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CENTRO DE ENERGIA NUCLEAR NA AGRICULTURA

KÉLIN SCHWARZ

Bioactive compounds and physical and chemical characteristics of mini tomatoes: influence of postharvest treatments

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and to my brother, Gleisson.

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"The task is not so much to see what no one yet has seen, but to think what nobody yet has thought about that which everybody sees".

(A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo mundo vê).

Arthur Schopenhauer

"I do not know what I may appear to the world, but to myself I seem to have been only like a boy playing on the sea-shore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me".

(Tenho a impressão de ter sido uma criança brincando à beira-mar, divertindo-me em descobrir uma pedrinha mais lisa ou uma concha mais bonita que as outras, enquanto o imenso oceano da verdade continua misterioso diante de meus olhos).

Isaac Newton

ABSTRACT

SCHWARZ, K. **Bioactive compounds and physical and chemical characteristics of mini tomatoes: influence of postharvest treatments**. 2016. 128 p. Thesis (PhD) – Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2016.

Tomatoes are among the most cultivated and used vegetables in the world. They are very succeptible to post harvest losses due to high perishability, therefore the use of postharvest treatments may contribute to conservation of this fruit, however the treatments might affect significantly physico-chemical, sensory and nutritional characteristics of tomatoes. Given the perishability of tomato and the economic importance of small tomato fruits, the purpose of the present study was to determine the effect of gamma radiation, carnauba coating and 1-MCP treatments on tomato fruit quality during storage. The study may be divided into two parts. In the first, mini tomatoes cv. Sweet Grape were harvested at breaker stage, divided into 4 grous and treated with gamma radiation (0.6 kGy), carnauba coating (1 L 1000 kg^{-1}) and 1-MCP (500 nL L^{-1}) and then stored at 25±2°C for 30 days with a control group of tomatoes. In the seconnd part, tomatoes harvested at light-red stage were submitted to the same treatments and storage period. Every 6 days tomatoes were evaluated for color modifications, fruit firmness, souble and total pectin (only for light-red tomatoes), mass loss, titratable acidity (TA), soluble solids (SS), SS/TA ratio, carotenoids profile, formation of lycopene isomers, total phenolic compounds, ascorbic acid and antioxidant capacity. For tomatoes harvested at breaker stage and submitted to the treatments the results showed mass loss was delaying mainly by carnauba wax, and to a lesser extend by 1-MCP. Fruit firmness were better retained for 1-MCP treated fruits and carnauba treatment showed a transient effect in preserving fruit firmness. SS/TA of tomatoes treated with gamma radiation and carnauba presented no differences from control values, and were lower with the application of 1-MCP. Color was negatively affected by 1-MCP and earlier changed (6^{th} day) when gamma radiation was applied. In relation to bioactive compounds of tomatoes harvest at breaker stage, results indicated gamma radiation and 1-MCP decreased the final content of lycopene and produced more (*Z*)-isomers of lycopene. Gamma radiation also induced a decreased in β-carotene and an increased in phenolic compounds by the end of storage period. 1-MCP treatment promoted a slow down increase in ascorbic acid content during storage. Antioxidant capacity of the hydrophilic fraction was not dramatically affected by treatments and the lipophilic fraction was lower, especially for 1-MCP fruits. In addition, contents of β-carotene, lycopene, (*Z*)-isomers of lycopene, ascorbic acid and antioxidant capacity increased during the period of storage while contents of lutein and phenolic compounds tended to decrease. Regarding tomatoes harvest at light-red stage, the most effective treatments for delaying fruit firmness and mass loss was carnauba and 1-MCP, while gamma radiation was the treatment with higher mass loss and the less fruit firmness, which could be associated with the higher solubilization of pectins promoted by radiation treatment. Color (L^* and Hue) was mainly affected by 1-MCP treatment which delayed color development, however, by the end of storage, the values were not different from the other treatments. SS/TA ratio was lower for fruits treated with 1-MCP and TA was not so dramatically affected by treatments. Furthermore, mini tomatoes harvested at light-red stage, demonstrated irradiation induced changes in the final content of lycopene, increasing it, and formed less (13*Z*)-lycopene, while 1-MCP and carnauba coating slow down the increase in lycopene and slown down the decrease of ascorbic acid and phenolic compounds. Antioxidant capacity of lipophilic fraction was not affected by treatments and the hydrophilic fraction was lower for irradiated fruits only on day 0 as well as phenolic compounds. In the other days, no differences among treatments were observed for hydrophilic antioxidant capacity. Considering the results, the

best combination of SS and TA and fruit preservation for mini tomatoes harvest at breaker stage was promoted by carnauba coating, which seems to be the treatment that causes fewer changes in bioactive compounds of breaker tomatoes. However, when mini tomatoes were harvested at light-red stage, SS/TA ratio and color were better and, to preserve the quality of these fruits, besides carnauba coating, 1-MCP also could be indicated.

Keywords: *Solanum lycopersicum*. Gamma radiation. Carnaúba coating. 1-MCP. Fruit quality. Carotenoids. Lycopene isomers. Antioxidant capacity.

RESUMO

SCHWARZ, K. **Compostos bioativos e características físico-químicas de mini tomates: influência de tratamentos pós-colheita**. 2016. 128 p. Tese (Doutorado) – Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2016.

Os tomates estão dentre as hortaliças mais cultivadas e consumidas no mundo, porém os frutos do tomateiro são muito sucetíveis a perdas após a colheita devido a alta perecibilidade, por isso, o uso de tratamentos pós-colheita pode contribuir para a conservação dos frutos. Entretanto, o uso destes tratamentos pode afetar significativamente características físico-químicas, sensorias e nutricionais dos tomates. Dada a perecibilidade e a importância econômica dos mini tomates, o objetivo deste estudo foi determinar o efeito da radiação gama, revestimento à base de cera de carnaúba e 1-metilciclopropeno (1-MCP) na qualidade do tomate durante o armazenamento. O estudo pode ser dividido em duas partes. Na primeira, mini tomates cv. Sweet grape colhidos no estádio de maturação breaker foram divididos em quarto grupos e tratados com radiação gama (0.6 kGy), cera de carnaúba (1 L 1000 kg^{-1}) e 1-MCP (500 nL L^{-1}) e então armazenados a 25±2°C por 30 dias, juntamente com um grupo de frutos controle. Na segunda parte, tomates colhidos no estádio de maturação vermelho-claro foram submetidos aos mesmos tratamentos e período de armazenamento. A cada seis dias os frutos foram avaliados para modificações nas características físico-químicas: coloração, firmeza, pectina solúvel e total (somente para os frutos vermelho-claro), perda de massa, acidez titulável (AT), sólidos solúveis (SS), relação SS/AT; e compostos bioativos: perfil de carotenoides, formação de isômeros de licopeno, compostos fenólicos totais, ácido ascórbico, e capacidade antioxidante. Para os tomates colhidos no estádio breaker, os resultados mostraram que a perda de massa foi retardada, principalmente pelo uso de cera de carnauba e de 1-MCP, este último em menor proporção. A firmeza dos frutos foi melhor retida para os frutos tratados com 1-MCP e, a cobertura com carnauba mostrou um efeito transitório em preservar a firmeza dos frutos. A relação SS/AT dos frutos irradiados e cobertos com carnauba não apresentaram diferenças dos valores do controle, mas foram menores com a aplicação de 1-MCP. A coloração foi negativamente afetada pelo uso do 1-MCP e precocemente modificada (no 6° dia) quando a radiação gama foi aplicada. Em relação aos compostos bioativos destes mesmos frutos, os resultados indicaram que a radiação gama e o 1-MCP diminuiram o conteúdo final de licopeno e produziram mais (*Z*)-isômeros de licopeno nos frutos. A radiação gama também induziu a diminuição de β-caroteno e o aumento dos compostos fenólicos no final do período de armazenamento. A aplicação de 1-MCP promoveu desaceleração no aumento do conteúdo de ácido ascórbico durante o armazenamento. A capacidade antioxidante da fração hidrofílica não foi muito afetada pelos tratamentos e, a fração lipofílica foi menor para os frutos tratados com 1-MCP. Além disso, os teores de β-caroteno, licopeno, (*Z*)-isômeros de licopeno, ácido ascórbico e capacidade antioxidante aumentaram durante o armazenamento, enquanto que, o teor de luteína e compostos fenólicos tenderam a diminuir. A respeito dos tomatates colhidos no estádio de maturação vermelho claro, os tratamentos mais efetivos para retardas a perda demassa e a firmeza foram a cobertura de carnauba e 1-MCP, enquanto o tratamento com radiação gama apresentou a maior perda de massa e de firmeza de frutos, o que pode ser associado a maior solubilização de pectinas promovida pela radiação gama. A cor (L^* e Hue) foi principalmente afetada pelo tratamento com 1-MCP, que retardou o desenvolvimento da mesma, porém no final do período de armazenamento, os valores não diferiram dos outros tratamentos. A relação SS/AT foi menor para os frutos tratados com 1-MCP e a AT não foi muito afetada pelos tratamentos. Além disso, mini tomates colhidos no estádio vermelho-claro demosntraram que a irradiação induziu modificações no teor final de licopeno, aumentando-o e houve menor formação de

(13*Z*)-licopeno, enquanto que 1-MCP e carnauba desaceleraram o aumento no teor de licopeno e desaceleraram a diminuição de ácido ascórbico e compostos fenólicos. A capacidade antioxidante da fração lipofílica não foi afetada pelos tratamentos e a fração hidrofílica foi menor para os tomates irradiados somente no dia 0, assim como para compostos fenólicos. Nos demais dias não houve diferenças entre os tratamentos. Considerando os resultados, a melhor combinação de SS/AT e preservação de mini tomates colhidos no estádio breaker foi promovida pela cobertura de carnaúba, a qual pareceu ser o tratamento que causou menores mudanças nos compostos bioativos dos tomates beaker. Entretanto, quando os mini tomates são colhidos no estádio vermelho-claro, SS/AT e coloração são melhor desenvolvidas e, para preservar a qualidade destes frutos, além da cera de carnaúba, o 1-MCP também pode ser indicado.

