UNIVERSIDADE FEDERAL RURAL DO SEMI-ÁRIDO
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM FITOTECNIA
MESTRADO EM FITOTECNIA

JOÃO CLAUDIO VILVERT

CHITOSAN AND GRAPHENE OXIDE-BASED BIODEGRADABLE PACKAGING FOR MAINTAINING POSTHARVEST QUALITY OF MANGO

MOSSORÓ
2021

JOÃO CLAUDIO VILVERT

CHITOSAN AND GRAPHENE OXIDE-BASED BIODEGRADABLE PACKAGING FOR MAINTAINING POSTHARVEST QUALITY OF MANGO

Dissertation submitted to the Federal Rural University of the Semi-Arid Region, as a requirement for the degree of Master of Science in Plant Science

Research area: Postharvest Technology

Advisor: Edna Maria Mendes Aroucha, Prof. Dr.

Co-advisor: Sérgio Tonetto de Freitas, PhD.

MOSSORÓ
2021

© Todos os direitos estão reservados a Universidade Federal Rural do Semi-Árido. O conteúdo desta obra é de inteira responsabilidade do (a) autor (a), sendo o mesmo, passível de sanções administrativas ou penais, caso sejam infringidas as leis que regulamentam a Propriedade Intelectual, respectivamente, Patentes: Lei n° 9.279/1996 e Direitos Autorais: Lei n° 9.610/1998. O conteúdo desta obra tomar-se-á de domínio público após a data de defesa e homologação da sua respectiva ata. A mesma poderá servir de base literária para novas pesquisas, desde que a obra e seu (a) respectivo (a) autor (a) sejam devidamente citados e mencionados os seus créditos bibliográficos.

V763c Vilvert, João Claudio.
Chitosan and graphene-oxide based biodegradable packaging for maintaining postharvest quality of mango / João Claudio Vilvert. - 2021.
65 f. : il.

Orientadora: Edna Maria Mendes Aroucha.
Coorientador: Sérgio Tonetto de Freitas.
Dissertação (Mestrado) - Universidade Federal Rural do Semi-árido, Programa de Pós-graduação em Fitotecnia, 2021.

1. Mangifera indica. 2. Biopolymer. 3. Modified atmosphere. 4. Nanoparticles. 5. Postharvest. I. Aroucha, Edna Maria Mendes, orient. II. Freitas, Sérgio Tonetto de, co-orient. III. Título.

O serviço de Geração Automática de Ficha Catalográfica para Trabalhos de Conclusão de Curso (TCC´s) foi desenvolvido pelo Instituto de Ciências Matemáticas e de Computação da Universidade de São Paulo (USP) e gentilmente cedido para o Sistema de Bibliotecas da Universidade Federal Rural do Semi-Árido (SISBI-UFERSA), sendo customizado pela Superintendência de Tecnologia da Informação e Comunicação (SUTIC) sob orientação dos bibliotecários da instituição para ser adaptado às necessidades dos alunos dos Cursos de Graduação e Programas de Pós-Graduação da Universidade.

JOÃO CLAUDIO VILVERT

CHITOSAN AND GRAPHENE OXIDE-BASED BIODEGRADABLE PACKAGING FOR MAINTAINING POSTHARVEST QUALITY OF MANGO

Dissertation submitted to the Federal Rural University of the Semi-Arid Region, as a requirement for the degree of Master of Science in Plant Science

Research area: Postharvest Technology

Approved in: 11/03/2021.

COMMITEE MEMBERS

Edna Maria Mendes Aroucha, Prof. Dr. (UFERSA)
Advisor

Sérgio T. F.

Sérgio Tonetto de Freitas, PhD. (Embrapa Semiárido)
External committee member

Fco Klebson G. Santos

Francisco Klébson Gomes dos Santos, Prof. Dr. (UFERSA)
External committee member

Ricardo Henrique de Lima Leite, Prof. Dr. (UFERSA)
External committee member

ACKNOWLEDGEMENTS

I am indebted to Dr. Edna Maria Mendes Aroucha for her guidance, patience, and support. I am obliged to you for believing in me with my research. My very special thanks for the conversations we had about the research, and for being so kind.

I am deeply grateful to Dr. Sérgio Tonetto de Freitas for his encouragement, patience, friendship, and for being great inspirer as a researcher. I would like to thank Dr. Freitas for his numerous valuable suggestions about my research throughout the course of this study and concerns about my professional career.

I must acknowledge Dr. José Gustavo Lima de Almeida for supporting me in this work, and for sharing knowledge about biopolymers. I also would like to thank for teaching me the techniques about biodegradable bags.

I wish to thank the members of my qualifying exam committee, Dr. Francisco Klebson Gomes dos Santos and Dr. Ricardo Henrique de Lima Leite, for their support and valuable considerations about the work.

I would like to thank the Plant Science Department staff at the Federal Rural University of the Semi-Arid Region (UFERSA) and the professors Dr. Maria Z. Negreiros, Aurélio P. Barros Junior, and Leilson C. Grangeiro, for helping me to improve my knowledge. I also would like to thank the professors Dr. Adriano N. Simões (UFRPE) and Vespasiano B. Paiva Neto (UNIVASF) for pleasantly and easily sharing their knowledge in Plant Physiology, stimulating curiosity in students. My thanks to Dr. Erika C. Reis for supervising me in the Teaching Assistant program.

My thanks to the National Council for Scientific and Technological Development (CNPq) and the Brazilian Agricultural Research Corporation (Embrapa) for the financial support. I would also thank the company Muranaka Comércio Imp. e Exp. EIRELI for providing the fruit for the experimental work.

I would like to demonstrate my gratitude to Maria A. Ferreira for her friendship, pleasant conversations, and kind help with the experimental work.

Many thanks to my fellow researchers Ianca Ferreira, Itala Guimarães, Luana Ferreira, Eleonora Santiago, Liziane Marques, Cristiane Paiva, Bárbara Sobreira, Vanda Aquino, Cristina Costa, Phetrus Bittar, Alberto Soares Filho and Matheus Ayres, for their support, and exchanges of ideas.

My special thanks to my friends from Mossoró, Jeová, Andrezza, André, Mauricio, Wirley and Silvana, for all the support during my stay in the city, and for the incredible moments

shared. I also thank to my friends Erica, Camyla, Isadora, Eloiza, Mariana and Bethania, for always being by my side and for being kind, loving and supportive friends.

I would like to express my gratitude to my boyfriend Bruno Felipe for supporting me throughout the master's journey and for his love, patience, encouragement and constant presence on my side.

Finally, none of this would be possible without the support and love of my parents, sisters, nephews and grandparents.

“Somewhere, something incredible is waiting to be known”.

Carl Sagan

ABSTRACT

VILVERT, J. C. **Chitosan and graphene-oxide based biodegradable packaging for maintaining postharvest quality of mango**. 2021. 65 p. Dissertation (Master in Plant Science) – Federal Rural University of the Semi-Arid Region (UFERSA). Mossoró-RN, 2021.

Mango is a highly appreciated tropical fruit with high respiration rate and ethylene production, which makes it highly perishable after harvest and limits fruit long-distance marketing. Thus, postharvest technologies are essential to reduce metabolic activity and extend postharvest life of mangoes shipped to distant markets. Modified atmosphere is one of the most used technologies for fruit conservation, generally applied through the use of synthetic petroleum-based bags. Despite their effectiveness, these synthetic packaging are non-biodegradable and slow to decompose, causing several impacts on environmental ecosystems and on human health. Chitosan is a natural, renewable and biodegradable polymer that has been extensively studied in fruit postharvest conservation, including mangoes. Despite its several advantages, the use of chitosan is limited by its hydrophilic nature, which tend to be characterized as an ineffective barrier to the loss of moisture from the fruit to the environment, and by its fragility. Thus, the addition and incorporation of nanoparticles to the chitosan biopolymeric matrix is an alternative to improve the properties of the films based on this polymer. The objective of this study was to evaluate the effect of chitosan and graphene oxide-based biodegradable bags on the postharvest quality of ‘Tommy Atkins’ and ‘Palmer’ mangoes during cold storage. Chitosan (2%) and graphene oxide (0.25%) based biodegradable bags were prepared following the casting method and evaluated for physicochemical, colorimetric and mechanical properties. The fruit were harvested at the recommended maturity stage and were stored for 42 (‘Tommy Atkins’) and 56 days (‘Palmer’) at 12° C without bagging (control), as well as in chitosan-based bag, chitosan-based bag with graphene oxide, and polyethylene-based bag. According to the results, the incorporation of graphene oxide into chitosan matrix improved the water vapor barrier and mechanical properties of the films by reducing their water vapor permeability and water vapor transmission rate in 37% and 35%, respectively, by reducing water solubility from 6.46% to 1.86%, and by increasing tensile strength and Young’s modulus in 21% and 19%, respectively. The bags evaluated in our study delayed mango ripening, maintaining external appearance, skin and pulp colors, firmness, soluble solids (SS), titratable acidity (TA), SS/TA ratio, β-carotene and chlorophyll contents in the fruit. In addition, bagging the fruit reduced weight loss, respiration rate and anthracnose (*Colletotrichum gloeosporioides*) incidence and severity during storage. Our results suggest that chitosan-based biodegradable bags are an ecological and effective alternative for maintaining postharvest quality of ‘Tommy Atkins’ and ‘Palmer’ mangoes during cold storage.

Keywords: *Mangifera indica*. Biopolymer. Modified atmosphere. Nanoparticles. Postharvest.

RESUMO

VILVERT, J. C. **Embalagens biodegradáveis à base de quitosana e óxido de grafeno para manutenção da qualidade pós-colheita de manga**. 2021. 65 p. Dissertação (Mestrado em Agronomia - Fitotecnia) – Universidade Federal Rural do Semi-Árido (UFERSA). Mossoró-RN, 2021.

A manga é uma fruta tropical altamente apreciada com elevada taxa respiratória e produção de etileno, o que a torna altamente perecível após a colheita e limita a comercialização dos frutos em mercados distantes. Assim, tecnologias pós-colheita são essenciais para reduzir a atividade metabólica e estender a vida pós-colheita das mangas comercializadas em mercados distantes. A atmosfera modificada é uma das tecnologias mais utilizadas para a conservação de frutas, geralmente aplicada através do uso de sacolas sintéticas à base de petróleo. Apesar da sua eficácia, essas embalagens sintéticas não são biodegradáveis e se decompõem lentamente, causando diversos impactos nos ecossistemas ambientais e na saúde humana. A quitosana é um polímero natural, renovável e biodegradável que tem sido extensamente utilizado na conservação pós-colheita de frutas. Apesar de suas diversas vantagens, o uso da quitosana é limitado pela sua natureza hidrofílica, que tende a ser caracterizada como uma barreira inefetiva contra a perda de água dos frutos para o ambiente, e pela sua fragilidade. Assim, a adição e incorporação de nanopartículas à matriz biopolimérica de quitosana é uma alternativa para melhorar as propriedades dos filmes à base deste polímero. O objetivo deste estudo foi avaliar o efeito de sacolas biodegradáveis à base de quitosana e óxido de grafeno na qualidade pós-colheita de mangas ‘Tommy Atkins’ e ‘Palmer’ durante o armazenamento refrigerado. As sacolas biodegradáveis à base de quitosana (2%) e óxido de grafeno (0,25%) foram preparadas seguindo o método *casting* e avaliadas quanto às propriedades físico-químicas, colorimétricas e mecânicas. Os frutos foram colhidos no estágio de maturação recomendado e armazenados por 42 (‘Tommy Atkins’) e 56 dias (‘Palmer’) à 12º C sem embalagem (controle), bem como em sacolas à base de quitosana, quitosana com óxido de grafeno e polietileno. De acordo com os resultados, a incorporação de óxido de grafeno na matriz de quitosana melhorou as propriedades de barreira ao vapor de água e mecânicas dos films por reduzir a permeabilidade ao vapor de água e a taxa de transmissão de vapor de água em 37% e 35%, respectivamente, por reduzir a solubilidade em água de 6,46% para 1,86%, e por aumentar a resistência à tração e o módulo de Young em 21% e 19%, respectivamente. As sacolas avaliadas no estudo retardaram o amadurecimento das mangas, mantendo a aparência externa, cor de casca de polpa, firmeza, sólidos solúveis (SS), acidez titulável (AT), relação SS/AT e os conteúdos de β-caroteno e clorofila dos frutos. Além disso, a embalagem dos frutos reduziu sua perda de massa, taxa respiratória e incidência e severidade de antracnose (*Colletotrichum gloeosporioides*) durante o armazenamento. Os resultados sugerem que as embalagens biodegradáveis à base de quitosana são uma alternativa ecológica e eficaz para manter a qualidade pós-colheita de mangas ‘Tommy Atkins’ e ‘Palmer’ durante o armazenamento refrigerado.

Palavras-chave: *Mangifera indica*. Biopolímero. Atmosfera modificada. Nanopartículas. Pós-colheita.

LIST OF FIGURES

Article 1

Article 2

LIST OF TABLES

Article 1

Article 2

TABLE OF CONTENTS

1 INTRODUCTION AND LITERATURE REVIEW

Mango (*Mangifera indica* L.) is the second most consumed tropical fruit in the world, either as fresh or processed products such as pulp, juice, nectar, candy or jelly (CANUTO; SOUZA NETO; GARRUTI, 2009). The high mango consumption is mainly due to its high nutritional value, pleasant taste, aroma and good marketing strategies (SINGH et al., 2013), Currently, about 103 countries produce mangoes, together being responsible for 50,649,147 t produced every year (FAO, 2019).

Despite all its commercial advantages, mangoes are highly perishable, with reduced shelf life and postharvest losses close to 50% (AMBUKO et al., 2017). In this context, the use of postharvest techniques is essential to prolong shelf life maintaining quality until the fruit reaches the final market (SIGRIST, 2004; RIBEIRO et al., 2009).

Among the postharvest techniques most used in the conservation of mangoes are the cold storage that reduces fruit metabolism and respiration; use of ethylene inhibitors, such as 1-methylcyclopropene (1-MCP); heat treatment, used to control postharvest diseases caused by fungi and insects; modified atmosphere (MA); and controlled atmosphere (CA). The last two are characterized by an increase in the CO_2 concentration and a reduction in the levels of O_2 available for the fruit, which reduces fruit respiration rate, and consequently increases shelf life.

MA can be obtained through the use of plastic films, such as polyethylene (PE) and polyvinyl chloride (PVC), or biodegradable polymers based on polysaccharides, waxes and proteins (ASSIS; BRITTO, 2014; RIBEIRO et al., 2009). The use of MA in mango storage has been effective in prolonging shelf life, delaying ripening and its related physiological and biochemical changes, as well as reducing the incidence of postharvest diseases, preserving fruit quality (NTSOANE et al., 2019).

In addition, the use of biodegradable polymers in fruit conservation is a technology that has been growing in recent years, especially due to the low cost combined with the low environmental impact, which is highly valued in European countries (MACHADO et al., 2014), which are the most important importers of Brazilian mango.

In this context, the importance of studies that evaluate postharvest techniques and their effects on the final quality of the fruit is highlighted. In addition, the improvement of these techniques and the development of new technologies are fundamental for the commercialization of fruit with the physicochemical, organoleptic and nutraceutical attributes desired by consumers.

In this chapter, important concepts of this research will be approached in order to contextualize the terms and definitions used throughout the text, making them familiar, in addition to bringing this study together with others studies that have been accomplished in area so far.

1.1 MANGO PRODUCTION IN BRAZIL: IMPORTANCE AND ECONOMIC VALUE

Brazil is the 7th world largest producer of mango, with an estimated production of 1,547,606 tons of fruit per year (FAO, 2019). Most of the Brazilian production (74.7%) is concentrated in the Northeast (IBGE, 2019), especially in region known as the São Francisco Valley (SFV). This region is steadily expanding its cultivated area, production and yield, which associated with high quality of the fruit has been resulting in a high worldwide participation of the SFV in mango exports (MOUCO, 2010). In the SFV, the edaphoclimatic conditions are ideal for mango production year around with cultivars such as Tommy Atkins, Palmer, Haden and Keitt (MOUCO et al., 2010).

As the most commercialized cultivar in the world, ‘Tommy Atkins’ represents 90% of mango exports in Brazil. Among the most important quality traits, this cultivar has attractive yellow-orange skin covered with red and intense purple color; it is also resistant to anthracnose and mechanical damage; has high productivity; and high shelf life (PINTO, 2002; COSTA; SANTOS, 2019). In addition, ‘Tommy Atkins’ mangoes has high resistance to transport, favoring its export to distant markets (ARAUJO; GARCIA, 2012).

Even with several advantages, 'Tommy Atkins' mangoes have higher incidence of physiological disorders, lower soluble solids and flavor and higher fiber content, when compared to fruit of other cultivars (COSTA; SANTOS, 2019). Such negative aspects have caused a slight drop in the export of this cultivar, stimulating the introduction and acceptance of other cultivars in the market, such as Palmer that has greater pulp firmness, flavor and sugar content, as well as less fiber content, and greater resistance to some diseases and smaller seed than other cultivars, which are considered better quality traits for mangoes. In addition, ‘Palmer’ mangoes cultivation in Brazil has increase due to the fact that it is a late variety with good acceptance in the domestic market, good conservation capacity and promising prospects for export market (TEIXEIRA; DURIGAN, 2011).

1.2 RIPENING AND QUALITY OF MANGO

Currently, mango is one of the most important tropical fruit present in the diet due to its good nutritional composition. It is estimated that, in Brazil, the consumption of mangoes varies between 1.2 and 2.5 kg per capita per year (CANUTO; SOUZA NETO; GARRUTI, 2009; PINTO, 2002).

Mango contains several bioactive compounds, such as carotenoids (precursors of vitamin A) (CHEN; TAI; CHEN, 2004; VÁSQUEZ-CAICEDO et al., 2007), ascorbic acid (vitamin C) (VALENTE et al., 2011) and phenolic compounds (GUANDALINI; RODRIGUES; MARCZAK, 2019; LIU et al., 2019), distributed in the different parts of the fruit (seed, peel and pulp). These compounds have the ability to reduce oxidative damage caused by free radicals and reactive oxygen species to human cells (BALSANO; ALISI, 2009). Thus, regular consumption of mangoes is highly beneficial to human health due to its antioxidant (RIBEIRO; SCHIEBER, 2010), anti-inflammatory (DHANANJAYA; SHIVALINGAIAH, 2016), anti-carcinogenic (ABDULLAH et al., 2015; CORRALES-BERNAL et al., 2014; NGUYEN et al., 2016) and antidiabetic (LIN; LEE, 2014) actions. It is important to highlight that the content of these bioactive compounds in the fruit depends on several factors, such as cultivar, maturity stage and storage conditions (BERARDINI et al., 2005; VITHANA; SINGH; JOHNSON, 2019).

Mango is a climacteric fruit, therefore it has an increase in respiration and ethylene production during ripening (SITI; SINGH, 2011). This characteristic allows mango to be harvested before ripening, which occurs after harvest during shipping and marketing of the fruit. Therefore, harvest maturity is important because it determines mango shelf live, which is required to reach distant markets. These fruit, however, need to be harvested at the physiological maturity, since fruit harvested before this stage develop poor taste and flavor after reaching the final market (BRECHT; YAHIA, 2017).

