et al., 2018; Galvis-Sánchez, Sousa, Hilliou, Gonçalves & Souza, 2016). Pure L-ascorbic acid (AsA) added as a plasticizer resulted in mechanical and water barrier properties films superior to those of other films with polyol plasticizers (glycerol and xylitol) (Yoon, 2013). Additionally, AsA has a central role in the biological redox signalling during fruit development and stress, acting as a reducing agent and antioxidant (Decros et al., 2019), as well as an active ingredient to improve quality and functionality of fruits. The main force for the formation of AsA/ChCl-based NADES is the hydrogen bonding formation between the halide anion of ChCl and AsA (Liu, Zhang, Chen & Yu, 2018b). Despite the ability of AsA to donate electrons, so far there have been no reports of AsA/ChCl-based NADES used as plasticizer to the best of our knowledge.

In light of the above, it might be hypothesized that AsA/ChCl NADES-coating may have better mechanical properties then glycerol-coating, leading to a further reduced O_2 exchange and delayed ripening of the fruit. Thus, the objective of this study was to evaluate the effects of

starch-based coating with ascorbic acid/choline chloride deep eutectic solvent as a plasticizer on postharvest quality of mango fruit.

2 Materials and Methods

2.1 Fruit material and coating chemicals

Mature green mango fruit (*Mangifera indica* L. cv. Keitt) was purchased from Central Estadual de Abastecimento S/A (CEASA) in Juazeiro, Bahia State, Brazil. Fruit was selected for uniformity in size, color and without any blemishes and disease symptoms and transferred to the Laboratory.

The cassava starch (*Manihot esculenta* Crantz) was purchased from the street market in Areia, Paraíba State, Brazil. Choline chloride and Tween 40 were purchased from Sigma Aldrich (São Paulo, SP, Brazil). Glycerol was purchased from Dinâmica Química Contemporânea LTDA (São Paulo, SP, Brazil). L-ascorbic acid was purchased from Labsynth, Diadema (São Paulo, SP, Brazil).

2.2 Formulation of natural deep eutectic solvent (NADES) and coating solution

Based upon Liu et al. (2018b), ascorbic acid (AsA) and choline chloride (ChCl) were mixed in the molar ratio of 1:2 (1mol of AsA:2mol of ChCl) to obtain persistently liquid NADES at room temperature (24±2°C). The eutectic mixtures were formed by stirring the two components at 100 °C until a homogeneous colorless liquid was formed (Abbott, Boothby, Capper, Davies & Rasheed, 2004). About 1 h was needed to obtain liquid and pellucid NADES. The eutectic mixture was placed in a rotary evaporator to remove all water and then, kept at room temperature where it demonstrated stability for weeks without precipitation. A Fourier transform infrared (FT-IR) spectrometer (Spectrum™ 400) was used to analyze the obtained NADES at room temperature.

Cassava starch solution (3% w/v) was prepared by heating the starch-water mixture up to 70 °C using a hot plate magnetic stirrer. The plasticizers (glycerol or NADES, at 1% w/v) were added when the emulsions were at 60 °C under constant stirring. The coating solution was cooled to 20 °C and 0.03% Tween 40 was added.

2.3 Fruit coating and design of experiment

Fruit were dipped for 1 min in coating solution at 20 °C. A set of control fruit were immersed in distilled water for 1 min. Fruits were air-dried at room temperature, then placed in polystyrene

trays (each a 2 fruit samples) and stored at 23±2 °C with 92% relative humidity. The experiment comprised of three treatments: (1, Control) uncoated fruit, (2, S+NADES) starch-based coating with NADES as a plasticizer and (3, S+Glycerol) starch-based coating with glycerol as a plasticizer. Fruit were evaluated at 0, 3, 6, 9 and 12 days of storage. The experiment was arranged in a completely randomized design, with four repetitions (each experimental unit consisting of two fruits) in each treatment and storage day.

2.4 Concentrations of O_2 in chambers adhered over fruit surface

The reduced O_2 levels achieved with the coatings was determined from 1,5-mL chambers adhered to the fruit surface as described by Rajapakse, Banks, Hewett and Cleland (1990). Cylinders of 16-mm long and 14-mm diameter were sealed with a rubber septum (6-mm thick). Silicone grease was applied at the septum-glass interface to prevent leaks. Two chambers were attached to the fruit skin at an equatorial position, on opposite sides of 8 fruits in each treatment using silicone sealant (*Dow Corning*® PV-804 Neutral Sealant), see Fig. 1A. After sealing the chambers, fruit were left for 36 h before applying the coatings. A 0.1-mL gas sample was taken from each chamber at 0, 1, 3, 6, 9, and 12 d after coating the fruit. Gas samples were injected in oxygen analyzer PA-10a (Sable Systems International, Las Vegas, USA) operated in a flow-through mode with N_2 as the carrier gas and a flow rate of 100 mL min^{-1}. O_2 concentration was calculated relative to the certified gas standard noted previously.

2.5 Determination of fruit gloss and dehydration

Uncoated and coated mango were subjected to evaluation by twenty trained panellists. The analyze was performed under controlled temperature and lighting conditions in individual booths. Each panellist received three samples (corresponding to uncoated fruit, S+NADES, and S+Glycerol coated fruit) each sample having eight fruit. Fruit samples were presented simultaneously on white trays coded with a random three-digit number. Uncoated and coated fruit was assessed regarding the peel gloss and dehydration. For this, a 0-9 cm structured scale was used, which ranged from zero (no gloss; no dehydration) to nine (very glossy; very dehydrated).

2.6 Physical and physicochemical evaluations

Weight loss was measured by recording the fruit weight during the storage. Four trays per treatment were weighed 1 day after coating applications, when the coatings were completely

dried on the fruit surface, and after 3, 6, 9 and 12 days of storage. The percentage of weight loss was relative to the initial weight. Firmness was measured with digital fruit hardness tester PDBF-200 (Soil Control, São Paulo, Brazil) at two equational regions of each fruit at 0, 3, 6, 9 and 12 days of storage using a 6 mm diameter probe and results were expressed in Newton (N).