Palavras-chave: *Solanum lycopersicum*. Radiação gama. Revestimento de carnaúba. 1-MCP. Qualidade do fruto. Carotenoides. Isômeros de licopeno. Capacidade antioxidante.

CONTENTS

1 INTRODUCTION

People's daily routines have been featured by changes on feeding habit and lifestyle. The search for a healthier life has increased the demand for food that are fresh, healthy and rich in substances regarded as benefit to health (KRIS-ETHERTON et al., 2002; DORAIS et al., 2008; ZHANG et al., 2009). However, for consumers even more demanding, it is not enough that food may be rich nutritionally, it is necessary quality, both in visual aspect and sensory. In addition, consumers are worried with the source from these foods, which treatment or chemical agents were used and whether it brings benefits or harm to health and to the environment.

On healthy food matter, is inserted vegetables, consumed *in natura* or processed. Among these, it is highlighted tomato fruit (*Solanum lycopersicum*), one of the oleraceous more popular worldwide, whether by the production volume and socioeconomic value or even by its versatility in consuming, turning out to be present in feeding of great part of the population during whole year and contribute significantly for human nutrition by its vitamin, mineral and phytochemical content (SIMONNE et al., 2006; TOOR; SAVAGE, 2006).

Being fresh fruit or processed products, tomato fruit provides a great variety of nutrients and benefits to health (GIOVANUCCI, 1999; MOCO et al., 2006), thus, it is considered a functional food (ALSHATWI et al., 2010). Studies suggest that tomato fruit daily ingestion or its derivatives reduce risks of certain cancer types (NGUYEN; SCHWARTZ, 1999; GIOVANNUCCI, 1999; GIOVANUCCI et al., 2002) and cardiovascular diseases (WILLCOX; CATIGNANI; LAZARUS, 2003). These benefits are attributed, mainly to bioactive compounds and antioxidants from fruits, as ascorbic acid, phenolic compounds, tocopherol and carotenoids, particularly lycopene (MARTINEZ-VALVERDE et al., 2002; GEORGE et al., 2004), pigment responsible for the red color on tomato fruit.

Solely after potato, tomato fruit is the second vegetable more produced worldwide, reaching around 163 million ton in 2013/14. China, United States, India and Turkey are the greatest producers, while Brazil is the ninth in production volume, about 4 million ton produced per year (FAOSTAT, 2014).

Among several types of tomatoes produced, it is highlighted mini tomato, which is gaining space in Brazilian market, with 15 to 20% annual growing (JUNQUEIRA et al., 2011). Mini tomatoes fruit are products with high aggregated value and may have prices from 20 to 30% higher than traditional tomatoes. Probably due to these fruits presented great cooking versatility and popularity (ZHAO et al., 2010), the peel and pulp present

dark red color, being small (10 to 20g) (JUNQUEIRA et al., 2011) and characterized by high concentration of sugar and low acidity level (PICHA, 1986), which tastes differently. While traditional varieties of tomatoes fruit present soluble solid content around 4 to 6 °Brix, some varieties of mini tomato (cherry and grape) may easily reach soluble solid content above 8 °Brix (JUNQUEIRA et al., 2011). In addition, mini tomatoes fruit may present higher contents of carotenoids and other antioxidants than traditional tomatoes (RAFFO et al., 2002).

Being a climacteric fruit, tomato keeps ripening postharvest (GHORBANI; POOZESH; KHORRAMDEL, 2012). During this process, many modifications occur in these fruits, as color changing, texture, flavor and also chemical changes (JAVANMARDI; KUBOTA, 2006). Based on these modifications of firmness, color and flavor that consumers judge the quality of fruits at first moment (BROOKS et al., 2008; DAVILA-AVIÑA et al., 2011). Then, it is interesting that fruit quality must be kept postharvest in order to please the consumer and also extend the commercialization period.

Nevertheless, tomato is a fruit extremely perishable, which, often results in great loss postharvest (ZAPATA et al., 2008), whether quantitative or qualitative, besides limiting storage time of fruits. Particularly in countries under development and tropical weather, where the weather conditions (high temperatures, humidity) (BAILÉN et al., 2006), transportation conditions and fruits storage are not ideal, fruit deterioration is faster. According to data collection from several studies related to postharvest loss in vegetables, Kitinoja and Kader (2015) reported that loss in tomato fruit from Brazil may vary from 15 to 50%. For this reason the use of techniques which extend conservation, as well as the period of fruit marketing are desirable (CARON et al., 2013).

Thus, different methods of postharvest conservation have been tested in order to expand shelf life of fruits and preserve quality, such as cooling, controlled and modified atmosphere, ethylene antagonists, irradiation**,** edible coatings and so on.

Among postharvest techniques of conservation, stand out the use of gamma radiation, application of 1-methylcyclopropene (1-MCP) and edible waxes. Each of these techniques present different mechanism of action, but all of them are recognized by delaying the maturation process of tomatoes fruit (CHIUMARELLI; FERREIRA, 2006; DAVILA-AVIÑA et al., 2014; LARRIGAUDIÈRE et al., 1991; ASSI; HUBER; BRECHT, 1997; CASTRICINI et al., 2004; KUMAR et al., 2014; KRAMMES et al., 2003; GUILLÉN et al., 2005; HURR; HUBER; LEE, 2005; PUSHPALATHA et al., 2006).

The use of gamma radiation has been presenting satisfactory results in relation to the shelf life and delay of tomato ripening (LARRIGAUDIÈRE et al., 1991; ASSI et al., 1997;

CASTRICINI et al., 2004; KUMAR et al., 2014). Economic and technological viability as well as safety of food irradiation was widely proved and several researches have showed the correct use of this technique in food do not present risk to health (WHO, 1981).

Edible coatings also delay the deterioration and preserve the quality of fruits (DAVILA-AVIÑA et al., 2011), because the coat is able to modify the atmosphere around the fruit, acting as a barrier to oxygen, carbon dioxide and water vapor**,** thus, decreasing the respiration rate and water loss in fruits (MARTÍNEZ-ROMERO et al., 2006). Different materials may be used as coat, the most commons are natural proteins**,** lipids or polysaccharides (BAI et al., 2003). In Brazil, carnauba wax have been tested and used as edible coating in fruits and vegetable. This wax is obtained by a Brazilian palm tree and sold diluted in different concentrations. It is not toxic and besides diminishes the postharvest loss, also brings shine to the fruits (HAGENMEIER; BAKER, 1994).

The application of 1-MCP is also an alternative for the delay of fruit maturation. This synthetic compound reduce the action of ethylene, because is able to block the receptors of ethylene in fruits, preventing hormone action (WATKINS, 2002). As a result, the modifications in maturation are delayed and, consequently postharvest life expanded (BLANKENSHIP; DOLE, 2003). The use of this compound is considered safe for human, because quickly diffuses from the plant tissue after the treatment (BLANKENSHIP; DOLE, 2003; WATKINS, 2006).

However, these treatments may change fruit composition, leading to physical, chemical and nutritional changes. Doses, forms and application time of these techniques and/or products have been widely researched, but few studies are available about the effect of postharvest in bioactive compounds. Many publications draw attention in order to the development of more studies, to identify the real modification in bioactive compound from fruits.

Given the perishability and the economic importance of mini tomatoes fruit and, regarding this overview, the study was conducted with the objective of evaluate the effects of postharvest treatments (gamma radiation, carnauba coating and 1-MCP) in physicochemical characteristics and bioactive compounds of mini tomatoes during storage.

Thus, this thesis has originated five chapters. The first is an introduction about the study. In the second and third chapter are presented the results of application of postharvest treatments (radiation, 1-MCP and carnauba coating) for physical and chemical characteristics (Chapter 2) and bioactive compounds (Chapter 3) of mini tomatoes harvested at breaker stage of maturation (classification of USDA, 1991). The conduction of the experiment that

originated these two chapters was based on previous data collected in literature which verified that, mainly treatments with 1-MCP and irradiation, showed better results when applied in tomato fruit harvested in early stages of maturation (mature-green and breaker). Therefore, this was starting point of the research and in order to compare, these three mentioned treatments were applied in fruits harvested in breaker stage of maturation. From results of these two chapters, was found the following situations: the soluble solids content, as well as the ratio of soluble solids/titratable acidity did not reach the desirable values for these mini tomatoes fruits, due to the harvest in breaker stage, followed by the application of treatments. For mini tomatoes, the expected quality by the consumer is different from those expected for conventional tomatoes, is expected a sweetish fruit. Therefore, treatments were repeated and applied in fruits harvested in light-red stage (USDA, 1991). It caused higher soluble solid content, besides different results for the characteristics fruit firmness, loss of fresh mass, color, titratable acidity which is presented on chapter 4, as well different results for carotenoids profile, phenolic compounds, ascorbic acid and antioxidant capacity, described on chapter 5.

1.1 Introdução

O cotidiano das pessoas tem sido marcado pelas mudanças no hábito alimentar e no estilo de vida. A busca por uma vida mais saudável aumentou a demanda por alimentos frescos, saudáveis e ricos em substâncias consideradas benéficas à saúde (KRIS-ETHERTON et al., 2002; DORAIS et al., 2008; ZHANG et al., 2009). Entretanto, para os consumidores cada vez mais exigentes, não basta que o alimento seja rico nutricionalmente, é preciso que tenha qualidade, tanto no aspecto visual quanto no sensorial. Além disso, os consumidores estão preocupados com a procedência dos alimentos, quais tratamentos ou agentes químicos foram empregados e se isso traz benefícios ou malefícios à sua saúde e ao ambiente.