The physiological maturity corresponds to the stage of development at which maximum growth and adequate maturation occur, whose factors are determinants in chemical composition, nutritional value, flavor development and characteristic color of the fruit (SUWONSICHON et al., 2012). Therefore, if the fruit are harvested long before their physiological maturity, the fruit will not ripe properly, developing poor taste and flavor (ALVES et al., 2002; REES; FARRELL; ORCHARD, 2012). However, if harvested after the physiological maturity, the fruit will develop a good taste and flavor, but will have a shorter postharvest life and will not be adequate for distant markets.

1.3 MODIFIED ATMOSPHERE

Due to the high perishability of the fruit, many qualitative and quantitative losses occur during the postharvest life of mangoes (NTSOANE et al., 2019). In addition, improper handling of the fruit during production, harvest, and packing and transportation can result in great mango losses (CHOUDHURY; COSTA, 2004). Therefore, the use of postharvest techniques to maintain fruit quality is essential to guarantee that mangoes will reach the final market with good consumer quality. The main postharvest techniques available for preserving mango quality involve the reduction of ethylene synthesis, the hormone responsible for changes in color, texture, aroma, nutritional content and flavor of the fruit (ECCHER ZERBINI et al., 2015) and alteration in the concentrations of O_2 and CO_2, reducing the metabolism and respiration rate that extends mango shelf life.

1.4 EDIBLE COATINGS AND FILMS

Edible coatings are natural polymers (also called biopolymers) of animal or vegetable origin, applied to the fruit surface, forming a thin layer, which act as a barrier to reduce surface gas exchange and the excessive loss or gain of water, in addition to maintaining the aroma and flavor and improve the appearance of the fruit (ASSIS; BRITTO, 2014; ZHAO; MCDANIEL, 2005). It is an excellent postharvest conservation technique for fresh fruit, in addition to being a sustainable and eco-friendly technology, due to its biodegradability (KUMAR; SETHI, 2018).

Biopolymers, in general, have high availability in nature, low cost and have good chemical organization, therefore, they are suitable for the production of highly resistant materials (DICASTILLO et al., 2013). In addition, there is the possibility of incorporating ingredients in the polymeric matrix such as antioxidants, antimicrobials, nutrients, aromatic and coloring compounds, which reflects in pleasant physicochemical, nutritional and sensory attributes to the fruit (KUMAR; SETHI, 2018; ZHAO; MCDANIEL, 2005).

Edible coatings are often mistakenly called edible films. However, it is important to distinguish between the two terms: the film is a pellicle formed from drying of the solution containing the biopolymer, which is prepared separately from the food, and then later applied; the coating is a suspension or emulsion applied directly to the surface of the fruit, which after drying, leads to the formation of a film (PINHEIRO et al., 2010).

The use of edible coatings is indicated mainly for fruit with a high respiration rate (ASSIS; FORATO; BRITTO, 2008), such as mango. Thus, several studies have been carried out worldwide with the aim of evaluating the quality of mangoes from different cultivars with biopolymer-based edible coating formed by different constituents, such as chitosan (ESHETU et al., 2019), cassava starch (OLIVEIRA et al., 2018; SERPA et al., 2014), carnauba wax (DANG; SINGH; SWINNY, 2008), *Alloe vera* (DANG; SINGH; SWINNY, 2008), dextrin (RIBEIRO et al., 2009), starch, olive oil, cellulose derivatives, beeswax (SOUSA et al., 2021) and benzoate sodium (BIBI; BALOCH, 2014).

1.5 CHITOSAN

Chitosan is a polysaccharide produced through deacetylation of chitin, found in the crustacean exoskeleton. It is a natural, renewable and biodegradable product, therefore presenting great socio-environmental importance. Crustacean exoskeletons are abundant residues and are rejected by the fishing industry, which in many cases consider them polluting agents. Thus, its commercial use reduces the environmental impacts caused by its undesirable accumulation (AZEVEDO et al., 2007).

Chitosan has positive charges that interact with the negatively charged cell surface of microorganisms, altering cell activity and membrane permeability, resulting in the loss of intracellular components (FAI; STAMFORD; STAMFORD, 2008). Thus, the use of this biopolymer in postharvest fruit conservation is highly advantageous due to its bactericidal and fungistatic properties (SHAHID-UL-ISLAM; BUTOLA, 2018; SONI et al., 2018; BRINK; ŠIPAILIENĖ, LESKAUSKAITĖ, 2019; KONG et al., 2010).

In addition, several benefits were observed in the physicochemical quality of mangoes with chitosan-based coatings. In 'Tommy Atkins' mangoes, the chitosan coating delayed the ripening of semi-ripe fruit stored at 23º C, with the concentration of 1.5% providing better maintenance of color, firmness, ascorbic acid content, soluble solids, titratable acidity and SS/TA ratio (SOUZA et al., 2011). 2% chitosan reduced weight loss and delayed softening of ‘Tommy Atkins’ and ‘Apple’ mangoes (ESHETU et al., 2019). Carbon metabolism was strongly altered by chitosan coating in ‘Palmer’ mangoes, reducing ethylene production and respiration rate and preserving fruit quality (SILVA et al., 2017).

The coating based on chitosan at 1% concentration reduced mycelial growth and sporulation in 70% and completely inhibited the germination of conidia of *Alternaria alternata* (LÓPEZ-MORA et al., 2013). It is the causal agent of the fungal disease in mangoes known as black spot, characterized by either small black spots with dark center and diffusive borders, or dark lenticels (YAHIA; SINGH, 2009). Coatings combining 2% chitosan and 2% cassava starch delayed ripening of 'Tommy Atkins' mangoes, providing mangoes with purchase intention and flavor higher than the uncoated ones (AZERÊDO et al., 2016).

1.6 NANOPARTICLES IN BIOPOLYMERS

Despite the several advantages of using degradable packaging for fresh food conservation, its use is still restricted and limited by two main factors: the hydrophilic nature of polysaccharides, which tend to be characterized as an ineffective barrier to the loss of moisture in the fruit for the environment; and the fragility of these materials. Thus, the interest of several researches in improving the properties of polymers has grown through the addition and incorporation of nanoparticles to the biopolymeric matrix.

Nanoparticles are materials whose components have nanometric dimensions, that is, on a measurement scale corresponding to the billionth part of the meter. This nanoscale gives these materials unique functional properties, with great applicability to food packaging and coatings. Thus, when incorporated into the biopolymeric matrix of packaging, nanoparticles improve their physical, chemical and mechanical characteristics in terms of flexibility, durability, temperature and humidity stability and gas barrier properties, and may also increase antimicrobial and antioxidant properties of packaging (SHANKAR; RHIM, 2018; SORRENTINO; GORRASI; VITTORIA, 2007), which is highly favorable for the storage of fresh food, including fruit and vegetables.

Among the nanoparticles already studied as additives are some based on metals such as sulfur (SHANKAR; RHIM, 2018), silver, titanium (CHI et al., 2019) and zinc, in addition to α-tocopherol (YAN et al., 2019), clay (CASARIEGO et al., 2009), tripolyphosphate (MOURA et al., 2009), xanthan gum (ZAMBRANO-ZARAGOZA et al., 2013) and microcrystalline cellulose (BILBAO-SÁINZ et al., 2010), which presented positive effect in improving the properties of packaging and biopolymer films.

In recent years, attention has been given to graphene (GEIM; NOVOSELOV, 2007). Graphene is a single, extremely thin layer of graphite, with a single atomic layer of sp^2 carbon atoms (MARCANO et al., 2010). Graphene oxide (GO) is a chemically modified molecule of graphene that contains various oxygen functional groups such as epoxide, carbonyl, carboxyl, and hydroxyl (RAY, 2015; TIGINYANU; URSAKI; POPA, 2011).

Both molecules of graphene and graphene oxide have very high mechanical properties with well biocompatibility (STANKOVICH et al., 2007). These properties bring advantages to the incorporation of these nanoparticles in biopolymeric matrices. Interactions between the oxygen functional groups of GO and some biopolymers, including chitosan, result in improvements in the mechanical and barrier properties of polymers. Amide linkages tends to be formed between GO's carboxylic acid groups and chitosan's amine groups, being expected that GO incorporates well into biopolymeric matrix (ZUO et al., 2013). In addition, the oxidation of graphite to GO improves its dispersibility in water, which enables its incorporation into biopolymeric matrices and the fabrication of films using a solution-casting method (HAN LYN et al., 2019).

1.7 REFERENCES

ABDULLAH, A. S. H. et al. Induction of apoptosis and oxidative stress in estrogen receptor-negative breast cancer, MDA-MB231 cells, by ethanolic mango seed extract. **BMC Complementary and Alternative Medicine**, v. 15, n. 1, p. 1-7, 2015.

ALVES, R. E. et al. **A cultura da mangueira**. 1. ed. Brasília: Embrapa Informação Tecnológica, 2002. 452 p.

AMBUKO, J. et al. Application of off-season flower induction chemicals to address seasonality in mango fruiting and the associated postharvest losses. In: THE 1ST ALL AFRICA POST HARVEST CONGRESS & EXHIBITION, REDUCING FOOD LOSSES AND WASTE: SUSTAINABLE SOLUTIONS FOR AFRICA, 2017, Nairobi. **Proceedings...** Nairobi: University Of Nairobi, 2017. p. 57 - 59.

ARAÚJO, J. L. P.; GARCIA, J. L. L. Estudo do mercado de manga na União Européia. **Revista Econômica do Nordeste**, v. 43, n. 2, p. 289-308, 2012.

ASSIS, O. B. G.; BRITTO, D. Revisão: coberturas comestíveis protetoras em frutas: fundamentos e aplicações. **Brazilian Journal of Food Technology**, v. 17, n. 2, p. 87-97, 2014.

ASSIS, O. B. G.; FORATO, L. A.; BRITTO, D. Revestimentos comestíveis protetores em frutos minimamente processados. **Higiene Alimentar**, v. 22, n. 160, p. 99-106, 2008.

AZERÊDO, L. P. M. et al. Qualidade de manga 'Tommy Atkins' da produção integrada recoberta com fécula de mandioca associada a óleos essenciais e quitosana. **Revista Brasileira de Fruticultura**, v. 38, n. 1, p. 141-150, 2016.

AZEVEDO, V. V. C. et al. Quitina e Quitosana: aplicações como biomateriais. **Revista Eletrônica de Materiais e processos**, v. 2, n. 3, p. 27-34, 2007.

BALSANO, C.; ALISI, A. Antioxidant effects of natural bioactive compounds. **Current Pharmaceutical Design**, v. 15, n. 26, p. 3063-3073, 2009.

BERARDINI, N. et al. Screening of mango (*Mangifera indica* L.) cultivars for their contents of flavonol O- and xanthone C-glycosides, anthocyanins, and pectin. **Journal of Agricultural and Food Chemistry**, v. 53, n. 5, p. 1563-1570, 2005.

BIBI, F.; BALOCH, M. K. Postharvest quality and shelf life of mango (*Mangifera indica* L.) fruit as affected by various coatings. **Journal of Food Processing and Preservation**, v. 38, n. 1, p. 499-507, 2014.

BILBAO-SÁINZ, C. et al. Composite edible films based on hydroxypropyl methylcellulose reinforced with microcrystalline cellulose nanoparticles. **Journal of Agricultural and Food Chemistry**, v. 58, n. 6, p. 3753-3760, 2010.

BRECHT, J. K.; YAHIA, E. M. Harvesting and Postharvest Technology of Mango. In: BRECHT, J. K.; SIDHU, J. S. (Org.). **Handbook of Mango Fruit:** Production, Postharvest Science, Processing Technology and Nutrition**.** Oxford: Wiley Blackwell, 2017. p. 105-130.

BRINK, I.; ŠIPAILIENĖ, A.; LESKAUSKAITĖ, D. Antimicrobial properties of chitosan and whey protein films applied on fresh cut turkey pieces. **International Journal of Biological Macromolecules**, v. 130, p.810-817, 2019.

CANUTO, K. M.; SOUZA NETO, M. A.; GARRUTI, D. S. Composição química volátil, em diferentes estádios de maturação, de manga “Tommy Atkins” produzida no Vale do São Francisco. **Química Nova**, v. 32, n. 9, p. 2377-2381, 2009.

CASARIEGO, A. et al. Chitosan/clay films’ properties as affected by biopolymer and clay micro/nanoparticles’ concentrations. **Food Hydrocolloids**, v. 23, n. 7, p. 1895-1902, 2009.

CHEN, J. P.; TAI, C. Y.; CHEN, B. H. Improved liquid chromatographic method for determination of carotenoids in Taiwanese mango (*Mangifera indica* L.). **Journal of Chromatography A**, v. 1054, n. 1-2, p. 261-268, 2004.

CHI, H. et al. Effect of PLA nanocomposite films containing bergamot essential oil, TiO_2 nanoparticles, and Ag nanoparticles on shelf life of mangoes. **Scientia Horticulturae**, v. 249, p. 192-198, 2019.

CHOUDHURY, M. M.; COSTA, T. S. **Perdas na cadeia de comercialização da manga**. Petrolina: Embrapa Semiárido-Documentos, 2004. 44 p.

CORRALES-BERNAL, A. et al. In vitro and in vivo effects of mango pulp (*Mangifera indica* cv. Azucar) in colon carcinogenesis. **Archivos Latinoamericanos de Nutricion**, v. 64, n. 1, p. 16-23, 2014.

COSTA, J. G. da; SANTOS, C. A. F. **Cultivo da Mangueira.** Disponível em: <http://www.cpatsa.embrapa.br:8080/sistema_producao/spmanga/cultivares.hm>. Acesso em: 21 out. 2020.

DANG, K. T. H.; SINGH, Z.; SWINNY, E. E. Edible coatings influence fruit ripening, quality, and aroma biosynthesis in mango fruit. **Journal of Agricultural and Food Chemistry**, v. 56, n. 4, p. 1361-1370, 2008.

DHANANJAYA, B. L.; SHIVALINGAIAH, S. The anti-inflammatory activity of standard aqueous stem bark extract of *Mangifera indica* L. as evident in inhibition of Group IA sPLA2. **Anais da Academia Brasileira de Ciências**, v. 88, n. 1, p. 197-209, 2016.

DICASTILLO, C. L. et al. Immobilization of green tea extract on polypropylene films to control the antioxidant activity in food packaging. **Food Research International**, v. 53, n. 1, p. 522-528, 2013.

ECCHER ZERBINI, P. et al. Optical properties, ethylene production and softening in mango fruit. **Postharvest Biology and Technology**, v. 101, p. 58-65, 2015.

ESHETU, A. et al. Effect of beeswax and chitosan treatments on quality and shelf life of selected mango (*Mangifera indica* L.) cultivars. **Heliyon**, v. 5, n. 1, p. e01116, 2019.

FAI, A. E. C.; STAMFORD, T. C. M.; STAMFORD, T. L. M. Potencial biotecnológico de quitosana em sistemas de conservação de alimentos. **Revista Iberoamericana de Polímeros**, v. 9, n. 5, p. 435-451, 2008.

FAO Statistical Database. **Food and agricultural data**. Disponível em: <http://www.fao.org/faostat/en/#data/QC>. Acesso em: 3 out. 2020.

GEIM, A. K.; NOVOSELOV, K. S. The rise of graphene. In: **Nanoscience and technology**: a collection of reviews from nature journals. 2010. p. 11-19.

GUANDALINI, B. B. V.; RODRIGUES, N. P.; MARCZAK, L. D. F. Sequential extraction of phenolics and pectin from mango peel assisted by ultrasound. **Food Research International**, v. 119, p. 455-461, 2019.

HAN LYN, F. et al. Effect of oxidation degrees of graphene oxide (GO) on the structure and physical properties of chitosan/GO composite film. **Food Packaging and Shelf Life**, v. 21, p. 100373, 2019.

IBGE. **Produção Agrícola Municipal 2019.** Disponível em: <https://sidra.ibge.gov.br/pesquisa/pam/tabelas/>. Acesso em: 2 jan. 2021.

KONG, M. et al. Antimicrobial properties of chitosan and mode of action: a state of the art review. **International Journal of Food Microbiology**, v. 144, n. 1, p. 51-63, 2010.

KUMAR, P.; SETHI, S. Edible coating for fresh fruit: A review. **International Journal of Current Microbiology and Applied Sciences**, v. 7, n. 05, p. 2619-2626, 2018.

LIN, Y. S.; LEE, S. S. Flavonol glycosides with α-Glucosidase inhibitory activities and new flavone C-diosides from the leaves of machilus konishii. **Helvetica Chimica Acta**, v. 97, n. 12, p. 1672-1682, 2014.

LIU, Y. et al. Ultrasound-assisted thawing of mango pulp: Effect on thawing rate, sensory, and nutritional properties. **Food Chemistry**, v. 286, p. 576-583, 2019.

LÓPEZ-MORA, L. I. et al. Evaluation of antifungal activity of chitosan in *Alternaria alternata* and in the quality of “Tommy Atkins” mango during storage. **Revista Chapingo Serie Horticultura**, v. 19, n. 3, p. 315-331, 2013.

MACHADO, B. A. S. et al. Tendências tecnológicas de embalagens biodegradáveis através da prospecção em documentos de patentes. **Cadernos de Prospecção**, v. 5, n. 3, p. 132-140, 2014.

MARCANO, D. C. et al. Improved synthesis of graphene oxide. **ACS nano**, v. 4, n. 8, p. 4806-4814, 2010.

MOUCO, M. A. do. **Cultivo da mangueira**. 2 ed. Petrolina: Embrapa Semiárido, 2010. 111 p.

MOURA, M. R. et al. Improved barrier and mechanical properties of novel hydroxypropyl methylcellulose edible films with chitosan/tripolyphosphate nanoparticles. **Journal of Food Engineering**, v. 92, n. 4, p. 448-453, 2009.

NGUYEN, H. X. et al. Chemical constituents of *Mangifera indica* and their antiausterity activity against the PANC-1 human pancreatic cancer cell line. **Journal of Natural Products**, v. 79, n. 8, p. 2053-2059, 2016.

NTSOANE, M. L. et al. Quality assesment and postharvest technology of mango: A review of its current status and future perspectives. **Scientia Horticulturae**, v. 249, p. 77-85, 2019.

OLIVEIRA, T. A. et al. Postharvest quality of Tommy Atkins mangoes coated with cassava starch and chitosan-based coatings. **Journal of Agricultural Science**, v. 10, n. 11, p. 401, 2018.

PINHEIRO A. C. et al. Utilização de revestimentos/filmes comestíveis para aplicações alimentares. **Boletim de Biotecnologia**. Portugal, 2010.

PINTO, A. C. de Q. Capa: a produção, o consumo e a qualidade da manga no Brasil. **Revista Brasileira de Fruticultura**, v. 24, n. 3, p. 0, 2002.

RAY, S. S. Application and Uses of Graphene Oxide and Reduced Graphene Oxide. In: ______. **Applications of Graphene and Graphene-Oxide based Nanomaterials**. Norwich: William Andrew, 2015. p. 39-55.

REES, D.; FARRELL, G.; ORCHARD, J. (Ed.). **Crop Post-Harvest:** Science and Technology, Volume 3: Perishables. Nova Iorque: John Wiley & Sons, 2012. 464 p.

RIBEIRO, S. M. R.; SCHIEBER, A. Bioactive compounds in mango (*Mangifera indica* L.). **Bioactive Foods in Promoting Health**, p. 507-523, 2010.