Chlorophyll fluorescence was performed using the Pocket Pea chlorophyll fluorometer (Hansatech Instruments, King's Lynn, England). The maximal Photosystem II quantum yield, Fv/Fm, was determined after 60 min dark adaptation and was calculated according to the equation: Fv/Fm = (Fm - Fo)/Fm, where Fv is variable fluorescence (fluorescence increase induced by a saturation pulse), Fm is 250 the maximal fluorescence yield and Fo stands for dark fluorescence yield (Luo, Hu, Zhang, Sun & Lu, 2012). Mango pulp color was evaluated with a color reader CR-10 (Konica Minolta Sensing, Inc., Osaka, Japan), using L* (lightness), a* (redness and greenness) and b* (yellowness and blueness) scale (CIELAB system) equipped with an 8-mm measuring head.

The following physicochemical characteristics were determined according to AOAC (2012): Total soluble solids was determined with abbe refractometer RMI/RMT (BEL Engineering®, Piracicaba, Brasil) with temperature control (20 °C) and expressed as percentage; acidity was determined by titration with 0.1 M NaOH and was expressed as g of citric acid per 100 g of fresh weight (fw); pH was measured from the acidity extract before titration with a pH-meter DM 22 (Digimed, São Paulo, Brasil); SS/TA ratio was obtained by the relation between the soluble solids and titratable acidity. Ascorbic acid were determined in one gram of pulp homogenized with 50 mL of 0.5% oxalic acid and titrated with DFI solution (2,6-dichlorophenolindophenol 0.002%) until a pink color remained for 15 s.

2.7 Extraction and activities of APX, POD, and SOD

All enzymatic assays were performed from the same crude extract. Two grams of the sample was taken and homogenized with 10 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, and 0.1 g of PVP. After being filtered and centrifuged at 9000 rpm for 25 min at 4 °C, the supernatant was used as enzymatic extract (Lv, Lin, Zhang & Hua, 2011).

APX (EC 1.11.1.11) activity was determined according to Nukuntornprakit, Chanjirakul, van Doorn and Siriphanich (2015) with modifications. The reaction mixture contained 1.3 mL of 50 mM potassium phosphate buffer (pH 7.0), 50 µL of 30 mM H_2O_2, 50 µL of 9.0 mM ascorbic acid, and 100 µL of the enzymatic extract. The decrease in absorbance was recorded for 3 min at

290 nm, and the enzymatic activity was calculated according to an extinction coefficient of 2.8 mM^{-1} cm^{-1}. One unit of APX activity was defined as the amount of the enzyme that oxidizes 1.0 µmol of ascorbic acid per g of fresh weight per min (U g^{-1} FW).

POD (EC 1.11.1.7) activity was assayed based on guaiacol oxidation using H_2O_2 and an extinction coefficient of 26.6 mM^{-1} cm^{-1}, following the method of Wu, Cui, Tao and Yang (2010). The reaction mixture contained 1.2 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.1 mL of 0.5 M H_2O_2, 0.1 mL of 3% guaiacol, and 0.1 mL of the enzymatic extract. The increase in absorbance was monitored for 60 s at 470 nm. One unit of POD activity was defined as the amount of enzyme that catalyzes the peroxidation of 1.0 µmol of guaiacol per g of fresh weight per min (U mg^{-1} FW).

SOD (EC 1.15.1.1) activity was assayed to quantify the ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), according to Lv et al. (2011). The reaction mixture (1.5 mL) contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 2.0 mM riboflavin, 50 mM EDTA, 75 mM NBT, and 50 µL of crude enzymatic extract. After adding riboflavin, the reaction was triggered by turning on two 30 W fluorescent lights, kept it for 10 min, and then the lights were turned off. The absorbance of the assay mixture was recorded at 560 nm. One unit of SOD was defined as the amount of enzyme necessary to inhibit the photoreduction of NBT at 50% under the assay conditions, per g of fresh weight per min (U mg^{-1} FW).

2.8 Statistical analysis

Experimental data were subjected to two-way analysis of variance using software R 4.00. Means of treatments were separated using Tukey's test ($P \leq 0.05$). Data were presented as mean ± standard error means in the figures.

3 Results and discussion

3.1 Characterization of the NADES

The natural deep eutectic solvent (NADES) were prepared through the complexation of ascorbic acid (AsA) with choline chloride (ChCl) at 1:2 molar ratio (AsA:ChCl). The NADES were persistently liquid at room temperature. The IR spectrum of the obtained NADES is shown in Fig 1. The peak at 950 cm^{-1} has been attributed to the C-N vibration of ChCl, and the peaks at 1677 cm^{-1} and 1759 cm^{-1} belongs to the C=C and C=O, respectively, characteristic vibrations

from AsA (Liu et al., 2018b). The indications of the formation of hydrogen bonding between AsA and ChCl in the IR spectrum of the NADES is the O–H stretching vibration appearing at 3250 cm^{-1} (Ling, Ho, San Chan, Nandong, & Chin, 2020), with a broad and wide peak in the range of 3100 cm^{-1} and 3600 cm^{-1} associated with either O-H and C-H stretching vibrations (Liu et al., 2018b; Ling et al. 2020), which is shown here (Fig. 1).

NADES have been largely applied in green chemistry because it can potentially preserve the environment and human health. Actually, they are composed of two or more plant-based primary metabolites which became liquid at certain temperatures when mixed in a proper ratio (Gómez et al., 2019). The AsA/ChCl-based NADES is non-toxic on HN-5 cells, creates a green environment for the cells, and also has a therapeutic potential effect against human cancer (Mokhtarpour, Shekaari, & Shayanfar, 2020). In turn, it can enhance the solubility and antioxidant properties of antioxidant extracts from fruit (Ling et al. 2020).