No contexto dos alimentos saudáveis inserem-se as hortaliças, consumidas na forma *in natura* ou processada. Dentre as hortaliças destaca-se o tomate (*Solanum lycopersicum*), uma das olerícolas mais difundidas no mundo, seja pelo volume de produção e valor socioeconômico ou por sua versatilidade de consumo, fazendo com que esteja presente na alimentação de grande parte da população durante o ano inteiro e contribua significativamente para a nutrição humana por seu conteúdo de vitaminas, minerais e fitoquímicos (SIMONNE et al., 2006; TOOR; SAVAGE, 2006). Seja na forma de fruto fresco ou produtos processados, o tomate provém uma grande variedade de nutrientes e benefícios à saúde (GIOVANUCCI,

1999; MOCO et al., 2006), por isso tem sido considerado um alimentos funcional (ALSHATWI et al., 2010). Estudos sugeriram que a ingestão diária de tomate ou seus produtos derivados reduzem riscos para determinados tipos de câncer (NGUYEN; SCHWARTZ, 1999; GIOVANNUCCI, 1999; GIOVANUCCI et al., 2002) e doenças cardiovasculares (WILLCOX; CATIGNANI; LAZARUS, 2003). Estes benefícios são atribuídos, principalmente aos compostos antioxidantes dos frutos, como ácido ascórbico, compostos fenólicos, tocoferol e carotenoides, em especial o licopeno (MARTINEZ-VALVERDE et al., 2002; GEORGE et al., 2004), pigmento responsável pela coloração vermelha característica dos tomates.

Atrás apenas da batata, o tomate é a segunda hortaliça mais produzida no mundo, alcançando aproximadamente 163 milhões de toneladas na safra 2013/2014 em todo o mundo. China, Estados Unidos, Índia e Turquia são os maiores produtores, enquanto o Brasil é o nono em volume de produção, com aproximadamente 4 milhões de toneladas produzidas por ano (FAOSTAT, 2014).

Dentre os muitos tipos de tomate produzidos merece destaque o mini tomate, que vem ganhando espaço no mercado brasileiro com crescimento de 15 a 20% no cultivo anualmente (JUNQUEIRA et al., 2011). Os mini tomates são produtos com alto valor agregado e podem ter preços de mercado 20 a 30% superiores aos dos tomates tradicionais. Isso porque estes frutos apresentam grande versatilidade culinária e popularidade (ZHAO et al., 2010), a casca e a polpa apresentam coloração vermelha escura, os frutos são pequenos (10 a 20 g) (JUNQUEIRA et al., 2011) e são caracterizados pela alta concentração de açúcares e baixo teor de acidez (PICHA, 1986), o que proporciona sabor diferenciado ao fruto. Enquanto variedades tradicionais de tomate apresentam teor de sólidos solúveis em torno de 4 até 6 °Brix, algumas variedades de mini tomate (cereja e uva) podem facilmente atingir teor de sólidos solúveis acima de 8 °Brix (JUNQUEIRA et al., 2011). Ainda, os mini tomates, podem apresentar maior teor de carotenoides e outros antioxidantes do que os tomates tradicionais (RAFFO et al., 2002).

O tomate é um fruto climatérico e continua a amadurecer após a colheita (GHORBANI; POOZESH; KHORRAMDEL, 2012). Durante este processo, várias modificações ocorrem nos frutos, como a transformação da coloração, da textura, do sabor e também modificações químicas (JAVANMARDI; KUBOTA, 2006). É com base nessas modificações de firmeza, cor e sabor que os consumidores julgam a qualidade dos frutos em um primeiro momento (BROOKS et al., 2008; DAVILA-AVIÑA et al., 2011).

Neste sentido, é interessante que a qualidade do fruto seja mantida após a colheita de forma a agradar o consumidor e também ampliar o período de comercialização.

Porém, o tomate é um fruto de alta perecibilidade o que pode resultar em grandes perdas pós-colheita (ZAPATA et al., 2008), sejam elas quantitativas ou qualitativas, além de limitar o tempo de armazenamento dos frutos. Particularmente em países em desenvolvimento e de clima tropical, onde as condições climáticas (altas temperaturas, umidade) (BAILÉN et al., 2006) e as condições de transporte e armazenamento dos frutos não são ideais, a deterioração dos frutos é acelerada. De acordo com o levantamento de dados a partir de vários estudos relacionados a perdas pós-colheita em vegetais, Kitinoja e Kader (2015) reportaram que as perdas de tomate no Brasil podem variar de 15 até 50%. Por essa razão o uso de técnicas que ampliem a conservação, bem como o período de comercialização dos frutos é desejável (CARON et al., 2013). Neste sentido, diferentes métodos de preservação pós-colheita tem sido testados de forma a estender a vida de prateleira dos frutos e preservar a qualidade, tais como refrigeração, atmosfera modificada e controlada, antagonistas de etileno, irradiação, películas comestíveis e assim por diante.

Dentre as técnicas de preservação pós-colheita, merecem destaque o uso de radiação gama, a aplicação de 1-metilciclopropeno (1-MCP) e ceras comestíveis. Cada um destes tratamentos apresenta mecanismos diferentes de ação, mas todos são reconhecidos por atrasarem o amadurecimento em tomates (CHIUMARELLI; FERREIRA, 2006; DAVILA-AVIÑA et al., 2014; LARRIGAUDIÈRE et al., 1991; CASTRICINI et al., 2004; KUMAR et al., 2014; GUILLÉN et al., 2005; 2007; PUSHPALATHA et al., 2006).

O emprego da radiação gama têm apresentado resultados satisfatórios em relação à extensão de vida de prateleira e retardo do amadurecimento em tomates (LARRIGAUDIÈRE et al., 1991; ASSI; HUBER; BRECHT, 1997; CASTRICINI et al., 2004; KUMAR et al., 2014). A viabilidade econômica, tecnológica e de segurança da irradiação de alimentos já foi extensamente comprovada e diversas pesquisas mostraram que o uso correto da irradiação em alimentos não apresenta risco para a saúde (WHO, 1981).

Ceras comestíveis também retardam a deterioração e preservam a qualidade dos frutos (DAVILA-AVIÑA et al., 2011), pois a película é capaz de modificar a atmosfera ao redor do fruto, atuando como uma barreira para o oxigênio, dióxido de carbono e vapor de água, diminuendo assim a taxa respiratória e a perda de água nos frutos (MARTÍNEZ-ROMERO et al., 2006). Diferentes materiais podem ser utilizados como películas, os mais comuns são proteínas naturais, lipídios ou polissacarídeos (BAI et al., 2003). No Brasil, a cera de carnaúba tem sido testada e utilizada como cobertura comestível em frutas e hortaliças.

Esta cera é obtida de uma palmeira brasileira e comercializada diluída em diferentes concentrações. Não é tóxica e além de diminuir as perdas pós-colheita, também confere brilho aos frutos (HAGENMEIER; BAKER, 1994).

A aplicação de 1-MCP também é uma alternativa para o atraso do amadurecimento em frutos. Este composto reduz a ação do etileno, pois é capaz de bloquear os receptores de etileno nos frutos, impedindo a ação do hormônio (WATKINS, 2002). Como resultado, as modificações do amadurecimento são atrasadas e, consequentemente a vida pós-colheita estendida (BLANKENSHIP; DOLE, 2003). O uso deste composto é considerado seguro para o ser humano, pois rapidamente se difunde dos tecidos da planta após o tratamento (BLANKENSHIP; DOLE, 2003; WATKINS, 2006).

Entretanto, estes tratamentos podem alterar a composição dos frutos, levando a transformações físicas, químicas e nutricionais. Doses, formas e tempos de aplicação destas técnicas e/ou produtos têm sido largamente estudados, porém poucos são os estudos disponíveis sobre o efeito dos tratamentos pós-colheita nos compostos bioativos. Várias publicações, inclusive, chamam a atenção para que mais estudos neste sentido sejam desenvolvidos, a fim de identificar as reais modificações nos compostos bioativos dos frutos.

Dada a perecibilidade e a importância econômica dos mini tomates e, considerando este panorama, o estudo foi conduzido com o objetivo de avaliar os efeitos dos tratamentos pós-colheita (radiação gama, revestimento comestível de carnaúba e 1-MCP) nas características físico-químicas e compostos bioativos de mini tomates durante o armazenamento.

Assim, a tese originou cinco capítulos. O primeiro é uma introdução sobre o estudo. No segundo e terceiro capítulos estão apresentados os resultados da aplicação dos tratamentos pós-colheita (irradiação, 1-MCP e cera de carnaúba) para as características físico-químicas (capítulo 2) e compostos bioativos (capítulo 3) de mini tomates colhidos no estádio de maturação breaker (classificação da USDA, 1991). A condução do experimento que originou estes dois capítulos fundamentou-se em um prévio levantamento de dados na literatura que verificou que, principalmente os tratamentos com 1-MCP e irradiação apresentaram melhores resultados quando aplicados em tomates colhidos nos estádios iniciais de maturação (mature-green e breaker). Sendo assim, este foi o ponto de partida da pesquisa e, com o intuito de comparar, os três tratamentos mencionados foram aplicados em frutos colhidos no estádio de maturação breaker. A partir dos resultados destes dois capítulos, deparamo-nos com a seguinte situação: o teor de sólidos solúveis, bem como a relação sólidos solúveis acidez titulável não atingiram os valores desejáveis para estes mini tomates em função da colheita no

estádio breaker seguida da aplicação dos tratamentos. Para os mini tomates, a qualidade esperada pelo consumidor é diferente daquela esperada para tomates convencionais, pois espera-se um fruto mais adocicado. Neste sentido, os tratamentos foram repetidos e aplicados em frutos colhidos no estádio vermelho-claro (USDA, 1991). Isso ocasionou teores mais elevados de sólidos solúveis, além de resultados diferentes para as características de firmeza, perda de massa fresca, coloração e acidez titulável que estão apresentadas no capítulo 4, bem como diferentes resultados para o perfil de carotenoides, compostos fenólicos totais, ácido ascórbico e capacidade antioxidante, descritos no capítulo 5.