RIBEIRO, T. P. et al. Uso de revestimentos à base de dextrina na conservação pós-colheita de manga “Tommy Atkins”. **Revista Brasileira de Fruticultura**, v. 31, n. 2, p. 343-351, 2009.

SERPA, M. F. P. et al. Conservação de manga com uso de fécula de mandioca preparada com extrato de cravo e canela. **Revista Ceres**, v. 61, n. 6, p. 975-982, 2014.

SHAHID-UL-ISLAM; BUTOLA, B.S. Recent advances in chitosan polysaccharide and its derivatives in antimicrobial modification of textile materials. **International Journal of Biological Macromolecules**, v. 121, p.905-912, 2018.

SHANKAR, S.; RHIM, J. W. Preparation of sulfur nanoparticle-incorporated antimicrobial chitosan films. **Food Hydrocolloids**, v. 82, p. 116-123, 2018.

SIGRIST, J. M. M. Tecnologia pós-colheita para comercialização de manga in natura. In: ROZANE, D.E. et al. (Ed.). **Manga**: produção integrada, industrialização e comercialização. Viçosa: UFV, 2004. p. 553-570.

SILVA, G. M. C. et al. The chitosan affects severely the carbon metabolism in mango (*Mangifera indica* L. cv. Palmer) fruit during storage. **Food Chemistry**, v. 237, p. 372-378, 2017.

SINGH, Z. et al. Mango - Postharvest Biology and Biotechnology. **Critical Reviews in Plant Sciences**, v. 32, n. 4, p. 217-236, 2013.

SITI, Z. S.; SINGH, Z. Post-harvest fumigation with nitric oxide at the pre-climacteric and climacteric-rise stages influences ripening and quality in mango fruit. **The Journal of Horticultural Science and Biotechnology**, v. 86, n. 6, p. 645-653, 2011.

SONI, B. et al. Physicochemical, antimicrobial and antioxidant properties of chitosan/TEMPO biocomposite packaging films. **Food Packaging and Shelf Life**, v. 17, p. 73-79, 2018.

SORRENTINO, A.; GORRASI, G.; VITTORIA, V. Potential perspectives of bio-nanocomposites for food packaging applications. **Trends in Food Science and Technology**, v. 18, n. 2, p. 84-95, 2007.

SOUSA, F. F. et al. Conservation of 'Palmer' mango with an edible coating of hydroxypropyl methylcellulose and beeswax. **Food Chemistry**, p. 128925, 2020.

SOUZA, M. L. de et al. Pós-colheita de mangas “Tommy Atkins” recobertas com quitosana. **Revista Brasileira de Fruticultura**, v. 33, n. spe1, p. 337-343, 2011.

STANKOVICH, S. et al. Synthesis of graphene-based nanosheets via chemical reduction of exfoliated graphite oxide. **Carbon**, v. 45, n. 7, p. 1558-1565, 2007.

SUWONSICHON, S. et al. Sensory lexicon for mango as affected by cultivars and stages of ripeness. **Journal of Sensory Studies**, v. 27, n. 3, p. 148-160, 2012.

TEIXEIRA, G. H. de A.; DURIGAN, J. F. Storage of ‘Palmer’ mangoes in low-oxygen atmospheres. **Fruits**, v. 66, p. 279-289, 2011.

TIGINYANU, I.; URSAKI, V.; POPA, V. Ultra-thin membranes for sensor applications. In: MAKHLOUF, A. S. H.; TIGINYANU, I. **Nanocoatings and Ultra-Thin Films**. Amsterdam: Elsevier, 2015. p. 330-354.

VALENTE, A. et al. Ascorbic acid content in exotic fruits: A contribution to produce quality data for food composition databases. **Food Research International**, v. 44, n. 7, p. 2237-2242, 2011.

VÁSQUEZ-CAICEDO, A. L. et al. Impact of packaging and storage conditions on colour and β-carotene retention of pasteurised mango purée. **European Food Research and Technology**, v. 224, n. 5, p. 581-590, 2007.

VITHANA, M. D. K.; SINGH, Z.; JOHNSON, S. K. Harvest maturity stage affects the concentrations of health-promoting compounds: Lupeol, mangiferin and phenolic acids in the pulp and peel of ripe 'Kensington Pride' mango fruit. **Scientia Horticulturae**, v. 243, p. 125-130, 2019.

YAHIA, E. M.; SINGH, S. P. Tropical fruits. In: YAHIA, E. M. (Ed.). **Modified and Controlled Atmospheres for the Storage, Transportation, and Packaging of Horticultural Commodities**. Flórida: CRC Press, 2009. p. 397-344.

YAN, W. et al. Preparation of α-tocopherol-chitosan nanoparticles/chitosan/montmorillonite film and the antioxidant efficiency on sliced dry-cured ham. **Food Control**, v. 104, p. 132-138, 2019.

ZAMBRANO-ZARAGOZA, M. L. et al. Use of solid lipid nanoparticles (SLNs) in edible coatings to increase guava (*Psidium guajava* L.) shelf-life. **Food Research International**, v. 51, n. 2, p. 946-953, 2013.

ZHAO, Y.; MCDANIEL, M. Sensory quality of foods associated with edible film and coating systems and shelf-life extension. **Innovations in Food Packaging**, p. 434-453, 2005.

ZUO, P.-P. et al. Fabrication of biocompatible and mechanically reinforced graphene oxide-chitosan nanocomposite films. **Chemistry Central Journal**, v. 7, n. 1, p. 1-11, 2013.

2 ARTICLE 1 – EFFECT OF GRAPHENE OXIDE ON THE PROPERTIES OF CHITOSAN FILMS AND THEIR APPLICATION AS BIODEGRADABLE BAGS FOR CONSERVATION OF ‘TOMMY ATKINS’ MANGO[1]

Abstract

This study aimed to evaluate the effects of graphene oxide (GO) on the properties of chitosan-based films and their application as biodegradable bags for ‘Tommy Atkins’ mango conservation. Chitosan-based (2%) films with and without GO (0.25%) were obtained by the casting method and bags were made by overlapping two films and sealing the edges. ‘Tommy Atkins’ mangoes were stored for 42 days at 12°C without bagging (control), as well as in chitosan-based bags with and without GO and petroleum-based synthetic bag. The incorporation of GO in chitosan-based films reduced bag water vapor transmission rate, thickness, lightness and a^* and increased bag tensile strength, Young’s modulus, opacity and b^*. Chitosan and GO-based biodegradable bags delayed the ripening of ‘Tommy Atkins’ mangoes by reducing respiration rate, weight loss and softening, delaying changes in color, soluble solids content and titratable acidity and preserving the visual appearance of the fruit.

Keywords: *Mangifera indica* L., water vapor permeability, nanocomposite, postharvest

2.1 INTRODUCTION

Since its creation, plastic has been studied and improved, once it is a durable material with low weight and production cost and that can be easily molded into different shapes and forms for several applications. In food industry, the petroleum-based synthetic plastic is an effective alternative for separating food from the external environment and for extending the shelf-life of fresh food against foodborne pathogens and fungal attack, which causes damage to the food. Estimations indicate that 8% of the world’s fossil fuel production is used as raw materials or to provide energy for the manufacturing of plastics [1].

The increase of the production and use of petroleum-based plastics, which decomposition time can reach 400 years, leads to negative consequences for ecosystems and for the health of humans and animals [2]. Being resistant to degradation, synthetic plastics tend to accumulate and create a serious environmental problem of plastic waste management, including their breakdown into microplastics, which can easily enter the food chain when consumed by animals such as fish, leading to bio accumulation [3,4].

The interest on biopolymers as eco-friendly and sustainable substitutes for petroleum-based plastics is strongly increasing because of their high biodegradability, low cost and easy to obtain from renewable resources [4–7]. In developed countries, the use of biopolymer-based

---

[1] Paper submitted to Materials Research - ISSN: 1980-5373 - JCR Impact Factor: 1.468 (2020)

materials as food packaging seems to be a promising alternative on the rise, once they can retard deterioration, extend shelf life, and maintain quality and safety of packaged food, without threats to the environment [3].

Chitosan is a low-cost and multipurpose animal-derived polymer with several desired properties, which has been tested for applications in agriculture, pharmacy, and biomedicine industries [8]. Use of chitosan for food packaging has been successfully gaining attention due to its excellent film-forming ability associated to antioxidant and antimicrobial properties [9]. Besides many advantages over synthetic polymers, chitosan may have weak mechanical and moisture-barrier properties [10], which raised the interest of researchers in improving these properties by incorporation of nanoparticles to the film-forming solutions.

Graphene oxide (GO) is derived from graphene by chemical modification of graphite and it contains various oxygen functional groups such as epoxide, carbonyl, carboxyl [11]. Amide linkages tend to be formed between the chitosan's highly reactive amine groups and GO’s carboxylic acid groups, being expected that GO incorporates well into biopolymeric matrix [6,12]. A homogeneous distribution and incorporation of GO into chitosan matrix can enhance the properties of the chitosan films, as reported previously [5,13–15].

This study aimed to evaluate the water vapor transmission rate, as well as mechanical and colorimetric properties of chitosan-based films incorporated with GO, and to investigate their use as biodegradable bags for conservation of ‘Tommy Atkins’ mango.

2.2 MATERIAL AND METHODS

2.2.1 Material

Chitosan was obtained from Polymar Indústria e Comércio Importação e Exportação Ltda. (Brazil), with a degree of deacetylation of 85%. GO with 15-20 sheets was provided by Sigma-Aldrich Inc. (USA). Glacial acetic acid with purity of 99.8% was purchased from Proquímicos (Brazil). ‘Tommy Atkins’ mangoes were harvested from a commercial orchard in Petrolina, PE, Brazil (9°03’04.6’’S, 40°17’46.5’’W). Synthetic plastic bags (Freshmama®) were provided by Nissan Steel Industry Co., Ltd. (http://freshmama.jp/en/) (Kyoto, Japan).

2.2.2 Preparation of the biopolymeric films

The film-forming solutions were prepared by dissolving chitosan (2%, w/v) in a solution of acetic acid (1%, v/v) and distilled water, under moderate stirring for 12 h at room temperature

(29º C). GO was incorporated into filmogenic solutions at 0 and 0.25% (w/w) in relation to the dry mass of the biopolymer, and the solutions were again subjected to stirring. The final film-forming solutions was immersed in the ultrasonic bath for 10 min to eliminate bubbles and guarantee a homogeneous mixing. Then, 240 mL of each solution were transferred to an acrylic plate (32 × 32 cm) and dried at 50 °C for 10 h, by the solution-casting method. The bags consist of two overlapped films that were sealed on the edges with the fruit inside.

2.2.3 Characterization of the biopolymeric films

The water vapor transmission rate (WVTR) of the films was determined through the gravimetric method described by Sun et al. [16], in accordance to the ASTM E96-00 method. Square film samples (2 cm x 2 cm) were deposited over the permeation measuring cells, with water level up to 1 cm below the film. The cell-film sets were weighed and then placed in a desiccator containing silica, at 25 °C and 50% relative humidity. Cell weight was recorded every hour for eight hours. The analysis was performed in quintuplicates, and the WVTR was calculated by Eq. (1):

Eq. (1): $WVTR = W / (A.t)$

Where WVTR is the water vapor transmission rate (g m^{-2} s^{-1}); W is the weight of water permeating through the film (g); A is the permeation area (m^2); and t is the permeation time (s).

The thickness of films was measured using a digital hand-held micrometer (model MDC-25M, MFG, Mitutoyo Corp., Japan) with a precision of 0.001 mm. Five random measures were performed throughout each film, which were used to calculate the average film thickness.

The mechanical properties tensile strength (σ, in MPa), elongation at break (ε, in %) and Young’s modulus (€, in MPa) of the films were measured using a mechanical testing machine (model DL5000/10000, Brazil), which operated according to ASTM D882-8312 at a test speed of 5 mm min^{-1} and an application force of 5 kN. The samples were cut into strips of 50 × 5 mm (length × width).

Colorimetric properties were determined using a colorimeter model CR-10 (Konica Minolta, Japan). Color was expressed through parameters L* (lightness), a* (negative - green; positive - red) and b* (negative - blue; positive - yellow). The opacity (%) was calculated as the ratio between lightness of the films against black and white background patterns. In both analyses, five random measures were accomplished throughout each film to calculate the average value.

2.2.4 Preparation and characterization of mangoes

'Tommy Atkins' mangoes were harvested at the maturity stage 2, represented by physiologically mature fruit with full shoulders at the stem end and a predominant light green skin color [17]. Fruit were washed and sanitized by immersion in a 0.1% sodium hypochlorite solution for 5 minutes, followed by drying at room temperature (25 °C). The selection of fruit was based on uniformity of size, color and shape, and on the absence of injuries and diseases.

Fruit were divided in four treatments, being two chitosan-based biodegradable bags (with and without GO), one synthetic plastic-based bag and control (unbagged fruit). Two fruit were packed and sealed in each bag, which were then stored at 12.0 ± 0.5 °C with 89 ± 3% RH for 42 days, simulating long distance shipping. Every 14 days, six fruit per treatment were randomly sampled for physicochemical analysis.

Fruit respiration rate was measured individually with a gas analyzer model PA 7.0 (WITT-Gasetechnik GmbH & Co KG, Germany), after the fruit were kept for 1 h in an airtight container. Respiration rate was expressed as mg CO_2 kg^{-1} h^{-1}. The weight loss (%) was calculated by the difference between the initial weight and the final weight at the end of the storage time. Fruit external appearance was evaluated with a 9-point visual scale [18], where higher grades represent best appearance and lowest incidence of injuries, spots or rots, and 5 is the acceptability cutoff, expressing fruit with 10% presence of spots.

Fruit color was determined with a Konica Minolta colorimeter (model CR-400, Konica Minolta, Japan), with readings expressed as lightness (L*), chromaticity (C*) and hue angle (ºh). The pulp firmness was measured with a texture analyzer (model TA.XTplus, Stable Micro Systems, UK), accoupled to a 6 mm diameter spherical stainless-steel probe, with a penetration distance of 10 mm. Firmness (N) represents the average of two measurements performed in the equatorial region of each fruit without the skin. Soluble solids (SS) content (º Brix) was determined in about 2 g of mango juice with a digital refractometer (model PAL-1, Atago, Japan). Titratable acidity (TA) was determined in 5 mL of mango juice diluted in 45 mL of distilled water, which was then titrated with a solution of 0.1 N NaOH until pH 8.1. Titration was performed with an automatic titrator (model 848, Metrohm, Switzerland), and the results were expressed in g of citric acid per 100 g of fruit.

2.2.5 Statistical analysis

Chitosan-based films with and without addition of GO were compared by F test ($p \leq 0.05$) for water vapor transmission rate, mechanical and colorimetric properties.

The experiment with mangoes was set up in a completely randomized design, in a split-plot arrangement (4 × 4), with three replications and two fruit per replication. The plots consisted of four packaging conditions (chitosan, chitosan and graphene oxide, synthetic and control), and the subplots were represented by four storage times (0, 14, 28 and 42 days). The data were submitted to analysis of variance, and the packages were compared within each storage time by LSD's test ($p \leq 0.05$).

A principal component analysis (PCA), based on a correlation matrix among the physicochemical attributes of the fruit, was applied to summarize the data into two principal components.

2.3 RESULTS AND DISCUSSION

2.3.1 Film properties

The water vapor transmission rate, as well as mechanical and colorimetric properties of the films are shown in Table 1. The addition of GO into the chitosan matrix caused significative changes in all properties of the films ($p \leq 0.05$), except for elongation at break.

Incorporation of GO reduced water vapor transmission rate (WVTR) of chitosan-based films by 35% ($p \leq 0.05$). The WVTR represents the ability of a film to block the passage of water across its surface. This property is influenced by environmental factors such as temperature and relative humidity, film structure, including its thickness and area, difference in pressure, or concentration gradient across the film [10]. Low WVTR values are favorable for the development of eco-friendly coatings or films to enhance shelf life of food products, including fresh fruit [16]. Molecules of GO tends to fill up the structural gaps when incorporated in the chitosan polymeric chain [19]. According to Ahmed et al. [13], the decrease in the diffusion of water vapor through the film is related to the creation of a tortuous path due to the excellent dispersion of GO in polymeric matrix.

The thickness of chitosan film had a tiny but significative decrease ($p \leq 0.05$) due to the addition of the GO nanoparticles (Table 1). This probably occurred due to the interactions between polymer chains and nanostructures, which may favor greater matrix compaction [20].

The tensile strength and the Young's modulus of the chitosan film were significantly increased ($p \leq 0.05$) in 21 and 19% by GO (Table 1), respectively, confirming previous results by Paiva et al. [21]. The elongation of break was not influenced by addition of GO ($p > 0.05$), which averaged 5.70 and 5.81% in the films with 0 and 0.25% of GO, respectively. Mechanical properties of films represent their resistance to reach the breaking point, and consequently reflect their potential to protect the food from contact with the external environment [10]. For fresh fruit and vegetables, the packaging is also important to reduce gas exchanges with the external environment, thus reducing fruit respiration and metabolism and extending their shelf life.

Even at a low concentration of 0.25% over chitosan dry mass, the GO decreased lightness and increased opacity of the chitosan-based films ($p \leq 0.05$), confirming the results obtained in a previous study [21]. Both films showed a typical yellow color due to the chitosan, represented by the high b^* values of 12.46 and 14.34 in CH and CH/GO films, respectively. Negative a^* values of -3.16 (CH/GO) and -2.74 (CH) indicate a slightly green color (Table 1).

The color of the packaging plays an important role on the visual acceptance of the product by consumers [9,22]. In this context, the chitosan-based biodegradable bags are more suitable for use throughout refrigerated transportation, as a way to preserve the quality of the mangoes until they arrive to their final destination, than for the transport of the fruit by the consumer. In this case, the biodegradable bags do not require mechanical strength or color equivalent to commercial polyethylene-based plastic bags for transport use, as indicated by Paiva et al. (2020).

Table 1. Water vapor transmission rate, colorimetric and mechanical properties of chitosan-based films.

Film	WVTR (g m^{-2} s^{-1})	Th (μm)	σ (×10^{-3} MPa)	ε (%)	Є (×10^{-3} MPa)	*L**	*a**	*b**	Op (%)
0% GO	52.73 ± 3.49 a	56.0 ± 5.0 a	31.2 ± 2.5 b	5.70 ± 0.27 a	5.47 ± 0.29 b	82.84 ± 0.34 a	-2.74 ± 0.21 a	12.46 ± 0.23 b	44.30 ± 0.53 b
0.25% GO	35.65 ± 2.42 b	49.0 ± 3.0 b	37.9 ± 4.5 a	5.81 ± 0.32 a	6.52 ± 0.77 a	72.04 ± 0.76 b	-3.16 ± 0.17 b	14.34 ± 0.32 a	46.92 ± 0.45 a

CH: film prepared with 2% chitosan; CH/GO: film prepared with 2% chitosan and 0.25% graphene oxide over chitosan dry mass. WVTR: water vapor transmission rate; *L**: lightness; Op: opacity; Th: thickness; σ: tensile strength; ε: elongation at break; Є: Young's modulus. Different letters in the column indicate statistical difference by the F test ($p \leq 0.05$).

Table 2. F-value of analysis of variance for physicochemical quality parameters of 'Tommy Atkins' mangoes stored in different packaging conditions at 12 °C for 42 days.