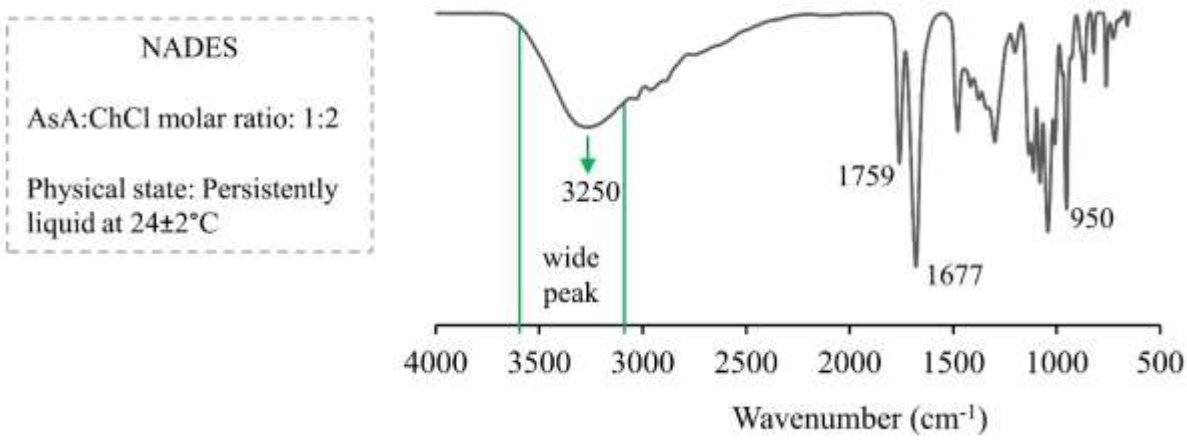

Fig 1. FT-IR spectra of the NADES obtained with ascorbic acid (AsA) and choline chloride (ChCl).

The potential of NADES as green plasticizers for starch (Zdanowicz and Johansson, 2016; Zdanowicz et al., 2019), pectin (Gouveia et al., 2019), and chitosan (Galvis-Sánchez et al., 2018; Almeida et al., 2018) based films have been investigated. Especially on starch films, NADES can form stronger interactions with starch than glycerol, therefore leading to better mechanical properties and inhibited tendency to starch recrystallization (Zdanowicz et al., 2019).

3.2 O_2 concentrations

Immediately prior to coating application, the partial pressure of O_2 ($P^i_{O_2}$) inside the chambers attached to the mangoes surface was 10.14 kPa (Fig. 2B). Thus, surface coatings significantly reduced the internal O_2 concentration inside the mangoes, since $P^i_{O_2}$ decreased rapidly after the fruit was coated, reaching 3.6 kPa and 2.93 kPa of O_2 in S+Glycerol and S+NADES coated mangoes, respectively, at the 1st d of storage.

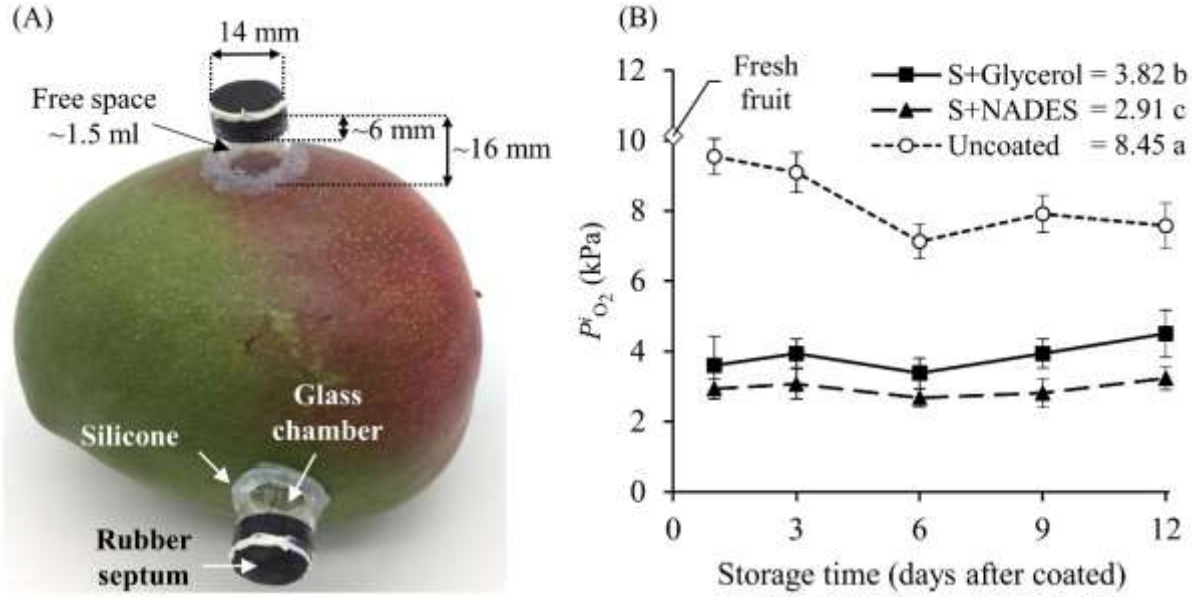

Fig. 2. System set for measuring the O_2 concentration beneath the skin of the coated mangoes (A), and internal partial pressure of O_2 ($P^i_{O_2}$) inside the chambers adhered on Keitt mango surface as affected by starch-based coating with natural deep eutectic solvent (S-NADES) or glycerol (S-Glycerol) as plasticizers during 12 days storage at room condition (23±2 °C; 78 ± 2% RH; B). Vertical bars represent standard error means (n = 4). Different letters indicate statistical difference among treatments according to Tukey's range test ($P \leq 0.05$).

Direct reduction in internal O_2 levels in coated fruit can be due to a large number of pores blocked by the coating, which reduces O_2 permeance (Amarante & Banks, 2010; Lima et al., 2010). In practice, reduced O_2 levels during mango storage (2 kPa > O_2 ≤ 5 kPa) reduce ethylene production and, therefore, respiratory activity, fruit softening rate, retards chlorophyll degradation (green color) and maintain organic acids (Ntsoane et al., 2019). Independently of storage time, S-NADES coated fruit showed significantly lower $P^i_{O_2}$ than S-Glycerol coated fruit.

Furthermore, it is worth to mention that O_2 concentration in coated fruit was stable from 1 to 12 d storage, indicating coatings' ability to establish an equilibrium condition at an O_2 target level and to sustain it throughout the mango storage period.

3.3 Weight loss, firmness, gloss, and dehydration

Weight loss of uncoated fruit was significantly higher than that of coated fruit from 9 d onward (Fig. 3A). Followed 12 d storage, uncoated mangoes showed 4.13% loss of weight, whereas the S+Glycerol and S+NADES coated mangoes lost 3.49% and 3.42%, respectively. Starch-based polymers have a high water absorption capacity (Yoon, 2013), which leads to starch-based coatings with smaller degree effects on fruit transpiration and weight loss then the effects on gasses exchanges. Since both of the coatings were composed of hydrophilic molecules (starch, glycerol, and AsA/ChCl-based NADES as well), is expected their effects on preventing water vapor mass transfer should have been similar (Lima et al., 2010).