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2 EFFECT OF GAMMA RADIATION, CARNAUBA COATING AND 1-MCP ON POSTHARVEST QUALITY OF MINI TOMATOES HARVEST AT BREAKER STAGE

Abstract

This study aimed to evaluate the changes in physical and chemical characteristics of mini tomatoes as a function of postharvest treatments during storage. Mini tomatoes cv. Sweet Grape harvested at breaker stage were treated with gamma radiation (0.6 kGy), carnauba coating (1 L 1000 kg^{-1}) and 1-MCP (500 nL L^{-1}) and then stored at 25±2°C for 30 days with a control group tomatoes. Color modifications, fruit firmness, mass loss, titratable acidity, soluble solids and SS/TA ratio were evaluated. Mass loss was delaying mainly by carnauba wax, and to a lesser extend by 1-MCP. Fruit firmness were better retained for 1-MCP treated fruits and carnauba treatment showed a transient effect in preserving fruit firmness. SS/TA of tomatoes treated with gamma radiation and carnauba presented no differences from control values, but were lower with the application of 1-MCP. Color was negatively affected by 1-MCP and earlier changed (6^{th} day) when gamma radiation was applied. The best combination of SS/TA ratio and fruit preservation for mini tomatoes harvest at breaker stage was promoted by carnauba coating.

Keywords: *Solanum lycopersicum*, soluble solids, titratable acidity, color, fruit firmness, mass loss

2.1 Introduction

Tomato (*Solanum lycopersicum*) is one of the most cultivated vegetable in the world. Second only to potato, tomato reached an annual production nearly to 163 million tons of fresh fruit worldwide in 2013 (FAOSTAT, 2014). China, USA and India are the leading producers, while Brazil is the ninth largest producer with about 4 million tons annually (FAOSTAT, 2014). In addition to its economic importance, tomato is a versatile vegetable that is consumed either fresh or as processed products (TOOR; SAVAGE, 2005), by a large population throughout the year. Its consumption has been associated to health benefits, because of the content of antioxidants such as lycopene, β-carotene, flavonoids, vitamin C and many essential nutrients (BEUTNER et al., 2001).

Among several types of tomatoes produced, mini tomato stands out as a product with high aggregated value, whose market price could be 20-30% higher than traditional tomatoes (JUNQUEIRA; PEETZ; ONODA, 2011). Great culinary versatility, dark red color of peel and pulp, small size of the fruits (10-20 g) and the high concentration of sugars are probably the reasons (PICHA, 1986; JUNQUEIRA; PEETZ; ONODA, 2011). Whereas the common

varieties of tomato has soluble solids content between 4 and 6 °Brix, varieties of mini tomatoes (cherry and grape) has concentrations of sugars enough to reach between 9 and 12 °Brix. (JUNQUEIRA; PEETZ; ONODA, 2011). These characteristics considerably affect the fruit flavor (BECKLES, 2012), which is more appreciated by consumers. Furthermore, mini tomatoes may present higher levels of antioxidants than traditional tomatoes (RAFFO et al., 2002).

For consumers, fresh tomatoes quality are judged by their firmness, color and taste, which are related to ripeness and shelf life (BROOKS; EL-HANA; GHALY, 2008; DAVILA-AVIÑA et al., 2011). As a climacteric fruit, tomato continues to ripen after harvest (GHORBANI; POOZESH; KHORRAMDEL, 2012). During this process, several modifications occurred in tomatoes such as changes in color, texture, flavor, and chemical compositions (JAVANMARDI; KUBOTA, 2006). Due to high perishability, tomato fruits has a relatively short postharvest life and great losses (ZAPATA et al., 2008) either quantitative or qualitative may happen. Several factors are associated to losses and may limit the storage life of fruits including transpiration, postharvest diseases, increased ripening and senescence (ALI et al., 2010). Particularly in tropical countries where the temperatures are higher, an important factor is the increase in respiration, which results in faster fruit ripening and deterioration of fruit quality (BAILÉN et al., 2006). In Brazil, for example, tomato losses may reach 15% to 50% (KITINOJA; KADER, 2015). The fast changes related to ripening can be a limitation for marketing (GUILLEN et al., 2006), wherefore tomato ripening has been extensively studied with the objective to extend tomato consistency, color and shelf life (JAVANMARDI; KUBOTA, 2006).

In order to control qualitative and quantitative losses in tomatoes, postharvest technologies that action extending shelf life have been developed and tested. Among these technologies stands out gamma radiation, application of 1-methylcyclopropene (1-MCP) and edible coatings. Each treatment has a different mechanism of action, but all are recognized by delaying ripening in tomatoes (CHIUMARELLI; FERREIRA, 2006; DAVILA-AVIÑA et al., 2011; LARRIGAUDIÈRE et al, 1991; ASSI; HUBER; BRECHT, 1997; CASTRICINI et al., 2004; KUMAR et al., 2014; KRAMMES et al., 2003; GUILLÉN et al., 2005; HURR; HUBER; LEE, 2005; PUSHPALATHA et al., 2006).

Gamma radiation has been employed as a postharvest food preservation process in several countries. The utilization of this technique had great results in relation to the extension of shelf life and delay ripening in tomatoes (LARRIGAUDIÈRE et al, 1991; ASSI et al., 1997; CASTRICINI et al., 2004; KUMAR et al., 2014). Safety and efficiency of food

irradiation has been approved by several authorities as World Health Organization (WHO), Food and Agriculture Organization (FAO) and International Atomic Energy Agency (IAEA) (WHO, 1981). 1-MCP treatment is also an alternative for delaying senescence of fruit and has a high potential for commercial use since it reduces the action of ethylene in plant tissues, resulting in longer preservation of postharvest (WATKINS, 2008; HUBER, 2008; GUILLÉN et al., 2005; HURR; HUBER; LEE, 2005). Another technique that has been used in postharvest fruit is the application of edible coatings commonly based on natural proteins, lipids or polysaccharides (BAI et al., 2003). Coatings act generating a modified atmosphere by creating a barrier against water loss, oxygen and carbon dioxide, reducing respiration and oxidation reaction rates (MARTÍNEZ-ROMERO et al., 2006). These effects contribute to minimize the fresh mass loss and the number of discarded fruit due to mechanical injury and diseases (CHIUMARELLI; FERREIRA, 2006; DAVILA-AVIÑA et al., 2014).

However, these postharvest treatments might affect significantly physico-chemical and sensory characteristics of foods. Given the perishability of tomato and the economic importance of small tomato fruits, the purpose of the present study was to determine the effect of gamma radiation, carnauba coating and 1-MCP treatments on tomato fruit quality during storage.

2.2 Material and Methods

2.2.1 Plant material

Mini tomatoes (*Solanum lycopersicum*) cv. Sweet Grape (Sakata Seed Sudamerica) harvest at the breaker stage of ripening according to the USDA standard tomato color classification chart (USDA, 1991) were obtained from greenhouses in Crop Science Department of University of São Paulo (USP) in Piracicaba, SP, Brazil (22º42’30”S, 47º38’01”W). The fruit were visually selected for uniformity in size, color, absence of physical defects and rots, and transported to the laboratory in Piracicaba, SP. Before treatments application, fruit were washed with chlorinated water (200 ppm) for 2 min, and air-dried at room temperature.

2.2.2 Post harvest treatments

Mini tomatoes were divided into four groups of 5 kg each for the following treatments: 1. control; 2. gamma radiation; 3. carnauba coating and, 4. 1-MCP. The treatments were performed within 24h after harvest and the analysis started at the same time for all treatments.

For gamma radiation treatment tomatoes were transported to Nuclear and Energy Research Institute (IPEN) in São Paulo, SP after having been left at room temperature (25±2°C) overnight. Samples were irradiated in a Compact Multiporpuse Irradiator (^{60}Co, C-188 model, MDS Nordion Canada) at a dose of 0.6 kGy. The dosage was established taking into account previous studies that suggested 0.6 kGy is within a range considered as effective to delay fruit ripening in tomatoes (ABREU; SOARES; JESUS, 1997; CASTRICINI et al., 2004; FABBRI et al., 2011; AKTER; KHAN, 2012; KUMAR et al., 2014). Dosimetric studies were performed using a gammachrome YR dosimeter to monitor the dose and estimate the dose rate (3.21 kGy h^{-1}). After irradiation, fruits were transported back to the laboratory in Piracicaba, SP.

The application of 1-MCP was performed in the Laboratory of Physiology and Biochemistry Postharvest of "Luiz de Queiroz" College of Agriculture (ESALQ/USP) in Piracicaba, SP. 1-MCP gas was prepared from SmartFresh (Agrofresh, Philadelphia) commercial powder (active ingredient 0.14%) at concentration of 500 nL L^{-1}. Predetermined amount of Smartfresh® were placed in flasks with lids and 5 mL of distilled water were added, flasks were shaken until complete dissolution. Then flasks were opened inside hermetic chambers containing the tomatoes. Fruit were treated for 12 h at room temperature (25±2°C). 1-MCP concentration is in accordance with recommendations for tomatoes of SmartFresh® and previous studies (GUILLEN et al., 2007; GUILLEN et al., 2006; CANTWELL et al., 2009).

Commercial carnauba coating Megh Wax ECF-124 (composed of carnauba wax emulsion, anionic surfactant, preservative and water) was provided by Megh Indústria e Comércio Ltda (SP, Brazil). Carnauba coating was manually applied using brushes with the original concentration according to manufacturer's recommendations (1 L 1000 kg^{-1}) and tomatoes were air-dried at room temperature overnight. Previous studies support carnauba coating as an alternative to maintain postharvest quality in tomatoes (CHIUMARELLI; FERREIRA, 2006; DAVILA-AVIÑA et al., 2011). The procedure was realized in the Laboratory of Human Nutrition and Bromatology, in Piracicaba, SP.