Source of variation	Weight loss	Respiration rate	Skin hue	Pulp hue	External appearance	Pulp firmness	SS	TA	SS/TA ratio
Packages (P)	94.89***	62.99***	112.99***	39.96***	17.44***	18.50***	11.17**	14.96**	76.90***
Storage (S)	275.24***	234.16***	205.19***	151.90***	72.17***	656.78***	578.23***	462.64***	885.58***
P x S	23.93***	8.37***	7.21***	2.64*	4.44**	2.62*	4.84***	3.89**	48.09***

2.3.2 Fruit quality

The analysis of variance is shown in Table 2. The effects of different bagging conditions on the physicochemical parameters of ‘Tommy Atkins’ mangoes during 42 days of storage are presented in Figures 1 and 2.

As climacteric fruit, mangoes of all treatments increased their respiration rate over storage; however, from the 14th day the unbagged fruit showed a higher respiration rate when compared to bagged ones ($p \leq 0.05$). With a respiration rate of 30.4 CO_2 kg^{-1} h^{-1} at harvest, control fruit showed 157.5 CO_2 kg^{-1} h^{-1} at the 42th day of storage, while bagged fruit had an average respiration rate 40% lower on the same day (Figure 1A).

The packaging resulted in reduced weight loss ($p \leq 0.05$) compared with the control treatment. Synthetic bags provided an incredibly lower weight loss when compared to the other treatments ($p \leq 0.05$), with 1.22% at 56 days. At the same day, weight losses of mangoes stored in CH and CH/GO-based bags were 5.62 and 5.03%, respectively, values statistically lower ($p \leq 0.05$) than those observed in control fruit (7.99%) (Figure 1B).

Weight loss occurs naturally in the fruit after harvest, as a result of ripening and transpiration processes, but promotes wrinkling and wilting and reduces fruit shelf life [23]. A reduced weight loss of ‘Tommy Atkins’ mangoes stored under modified atmosphere using chitosan has been also reported in other studies [23–28]. The lower weight loss of bagged fruit is mainly related to the water vapor barrier provided by the bags [24]. In this context, mangoes stored in petroleum-based synthetic bags tend to lose less weight due to the hydrophobic nature of these polymers, which is an advantage over the hydrophilicity of the chitosan-based biodegradable bags.

The hue angle of both the skin and pulp of ‘Tommy Atkins’ mangoes decreased over storage. This decrease, however, was more intense in unpackaged fruits. When harvested, unbagged mangoes had a green skin color ($h^o = 114.3^o$), which gradually changed to yellow ($h^o = 82.6$) after 42 days of storage, while packed fruit showed an average $h^o = 94.2$ on the last day of storage, being statistically higher than control ($p \leq 0.05$) and indicating a yellowish-green color (Figure 1C). Pulp h of control mangoes decreased from 93.4° at harvest to 82.0° at 42 days of storage, which was statistically lower ($p \leq 0.05$) than the average 85.2° found in bagged fruit at 42 days of storage (Figure 1D).

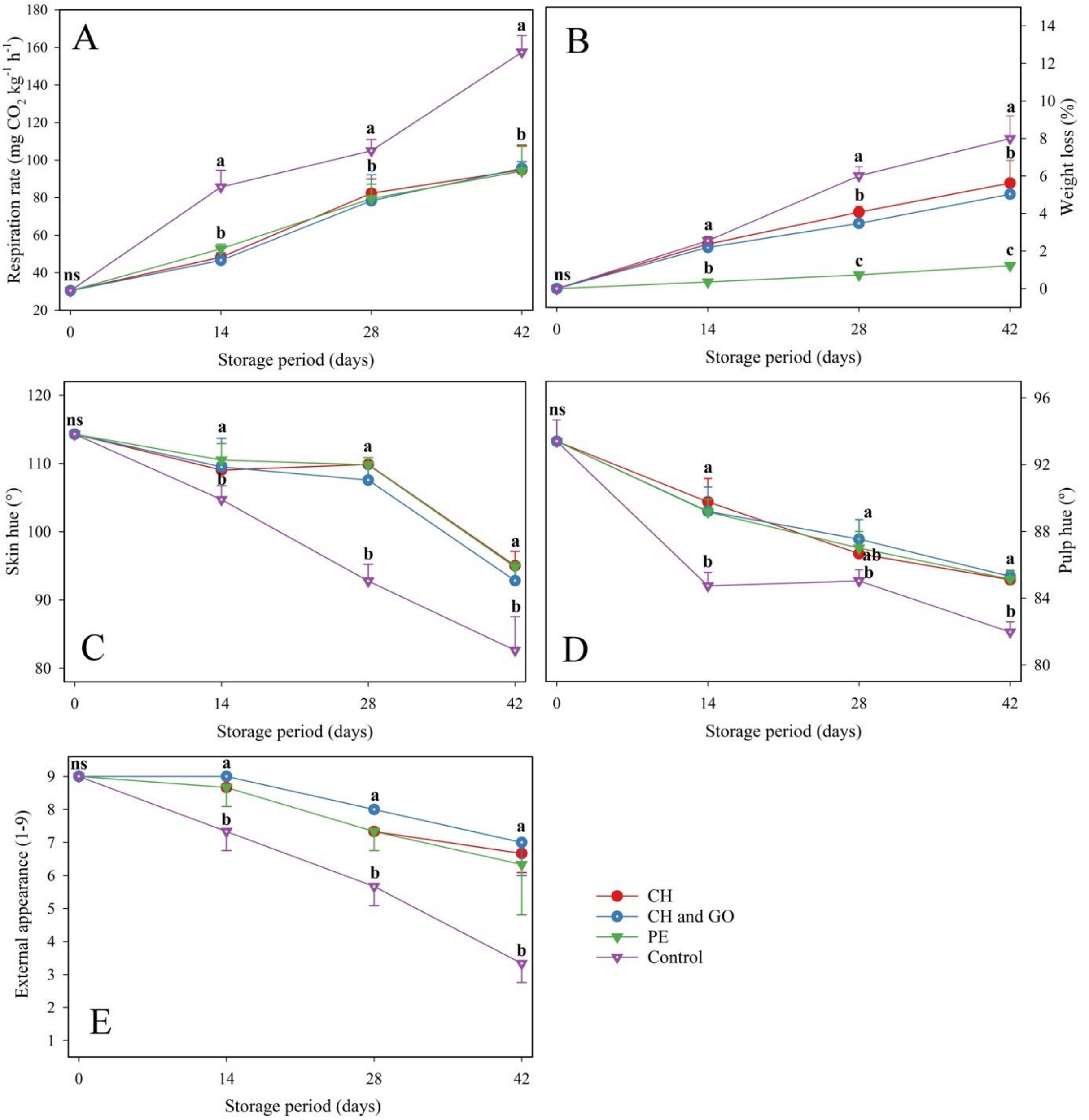

Figure 1. Respiration rate (A), weight loss (B), skin and pulp color (C and D) and external appearance (E) of ‘Tommy Atkins’ mangoes stored in different packaging conditions at 12 ± 0.5º C for 42 days.

CH: chitosan; GO: chitosan/graphene oxide; PE: polyethylene.
Means followed by the same letter, in the same storage day, do not differ statistically by the LSD test ($p \leq 0.05$). Vertical bars represent the standard deviation of the means (n = 3). External appearance = 9 - extremely good appearance - without injuries, spots or rots; 8 - very good - free of spots with slight loss of turgidity; 7 - good - slight presence of spots and slight loss of turgidity (5% of the fruit); 6 - regular - slight presence of spots (5%) and wrinkles (5%); 5 - acceptable - 10% presence of spots; 4 - bad - 25% presence of spots; 3 - very bad - 50% of spots and/or wrinkling; 2 - extremely bad - 75% of spots, injuries or wrinkles and apparent softening; 1 - terrible - over 75% damage, unacceptable (Lima et al., 2012).

The ripening of mangoes is characterized by changes in the external and internal color of the fruit. These changes represent the concomitant degradation of chlorophylls and synthesis of carotenoids. Higher *h*º in skin and pulp of bagged fruit indicate a delayed ripening in relation to unbagged ones.

On the 14th day of storage, the unpacked fruit already presented an external appearance inferior to the packed ones ($p \leq 0.05$), which occurred until the end of storage. On the last day of storage, the control fruit had an average score of 3.3 for external appearance, classifying them as unsuitable for commercialization due to the high severity of spots, injuries and wrinkles. On the same evaluation day, bagged fruit had external appearance classified as regular/good, averaging 6.7 (Figure 1E).

Pulp firmness decreased over storage in all treatments, but this reduction was more intense in control fruit, which differed at 14th from bagged ones ($p \leq 0.05$). Mangoes have shown a pulp firmness of 73.0 N at harvest, decreasing to 2.8 and 5.0 N after 42 days of storage in unbagged and bagged fruit, respectively (Figure 2A). The softening of mangoes during ripening is mainly related to three factors: reduction of cell turgor by water loss through transpiration, starch breakdown into sugars and high activity of cell wall degrading enzymes. Faster changes in bagged fruit might be attributed to enhanced ripening that results in faster softening [27].

Starting at 7.8º Brix at harvest, the soluble solids (SS) content increased until 14.5 and 13.8º Brix in unbagged and bagged mangoes, respectively (Figure 2B). Bagged fruit have shown a lower increase of SS content at 14 and 28 days of storage ($p \leq 0.05$), compared to control fruit, which can indicate a reduction of the enzymatic metabolism responsible for the conversion of starch into sugar, characteristic of fruit ripening [29].

Titratable acidity (TA) decreased in all treatments during storage. However, this reduction was faster in unbagged fruit, differing from other treatments at 28 and 42 days of storage ($p \leq 0.05$) (Figure 2C). ‘Tommy Atkins’ mangoes were harvested with 1.29 g of citric acid per 100 g of fruit, which decreased to 0.20 and 0.38 g of citric acid per 100 g of fruit after 42 days of storage in unbagged and bagged fruit, respectively.

The SS/TA ratio increased in all treatments, but unbagged fruit increased 1126%, while bagged mangoes showed an increase of 546%, 489%, and 492%, for fruit in CH, CH/GO and synthetic bags, respectively (Fig. 2D). Lower SS/TA ratio has also been reported in ‘Tommy Atkins’ mangoes coated with chitosan [26,27].

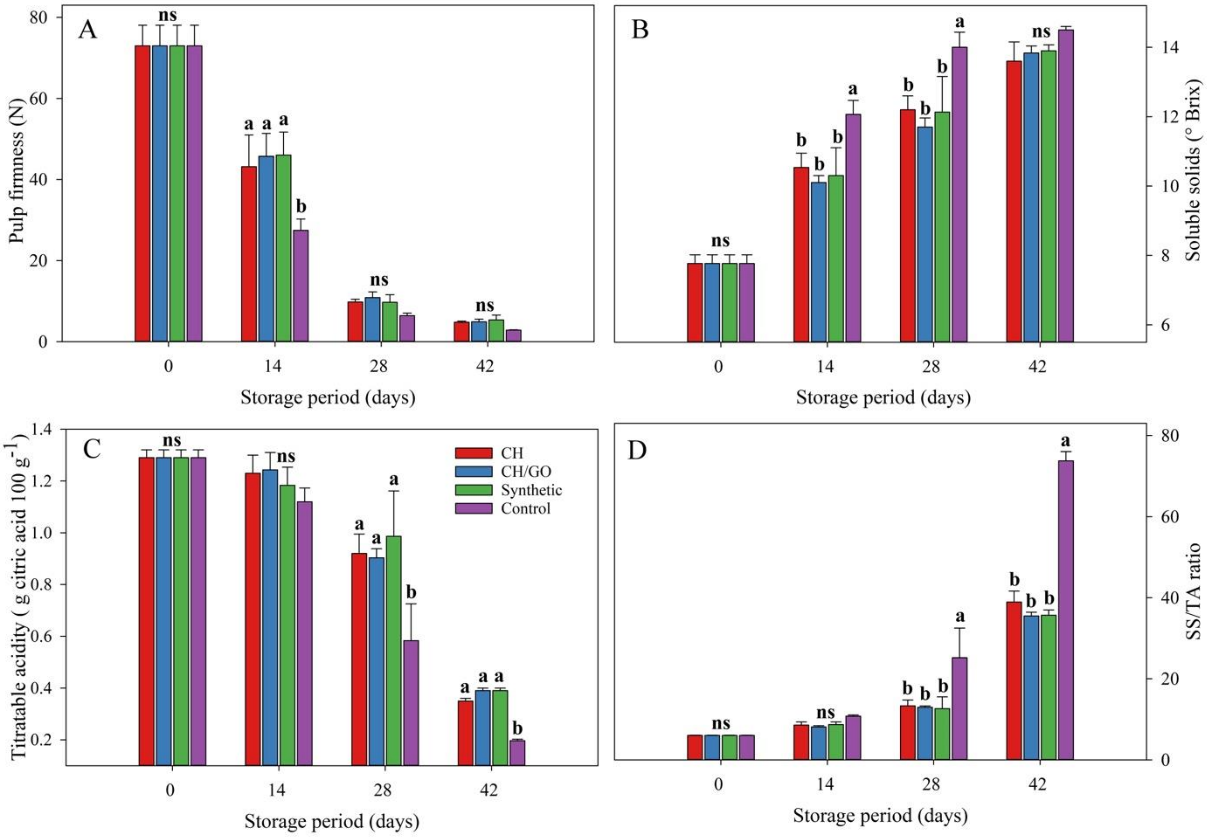

Figure 2. Pulp firmness (A), soluble solids (B), titratable acidity (C) and SS/TA ratio (D) of ‘Tommy Atkins’ mangoes stored in different packaging conditions at 12 ± 0.5º C for 42 days.

CH: chitosan; GO: chitosan/graphene oxide. Means followed by the same letter, in the same storage day, do not differ statistically by the LSD test ($p \leq 0.05$). Vertical bars represent the standard deviation of the means (n = 3).

2.3.3 Principal component analysis

A principal component analyses (PCA) was applied to summarize the great quantity of information obtained from all physicochemical parameters of ‘Tommy Atkins’ mangoes stored in different packaging conditions for 42 days at 12º C (Figure 3). The eigenvalues of the covariance matrix showed that the first two principal components (PCs) accounted for most of the variability observed for external appearance, skin and pulp hue, titratable acidity and flesh firmness; on the negative axis, variables with higher quantity in ripe fruit, including soluble solids, respiration rate, weight loss and SS/TA ratio (Table 4).

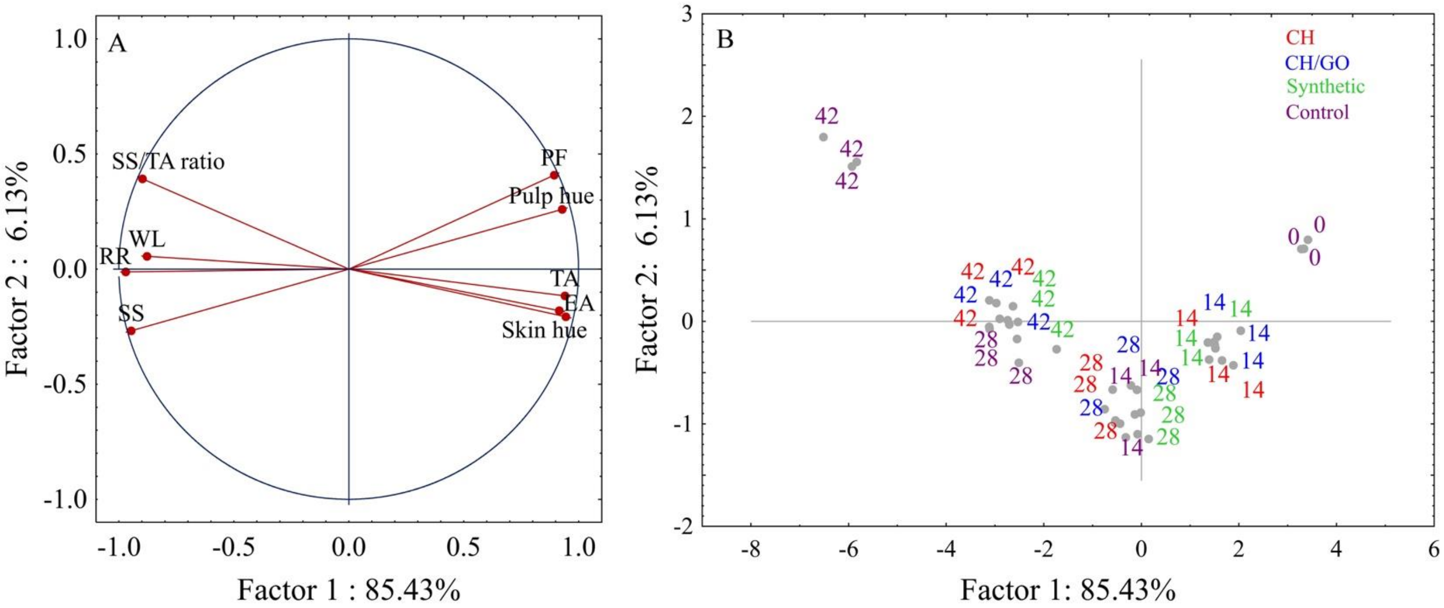

Figure 3. Principal component analysis (PCA): factor loading plot of physicochemical variables of mangoes along PC1 and PC2 (A) and distribution of the samples on score plots (B).

RR: respiration rate; WL: weight loss; EA: external appearance; PF: pulp firmness; SS: soluble solids; TA: titratable acidity

Table 3. Eigenvalues and variances of the principal components that represent 9 variables evaluated in ‘Tommy Atkins’ mangoes stored in different packaging conditions for 42 days at 12 °C.

Principal component	Eigenvalue	Variance (%)	Cumulative variance (%)
PC1	7.688	85.43	85.43
PC2	0.551	6.13	91.55
PC3	0.264	2.94	94.49
PC4	0.231	2.56	97.05
PC5	0.106	1.18	98.23
PC6	0.074	0.83	99.06
PC7	0.040	0.44	99.51
PC8	0.027	0.30	99.80
PC9	0.018	0.20	100.00

Table 4. Loadings in the principal components PC1 and PC2, for the 9 variables evaluated in ‘Tommy Atkins’ mangoes stored in different packaging conditions for 42 days at 12 °C.

Variable	PC1
WL	-0.878
CO2	-0.971
Skin hue	0.944
Pulp hue	0.928
PF	0.894
SS	-0.947
TA	0.940
SS/TA ratio	-0.898
EA	0.916

RR: respiration rate; WL: weight loss; EA: external appearance; PF: pulp firmness; SS: soluble solids; TA: titratable acidity.

Mangoes at harvest and bagged mangoes at 14 days of storage showed the highest correlation with the positive axis, indicating unripe fruit. Conversely, fruit of the control treatment at 42 days of storage showed the strongest correlation with variables of negative eigenvalues, which represent a more advanced ripening stage. These results confirm the delay that the packages provided on ‘Tommy Atkins’ mangoes ripening.

2.4 CONCLUSIONS

The incorporation of graphene-oxide in chitosan-based films reduced their water vapor transmission rate, thickness, lightness and a^* and increased their tensile strength, Young’s modulus, opacity and b^*. Chitosan and graphene oxide-based biodegradable bags delayed the ripening of ‘Tommy Atkins’ mangoes by reducing respiration rate, weight loss and softening, delaying changes in color, soluble solids content and titratable acidity and preserving the visual appearance of fruit.