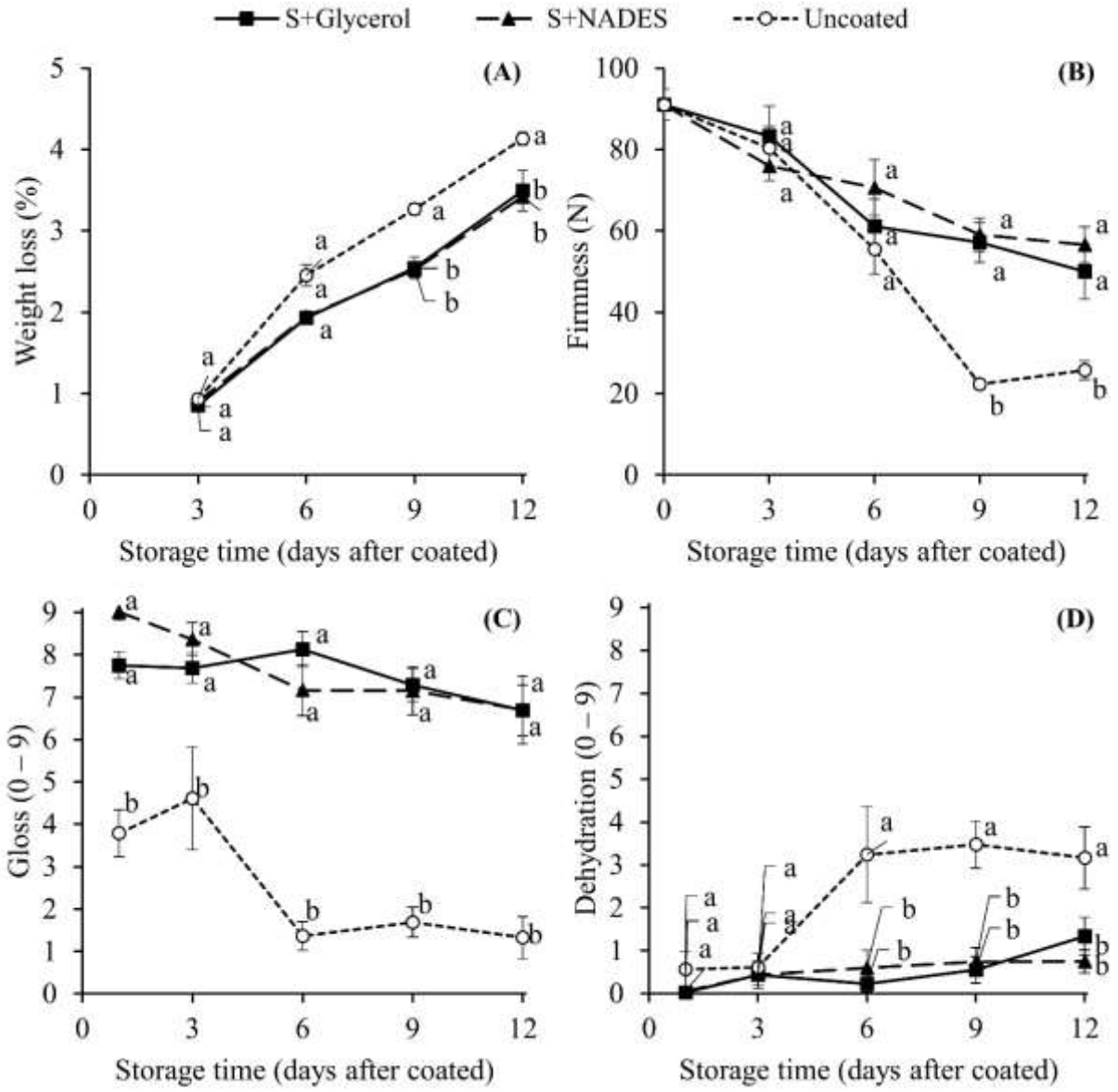

Fig. 3. Changes in weight loss (A), firmness (B), brightness (C) and dehydration (D) of the 'Keitt' mango fruit under starch-based coating with glycerol (S+Glycerol) or natural deep eutectic solvent (S+NADES) as plasticizers during 12 days storage at room condition (23±2 °C; 78 ± 2% RH). Vertical bars represent standard error means (n = 4). Different letters within the same period indicate statistical difference between treatments according to Tukey's range test ($P \leq 0.05$).

After 12 days of storage at room conditions, all mangoes became softer, especially those uncoated (Fig. 3B). From the 9th d after coating, S+NADES and S+Glycerol coated fruit retained more initial firmness in contrast to uncoated fruit. However, S+NADES and S+Glycerol coated

fruit reached similar values of firmness at the end of storage. The reduction of firmness occurs due to the degradation of pectin contents due to an increase of the activity of the cell wall degrading enzymes (Khaliq et al., 2015). This degradation is intensified during mango ripening triggered by ethylene production and action (Lawson et al., 2019; Jongsri et al., 2016). Thus, coated mangoes showed higher firmness than control fruits due to the much lower internal O_2 levels and high CO_2, which impacted in reducing ripening rate in coated mango.

The first evaluation of the gloss and dehydration was carried out from the 1st d after coatings application. Both of the coatings promoted greater intensity of gloss in the epidermis when compared with uncoated fruits (Fig. 3C). Peel of Tommy Atkins mango coated with chitosan and *Mentha piperita* L. essential oil presented higher *L** value than peel of uncoated fruit at the beginning of storage (just after coating application), indicating added brightness to the fruit surface (Oliveira et al., 2019). Regarding the glossy appearance of mango Keiit, starch-based coatings with NADES or Glycerol as plasticizers also appear to enhance the natural brightness and/or promote an additional glow to the fruit epidermis.

From 6 to 12 d storage, dehydration of uncoated fruit was significantly higher than coated fruits (Fig. 3D). The result confirms that coatings act as barriers on the fruit surface, limiting water transfer and preventing fruit dehydration (Khaliq et al., 2015).