Control group received no treatment and was maintained at room temperature until the other treatments were performed (within 24 hours after harvest).

Subsequently to treatments, tomato samples were packed on 300 g capacity commercial packages (polyethylene terephthalate, PET) commonly used for tomatoes, except for gamma radiation treated tomatoes that were package before treatment, and stored at room temperature (25±2°C) for 30 days. During storage, fruits samples of each group were taken on

days 0, 6, 12, 18, 24 and 30 after postharvest treatments to analyze fruit firmness, color, soluble solids and titratable acidity.

To analyze mass loss, tomato fruits from each treatment were separated in different packages, in order to asses de same samples during storage.

2.2.3 Mass loss

Mass loss was determined by calculating the difference between the initial mass of fresh fruits and the mass at the time of each assessment, measured by semi-analytical scales. The results were reported as mass loss percentage. Four replications with ten fruits were used per treatment.

2.2.4 Fruit firmness

In order to assess firmness, four replications with five fruits were sampled per treatment per day of assessment. Firmness was determined by the flattening method proposed by Calbo and Nery (1995), with fruits being evaluated over a 30-day period, at six-day intervals. In an horizontal flattener, fruit receive pressure from a test point of 0.902 kg. In the test point basis, a small acrylic plate horizontally acts directly on the surface of the fruit, always at the same point previously marked in the equatorial region, where it remain for 15 seconds. The direct pressure on the fruit promotes the formation of a contact surface with ellipsoidal shape. By a digital caliper the smaller (a) and larger diameter (b) of the ellipsoid delineated were measured and the surface area was calculated by the expression A = a x b x 0.7854. The firmness was then determined by dividing the test point and flat area (A). The results of this relationship were expressed in N m^{-2}.

2.2.5 Color

External color was determined from 16 fruit per treatment each day of assessment using a Minolta colorimeter model CR-400 (Minolta Co., Japan). Three-color measurements were taken on each tomato, ensuring that a color measurement was taken on the top, half and bottom of each fruit. The values were obtained on a CIELAB scale (L*, a*, b*); (L) lightness (0 = black and 100 = white), a^* ranging from green (a-) to red (a+), b^* ranging from blue (b-) to yellow (b+). Hue angle (°Hue) and chroma (C) values were calculated by the equations: °Hue = arctg b*/a*; C = $[(a^*)2 + (b^*)2\ ]^{1/2}$.

2.2.6 Soluble solids and titratable acidity

Ten tomatoes from each treatment were ground in a blender in triplicate (n=3) and the grounded pulp was used to determine the soluble solids (SS) concentration and titratable acidity (TA). Total Soluble Solid (SS) content of tomato fruits was determined by using an Abbe refractrometer (Gehaka, Brazil) by placing a drop of filtered pulp solution on its prism. The TSS was obtained from direct reading of the refractrometer and temperature correction was calculated as described by Rangana (1979). Results were expressed in percentage.

Titratable acidity (TA) was determined by potentiometric titration with 0.1 mol L^{-1} NaOH up to pH 8.1, using 10 g of diluted pulp in 100 mL distilled water (AOAC, 2000). The results were expressed in percentage of citric acid in the pulp. The ratio between SS and TA was also calculated.

2.2.7 Statistical analysis

Statistical analysis were performed using SAS software version 9.0 (SAS Institute, Cary, NC, USA). The data were submitted to the Shapiro-Wilk and Box-Cox tests to verify the normality and homogeneity of variance among the treatments. Then analysis of variance (ANOVA) was carried out by the F test ($P<0.05$ and $P<0.01$) in order to study the factors - treatments and periods of storage - as well as the interaction between them. According to the significance, the means were compared by the Tukey test ($P<0.05$). When appropriate the means of the quantitative data were submitted to regression analysis ($P<0.05$). The values were recorded as means ± standard deviations.

2.3 Results and Discussion

The mass loss in tomato fruits increased linearly and gradually during the storage period for all treatments as shown in Figure 1a. Increasing in mass loss is a normal process since fruits are living tissues and continue to respire and transpire during storage (RAMASWAMY, 2014). The main mechanism of mass loss from fresh fruit is by vapor pressure at different locations (YAMAN; BAYOINDIRLI, 2002), although respiration also causes a weight reduction (PAN; BHOWMILK, 1992). The magnitude of these losses, however, varied according to the treatment (Figure 1a). The most effective treatment for delaying the mass loss was carnauba coating. Whereas control, 1-MCP and gamma radiation treated fruits lost about 7.5% of mass by the end of storage, carnauba coated fruits lost only 4.58% by the end of the 30 days. This mass loss reduction probably occurred due to the coating act as a barrier against O_2, CO_2, moisture and movement of solutes, promoting a

reduction in respiration, water loss and oxidation reaction rates (MARTÍNEZ-ROMERO et al., 2006). Similarly, Zhuang and Huang (2003), Chiumarelli and Ferreira (2006) and Davila-Aviña et al. (2011) reported that wax coating widely contributed to the reduction of the mass loss in tomatoes.

1-MCP treatment showed a moderate effect in reducing mass loss in tomato fruits compared to carnauba coating. Although tomatoes treated with 1-MCP had similar mass loss to control and gamma radiation fruits by the end of storage ($P>0.05$), on the 12^{th}, 18^{th} and 24^{th} days of storage, fruits with 1-MCP application were the second in reduction of mass loss and differed from control. On the 6^{th} day of storage, mass loss of 1-MCP treated fruits did not differ significantly from mass loss of carnauba treated tomatoes. Guillen et al. (2007) reported similar effects for tomatoes treated with 1-MCP (0.5 μL L^{-1}); in comparison to untreated tomatoes, those treated with 1-MCP had low weight losses. They attributed this effect to the low respiration rate observed in 1-MCP treated tomatoes. Furthermore, gamma radiation treatment did not influenced mass loss in tomatoes. On the contrary, Adam et al. (2014) showed gamma radiation at the doses 0.25, 0.5 and 1 kGy reduced mass loss in tomatoes (conventional size) harvest at mature green and storage under refrigeration.

Texture is one of the major aspects that defines the quality of fruit and influences consumer acceptability (GONZALEZ-AGUILAR et al., 2008). In the present study, fruit firmness was higher for all treatments on day 0 and then declined continuously during the storage period, presenting second-degree polynomial performance (Figure 1b). This softening process is normal during ripening and occurred due to deterioration of the cell structure, cell wall composition and intracellular materials (SEYMOUR et al., 1993). The process involves the hydrolysis of pectin and starch by the action of wall hydrolases, such as pectinesterase and polygalacturonase, which increase their activities during ripening (YAMAN; BAYOINDIRLI, 2002). A typical softening process was observed in control and gamma radiation tomatoes that reduction 42.7 and 39.1% from day 0 to the 30^{th} day of storage. Although the firmness reduction was lower in gamma radiation tomatoes than in control, there were no differences between treatments ($P>0.05$), indicating gamma radiation did not affect fruit firmness. In contrast, the application of 1-MCP was effective on delaying firmness loss, with the most pronounced effect in comparison to other treatments during all the storage period. Further, fruit treated with carnauba coating exhibited a transient effect in fruit firmness due to present higher firmness than control (but lower than 1-MCP) on the 6^{th}, 12^{th} and 18^{th} days of storage.

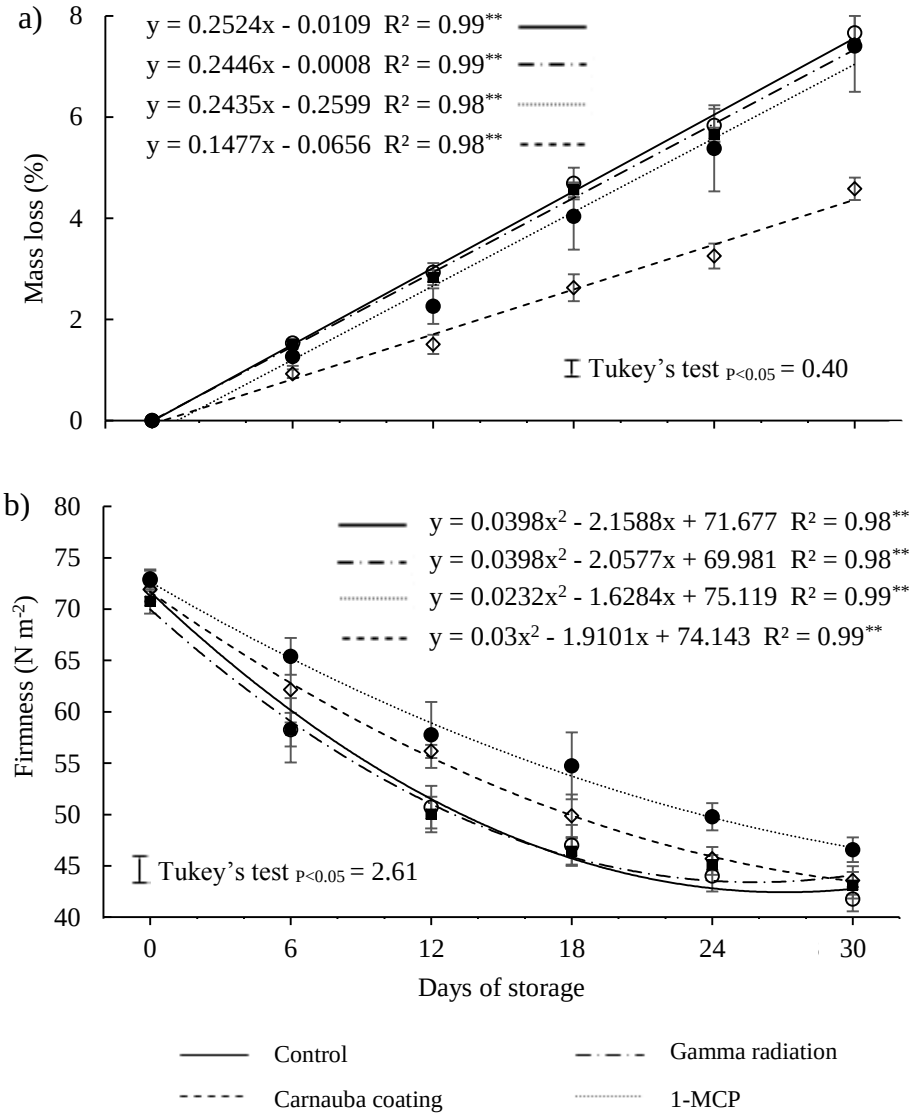

Figure 1 – Mass loss (%) (a) and fruit firmness (N m^{-2}) (b) of mini tomatoes treated with gamma radiation, 1-MCP and carnauba coating during storage. Vertical bar indicate least significant difference by Tukey's test ($P<0.05$) among treatments. Each observation is mean ± Standard Deviation (n=4)