2.5 REFERENCES[2]

1. Andrady AL. Plastic products. In: Plastics and Environmental Sustainability. Hoboken: John Wiley & Sons; 2015. p. 88-119.
2. Danso D, Chow J, Streita WR. Plastics: Environmental and biotechnological perspectives on microbial degradation. Applied and Environmental Microbiology 2019;85(19):e01095-19.

---

[2] The references were formatted with Vancouver citation style, according to the journal's standards.

3. Ncube LK, Ude AU, Ogunmuyiwa EN, Zulkifli R, Beas IN. Environmental impact of food packaging materials: A review of contemporary development from conventional plastics to polylactic acid based materials. Materials 2020;13(21):4994.
4. Gomes ÁVR, Leite RH, Silva Júnior MQ, Santos FKG, Aroucha EMM. Influence of composition on mechanical properties of cassava starch, sisal fiber and carnauba wax biocomposites. Materials Research 2019;22(1): e20180887.
5. Maraschin TG, Correa RS, Rodrigues LF, Balzarettid NM, Malmonge JA, Galland GB, et al. Chitosan nanocomposites with graphene-based filler. Mater Res. 2019;22(1): e20180829.
6. Das L, Das P, Bhowal A, Bhattachariee C. Synthesis of hybrid hydrogel nano-polymer composite using Graphene oxide, Chitosan and PVA and its application in waste water treatment. Environmental Technology & Innovation 2020;18:100664.
7. Bedor PBA, Caetano RMJ, Souza FG, Leite SGF. Advances and perspectives in the use of polymers in the environmental area: A specific case of PBS in bioremediation. Polimeros 2020;30(2):1-10.
8. Yu J, Wang D, Geetha N, Mahmood K, Jogaiah S, Mujtaba M. Current trends and challenges in the synthesis and applications of chitosan-based nanocomposites for plants: A review. Carbohydrate Polymers 2021;261:117904.
9. Han Lyn F, Tan CP, Zawawi RM, Nur Hanani ZA. Physicochemical properties of chitosan/ graphene oxide composite films and their effects on storage stability of palm-oil based margarine. Food Hydrocolloids 2021;117:106707.
10. Jakubowska E, Gierszewska M, Nowaczyk J, Olewnik-Kruszkowska E. The role of a deep eutectic solvent in changes of physicochemical and antioxidative properties of chitosan-based films. Carbohydrate Polymers 2021;255:117527.
11. Bagri A, Mattevi C, Acik M, Chabal YJ, Chhowalla M, Shenoy VB. Structural evolution during the reduction of chemically derived graphene oxide. Nature Chemistry 2010;2(7):581-7.
12. Zuo P-P, Feng H-F, Xu Z-Z, Zhang L-F, Zhang Y-L, Xia W, et al. Fabrication of biocompatible and mechanically reinforced graphene oxide-chitosan nanocomposite films. Chemistry Central Journal 2013;7(1):39.
13. Ahmed J, Mulla M, Arfat YA, Thai T LA. Mechanical, thermal, structural and barrier properties of crab shell chitosan/graphene oxide composite films. Food Hydrocolloids 2017;71:141-8.
14. Han D, Yan L, Chen W, Li W. Preparation of chitosan/graphene oxide composite film with enhanced mechanical strength in the wet state. Carbohydrate Polymers 2011;83(2):653-8.
15. Yang X, Tu Y, Li L, Shang S, Tao X. Well-dispersed chitosan/graphene oxide nanocomposites. Applied Materials and Interfaces 2010;2(6):1707-13.
16. Sun Q, Sun C, Xiong L. Mechanical, barrier and morphological properties of pea starch and peanut protein isolate blend films. Carbohydrate Polymers 2013;98(1):630-7.
17. National Mango Board. Mango maturity and ripeness guide [Internet]. 2010 [cited 2021 Apr 20]. Available from: mango.org
18. Lima AB, Silva SM, Rocha A, Nascimento LC, Ramalho FS. Conservação pós-colheita de manga “Tommy Atkins” orgânica sob recobrimentos bio-orgânicos. Revista Brasileira de Fruticultura 2012;34(3):704-10.

19. Rambabu K, Bharath G, Banat F, Show PL, Cocoletzi HH. Mango leaf extract incorporated chitosan antioxidant film for active food packaging. International Journal of Biological Macromolecules 2019;126:1234-43.
20. Goh PS, Ismail AF, Ng BC. Directional alignment of carbon nanotubes in polymer matrices: Contemporary approaches and future advances. Composites: Part A 2014;56:103-26.
21. Paiva CA, Vilvert JC, Menezes FLG, Leite RHL, Santos FKG, Medeiros JF, et al. Extended shelf-life of melons using chitosan and graphene oxide based biodegradable bags. Journal of Food Processing and Preservation 2020;44:e14871.
22. Ren L, Yan X, Zhou J, Tong J, Su X. Influence of chitosan concentration on mechanical and barrier properties of corn starch/chitosan films. International Journal of Biological Macromolecules 2017;105:1636-43.
23. Costa JDS, Figueiredo Neto A, Almeida FAC, Costa MS. Conservation of “Tommy Atkins” mangoes stored under passive modified atmosphere. Revista Caatinga 2018;31(1):117-125.
24. Medeiros BGS, Pinheiro AC, Carneiro-da-cunha MG, Vicente AA. Development and characterization of a nanomultilayer coating of pectin and chitosan - Evaluation of its gas barrier properties and application on ‘Tommy Atkins’ mangoes. Journal of Food Engineering 2012;110(3):457-64.
25. Catamari FOS, Santana LCLA, Carnelossi MAG, Alexandre APS, Nunes ML, Goulart MOF, et al. Impact of edible coatings based on cassava starch and chitosan on the post-harvest shelf life of mango (*Mangifera indica*) ‘Tommy Atkins’ fruits. Food Science and Technology 2018;38(1):86-95.
26. Souza ML de, Morgado CMA, Marques KM, Mattiuz CFM, Mattiuz B-H. Pós-colheita de mangas “Tommy Atkins” recobertas com quitosana. Revista Brasileira de Fruticultura 2011;33(1):337-43.
27. Eshetu A, Ibrahim AM, Forsido SF, Kuyu CG. Effect of beeswax and chitosan treatments on quality and shelf life of selected mango (*Mangifera indica* L.) cultivars. Heliyon 2019;5(1):e01116.
28. Oliveira TA, Paiva CA, Silva AC, Nascimento L V., Leite RHL, Aroucha EMM. Postharvest quality of Tommy Atkins mangoes coated with cassava starch and chitosan-based coatings. Journal of Agricultural Science 2018;10(11):401.
29. Sousa FF, Pinsetta Junior JS, Oliveira KTEF, Rodrigues ECN, Andrade JP, Mattiuz B-H. Conservation of ‘Palmer’ mango with an edible coating of hydroxypropyl methylcellulose and beeswax. Food Chemistry 2021;346:128925.

3 ARTICLE 2 - CHITOSAN AND GRAPHENE OXIDE-BASED BIODEGRADABLE BAGS: AN ECO-FRIENDLY AND EFFECTIVE PACKAGING ALTERNATIVE TO MAINTAIN POSTHARVEST QUALITY OF 'PALMER' MANGO[3]

Abstract

Mango is a highly appreciated tropical fruit with high respiration rate and ethylene production, which makes it highly perishable after harvest. The objective of this study was to evaluate the effect of chitosan and graphene oxide-based biodegradable bags on the postharvest quality of 'Palmer' mangoes during cold storage. The fruit were harvested at the recommended maturity stage and were stored for 56 days at 12° C without bagging (control), as well as in chitosan-based bag, chitosan-based bag with graphene oxide, and polyethylene-based bag. According to the results, the bags evaluated in our study delayed mango ripening, maintaining external appearance, skin and flesh colors, firmness, soluble solids (SS), titratable acidity (TA), SS/TA ratio, β-carotene and chlorophyll contents in the fruit. In addition, bagging the fruit reduced weight loss, respiration rate and anthracnose (*Colletotrichum gloeosporioides*) incidence and severity during storage. Our results suggest that chitosan-based biodegradable bags are an ecological and effective alternative to maintain postharvest quality of 'Palmer' mango during cold storage.

Keywords: *Mangifera indica*; biopolymer; modified atmosphere packaging; nanoparticles; postharvest.

3.1 INTRODUCTION

Mango (*Mangifera indica* L.) is the second most traded tropical fruit globally, mainly due to its unique features, such as succulent and delicious taste and exotic flavor (Mwaurah et al., 2020; Rastegar, Hassanzadeh Khankahdani, & Rahimzadeh, 2019). Mango is often referred to as the 'king of the fruit' (Tharanathan, Yashoda, & Prabha, 2006), because in addition to the sensory attributes desired by consumers, it also has a high nutritional value and health benefits due to bioactive compounds, such as vitamin C, provitamin A, carotenoids and phenolic compounds (Maldonado et al., 2019).

---

[3] Paper submitted to LWT - ISSN: 0023-6438 - JCR Impact Factor: 4.006 (2020)

Mango has been produced in more than 100 countries to attend the increasing fruit demand in the global market (Evans, Ballen, & Siddiq, 2017). Asia is the center of origin and the major producing region of mangoes, contributing to about 73% of the world's mango production (FAO, 2019). In the Americas, the most important mango producing and exporting countries are Mexico, Brazil and Peru, which have supplied mango to the market by improving cultural practices, investing in high technology production and marketing systems, and focusing exports on a few varieties that have the greatest international appeal (Evans et al., 2017).

Mango is a climacteric fruit with high respiration rate and ethylene production, which makes it highly perishable and limits fruit long-distance marketing. Therefore, postharvest technologies are essential to reduce metabolic activity and extend postharvest life of mangoes shipped to distant markets (Rastegar et al., 2019). Refrigerated containers is the most important technology commercially used to maintain quality and ship mangos overseas (Rosalie, Léchaudel, Dhuique-Mayer, Dufossé, & Joas, 2018). However, depending on the shipping distance, the use of low temperatures alone is not enough to guarantee high mango quality in the market, requiring other technologies associated with low temperatures to further reduce metabolic activity and increase fruit postharvest life (Ntsoane, Zude-Sasse, Mahajan, & Sivakumar, 2019).

Modified atmosphere packaging (MAP) can potentially increase fruit postharvest life by reducing oxygen and increasing carbon dioxide concentrations around the fruit, which inhibit fruit respiration rate and metabolic activity. MAP is usually applied by packing the fruit in hermetically sealed polyethylene bags. Polyethylene is a polymer with several desirable properties, including flexibility, strength, lightness, stability, moisture and chemical resistance, and easy processability (Marsh & Bugusu, 2007). However, for being a petroleum-based polymer, the polyethylene is non-biodegradable and slow to decompose, which raises health and environmental concerns about the solid waste after using this polymer (Ren, Yan, Zhou, Tong, & Su, 2017).

Chitosan is a natural polysaccharide derived from the deacetylation of chitin, obtained from crustacean exoskeleton, which has been widely used as fruit coatings. Chitosan is a promising alternative as an environmentally friendly packaging material, which can provide modified atmosphere conditions to different fruit species, reducing gas exchange and delaying ripening, as reported previously for melons (Paiva et al., 2020).

The objective of this study was to evaluate the effect of chitosan and graphene oxide-based biodegradable bags on the postharvest quality of 'Palmer' mangoes during cold storage.

3.2 MATERIAL AND METHODS

3.2.1 Material

Chitosan (degree of deacetylation 85%) was obtained from Polymar Indústria e Comércio Importação e Exportação Ltda. (Fortaleza, Brazil). Graphene oxide (15-20 sheets) was provided by Sigma-Aldrich Inc. (St. Louis, USA). Synthetic polyethylene-based bags (Freshmama®) were provided by Nissan Steel Industry Co., Ltd. (http://freshmama.jp/en/) (Kyoto, Japan).

‘Palmer’ mangoes (*Mangifera indica* L.) were produced in a commercial orchard in the São Francisco Valley, Petrolina, PE, Brazil (latitude 9°03’04.6’’S, longitude 40°17’46.5’’W) and were harvested at the commercial maturity stage 2 (Costa, Figueiredo Neto, Almeida, & Costa, 2018). After harvest, the fruit were washed, dried at room temperature (25 °C) and selected based on uniformity of size, color and shape, as well as based on the absence of injuries and diseases.

3.2.2 Preparation of the biodegradable bags

The bags were prepared according to Paiva et al. (2020). The filmogenic solutions were prepared by dissolving chitosan (2%, w/v) in an aqueous solution of glacial acetic acid (1%, v/v), which was kept stirring for 12 h. In the bags with graphene oxide, 0.25% of graphene oxide, based on chitosan dry weight, was added to the filmogenic solution before stirring. The solutions were immersed in the ultrasonic bath for 10 min to eliminate bubbles and guarantee a homogeneous mixing. 240 g of filmogenic solutions were deposited in an acrylic plate and dried at 50 °C for 10 h, following the solution-casting method. The bags consist of two overlapped films that were sealed on the edges with the fruit inside.

3.2.3 Characterization of water-barrier properties of biodegradable bags

3.2.3.1 Water vapor permeability (WVP)

The water vapor permeability (WVP) of the films was determined, in triplicates, through the gravimetric method described by ASTM E96/E96M-12, according to Monteiro et al. (2017). Film samples (2 cm x 2 cm) were deposited over the permeation measuring cells, with water level up to 1 cm below the film. The system was placed in a desiccator containing silica, at 25 °C and 50% relative humidity. Cell weight was recorded every hour for eight hours. The WVP through the samples was calculated by Eq. (1):

$$\text{Eq. (1): } WVP = \frac{\mathrm{W.L}}{\mathrm{A.t.\Delta P}}$$

Where WVP is the water vapor permeability (g m^1 m^{-2} s^{-1} Pa^{-1}); W is the weight of water permeating through the film (g); L is the film thickness (m); A is the permeation area (m^2); t is the permeation time (s) and ΔP is the pressure difference to water vapor between the two sides of the film (Pa).

3.2.3.2 Water solubility

Water solubility was measured, in triplicates, according to Ge et al. (2015), with slight modifications. Film samples were dried at 105 °C for 1 h and weighed (initial mass). Then, film samples were immersed in 50 mL of distilled water at 25 °C and kept under stirring for 24 h. Later, the discs were dried and weighed again (final mass). The solubility was calculated by Eq. (2):

$$\text{Eq. (2): Water solubility (\%)} = (\text{Initial - final mass} \times 100) / \text{Initial mass}$$

3.2.4 Fruit treatment and physico-chemical analysis

A total of 120 fruit were stored under four conditions of modified-atmosphere packaging (MAP): (i) chitosan-based biodegradable bag (CH), (ii) chitosan-based biodegradable bag with graphene oxide (CH/GO), (iii) non-biodegradable polyethylene-based bag and (iv) control (without bag). Fruit were packed and sealed in each bag with a sealing machine. After packing, the fruit were stored at 12.0 ± 0.5 °C with 89 ± 3% RH for 56 days, which was used to simulate long distance shipping. During storage, 24 fruit were randomly sampled every 14 days for physico-chemical analysis.

3.2.4.1 In-pack gaseous composition and respiration rate

The in-pack concentrations of O_2 and CO_2 were weekly analyzed in all packages during storage, using a gas analyzer model PA 7.0 (WITT-Gasetechnik GmbH & Co KG, Germany).

Fruit respiration rate was determined based on the CO_2 production rate. The measurement was carried out individually with a gas analyzer model PA 7.0 (WITT-Gasetechnik GmbH & Co KG, Germany), after the fruit were kept for 1 h in an airtight container. CO_2 production rate was expressed as mg kg^{-1} h^{-1}.

3.2.4.2 Weight loss

The weight loss was measured as the difference between the initial weight of the fruit and its weight after storage. The results were expressed in percentage.

3.2.4.3 External appearance and anthracnose incidence and severity

Fruit external appearance was evaluated based on the visual scale proposed by Lima *et al.* (2012), which varies from 1 to 9, where: 9 - extremely good appearance - without injuries, spots or rots; 8 - very good - free of spots with slight loss of turgidity; 7 - good - slight presence of spots and slight loss of turgidity (5% of the fruit); 6 - regular - slight presence of spots (5%) and wrinkles (5%); 5 - acceptable - 10% presence of spots; 4 - bad - 25% presence of spots; 3 - very bad - 50% of spots and/or wrinkling; 2 - extremely bad - 75% of spots, injuries or wrinkles and apparent softening; 1 - terrible - over 75% damage, unacceptable. Fruit with a score below 5 were considered unacceptable for marketing.

The anthracnose [(*Colletotrichum gloeosporioides*) (Penz.) Sacc.] incidence was measured by counting diseased fruit in each timing. Disease incidence (%) was calculated as the ratio between the number of diseased fruit and total fruit on each sample.

Anthracnose severity was evaluated based on the scale of 1-5 proposed by Corkidi, Balderas-Ruíz, Taboada, Serrano-Carreón, & Galindo (2006), which is based on the percentage of infected area on each fruit surface, where: 1 (no disease) - 0 to 1% of infected area; 2 (slight disease) - 1 to 5%; 3 (moderate disease) - 6 to 9%; 4 (severe disease) - 10 to 49%; 5 (very severe disease) - 50% to 100%.

3.2.4.4 Skin and pulp color

Skin and pulp color were assessed with a Minolta colorimeter model CR-400 (Konica Minolta, Japan). The color values were determined using the CIE Lab system, which were expressed as lightness (L*) that varies from white (0) to black (100), chroma (C*) that varies from mate (0) to vivid (100) colors, and hue angle (°h) that varies from 0º to 360º, where 0º represents red, 90º represents yellowish green, 180º represents turquoise blue and 270º represents violet.

3.2.4.5 Pulp firmness

The pulp firmness was determined with a texture analyzer model TA.XTplus (Stable Micro Systems, UK). The measurements were performed with a 6 mm diameter spherical stainless-steel probe, with a penetration distance of 10 mm. Two measurements were accomplished per fruit in the equatorial region, which were used to calculate the average firmness. The results were expressed in Newton.

3.2.4.6 Soluble solids (SS), titratable acidity (TA) and SS/TA ratio

Soluble solids (SS) content was determined in about 2g of mango juice with a digital refractometer model PAL-1 (Atago, Japan), and the results were expressed in °Brix.

Titratable acidity (TA) was determined in 5 mL of mango juice diluted in 45 mL of distilled water, which was then titrated with a solution of 0.1 N NaOH until pH 8.1. Titration was performed with a Titrino Plus automatic titrator model 848 (Metrohm, Switzerland), and the results were expressed in g of citric acid per 100 g of fruit. The SS/TA ratios were calculated by dividing the SS value by its respective TA value.

3.2.4.7 Carotenoid and chlorophyll contents

Carotenoid and chlorophyll contents were determined according to the approach described by Nagata and Yamashita (1992). 1 g of mango pulp was dissolved in 10 mL of an acetone:hexane (4:6, v/v) extractor solution, and stirred for 1 min using an Ultra-turrax homogenizer model T18 digital (IKA, China). The supernatant was read at four wavelengths (663 nm, 645 nm, 505 nm and 453 n) on a UV-VIS spectrophotometer model Carry 50 (Varian, Australia).

The pigment contents were calculated using the following equations:

Chlorophyll a ($\mu g\ g^{-1}$) = ($0.999A_{663} – 0.0989A_{645}$) x 10
Chlorophyll b ($\mu g\ g^{-1}$) = ($-0.328A_{663} + 1.77\ A_{645}$) x 10
β-carotene ($\mu g\ g^{-1}$) = ($0.216\ A_{663} – 1.22\ A_{645} – 0.304\ A_{505} + 0.452\ A_{453}$) x 10

Where A_{663}, A_{645}, A_{505} e A_{453} refer to absorbances at 663 nm, 645nm, 505 nm and 453 nm, respectively.