3.4 Changes in chlorophyll fluorescence and pulp color

The Fv/Fm ratio represents the maximal Photosystem II (PSII) quantum yield. This chlorophyll fluorescence parameter is an indirect measurement of the physiological status of chlorophyll-containing tissues. From 6 d onward, Fv/Fm values were higher for S+NADES coated mangoes in contrast to uncoated fruits (Fig. 4A). In turn, the difference between uncoated and S+Glyceral coated fruit appeared from the 9th d after coating. The behavior indicates a loss of photosynthetic activity for uncoated fruit at the end of storage period, and it can be attributed to the degradation of chlorophyll and/or changes in chloroplast ultrastructure due to the advance of senescence processes. Moreover, in coated mangoes the delay of chlorophyll degradation can be due to higher internal CO_2 and lower internal O_2 levels provided by the coating's modified atmosphere, which retarded ripening and thus extends shelf-life (Srinivasa, Baskaran, Ramesh, Prashanth, & Tharanathan, 2002).

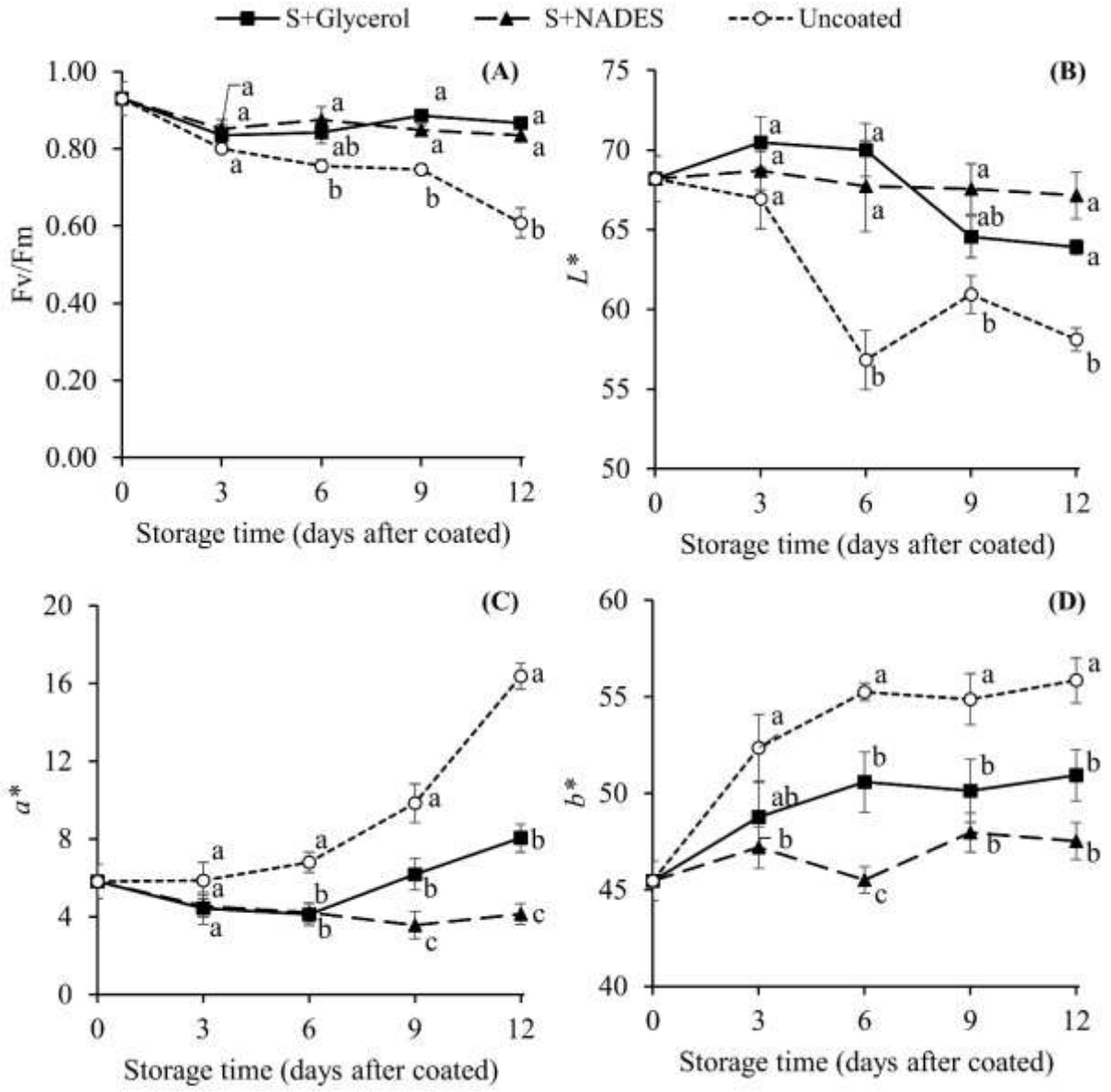

Fig. 4. Changes in Fv/Fm of skin (A), and *L** (B), *a** (C) and *b** (D) pulp color parameters of the 'Keitt' mango fruit under starch-based coating with glycerol (S+Glycerol) or natural deep eutectic solvent (S+NADES) as plasticizers during 12 days storage at room condition (23±2 °C; 78 ± 2% RH). Vertical bars represent standard error means (n = 4). Different letters within the same period indicate statistical difference between treatments according to Tukey's test ($P \leq 0.05$).

Color of mango pulp might also gave a clear indication of the state of fruit ripening. Uncoated mangoes lost the green-yellowish pulp color from 6 d of storage, as indicated by decreasing *L** (Fig. 4A), and increasing *a** (Fig. 4B) and *b** (Fig. 4D). Similar behavior was

observed in S+Glycerol coated fruit at 9 d of storage. Furthermore, at the 9th and 12th d storage *a** values in control and S+Glycerol coated fruits were significant higher when compared with S+NADES fruit (Fig. 4D). In fact, S+NADES coated fruit presented *L**, *a**, and *b** values constant throughout the storage period, indicating that this coating delayed ripening during 12 days of room storage.

3.5 Physicochemical quality

The soluble solids (SS) of mango accumulates gradually during storage due to the polysaccharides degradation during maturation (Liu et al., 2014; Khaliq et al., 2015; Xing et al., 2020). Thus, SS reaches its maximum when the mango is fully ripe, however, its declines as the consumption of soluble sugars increases as the respiration rate intensifies in the later stages of maturation/storage (Liu et al., 2014). Herein, SS of uncoated mango reached a peak in the 6th day, and after that was reduced up to the 12th d (Fig. 5A). However, by applying coating the rate of SS increases during storage was significantly reduced, also indicating a delay in ripening. In addition, S+NADES coated fruit had SS contents significantly lower than S+Glycerol coated fruit at 12th d.