During ripening evolution, one of the most ethylene-sensitive processes is the conversion of insoluble pectin in soluble pectin by pectinolytic enzymes, which promotes fruit softening (LELIEVRE et al., 1997). Thus, probably by inhibit ethylene action, 1-MCP treatment reduced the activity of pectinolytic enzymes decreasing the loss of firmness in tomatoes. In agreement with our results, Guillen et al. (2007), Zhang et al. (2009) and Guillen et al. (2006) reported higher firmness for tomatoes treated with 1-MCP in comparison to control. Delaying in fruit firmness has also been reported for tomatoes treated with edible coatings (ALI et al., 2010; CHIUMARELLI; FERREIRA, 2006) and low doses of gamma

radiation (0.25 and 0.5 kGy) maintained under refrigerated storage (CASTRICINI et al., 2004). In edible coatings, low levels of O_2 and CO_2 promoted by the coating barrier might limit the activity of pectinolytic enzymes, with the reduction in respiration rates allow retention of the firmness during storage (SALUNKHE et al., 1991). In addition, the barrier decrease the water vapor transmission rate, which prevents firmness reduction by preservation the cell turgor (PEREZ-GAGO; GONZALEZ-AGUILAR; OLIVAS, 2010). However, climacteric fruits submitted to gamma radiation may respond either a delay in ripening, as reported by Castricini et al. (2004) or an advance as observed by Assi, Huber and Brecht (1997) and Akter and Khan (2012) with doses below and above 1 kGy. These facts may occurred due to the temporary decrease in cellular functions caused by gamma radiation, delaying ripening or on the contrary, as gamma radiation response, ethylene synthesis is stimulated (LARRIGAUDIÈRE et al., 1991). Regarding fruit firmness, in the present study, none of these effects was observed in tomatoes treated with gamma radiation.

During ripening, mini tomatoes changed from green to red color. Figure 2 shows the effects of postharvest treatments and storage time on the color attributes (L*, °Hue and Chroma) of tomato fruits stored at room temperature (25±2°C), and Figure 3 shows the color aspect of fruits on days 0, 6 and 30 of storage. With respect to color change, the lightness (L*) gradually decreased during storage for all treatments (Figure 2a). The lowest decrease in lightness was observed in 1-MCP tomatoes, whose L^* values were significantly higher ($P<0.05$) than other treatments from the 6^{th} day by the end of storage. Significant ($P<0.05$) differences among other treatments were only observed on the 12^{th} day of storage when gamma radiation treated fruits showed lower L^* values than carnauba coated fruits, but they did not differ from control. In the other days, no differences among control, carnauba coating and gamma radiation fruits were observed.

Croma values increased from day 0 until the 12^{th} day of storage for control, gamma radiation and carnauba tomatoes and from day 0 until the 18^{th} day of storage for 1-MCP fruits, remaining constant thereafter (Figure 2b). Fruit treated with 1-MCP showed chroma values significantly higher ($P<0.05$) than other treatments in most days of storage, exception on the 0 and 12^{th} days of storage, when there were no differences among treatments. The increase in chroma value reflected increasing intensity of color vivacity in tomatoes, while the decrease in hue angle represents the change from green to red color.

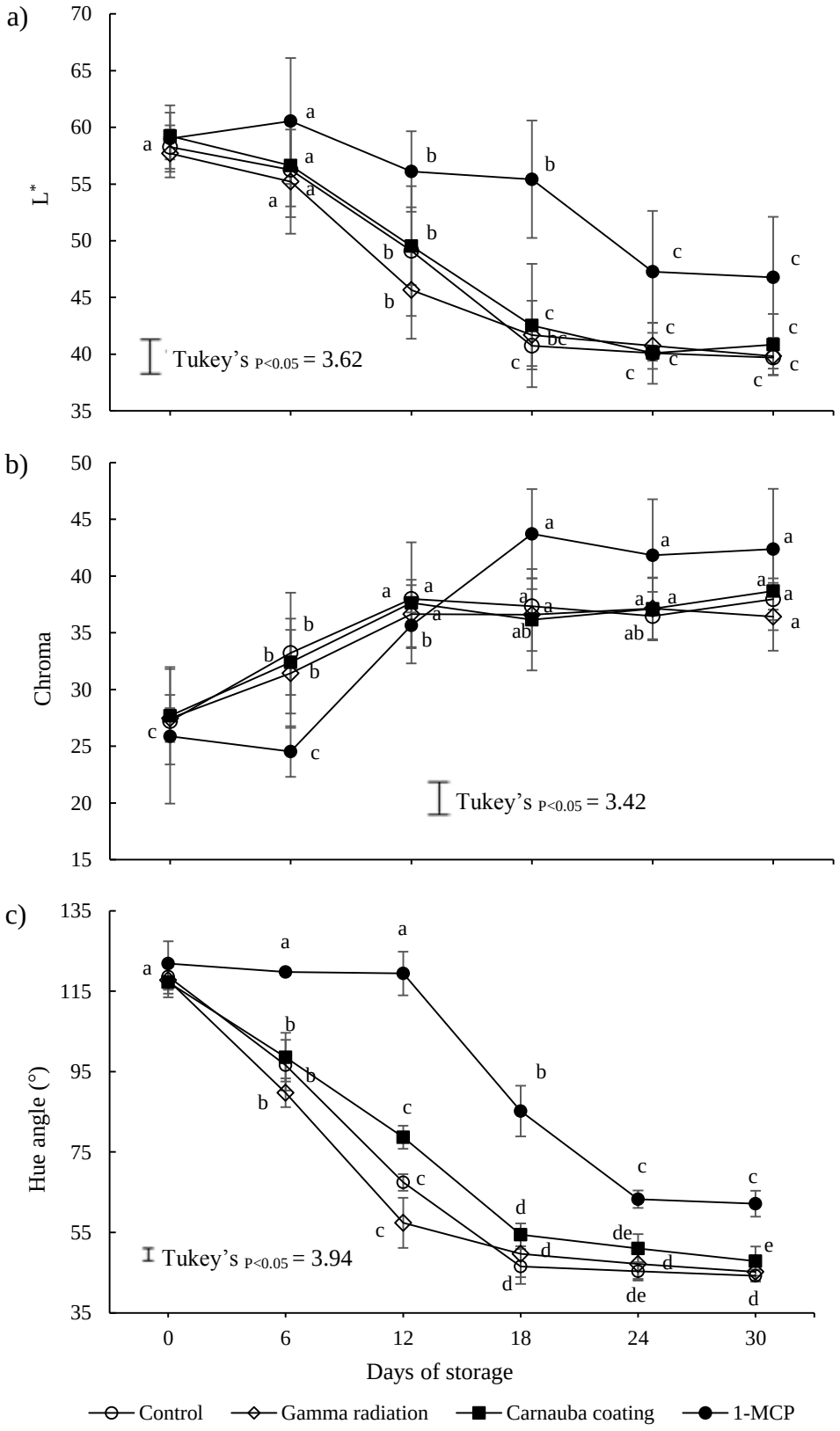

Figure 2 – Lightness (L^*) (a), Croma (b) and Hue angle (°) (c) of mini tomatoes treated with gamma radiation, 1-MCP and carnauba coating during storage. Different letters indicate significant differences among days of storage by Tukey's test ($P<0.05$). Vertical bars indicate least significant difference by Tukey's test ($P<0.05$) among treatments. Each observation is mean ± Standard Deviation (n=16).

Hue angle of tomato fruits declined during storage for all treatments (Figure 2c). However, the hue angle decline was significantly suppressed by 1-MCP treatment in comparison to other treatments. Tomatoes treated with 1-MCP reached 62.1° by the end of storage, while tomatoes treated with gamma radiation and carnauba or control fruits reached approximately 45° on the same day. During storage, 1-MCP treated fruits presented higher values for hue angle on all days, except in day 0 when 1-MCP fruits did not differ from control.

Loss of green peel color is due to chlorophyll molecule breakdown by the chlorophyllase enzyme, whose activity is related to ethylene production during fruit ripening (TUCKER, 1993). As 1-MCP blocks the ethylene action, a delaying in normal ripening process was observed, resulting in green tomatoes for longer periods. These results are consistent with those found for L^* values, once 1-MCP treated tomatoes had the highest values, indicating lighter color. Green color retention in tomatoes treated with 1-MCP has also been reported by Guillen et al. (2007), Ilic et al. (2013) and Zhang et al. (2009).