3.2.5 Statistical analysis

The experiment was set up in a completely randomized design, in a split-plot arrangement (4 × 5), with three replications containing two fruit per replication. The plots consisted of four packaging conditions (chitosan, chitosan and graphene oxide, polyethylene and control), and the subplots were represented by five storage times (0, 14, 28, 42 and 56 days). The data were submitted to analysis of variance, and the packages were compared within each storage time by Tukey’s test ($p \leq 0.05$). Alternatively, the nonparametric Kruskal–Wallis test ($p \leq 0.05$) followed by post-hoc Dunn’s test were applied for the ordinal variables (external appearance and anthracnose severity).

Principal component analysis (PCA) was applied to reduce the number of variables to two principal components that account for most of the variance, based on a correlation matrix among the original variables.

Statistical analyzes were performed using R version 4.0.2 (R Foundation for Statistical Computing, Austria), SigmaPlot version 14.0 (Systat Software Inc., USA) and Statistica version 10 (StatSoft Inc., USA).

3.3 RESULTS

3.3.1 Water-barrier properties of biodegradable bags

Incorporation of graphene oxide significantly reduced WVP of chitosan-based bags by 37% ($p \leq 0.05$). The water solubility of the chitosan-bags was significantly reduced from 6.46% to 1.86% by the addition of graphene oxide in the polymer matrix ($p \leq 0.05$) (Table 1).

3.3.2 In-pack gaseous composition and respiration rate

Changes in oxygen and carbon dioxide concentrations within packages containing ‘Palmer’ mangoes are shown in Fig. 1A and 1B. The in-pack oxygen composition was changed from 21% under normal atmospheric conditions to 14.6, 11.7 and 6.0%, respectively, in the CH-based, CH/GO-based and PE-based bags, after 56 days (Fig. 1A). In the same period, the carbon dioxide levels increased from 0.04% to 6.9, 9.4 and 6.3%, respectively (Fig. 1B).

Fruit of all treatments increased their respiration rate over storage, since mango is a climacteric fruit (Fig. 1C). However, unbagged fruit differed from bagged ones ($p \leq 0.05$) for their highest average respiration rate (58.40 mg CO_2 kg^{-1} h^{-1}). Between packages, the CH/GO-based promoted the lowest respiration rate (41.53 mg CO_2 kg^{-1} h^{-1}).

Table 1. Water-barrier properties of chitosan-based biodegradable bags incorporated with graphene oxide.

Bag	Water vapor permeability ($g\ m^{1}\ m^{-2}\ s^{-1}\ Kpa^{-1}$)	Solubility (%)
CH	0.391 ± 0.023 a	6.46 ± 0.82 a
CH/GO	0.248 ± 0.035 b	1.86 ± 1.27 b

CH: film prepared with 2% chitosan; CH/GO: film prepared with 2% chitosan and 0.25% graphene oxide over chitosan dry mass.

Table 2. F-value of analysis of variance for physicochemical quality parameters of ‘Palmer’ mangoes stored in different packaging conditions for 56 days at 12 °C.

Source of variation	WL	RR	Skin L	Skin C	Skin h	Pulp L	Pulp C	Pulp h	PF	SS	TA	Ratio
Packages (P)	149.75***	27.60***	12.73***	22.64***	32.08***	4.89*	6.79*	15.76**	13.99**	13.72**	21.61***	48.93***
Storage (S)	190.067***	65.40***	21.50***	10.16***	24.82***	30.97***	35.78***	115.04***	467.13***	900.65***	659.91***	482.69***
P x S	15.59***	1.84ns	1.68ns	1.07ns	2.76*	0.84ns	1.34ns	1.73ns	3.18**	6.94***	3.88**	11.98***

WL: weight loss; RR: respiration rate; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity.
ns: non-significant. *, ** and *** represents a significative F-value at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.

3.3.3 Fruit quality

The analysis of variance of mango physicochemical traits is presented in Table 2.

3.3.3.1 Weight loss

The packaging reduced weight loss of mango fruit compared to the control ($p \leq 0.05$), which was observed as early as 14 days of storage (Fig. 1D). The minimum weight loss at 56 days was recorded in PE bags (1.76%) followed by CH/GO (8.69%) and CH (9.22%) bags (Fig. 1D). Even so, the weight loss of fruit stored in biodegradable bags is still considered low ($p \leq 0.05$), compared to the control unpacked fruit (12.49%).

3.3.3.2 External appearance and anthracnose incidence and severity

At 56 days of storage, unbagged fruit showed the lowest ($p \leq 0.05$) external appearance (4.0), which represents fruit unacceptable for marketing due to the high presence of decay spots on the fruit (Fig. 1E). At the same day, fruit stored in CH/GO, PE e CH bags showed average grades of 8.3, 8.0 and 7.8, respectively, which characterizes a very good external appearance, as shown in Fig. 2.

In all treatments, there was no disease incidence until 42 days of storage, except for the 16.7% incidence recorded in unbagged fruit at 14 days and in CH-bag fruit at 42 days (data not shown). On the last day of storage, unbagged fruit reached 100% disease incidence, with average severity between 1 and 5% (Fig. 1F), in contrast to the absence of disease in bagged fruit ($p \leq 0.05$).

3.3.3.3 Skin and pulp color

The use of packages delayed color changes in mangoes, as observed by significant changes ($p \leq 0.05$) in all color parameters. The skin of unbagged fruit had highest lightness and chroma values and lowest hue angle ($p \leq 0.05$) throughout the storage period (Fig. 3A, C and E).

Lightness and hue angle were lower ($p \leq 0.05$) and chroma was higher ($p \leq 0.05$) in the pulp of unbagged mangoes (Fig. 3B, D and F).

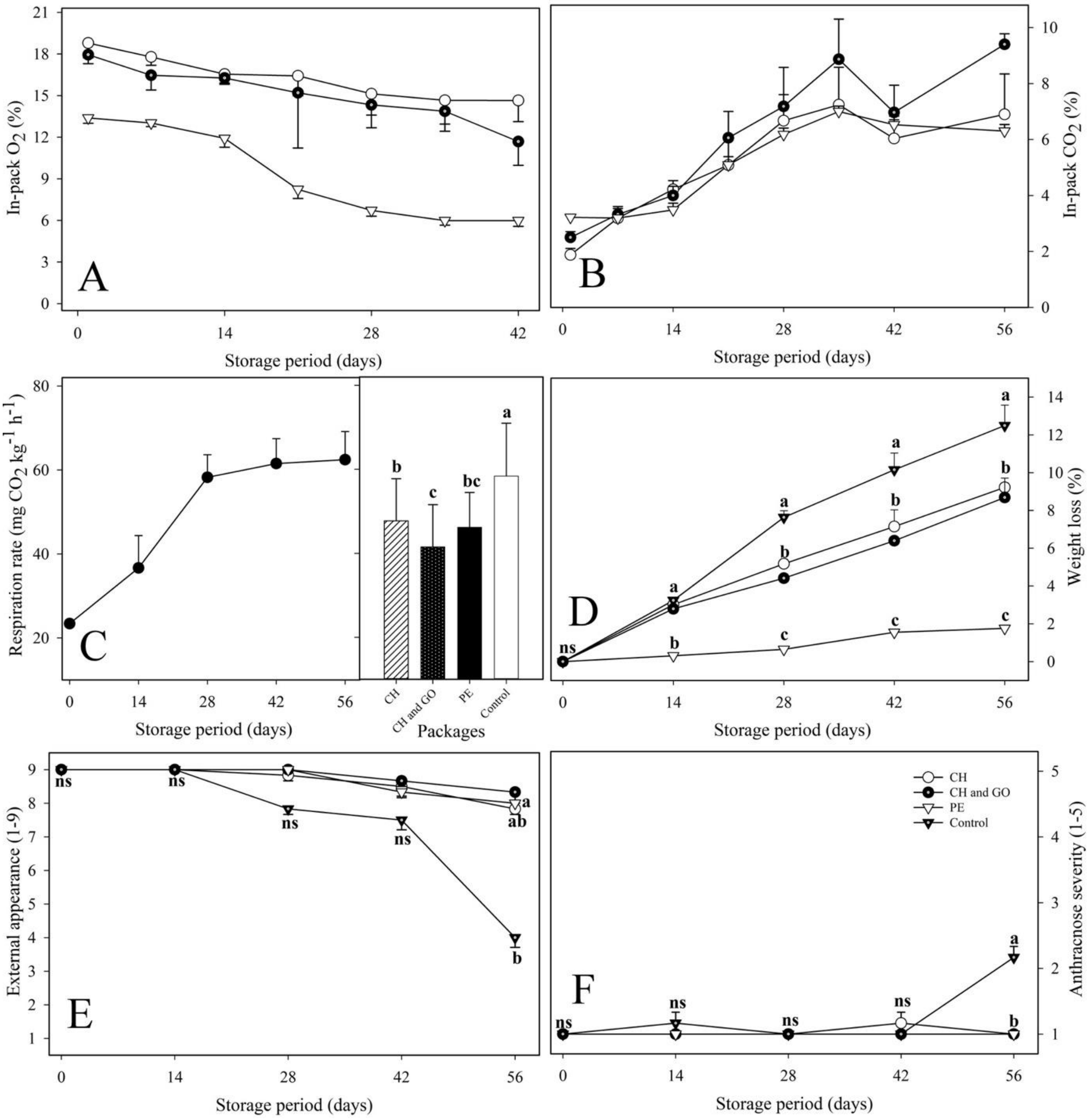

Figure 1. In-pack O_2 and CO_2 concentrations (A and B), respiration rate (C), weight loss (D), external appearance (E) and anthracnose severity (F) of 'Palmer' mangoes stored in different packaging conditions for 56 days at 12 °C.

CH: chitosan; GO: chitosan and graphene oxide; PE: polyethylene synthetic bag.

Means followed by the same letter, in the same storage day, do not differ statistically by the Tukey test ($p \leq 0.05$) for respiration rate and weight loss and by the Dunn's test ($p \leq 0.05$) for external appearance and anthracnose severity. Vertical bars represent the standard error of the means (n = 3). External appearance = 9 - extremely good appearance - without injuries, spots or rots; 8 - very good - free of spots with slight loss of turgidity; 7 - good - slight presence of spots and slight loss of turgidity (5% of the fruit); 6 - regular - slight presence of spots (5%) and wrinkles (5%); 5 - acceptable - 10% presence of spots; 4 - bad - 25% presence of spots; 3 - very bad - 50% of spots and/or wrinkling; 2 - extremely bad - 75% of spots, injuries or wrinkles and apparent softening; 1 - terrible - over 75% damage, unacceptable (Lima et al., 2012). Anthracnose severity = 1 (no disease) - 0 to 1% of infected area on fruit surface; 2 (slight disease) - 1 to 5%; 3 (moderate disease) - 6 to 9%; 4 (severe disease) - 10 to 49%; 5 (very severe disease) - 50% to 100% (Corkidi et al., 2006)

Figure 2. Visual aspect of 'Palmer' mangoes stored in different packaging conditions for 56 days at 12 °C

CH: chitosan; CH/GO: chitosan and graphene oxide.

3.3.3.4 Pulp firmness

Considering the pulp firmness of 95.5 N at harvest, an intense softness process was observed in unbagged mangoes, which showed firmness of 65.2 N and 13.9 N after 14 and 28 days of cold storage, respectively. These values are, on average, 30% and 65% lower ($p<0.05$) than those observed in bagged fruit, at the same period (Fig. 4A).

3.3.3.5 Soluble solids (SS), titratable acidity (TA) and SS/TA ratio

Soluble solids (SS) content of mangoes gradually increased in all treatments during storage, as an effect of fruit ripening. However, bagged fruit had lower SS content when compared to unbagged ones. As shown by Fig. 4B, fruit from control treatment had an increase of 142% from harvest (6.4° Brix) to the 28th day of cold storage (15.5° Brix). In the same day, fruit stored in PE, CH and CH/GO had lower ($p\leq0.05$) SS contents of 13.1, 13.2 and 12.7° Brix, respectively.

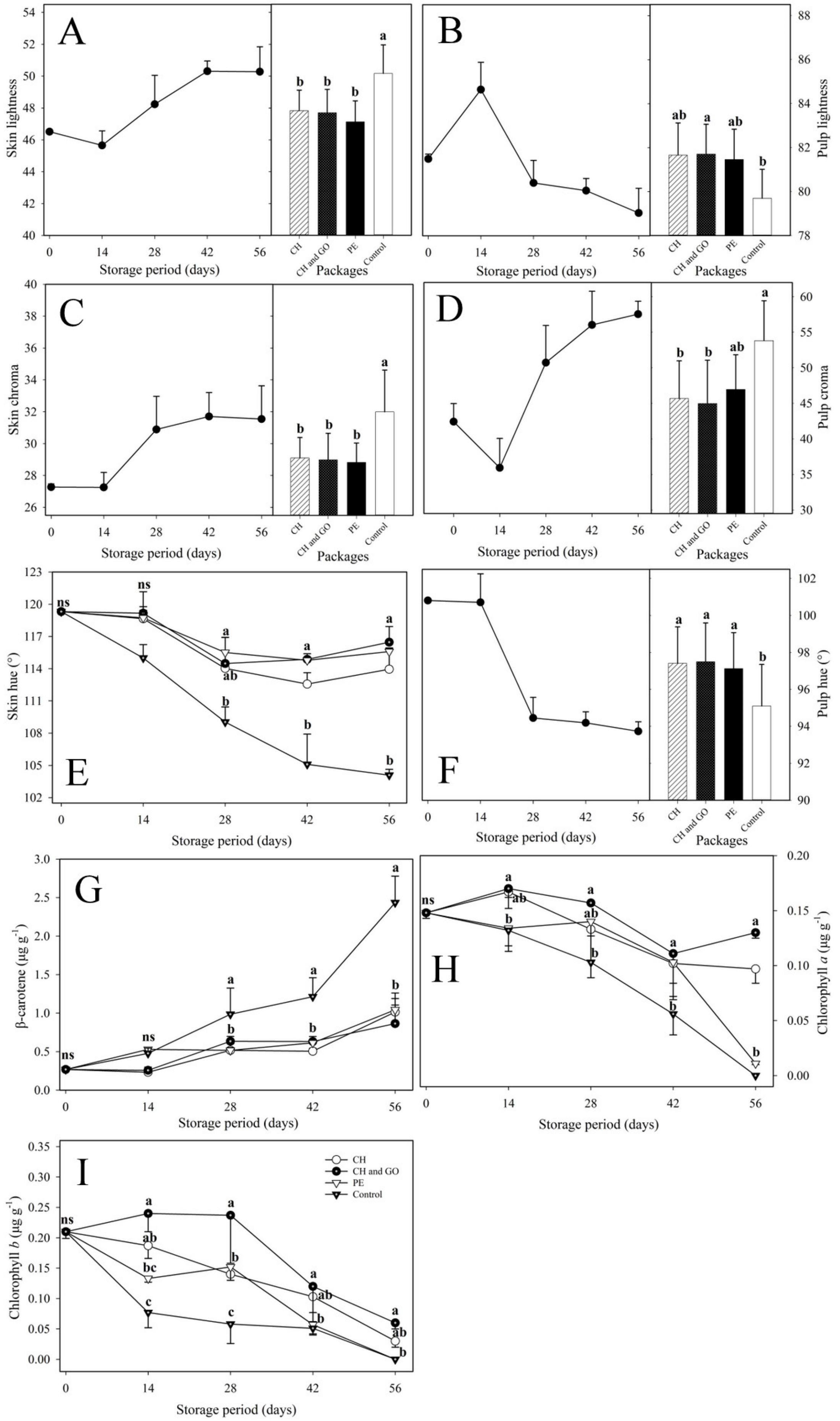
A
Skin lightness
Storage period (days)
Packages
CH
CH and GO
PE
Control
B
Pulp lightness
C
Skin chroma
D
Pulp croma
E
Skin hue (°)
F
Pulp hue (°)
G
β-carotene (µg g-1)
H
Chlorophyll a (µg g-1)
I
Chlorophyll b (µg g-1)
CH
CH and GO
PE
Control
0
14
28
42
56
a
b
ab
bc
c
ns

Figure 3. Color parameters in skin (A, C and E) and pulp (B, D and F), carotenoids (G) and chlorophylls (H and I) of ‘Palmer’ mangoes stored in different packaging conditions for 56 days at 12 °C

CH: chitosan; GO: chitosan and graphene oxide; PE: polyethylene synthetic bag.
Means followed by the same letter, in the same storage day, do not differ statistically by the Tukey test ($p \leq 0.05$).
Vertical bars represent the standard error of the means (n = 3).

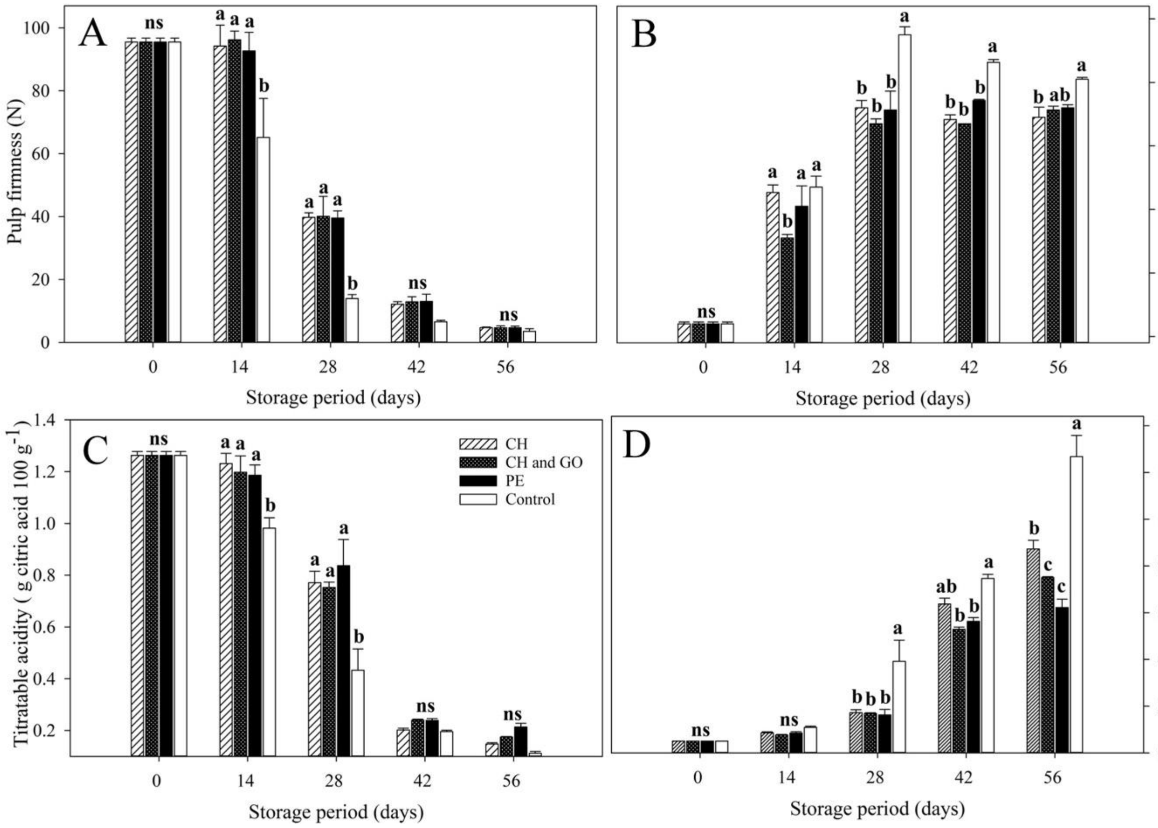

Figure 4. Pulp firmness (A), soluble solids (SS) (B), titratable acidity (TA) (C) and SS/TA ratio (D) of ‘Palmer’ mangoes stored in different packaging conditions for 56 days at 12 °C.