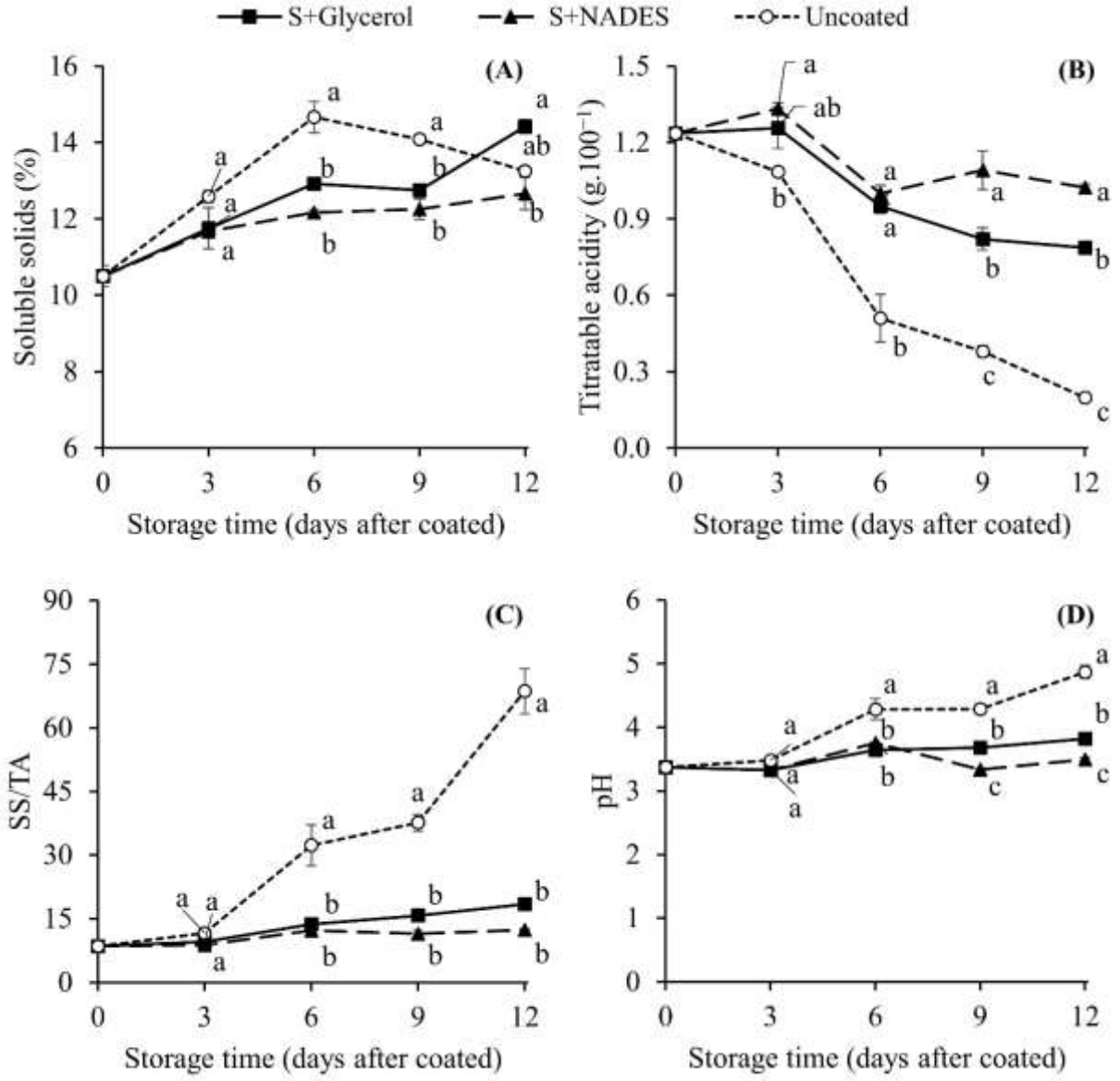

Fig. 5. Changes in soluble solids (SS; A), titratable acidity (TA; B), SS/TA (C) and pH (D) contents of the 'Keitt' mango fruit under starch-based coating with glycerol (S+NADES) or natural deep eutectic solvent (S+Glycerol) as plasticizers during 12 days storage at room condition (23±2 °C; 78 ± 2% RH). Vertical bars represent standard error means (n = 4). Different letters within the same period indicate statistical difference between treatments according to Tukey's test ($P \leq 0.05$).

Titratable acidity (TA) is an important parameter reflecting storage quality of fruits. The TA contents showed a declining trend, which was much sharper in uncoated and S+Glycerol coated

fruit reaching 0.20 and 0.79 g citric acid. $100g^{-1}$, respectively, at the 12^{th} day. Like S+NADES coated fruit, S+Glycerol coated fruit showed higher TA than uncoated fruit from the 6^{th} d after coatings, nevertheless, TA contents in S+NADES coated fruits were significantly higher than in S+Glycerol coated fruit from the 9^{th} d. A high decrease in titratable acidity indicates an intense activity of the Krebs's Cycle that results in higher consumption of organic acids in the respiratory metabolism, inducing faster senescence of fruits (Khaliq, Mohamed, Ghazali, Ding, & Ali, 2016; Liu et al., 2014).

A significantly increase in SS/TA (Fig. 5C) and pH (Fig. 5D) was observed in uncoated mangoes from the 6^{th} d storage, whereas in coated fruit the SS/TA and pH remained fairly unchanged. This behavior was mainly due to acidity decline that resulted in increases in both SS/TA and pH values during postharvest ripening in uncoated fruits.

In general, the application of coatings delayed ripening when compared to uncoated mangoes. Additionally, the effectiveness of S+NADES coating in delaying mango ripening were significant increased as related to S+Glycerol coating at the last days of storage when comparing the changes in the main maturity indices, such as flesh color, soluble solids, and titratable acidity. This trend is consistent with the internal levels of O_2 inside the fruits, which was lower for S+NADES coating (Fig. 2B), and therefore, its effects on preventing ripening and senescence were superior.