Interestingly, gamma radiation treatment promoted lower hue angles on the 6^{th} and 12^{th} days of storage differing from other treatments ($P<0.05$). This result suggests radiation treatment stimulated changes in peel color of breaker tomatoes first than other treatments or control. In agreement with the finding, Lee, McGlasson and Edwards (1968) described that tomatoes harvested more immature and irradiated (in this case with 400 krad) colored 2 or 3 days earlier than control tomatoes, which was not observed for tomatoes in more advanced mature stage. Pimentel and Walder (2004) and Ramli and Yusof (1992) also observed this phenomenon of earlier change color in irradiated papaya. For vegetables, but particularly for tomatoes, color is an important criterion of quality and consumer acceptability (AKED, 2000). Thus, if the earlier color change is not accompanied by loss of firmness as observed in this study, probably the commercialization of these tomatoes is facilitated, once they have better color.

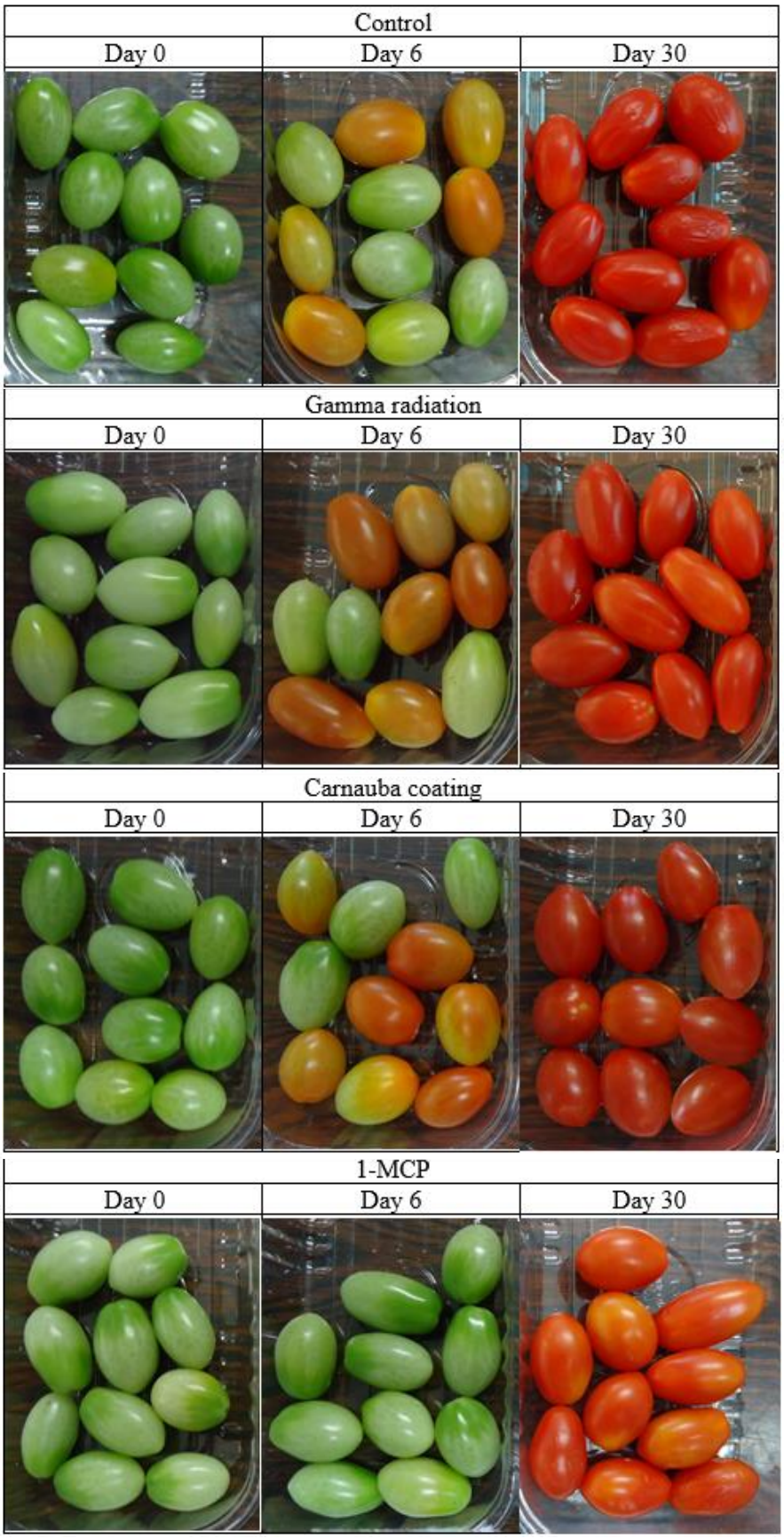

Figure 3 – Color aspects of mini tomatoes treated with gamma radiation, 1-MCP and carnauba coating on days 0, 6 and 30 of storage at 25±2°C. Copyright: the author.

The changes in soluble solids (SS), titratable acidity (TA) and ratio SS/TA of breaker tomatoes submitted to different postharvest treatments are presented in Table 1. In general, tomato SS content ranged from 4.8 to 4.14 °Brix, values lower than expected for cv. Sweet Grape. Probably the lower values are due to the maturity stage of fruits at harvest (breaker), because fruits import sugar during vine-ripening and when they are harvest more immature sugar import is curtailed (KADER et al., 1978; CARRARI et al., 2006; BECKLES, 2012). If harvested at more advanced stages of maturation, the trend is the fruits of this cultivar present soluble solids content above 7 °Brix (CUNHA et al., 2011; JUNQUEIRA; PEETZ; ONODA, 2011), but the shelf life may be limited (AUERSWALD et al., 1999).

There was a slight decrease in SS content for control, carnauba and gamma radiation tomatoes from day 0 to the 6^{th} day of storage, remaining constant by the complete storage period, while SS of 1-MCP treated tomatoes decreased from 6^{th} day to the 12^{th} day of storage, remaining constant thereafter (Table 1). Regarding differences among treatments, 1-MCP maintained higher SS values in tomatoes than control until the 24^{th} of storage, decreasing and equating to control in the last day of the storage period (day 30). SS content of carnauba coating and gamma radiation treated tomatoes did not significantly differ from control during storage.

Studies have suggested that application of 1-MCP may prevent quickly changes in SS in tomatoes due to the delaying in ripening process (GUILLEN et al., 2007), while gamma radiation (doses until 3 kGy) and edible coatings treatments may not significantly change SS from the untreated fruit (SHURONG et al., 2005; PRAKASH et al., 2002; MEJIA-TORRES et al., 2009).

In general, TA of tomatoes decreased during storage, except for tomatoes treated with 1-MCP that showed an increase in TA until the 12^{th} day of storage, decreasing thereafter. Decreasing in TA is a normal process related to organic acids reduction during fruit ripening by the oxidation process in order to produce energy (CHITARRA; CHITARRA, 2005). However, the TA increase observed in 1-MCP tomatoes could be due to the delaying in ripening maintaining the breaker stage for longer. Adam et al. (2014) reported breaker tomatoes showed an increase in titratable acidity shortly after breaker stage before decreased.

Table 1 - Soluble solids content (°Brix), titratable acidity (g citric acid 100 g^{-1}) and SS/AT ratio of mini tomatoes treated with gamma radiation, carnauba coating and 1-MCP during storage[1]

Treatment	Days of storage					
	0	6	12	18	24	30
	Soluble solids (°Brix)					
Control	4.70 ± 0.03 ^{Aa}	4.31 ± 0.03 ^{Bb}	4.29 ± 0.05 ^{Bb}	4.27 ± 0.06 ^{Bb}	4.35 ± 0.06 ^{Bb}	4.35 ± 0.03 ^{Ab}
Gamma radiation	4.75 ± 0.03 ^{Aa}	4.37 ± 0.12 ^{Bb}	4.26 ± 0.24 ^{Bb}	4.27 ± 0.06 ^{Bb}	4.33 ± 0.01 ^{Bb}	4.37 ± 0.10 ^{Ab}
Carnauba coating	4.80 ± 0.09 ^{Aa}	4.39 ± 0.09 ^{Bb}	4.39 ± 0.13 ^{ABb}	4.28 ± 0.05 ^{Bb}	4.33 ± 0.01 ^{Bb}	4.35 ± 0.03 ^{Ab}
1-MCP	4.71 ± 0.01 ^{Aa}	4.57 ± 0.06 ^{Aab}	4.51 ± 0.06 ^{Abc}	4.52 ± 0.06 ^{Abc}	4.50 ± 0.01 ^{Abc}	4.39 ± 0.14 ^{Ac}
	Titratable acidity (g citric acid 100 g^{-1})					
Control	0.76 ± 0.04 ^{Aa}	0.69 ± 0.03 ^{Bb}	0.61 ± 0.01 ^{Bc}	0.45 ± 0.01 ^{Bd}	0.38 ± 0.01 ^{Be}	0.34 ± 0.01 ^{Be}
Gamma radiation	0.75 ± 0.03 ^{Aa}	0.71 ± 0.03 ^{Ba}	0.54 ± 0.02 ^{Cb}	0.48 ± 0.01 ^{Bc}	0.39 ± 0.01 ^{Bd}	0.36 ± 0.01 ^{Bd}
Carauba coating	0.76 ± 0.05 ^{Aa}	0.67 ± 0.06 ^{Bb}	0.61 ± 0.05 ^{Bb}	0.49 ± 0.02 ^{Bc}	0.36 ± 0.01 ^{Bd}	0.33 ± 0.01 ^{Bd}
1-MCP	0.80 ± 0.05 ^{Ab}	0.84 ± 0.03 ^{Ab}	0.98 ± 0.03 ^{Aa}	0.70 ± 0.01 ^{Ac}	0.61 ± 0.01 ^{Ad}	0.51 ± 0.02 ^{Ae}
	Ratio SS/TA					
Control	6.18 ± 0.26 ^{Ae}	6.23 ± 0.34 ^{Ae}	7.00 ± 0.19 ^{Bd}	9.56 ± 0.28 ^{Ac}	11.50 ± 0.12 ^{ABb}	12.75 ± 0.17 ^{Aa}
Gamma radiation	6.34 ± 0.20 ^{Ae}	6.21 ± 0.38 ^{Ae}	7.93 ± 0.21 ^{Ad}	8.98 ± 0.38 ^{Bc}	11.03 ± 0.24 ^{Bb}	12.14 ± 0.34 ^{Aa}
Carauba coating	6.30 ± 0.36 ^{Ae}	6.58 ± 0.66 ^{Ade}	7.18 ± 0.68 ^{Bd}	8.54 ± 0.37 ^{Bc}	11.67 ± 0.41 ^{Ab}	12.58 ± 0.31 ^{Aa}
1-MCP	5.92 ± 0.31 ^{Acd}	5.39 ± 0.19 ^{Bd}	4.61 ± 0.17 ^{Ce}	6.49 ± 0.18 ^{Cc}	7.40 ± 0.16 ^{Cb}	8.60 ± 0.35 ^{Ba}

[1] Data are means ± Standard Deviation (n=3). Means followed by same capital letter on column (within the same compound) and small letter on line were not significantly different by Tukey's test (P>0.05).