CH: chitosan; GO: chitosan and graphene oxide; PE: polyethylene synthetic bag.
Means followed by the same letter, in the same storage day, do not differ statistically by the Tukey test ($p \leq 0.05$).
Vertical bars represent the standard error of the means (n = 3).

As shown by Fig. 4C, titratable acidity of mangoes decreased throughout storage, regardless the treatment. However, this reduction occurred more slowly in bagged fruit. In the first 14 days of storage, the titratable acidity dropped from 1.30 to 0.98 g citric acid 100 g^{-1} in unbagged fruit, which represents a 24% drop. In the same period, bagged fruit showed a smaller ($p \leq 0.05$) reduction in acidity, which varied between 5 and 9% in CH and PE bags, respectively. On the 28th day, bagged mangoes showed titratable acidity 45% higher ($p \leq 0.05$) than unbagged ones.

The SS/TA ratio increased over storage in all treatments, more markedly in unbagged fruit, starting in 5.1 at the beginning of experiment and reaching 126.8 after 56 days of storage

(Fig. 4D). This value is higher ($p \leq 0.05$) than the SS/TA ratios of 87.3, 75.0 and 62.2 found in fruit stored in CH, CH/GO and PE bags, respectively.

3.3.3.6 Carotenoids and chlorophylls

Pulp carotenoid content increased over storage (Fig. 3G). At 56 days of storage, bagged fruit showed an average increase from 0.30 to 0.97 µg g^{-1} on beta-carotene content. In the same period, unbagged fruit showed a significant increase ($p \leq 0.05$) of 800% on beta-carotene content, with 2.43 µg g^{-1} at 56 days (Fig. 3G).

Pulp chlorophylls contents decreased throughout storage, as a result of degradation. However, fruit stored in biodegradable bags maintained higher levels ($p \leq 0.05$) of these pigments after 56 days of storage, compared to control unbagged fruit (Fig. 3H and I).

3.3.4 Principal component analysis

The eigenvalues of the covariance matrix showed that the first two principal components (PCs) accounted for more than 78% of the total variance in the dataset (Fig. 5). PC1 explained 68.72% of the variance in the dataset, whereas PC2 was responsible for 9.89% (Table 3). PC1 was positively correlated with soluble solids, respiration rate (CO_2), pulp chroma, skin lightness and chroma, weight loss, SS/TA ratio and carotenoids (β-carotene). Conversely, the negative axis of PC1 was defined by external appearance, chlorophylls *a* and *b*, skin and pulp hue, pulp lightness, titratable acidity and flesh firmness (Table 4).

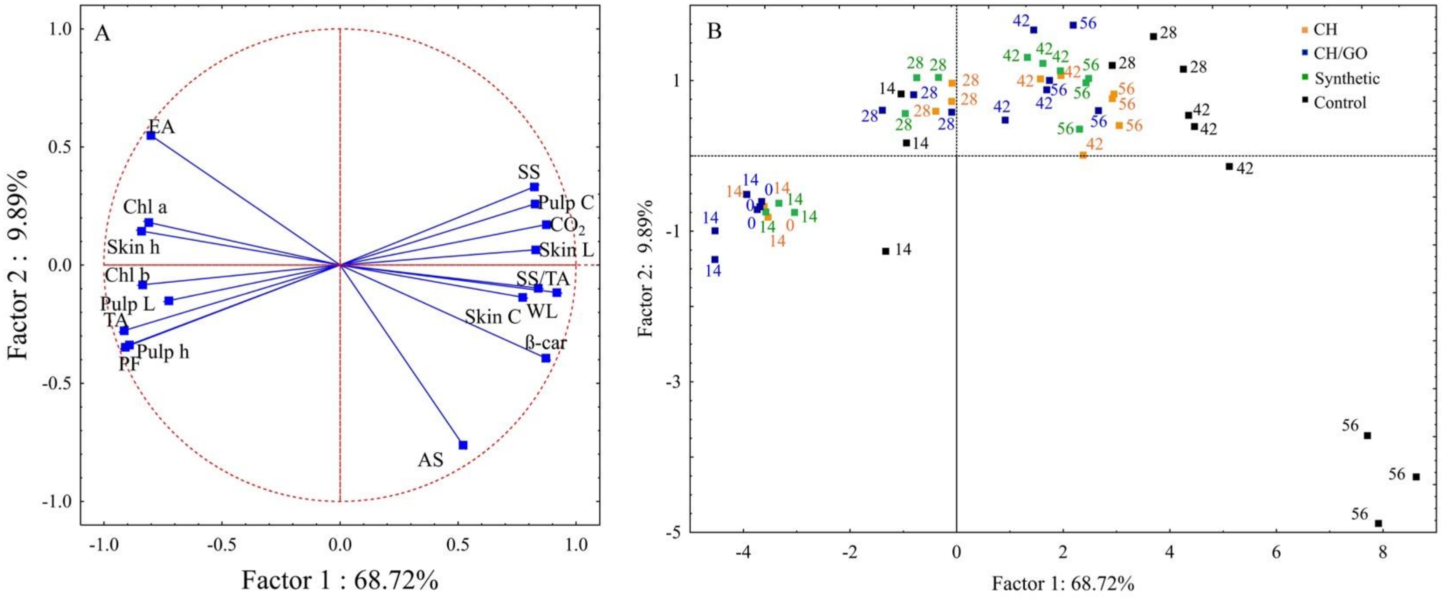

Figure 5. Principal component analysis (PCA): factor loading plot of physicochemical variables of mangoes along PC1 and PC2 (A) and distribution of the samples on score plots (B).

CO_2: respiration rate; WL: weight loss; EA: external appearance; AS: anthracnose severity; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; β-car: beta-carotene; chl: chlorophyll.

Fruit at harvest and bagged at 14 days of storage showed the highest correlation with the negative axes of PC1, indicating unripe fruit. In contrast, unbagged fruit at 28, 42 and 56 days of storage showed the strongest correlation with variables of positive eigenvalues of PC2, which represent a more advanced ripening stage (Fig. 5B). The variable anthracnose severity was better explained by PC2, in its negative axis. Control fruit at 56 days of storage were negatively correlated with PC2, while other samples had positive values in PC2.

Table 3. Eigenvalues and variances of the principal components that represent 17 variables evaluated in mangoes stored in different packaging conditions for 56 days at 12 °C.

Principal component	Eigenvalue	Variance (%)	Cumulative variance (%)
PC1	11.682	68.72	68.72
PC2	1.682	9.89	78.61
PC3	0.802	4.72	83.33
PC4	0.686	4.03	87.36
PC5	0.443	2.60	89.96
PC6	0.388	2.28	92.25
PC7	0.298	1.75	94.00
PC8	0.212	1.25	95.25
PC9	0.201	1.18	96.43
PC10	0.176	1.03	97.46
PC11	0.146	0.86	98.32
PC12	0.088	0.52	98.84
PC13	0.074	0.44	99.28
PC14	0.059	0.35	99.63
PC15	0.034	0.20	99.82
PC16	0.017	0.10	99.92
PC17	0.013	0.08	100.00

Table 4. Loadings in the principal components PC1 and PC2, for the 17 variables evaluated in mangoes stored in different packaging conditions for 56 days at 12 °C.

Variable	PC1	PC2
CO_2	0.874	0.172
WL	0.839	-0.097
EA	-0.802	0.549
AS	0.521	-0.761
Skin L	0.829	0.065
Skin C	0.773	-0.136
Skin h	-0.841	0.144

Pulp L	-0.726	-0.150
Pulp C	0.826	0.259
Pulp h	-0.893	-0.337
PF	-0.911	-0.347
SS	0.823	0.331
TA	-0.915	-0.277
SS/TA	0.918	-0.116
β-car	0.872	-0.393
Chl a	-0.811	0.181
Chl b	-0.837	-0.083

CO_2: respiration rate; WL: weight loss; EA: external appearance; L: lightness; C: chroma; h: hue angle; PF: pulp firmness; SS: soluble solids; TA: titratable acidity; β-car: beta-carotene; chl: chlorophyll.

3.4 DISCUSSION

The main feature of modified atmosphere packaging (MAP) technique is the reduced oxygen concentration coupled with an increase in carbon dioxide levels. This alteration in the atmospheric composition within the package occurs as a result of the metabolic activity (respiration) of the fruit (Cukrov, Brizzolara, & Tonutti, 2019). The MAP delays the ripening of mango fruit and its related physiological, biochemical and organoleptic transformations, such as increased respiration and ethylene production, changes in skin and pulp colors, softening, increased sugar content and reduced acidity (Singh, Singh, Sane, & Nath, 2013).

The addition of graphene oxide into chitosan matrix improved the water barrier properties of the films that make up the bags for mango storage.

The water vapor permeability (WVP) represent the ability of a film to withstand the passage of water molecules across its matrix (Han Lyn, Chin Peng, Ruzniza, & Nur Hanani, 2019; Meindrawan, Suyatma, Wardana, & Pamela, 2018). This parameter is greatly important when designing packaging materials and the lowest value is preferred, once the water vapor exchange plays a role in the reactions that accelerate the metabolism of the fruit thereby affecting the shelf life (Oliveira, Santos, Leite, Aroucha, & Silva, 2018). The bags used for mango storage act as a barrier that prevents the contact between the fruit and the external environment, reducing the gas exchange and consequently reducing the fruit transpiration and respiration, which cause a delay of ripening and a better preservation in the external appearance of the fruit.

Incorporation of graphene oxide reduced WVP of chitosan-based bags, which is in accordance with the previous study by Paiva et al. (2020), reporting that the addition of 0.25% graphene oxide into 2% chitosan-based bags decreased their WVP from 0.33 to 0.27 g mm h^{-1}

Kpa^{-1} m^{-2}. When incorporated to the films, graphene oxide fills up the structural gaps existing in the chitosan chain, chocking the channels and pathways for water transport across the film (Rambabu, Bharath, Banat, Show, & Cocoletzi, 2019).

The strong interaction between water molecules and the hydroxyl groups of chitosan results in swelling and weakening of intermolecular H-bondings (Han, Yan, Chen, & Li, 2011). With the addition of graphene oxide to chitosan matrix, the attraction between water molecules and carbon atoms are generally not strong enough to overcome the strong C−C bonds (Han Lyn et al., 2019), leading to the low water solubility of CH/GO-bags (Table 1).

After 56 days of storage, the in-pack oxygen composition was changed from 21% under normal atmospheric conditions to 14.6, 11.7 and 6.0%, respectively, in the CH-based, CH/GO-based and PE-based bags, while the carbon dioxide levels increased from 0.04% to 6.9, 9.4 and 6.3%, in the respective packages. The benefits of MAP vary on type of cultivar, maturity stage at harvest, initial quality, type and thickness of packaging materials, gaseous composition, temperature of storage and duration of exposure (Pal, Koley, & Singh, 2018). Stressing conditions by gaseous composition below or above the tolerance limits for mangoes can cause a shift from aerobic to anaerobic respiration, which contributes to susceptibility to decay, production of off-flavors and occurrence of physiological disorders (Yahia & Singh, 2009). In this study, we did not observe occurrence of fermentation or other physiological disorders due to the alterations in concentration levels of O_2 or CO_2. Teixeira, Santos, Cunha Júnior and Durigan (2018) studied the conservation of ‘Palmer’ mangoes under controlled atmosphere storage conditions and reported that levels of 5% of O_2 and up to 20% of CO_2 did not cause negative effects to fruit quality.

PE is one of the most used polymers for foods because of its transparency, flexibility, low density, low cost, low toxicity, and high processability (Heo, Choi, & Hong, 2019). However, PE is a poor barrier to gases because of its nonpolar nature (Raj, Jagadish, Srinivas, & Siddaramaiah, 2005), besides being non-biodegradable. Thus, a wide variety of biopolymers, specially polysaccharides like chitosan, have been studied to produce barrier materials, due to their good barrier properties against oxygen and carbon dioxide (Ferreira, Alves, & Coelhoso, 2016).

Unbagged fruit differed from bagged ones for their highest average respiration rate. Silva et al. (2017) reported a reduction in respiration of ‘Palmer’ mangoes under modified atmosphere conditions through chitosan-based edible coatings. MAP treatment can limit the diffusion of CO_2 out of the tissue (Meindrawan et al., 2018). Thus, high internal concentration of CO_2 in fruit leads to a reduction in ethylene biosynthesis and action, and subsequently delays fruit ripening (Botton, Tonutti, & Ruperti, 2019).

Weight loss of mangoes was reduced by use of packages, especially the PE-based one. This effect is related to the nonpolar nature of PE, which makes it a hydrophobic polymer, in contrast to the polar character of chitosan. Moreover, the technology of ethylene gas conversion into carbon dioxide and water was described by the manufacturer of the PE bags evaluated in this study (Freshmama®, Nissan Steel Industry Co., Ltd., Kyoto, Japan).

Even so, the weight loss of fruit stored in biodegradable bags is still considered low when compared to the control. Nunes, Emond, Brecht, Dea, & Proulx (2007) suggested a weight loss between 9 and 7% as a maximum acceptable for ‘Palmer’ mangoes before become unacceptable for sale.

The weight loss is a natural feature of horticultural crops during storage, and it is attributed to respiration, transpiration and other biological changes taking place in the fruit. High weight loss is associated with bad appearance of fruit, which tends to wilt, and thus, lose consumer’s acceptance. Several authors reported a reduction in weight loss of ‘Palmer’ mangoes by using MAP technique for fruit conservation (Rodrigues et al., 2020; Silva et al., 2017; Sousa et al., 2021).

The external appearance of a fruit is one of its main quality attributes, since it plays an important role in the consumer's decision by visual attraction. At 56 days of storage, unbagged fruit showed the lowest external appearance (4.0), resulting in fruit unacceptable for marketing due to the high presence of decay spots. At the same day, fruit stored in CH/GO, PE e CH bags had a very good external appearance. Several factors are associated with changes in the appearance of mangoes after harvest. Among them are the weight loss with consequent wilting and the microbial infection, which results in the early senescence of the fruit.

In mango crop, the anthracnose is the major disease caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Sacc. that has been known to cause the highest losses during storage (Singh et al., 2013). This disease occurs especially in mango-growing regions with high temperature and humidity, and is responsible for limiting productivity and quality by the presence of dark sunken patches at any point on the fruit surface, thus directly affecting mango export to the market (N. B. Lima et al., 2013).

On the last day of storage, there was an impressive indication of 100% disease incidence in unbagged mangoes, with average severity between 1 and 5% (Fig. 1F), in contrast to the absence of disease in bagged fruit. These results are related to the lower oxygen and high carbon dioxide conditions and slow ripening of mangoes kept in MAP, which are inhibiting factors for fungal growth and development in fruit (Jongsri, Wangsomboondee, Rojsitthisak, & Seraypheap, 2016).

Fruit color is a very important trait in determining consumer acceptance of mangoes (Ebrahimi & Rastegar, 2020). Moreover, mango pigments also contribute to fruit nutritional value and are reliable as harvest indexes due to their relationship with fruit maturity stage (Nordey, Joas, Davrieux, Génard, & Léchaudel, 2014). As a climacteric fruit with high perishability, mango is usually harvested at physiologically mature green stage and ripens quickly to achieve desired quality attributes for the fresh market (Evans et al., 2017).

The use of packages delayed color changes in mangoes, as observed by significant changes in all color parameters. The skin of unbagged fruit had the highest lightness and chroma values and the lowest hue angle throughout storage. Mature green ‘Palmer’ mangoes had a matte dark-greenish ground skin color due to the presence of chlorophylls. During ripening, the degradation of chlorophylls concomitant with the steadily increase of carotenoid synthesis results in the change in skin color, which tends to turn light green, and finally vivid yellow (Nunes et al., 2007; Solovchenko, Yahia, & Chen, 2019). Thus, these results suggest a delayed ripening in bagged fruit, as observed in ‘Palmer’ mangoes coated with chitosan (Silva et al., 2017), starch (Rodrigues et al., 2020), hydroxypropyl methylcellulose and beeswax (Sousa et al., 2021).

The pulp color is one of the main indicators of mango ripening, and consequently of fruit shelf life (Nordey, Davrieux, & Léchaudel, 2019). Pulp color changes due to chlorophyll degradation and synthesis of carotenoids, reflecting an intense yellow to orange color (Singh et al., 2013). The most abundant carotenoid of mango is β-carotene, which as quantified in our study to determine changes in fruit ripening.

Lightness and hue angle were lower and chroma was higher in the pulp of unbagged mangoes. These findings represent the natural shift in pulp color, which was more intense in control unbagged fruit, changing from matte light-yellow to vivid yellow-orange tones due to the carotenoid synthesis, eventually becoming darker due to senescence (Nunes et al., 2007).

Pulp carotenoid content increased over storage, mainly in unbagged fruit. Beta-carotene provides several health benefits, as a pro-vitamin A molecule with high antioxidant activity. The biosynthesis and accumulation of some carotenoids are regulated by the expression of the structural carotenoid pathway genes during fruit ripening (Ma et al., 2018). When storing mangoes in MAP conditions, the biosynthetic carotenoid pathway is triggered more slowly, but without compromising nutritional quality of the fruit. A delay in the carotenoid synthesis of

'Palmer' mangoes due to changes in the atmosphere composition was also reported in fruit coated with hydroxypropyl methylcellulose and beeswax (Sousa et al., 2021).

Both pulp chlorophylls decreased throughout storage, as a result of their degradation. However, fruit stored in biodegradable bags maintained higher levels of these pigments until 56 days of storage, compared to unbagged fruit. The reduction in chlorophyll content of fruit is attributed to the activity of the enzyme chlorophyllase, which is up-regulated by ethylene through the *de novo* synthesis of chlorophyllase protein (Mir, Curell, Khan, Whitaker, & Beaudry, 2001). The change in the gas composition provided by the bags modifies the fruit metabolism by inhibiting ethylene synthesis, delaying the degradation of chlorophylls, which in turn is a reasonable ripening indicator in mango flesh (Seifert, Pflanz, & Zude, 2014).

Fruit color is dependent on ethylene, since this hormone triggers the ripening process and the expression of several enzymes involved in both the chlorophyll breakdown and carotenoid biosynthesis (Singh et al., 2013). Conditions of high dioxide carbon and low oxygen concentrations provided by the use of bags promoted a reduction in ethylene synthesis, and consequently in ethylene-dependent pathways, such as the ones responsible for pulp color changes.

The reduction of pulp firmness during ripening is an irreversible process that occurs as a result of cell wall carbohydrate metabolism, enzymatic activity, starch hydrolysis and weight loss (Singh et al., 2013). However, in our study, this reduction on pulp firmness was delayed in response to MAP.