3.6 Ascorbic acid and enzymatic activity

It is well known that reactive oxygen species (ROS) tends to increase largely during mango ripening, as in many other fruits, leading to oxidative stress and damage of the cellular structures (Khaliq et al., 2016; Jongsri et al., 2016). ROS homeostasis is finely tuned over between their production and processing, which involves several enzymatic and non-enzymatic mechanisms (Decros et al., 2019). Ascorbic acid (AsA) plays a central role as an antioxidant, detoxifying different ROS, such as $O_2^{\bullet-}$, H_2O_2, and $OH^{\bullet}$, and hence reducing the oxidative stress (Khaliq et al., 2016; Decros et al., 2019). Ascorbic acid content in coated and uncoated mangoes progressively decreased during storage (Fig. 6A). However, this decreasing trend was much more pronounced in the uncoated and S+Glycerol coated fruits. From day 6^{th} to the end of storage period, mangoes coated with S+NADES presented significantly higher ascorbic acid content that of uncoated fruits. Furthermore, ascorbic acid content in S+NADES coated fruit was significantly

higher than in S+Glycerol coated at 9^{th} and 12^{th} d, indicating NADES-coating ability to prevent and/or reduce the ROS-induced damage by maintaining the higher content of ascorbic acid in these fruits. Since AsA is susceptible to decline during mango ripening, it was suggested that herein the S- NADES-coating might delay the ripening or senescence and thereby reduced the ascorbic acid losses (Khaliq et al., 2016). It might be hypostatized that ascorbic acid also has migrated from the S-NADES-coating to mangoes pulp, enriching fruit quality and functionality. Further studies are necessary to investigate the possible mechanism of the migration of ascorbic acid from surface coating through fruit skin, as suggested by Xing et al. (2020) regarding the migration of nano-TiO_2 from chitosan/Nano-TiO_2 composite coatings in mango fruit. When comparing the uncoated with S+Glycerol coated fruits, the first showed much lower ascorbic acid content at 12^{th} d storage. Similar results have also been reported, where coatings reduced ascorbic acid losses in mangoes (Liu et al., 2014; Khaliq et al., 2016; Jongsri et al., 2016).

SOD, APX, and POD are among the major antioxidant enzymes that rapidly process ROS (Decros et al., 2019). High antioxidant activities might be induced by oxidative stress during fruit ripening and senescence as a protective mechanism from cell damage (Jongsri et al., 2016). SOD activity in uncoated fruits increased during storage and were higher than in S+NADES coated ones from 6^{th} d onward (Fig. 6B). A slight increase in SOD activity of S+Glycerol coated fruit during storage were also seen, but it was constant in S+NADES coated ones throughout storage time. Importantly, SOD can be considered as both ROS-generating and ROS-processing components since dismutate rapidly $O_2^{\bullet-}$ into H_2O_2 (Decros et al., 2019). In addition, it is well known that both H_2O_2 content and $O_2^{\bullet-}$ production rate can steadily increase during mango storage (Khaliq et al., 2016). Therefore, the highest SOD activity in uncoated and S+Glycerol coated fruits may have led to H_2O_2 accumulation in mangos as compared to S+NADES coated ones.

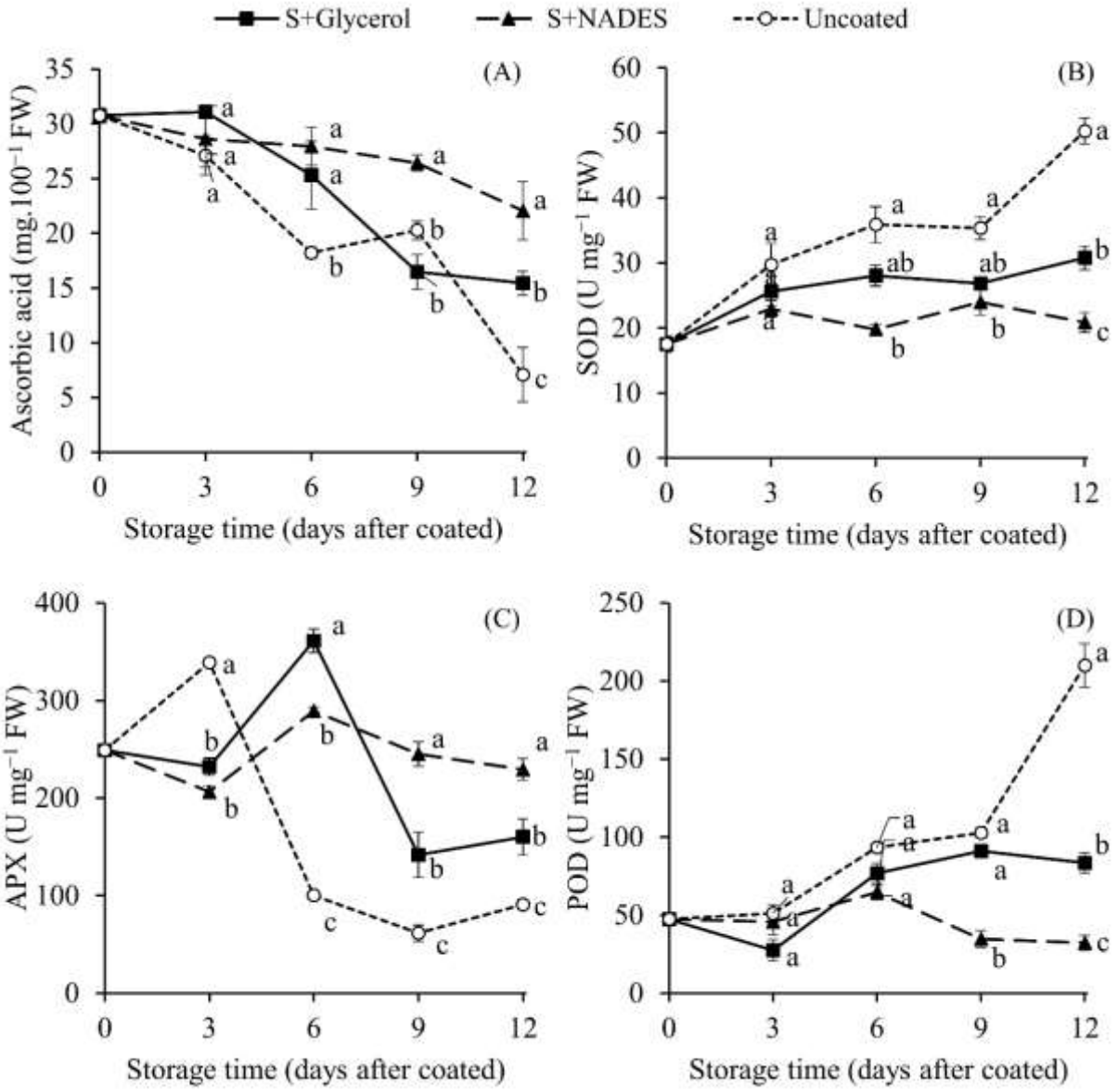

Fig. 5. Changes in ascorbic acid content (A), and SOD (B), APX (C) and POD (C) activities of 'Keitt' mango fruit under starch-based coating with glycerol (S+Glycerol) or natural deep eutectic solvent (S+NADES) as plasticizers during 12 days storage at room condition (23 ± 2 °C; 78 ± 2% RH). Vertical bars represent standard error means (n = 4). Different letters within the same period indicate statistical difference between treatments according to Tukey's range test ($P \leq 0.05$).

The highest peak activity of APX was observed in uncoated fruit at 3rd d and then declined until the 9th d (Fig. 6C). Fruit coated with S+Glycerol presented same peak at 6th d of storage and afterward the APX activity decreased. These results suggested that the faster ripening in uncoated

fruit induced the enzymatic antioxidant system to defense against oxidative stress earlier, at 3rd d of storage, while in S+Glycerol coated fruit it was induced at 6th d. Afterward, APX capacity of scavenging against ROS decreased. However, by applying S+NADES coating the APX activities in mangoes were maintained significantly highest at the last days of storage. Together, the highest AsA content with the constant SOD and APX activity during storage indicate a balanced reduction-oxidation (redox) homeostasis in S+NADES coated fruit (Decros et al., 2019).

High activity of POD has been associated with the development of off-flavors, texture loss and colour change in mango fruit (Oliveira et al., 2019). Uncoated and S+Glycerol coated fruits showed higher POD activity than that observed in the S+NADES coated at 9th and 12th days of storage (Fig. 6D). POD activity in uncoated fruits were also higher than S+Glycerol coated at 12th d. This behavior is supported by previous reports, in which uncoated mango presented higher POD activity than mango coated with chitosan added of *Mentha piperita* L. essential oil (Oliveira et al., 2019).

In this study, the findings revealed S+NADES coating capability to prevent and/or reduce the ROS-induced damage by maintaining the higher content of ascorbic acid in the fruit, even with higher APX activity in the last days of storage. Actually, the balanced activity of both SOD, APX, and POD during storage was another factor impacting to maintain shelf life and quality of S+NADES coated mangoes.

4 Conclusion

This study brings the first report of application of ascorbic acid/choline chloride-based natural deep eutectic solvent (NADES) as a plasticizer in starch-based coating for preventing fruit postharvest losses, to the best of our knowledge. The levels of O_2 immediately beneath the coated skin were significantly lower for starch plus NADES coating as compared to starch plus glycerol coating and uncoated fruits. The internal atmosphere ensured by starch plus NADES coating was effective in delaying mango ripening and maintaining higher qualities as related to both uncoated and starch plus glycerol coated fruits. Furthermore, starch plus NADES coating maintained the balance right to prevent and/or reduce the ROS-induced damage by maintaining a higher ascorbic acid content, and balanced SOD, POD, and APX activities in mango as compared to starch plus glycerol coating and uncoated fruit throughout storage time.

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6 GENERAL CONCLUSIONS

Coatings ability to adhere on different fruit surfaces were improved by adding surfactant and increasing starch concentration in the dispersions. Adding Tween 40 in a warmed coating dispersion, immediately after starch gelatinization, leads to weak interaction of the coating with the skin of fruits with high cutin and wax content (mandarins and peppers), in addition to a low proportion of coated surface. However, the addition of Tween in the cooled dispersion improved the coating ability to adhere to the surface of different fruits (mandarin, pepper, banana and papaya), leading to an increased blockage of the pores, which may be due to the increased adsorption of the coating to the fruit' surfaces without decreasing amylose-amylose interactions.

The quantification of the resistance to the diffusion of gases through the fruit' surface can be useful in defining the target internal atmosphere in coated fruits, especially considering the interaction between the coating and the peel. In defining changes in the composition of the internal atmosphere, the resistance to gas diffusion was the most appropriate variable than the respiratory rate, based on the strongest correlations, positive with the partial internal pressure of O_2 (P^i_{O2}) and negative with that of CO_2 (P^i_{CO2}) in relation to the respiratory rate during coated papaya storage. The relationship between the resistance of the coated skin to gas exchange and the P^i_{O2} and P^i_{CO2} in the tissues immediately beneath the skin and also in the fruit cavity was described for coated papaya.

Regarding the proportion of the coated surface area and the P^i_{O2} and P^i_{CO2} in starch-coated banana, the coated surface proportion increased with the increase of starch concentration from 1.5 to 3.0% in the coating. A 3.0%-starch coated the entire banana surface and resulted in anaerobiosis, indicated by a too low P^i_{O2} and a burst in P^i_{CO2} and increased respiratory quotient. The lower internal O_2 limit was assessed by plotting individual values of P^i_{CO2} against P^i_{O2}. The P^i_{O2} of 6 kPa in chambers attached on the fruit skin was the indicative of an adequate internal atmosphere was set for starch coated bananas, at which respiratory rate was minimized without development of anaerobiosis. Based on that, 1.5 and 2.0% starch coatings might be effective for delaying ripening, maintaining qualities, and extend shelf life of bananas. A proportion of 75% of coated surface was estimated as the limit for secure modified atmosphere for bananas.

The P^i_{O2} immediately beneath the coated skin was slightly lower in mangoes coated with starch + NADES compared to those with starch + glycerol and those not coated. The internal

atmosphere ensured by starch + NADES coating was effective in delaying mango ripening and maintaining the superior quality in relation to the other coatings. In addition, mangos with starch + NADES prevented and / or reduced the damage induced by ROS, maintaining a higher content of ascorbic acid and balanced SOD, POD and APX activities compared to those with starch + glycerol and those not coated during storage.