Firmness is one of the main factors that determine the quality of fresh mangoes and their postharvest shelf life. Higher firmness values are recommended, as they can increase the fruit resistance to transport and pathogen infection. The Mango Postharvest Best Management Practices Manual (Brecht, 2017) classifies mangoes under 2 lb (~ 9 N) as overripe, condition observed at 42 days in unbagged fruit and only at 56 days in bagged ones (Fig. 4A). Considering this fruit trait, the MAP used in our study extended the shelf life of ‘Palmer’ by 14 days.

Studies have shown that modification in the atmosphere composition inside the bags downregulates the expression of some genes coding for enzymes that disassemble the middle lamella and cell wall of fruit, such as polygalacturonase, β-galactosidase, β-xylosidase, α-arabinofuranosidase, pectate lyase, endoglucanase and pectinmethylesterase (Cukrov et al., 2019), delaying the loss of firmness in bagged mangoes, which can possibly help explaining the results observed in our study.

Soluble solids content of mangoes gradually increased in all treatments during storage, as an effect of fruit ripening. However, bagged fruit had lower SS content when compared to unbagged ones. During mango ripening, starch is almost completely hydrolyzed into simple sugars, mainly glucose, fructose and sucrose, increasing soluble solids content and providing the sweet taste to the fruit. However, the results suggest that this hydrolyzation process was delayed in bagged fruit. This effect occurs due to the low oxygen concentration around the fruit promoted by MAP, which reduces the starch breakdown into glucose with the inhibition of amylase and maltase activities (Singh et al., 2013).

Titratable acidity of mangoes decreased throughout storage, and this reduction occurred more slowly in bagged fruit. Mangoes are composed of organic acids, mainly citric and malic acid, which concentration decreases after harvest due to their consumption as respiratory substrates (Maldonado et al., 2019). Thus, fruit subjected to conditions that provide them lower respiration rates, like the MAP by the use of packages, tend to delay degradation of organic acids.

Fruit flavor is mainly represented by the balance between the content of sugars and organic acids (Medlicott & Thompson, 1985), quantified through SS/TA ratio. The SS/TA ratio increased over storage in all treatments, more markedly in unbagged fruit. These results corroborate with the delayed increase in SS/TA ratio found in ‘Palmer’ mangoes stored under fruit coating conditions with chitosan (Silva et al., 2017) and hydroxypropyl methylcellulose/beeswax (Sousa et al., 2021).

With the aim of evaluating the effect that packaging conditions and storage time have on the physicochemical traits of mango fruit, the principal component analyses (PCA) statistical approach was chosen to synthesize the great quantity of information obtained from all these parameters (Fig. 5). The principal component 1 clearly separated the physicochemical traits into two groups: on the left, variables present in greater value in mature green fruit, and on the right, the main variables in ripe fruit. Fruit at harvest and bagged at 14 days have shown the highest correlation with the negative axes of PC1, indicating unripe fruit. In contrast, the fruit of the control treatment at 28, 42 and 56 days showed the strongest correlation with variables of positive eigenvalues in PC1, which represent a more advanced ripening stage. Anthracnose severity was the only better explained by PC2, in its negative axis. Unbagged fruits at 56 days of storage were the only samples with negative axis in PC2, due to their higher disease severity in comparison to other treatments. These results ratify what was previously discussed about the delay that packages, whether biodegradable or synthetic, provide for the ripening of mango fruit. Although in our study the synthetic package showed similar benefits on maintaining postharvest mango quality, compared to biodegradable ones, the last ones have the advantage of being eco-friendly.

3.5 CONCLUSIONS

Chitosan and graphene-oxide based biodegradable bags are effective in the conservation of postharvest quality of ‘Palmer’ mangoes during cold storage. The bags delayed fruit ripening, reducing respiration rate, weight loss and disease incidence and severity, maintaining skin and pulp color and firmness, and preserving appearance of fruit.

3.6 REFERENCES[4]

Botton, A., Tonutti, P., & Ruperti, B. (2019). Biology and Biochemistry of Ethylene. In Elhadi M. Yahia (Ed.), *Postharvest Physiology and Biochemistry of Fruits and Vegetables* (pp. 93–112).

Brecht, J. K. (2017). *Mango Postharvest Best Management Practices Manual*. Gainesville.

Corkidi, G., Balderas-Ruíz, K. A., Taboada, B., Serrano-Carreón, L., & Galindo, E. (2006). Assessing mango anthracnose using a new three-dimensional image-analysis technique to quantify lesions on fruit. *Plant Pathology, 55*(2), 250–257. https://doi.org/10.1111/j.1365-3059.2005.01321.x

Costa, J. D. S., Figueiredo Neto, A., Almeida, F. A. C., & Costa, M. S. (2018). Conservation of “Tommy Atkins” mangoes stored under passive modified atmosphere. *Revista Caatinga, 31*(1), 117–125. https://doi.org/10.1590/1983-21252018v31n114rc

Cukrov, D., Brizzolara, S., & Tonutti, P. (2019). Physiological and Biochemical Effects of Controlled and Modified Atmospheres. In E. M. Yahia (Ed.), *Postharvest Physiology and Biochemistry of Fruits and Vegetables* (pp. 425–441). Elsevier Inc.

Ebrahimi, F., & Rastegar, S. (2020). Preservation of mango fruit with guar-based edible coatings enriched with Spirulina platensis and Aloe vera extract during storage at ambient temperature. *Scientia Horticulturae, 265*, 109258. https://doi.org/10.1016/j.scienta.2020.109258

Evans, E. A., Ballen, F. H., & Siddiq, M. (2017). Mango Production, Global Trade, Consumption Trends, and Postharvest Processing and Nutrition. In M. Siddiq, J. K. Brecht, & J. S. Sidhu (Eds.), *Handbook of Mango Fruit: Production, Postharvest Science, Processing Technology and Nutrition* (pp. 1–16).

---

[4] The references were formatted with APA citation style, according to the journal's standards.

FAO. (2019). Food and Agriculture Organization statistical database. Retrieved February 15, 2021, from http://www.fao.org/faostat/en/#data/QC/

Ferreira, A. R. V, Alves, V. D., & Coelhoso, I. M. (2016). Polysaccharide-Based Membranes in Food Packaging Applications. *Membranes*, *6*(2), 22. https://doi.org/10.3390/membranes6020022

Ge, L., Li, X., Zhang, R., Yang, T., Ye, X., Li, D., & Mu, C. (2015). Development and characterization of dialdehyde xanthan gum crosslinked gelatin based edible films incorporated with amino-functionalized montmorillonite. *Food Hydrocolloids*, *51*, 129–135. https://doi.org/https://doi.org/10.1016/j.foodhyd.2015.04.029

Goh, P. S., Ismail, A. F., & Ng, B. C. (2014). Directional alignment of carbon nanotubes in polymer matrices: Contemporary approaches and future advances. *Composites: Part A*, *56*, 103–126. https://doi.org/http://dx.doi.org/10.1016/j.compositesa.2013.10.001

Han, D., Yan, L., Chen, W., & Li, W. (2011). Preparation of chitosan/graphene oxide composite film with enhanced mechanical strength in the wet state. *Carbohydrate Polymers*, *83*(2), 653–658. https://doi.org/10.1016/j.carbpol.2010.08.038

Han Lyn, F., Chin Peng, T., Ruzniza, M. Z., & Nur Hanani, Z. A. (2019). Effect of oxidation degrees of graphene oxide (GO) on the structure and physical properties of chitosan/GO composite films. *Food Packaging and Shelf Life*, *21*, 100373. https://doi.org/10.1016/j.fpsl.2019.100373

Heo, J., Choi, M., & Hong, J. (2019). Facile Surface Modification of Polyethylene Film via Spray-Assisted Layer-by-Layer Self-Assembly of Graphene Oxide for Oxygen Barrier Properties. *Scientific Reports*, *9*(1), 1–7. https://doi.org/10.1038/s41598-019-39285-0

Jongsri, P., Wangsomboondee, T., Rojsitthisak, P., & Seraypheap, K. (2016). Effect of molecular weights of chitosan coating on postharvest quality and physicochemical characteristics of mango fruit. *LWT*, *73*, 28–36. https://doi.org/10.1016/J.LWT.2016.05.038

Lima, A. B., Silva, S. M., Rocha, A., Nascimento, L. C., & Ramalho, F. S. (2012). Conservação pós-colheita de manga “Tommy Atkins” orgânica sob recobrimentos bio-orgânicos. *Revista Brasileira de Fruticultura*, *34*(3), 704–710. https://doi.org/10.1590/s0100-29452012000300008

Lima, N. B., Batista, M. V. A., Morais Jr, M. A., Barbosa, M. A. G., Michereff, S. J., Hyde, K. D., & Câmara, M. P. S. (2013). Five Colletotrichum species are responsible for mango anthracnose in northeastern Brazil. *Fungal Diversity*, *61*, 75–88. https://doi.org/10.1007/s13225-013-0237-6

Ma, X., Zheng, B., Ma, Y., Xu, W., Wu, H., & Wang, S. (2018). Carotenoid accumulation and expression of carotenoid biosynthesis genes in mango flesh during fruit development and ripening. *Scientia Horticulturae, 237*, 201–206. https://doi.org/https://doi.org/10.1016/j.scienta.2018.04.009

Maldonado, M. E., Yahia, E. M., Bedoya, R., Landázuri, P., Loango, N., Aguillón, J., … Guerrero, J. C. (2019). Chemical composition of mango (Mangifera indica L.) fruit: nutritional and phytochemical compounds. *Frontiers in Plant Science*, *10*, 1073. https://doi.org/10.3389/fpls.2019.01073

Marsh, K., & Bugusu, B. (2007). Food packaging - Roles, materials, and environmental issues. *Journal of Food Science*, *72*(3), 39–55. https://doi.org/10.1111/j.1750-3841.2007.00301.x

Medlicott, A. P., & Thompson, A. K. (1985). Analysis of sugars and organic acids in ripening mango fruits (Mangifera indica L. var. Keitt) by high performance liquid chromatography. *Journal of Science and Food Agriculture*, *36*, 561–566. https://doi.org/https://doi.org/10.1002/jsfa.2740360707

Meindrawan, B., Suyatma, N. E., Wardana, A. A., & Pamela, V. Y. (2018). Nanocomposite coating based on carrageenan and ZnO nanoparticles to maintain the storage quality of mango. *Food Packaging and Shelf Life, 18*, 140–146. https://doi.org/10.1016/j.fpsl.2018.10.006

Mir, N. A., Curell, E., Khan, N., Whitaker, M., & Beaudry, R. M. (2001). Harvest maturity, storage temperature, and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of “Redchief Delicious” apples. *Journal of the American Society for Horticultural Science*, *126*(5), 618–624. https://doi.org/https://doi.org/10.21273/JASHS.126.5.618

Monteiro, M. K. S., Oliveira, V. R. L., Santos, F. K. G., Leite, R. H. L., Aroucha, E. M. M., Silva, R. R., & Silva, K. N. O. (2017). Analysis of Water Barrier, Mechanical and Thermal Properties of Nanocoposites Based on Cassava Starch and Natural Clay or Modified by Anionic Exchange. *Materials Research*, *20*(Suppl. 2), 69–76. https://doi.org/https://doi.org/10.1590/1980-5373-mr-2016-1087

Mwaurah, P. W., Kumar, S., Kumar, N., Panghal, A., Attkan, A. K., Singh, V. K., & Garg, M. K. (2020). Physicochemical characteristics, bioactive compounds and industrial applications of mango kernel and its products: A review. *Comprehensive Reviews in Food Science and Food Safety*, *19*(5), 1–26. https://doi.org/10.1111/1541-4337.12598

Nagata, M., & Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Society of Food Science and Technology*

(Nippon Shokuhin Kogyo Gakkaishi), *39*(102), 925–928. https://doi.org/10.3136/nskkk1962.39.925

Nordey, T., Davrieux, F., & Léchaudel, M. (2019). Predictions of fruit shelf life and quality after ripening: Are quality traits measured at harvest reliable indicators? *Postharvest Biology and Technology*, *153*, 52–60. https://doi.org/10.1016/j.postharvbio.2019.03.011

Nordey, T., Joas, J., Davrieux, F., Génard, M., & Léchaudel, M. (2014). Non-destructive prediction of color and pigment contents in mango peel. *Scientia Horticulturae*, *171*, 37–44. https://doi.org/10.1016/j.scienta.2014.01.025

Ntsoane, M. L., Zude-Sasse, M., Mahajan, P., & Sivakumar, D. (2019). Quality assesment and postharvest technology of mango: A review of its current status and future perspectives. *Scientia Horticulturae*, *249*, 77–85. https://doi.org/10.1016/J.SCIENTA.2019.01.033

Nunes, M. C. N., Emond, J. P., Brecht, J. K., Dea, S., & Proulx, E. (2007). Quality curves for mango fruit (cv. Tommy Atkins and Palmer) at chilling and nonchilling temperatures. *Journal of Food Quality*, *30*(1), 104–120. https://doi.org/https://doi.org/10.1111/j.1745-4557.2007.00109.x

Oliveira, V. R. L., Santos, F. K. G., Leite, R. H. L., Aroucha, E. M. M., & Silva, K. N. O. (2018). Use of biopolymeric coating hydrophobized with beeswax in post-harvest conservation of guavas. *Food Chemistry*, *259*, 55–64. https://doi.org/10.1016/j.foodchem.2018.03.101

Paiva, C. A., Vilvert, J. C., Menezes, F. L. G., Leite, R. H. L., Santos, F. K. G., Medeiros, J. F., & Aroucha, E. M. M. (2020). Extended shelf-life of melons using chitosan and graphene oxide based biodegradable bags. *Journal of Food Processing and Preservation*, *44*, e14871. https://doi.org/10.1111/jfpp.14871

Pal, R. K., Koley, T. K., & Singh, S. (2018). Strategies for low-temperature storage of vegetables. In B. Singh, S. Singh, & T. K. Koley (Eds.), *Advances in Postharvest Technologies of Vegetable Crops* (pp. 229–248).

Raj, B., Jagadish, R. S., Srinivas, P., & Siddaramaiah. (2005). Crosslinking of low-density polyethylene by diisocyanates for superior barrier properties. *Journal of Applied Polymer Science*, *96*(4), 1193–1199. https://doi.org/10.1002/app.21355

Rambabu, K., Bharath, G., Banat, F., Show, P. L., & Cocoletzi, H. H. (2019). Mango leaf extract incorporated chitosan antioxidant film for active food packaging. *International Journal of Biological Macromolecules*, *126*, 1234–1243. https://doi.org/10.1016/j.ijbiomac.2018.12.196

Rastegar, S., Hassanzadeh Khankahdani, H., & Rahimzadeh, M. (2019). Effectiveness of alginate coating on antioxidant enzymes and biochemical changes during storage of mango fruit. *Journal of Food Biochemistry*, *43*(11), 1–10. https://doi.org/10.1111/jfbc.12990

Ren, L., Yan, X., Zhou, J., Tong, J., & Su, X. (2017). Influence of chitosan concentration on mechanical and barrier properties of corn starch/chitosan films. *International Journal of Biological Macromolecules*, *105*, 1636–1643. https://doi.org/10.1016/j.ijbiomac.2017.02.008

Rodrigues, A. A. M., Santos, L. F., Costa, R. R., Félix, D. T., Nascimento, J. H. B., & Lima, M. A. C. (2020). Characterization of starch from different non-traditional sources and its application as coating in ‘ Palmer ’ mango fruit. *Ciencia e Agrotecnologia*, *44*, e011220. https://doi.org/http://dx.doi.org/10.1590/1413-7054202044011220

Rosalie, R., Léchaudel, M., Dhuique-Mayer, C., Dufossé, L., & Joas, J. (2018). Antioxidant and enzymatic responses to oxidative stress induced by cold temperature storage and ripening in mango (Mangifera indica L. cv. ‘Cogshall’) in relation to carotenoid content. *Journal of Plant Physiology*, *224–225*, 75–85. https://doi.org/10.1016/j.jplph.2018.03.011

Seifert, B., Pflanz, M., & Zude, M. (2014). Spectral shift as advanced index for fruit chlorophyll breakdown. *Food and Bioprocess Technology*, *7*, 2050–2059. https://doi.org/10.1007/s11947-013-1218-1

Silva, G. M. C., Silva, W. B., Medeiros, D. B., Salvador, A. R., Cordeiro, M. H. M., Silva, N. M., … Mizobutsi, G. P. (2017). The chitosan affects severely the carbon metabolism in mango (Mangifera indica L. cv. Palmer) fruit during storage. *Food Chemistry*, *237*, 372–378. https://doi.org/10.1016/j.foodchem.2017.05.123

Singh, Z., Singh, R. K., Sane, V. A., & Nath, P. (2013). Mango - Postharvest Biology and Biotechnology. *Critical Reviews in Plant Sciences*, *32*(4), 217–236. https://doi.org/10.1080/07352689.2012.743399

Solovchenko, A., Yahia, E. M., & Chen, C. (2019). Pigments. In E. Yahia (Ed.), *Postharvest Physiology and Biochemistry of Fruits and Vegetables* (pp. 225–253).

Sousa, F. F., Pinsetta Junior, J. S., Oliveira, K. T. E. F., Rodrigues, E. C. N., Andrade, J. P., & Mattiuz, B.-H. (2021). Conservation of ‘Palmer’ mango with an edible coating of hydroxypropyl methylcellulose and beeswax. *Food Chemistry*, *346*, 128925.

Teixeira, G. H. A., Santos, L. O., Cunha Júnior, L. C., & Durigan, J. F. (2018). Effect of carbon dioxide (CO2) and oxygen (O2) levels on quality of ‘Palmer’ mangoes under controlled atmosphere storage. *Journal of Food Science and Technology*, *55*(1), 145–156. https://doi.org/10.1007/s13197-017-2873-4

Tharanathan, R. N., Yashoda, H. M., & Prabha, T. N. (2006). Mango (Mangifera indica L.), "the king of fruits" - An overview. *Food Reviews International*, *22*(2), 95–123. https://doi.org/10.1080/87559120600574493

Yahia, E M, & Singh, S. P. (2009). Tropical fruits. In E M Yahia (Ed.), *Modified and Controlled Atmospheres for the Storage, Transportation, and Packaging of Horticultural Commodities* (pp. 397–344).

4 FINAL CONSIDERATIONS

Modified atmosphere is one of the best-known postharvest technologies due to its effectiveness on maintaining fruit and vegetables quality. However, synthetic petroleum-based packages traditionally used for this purpose represent an environmental problem due to the long degradation time of these packages. As an eco-friendly alternative, chitosan-films were prepared and tested as biodegradable bags for conservation of mangoes. The incorporation of graphene oxide improved water vapor barrier and mechanical properties of chitosan-based films, confirming the potential of these nanoparticles for this application.

Both chitosan and graphene-oxide based biodegradable bags and synthetic petroleum-based were effective on maintaining postharvest quality of 'Tommy Atkins' and 'Palmer' mangoes during cold storage. The bags delayed fruit ripening by reducing respiration rate, weight loss and softening, delaying changes in color, soluble solids content and titratable acidity and preserving visual appearance of fruit.

Based on the results obtained in this study, biodegradable bags are a potential new eco-friendly technology to improve postharvest management of mango in order to maintain the quality of the fruit intended to distant markets. New studies are recommended to improve the properties of the films, making them viable for commercial use.