In comparison to the other treatments TA of 1-MCP treated tomatoes was higher from the 6th day to the end of storage. This parameter influenced the most in SS/TA ratio for all treatments, because TA changes were more evident than SS changes. SS/TA ratio increased during storage for tomatoes treated with gamma radiation and carnauba and for untreated tomatoes. Whereas for 1-MCP treated tomatoes SS/TA ratio decreased until the 12th day of storage and tended to increase thereafter. It has been reported 1-MCP treatment may delayed the typical reduction in TA that occurs during tomato ripening causing a decrease in SS/TA ratio, an important quality parameter (GUILLEN et al., 2007; GUILLEN et al., 2006; WILLS; KU, 2002). This phenomenon may be pronounced when 1-MCP is applied in fruits harvest at more immature stages (WILLS; KU, 2002).

Retention of titratable acidity has also been reported for fruit treated with edible coatings (YAMAN; BAYOINDIRLI, 2002; TANADA-PALMU; GROSSO, 2005, ALI et al., 2010), because by reducing respiration rate, edible coatings may delay the utilization of organic acids (YAMAN; BAYOINDIRLI, 2002). However, in the present study this trend was not observed. As SS, titratable acidity was not strongly affected by gamma radiation treatment. This parameter only differed from control on the 12th day of storage, when was lower. Similarly, studies with tomatoes (PRAKASH et al., 2002), grapefruit (PATIL; VANAMALA; HALLMAN, 2004) and citrus (ZHANG et al., 2014) show no differences for titratable acidity between irradiated and non-irradiated fruits.

2.4 Conclusions

Mini tomatoes harvest at breaker stage and stored for 30 days showed changes in all evaluated characteristics, the magnitude of these changes, however, varied according to the treatment. The most effective treatment for delaying the mass loss was carnauba, second 1-MCP. Fruit firmness was better retained for 1-MCP treated fruits and carnauba treatment showed a transient effect in preserving fruit firmness. Whereas, color and SS/TA ratio were compromised by the application of 1-MCP, SS/TA of tomatoes treated with gamma radiation and carnauba presented no differences from control values. However, gamma radiation treated fruits showed earlier peel color change (from the 6th day of storage).

Considering acidity and soluble solids contents directly influence fruit flavor, our results suggests carnauba coating, gamma radiation and control fruits had better SS/TA ratio than 1-MCP fruits. Nevertheless, the best combination of SS/TA ratio and fruit preservation for mini tomatoes harvest at breaker stage was promoted by carnauba coating.

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3 EFFECT OF POSTHARVEST TREATMENTS ON BIOACTIVE COMPOUNDS OF MINI TOMATOES HARVEST AT BREAKER STAGE

Abstract

This work investigated the effects of postharvest treatments on the content of bioactive compounds such as ascorbic acid, phenolic compounds, carotenoids and antioxidant capacity in tomatoes during the storage. Mature green mini tomatoes (breaker stage) were treated with gamma radiation (0.6 kGy), carnaúba wax coating (1 L 1000 kg^{-1}) and 1-MCP (500 nL L^{-1}) and then stored at 25±2°C for 30 days with a control group tomatoes. Carotenoids profile, lycopene isomers, phenolic compounds, ascorbic acid and antioxidant capacity were evaluated in mini tomatoes, on days 0, 6, 12, 18, 24 and 30 posttreatment. Results indicate gamma radiation and 1-MCP decreased the final content of lycopene and produced more (*Z*)-isomers of lycopene. Gamma radiation also induced a decreased in β-carotene and an increased in phenolic compounds by the end of storage period. 1-MCP treatment promoted a slow down increase in ascorbic acid content during storage. Antioxidant capacity of the hydrophilic fraction was not so dramatically affected by treatments and the lipophilic fraction was lower, especially for 1-MCP fruits. Carnauba coating seems to be the treatment that causes less change in bioactive compounds of breaker tomatoes. In addition, contents of β-carotene, lycopene, (*Z*)-isomers of lycopene, ascorbic acid and antioxidant capacity increased during the period of storage while contents of lutein and phenolic compounds tended to decrease.

Keywords: *Solanum lycopersicum*, gamma radiation, carnauba coating, 1-methylcyclopropene, carotenoids, lycopene isomers, antioxidant capacity

3.1 Introduction

Consumers are becoming very health conscious, requiring food products that are not only safe, but that are also with optimal nutritional quality (SCALZO et al., 2005; EISSA; SHAHEEN; BROTOS, 2014; BRAVO et al., 2012). This attitude is supported by governments, which invest resources in promoting the consumption of fresh fruits and vegetables (EISSA; SHAHEEN; BROTOS, 2014), healthy products strongly associated with prevention of degenerative diseases (KRIS-ETHERTON et al., 2002; DORAIS et al., 2008; ZHANG et al., 2009). Tomatoes (*Solanum lycopersicum*) are one of the most consumed vegetables in the world, highly appreciated by consumers due to their versatility of consumption, attractive color, taste and nutritional quality, being an important constituent of the human diet either directly or as tomato-based food products. Tomato provides a wide variety of dietary antioxidants such as ascorbic acid, vitamin E, carotenoids and phenolic compounds (ABUSHITA et al., 1997; LENUCCI et al., 2006), which are able to exert a

protective role in reducing the risk of certain types of cancers (GIOVANNUCCI, 1999; STAHL; SIES, 2005) and cardiovascular diseases (WILLCOX; CATIGNANI; LAZARUS, et al., 2003). Particularly lycopene and β-carotene represent the major carotenoids of ripe tomatoes which are responsible for the characteristic color of them, conferring red and orange colors, respectively (LIU et al., 2009). In addition to influence the quality perception of fresh tomatoes, which is directly related to their marketing value (TIJSKENS; EVELO, 1994), there are plentiful evidences that these health-promoting compounds have become a decisive parameter of quality for consumers of fruits and vegetables (GIUNTINI et al., 2005; FIGUEIREDO et al., 2014).

The health benefits of eating tomato, as well as its low caloric value, make it a very attractive vegetable. However, the levels of bioactive compounds and the antioxidant activity of tomatoes are influenced by genotype differences, agricultural techniques, environmental conditions, ripening stage, harvest and postharvest manipulations (TOOR; SAVAGE; HEEB, 2006; HERNÁNDEZ; RODRÍGUEZ; DÍAZ, 2007; LENUCCI et al., 2009).

Tomatoes like most fruits and vegetables are considered as high perishability, because of its tendency to deteriorate rapidly after harvesting (FRAZIER; WESTHOLF, 1986; GONZALEZ-AGUILAR et al., 2009; GAJEWSKI et al., 2014), especially in developing countries (BARBITHA; KIRANMAYIA, 2010). Thus, postharvest treatments are of great importance to prevent both qualitative and quantitative losses in tomatoes (BARBITHA; KIRANMAYIA, 2010). Several techniques have been developed and tested to extend shelf life of fresh fruit (BICO et al., 2010). In tomatoes, postharvest treatments as gamma radiation, application of 1-methylcicloprope ne (1-MCP) and edible coatings have been extensively studied and the potential of these techniques in delaying fruit ripening has been reported (CHIUMARELLI; FERREIRA, 2006; DAVILA-AVIÑA et al., 2014; LARRIGAUDIÈRE et al, 1991; ASSI; HUBER; BRECHT, 1997; CASTRICINI et al., 2004; KUMAR et al., 2014; KRAMMES et al., 2003; GUILLÉN et al., 2005; HURR; HUBER; LEE, 2005; PUSHPALATHA et al., 2006). However, these treatments may change the composition of the fruit, leading to transformation or greater or lesser concentration of compounds with nutritional importance. It is well known that carotenoids and some vitamins are susceptible to degradation by heat, oxygen and light due to their unsaturated structure (SANTANA et al., 1998), but limited information is available on bioactive compounds affected by postharvest treatments.

Gamma radiation has been considered an effective alternative process to delay ripening, control firmness and consequently extend shelf life of perishable vegetables

(FIGUEIREDO et al., 2014). Economic and technological viability as well as safety of food irradiation has been proven by years, and studies of expert committee of Food and Agriculture Organization (FAO), World Health Organization (WHO) and International Atomic Energy Agency (IAEA) have shown that the proper use of irradiation in food presents no health risk (WHO, 1981). Edible coatings preserve fruit quality and delay senescence (DAVILA-AVIÑA et al., 2011) by modifying the atmosphere around the product, being a barrier to oxygen, carbon dioxide and water vapor, decreasing the respiration rate of the fruit and water loss (MARTÍNEZ-ROMERO et al., 2006). Different materials can be used as edible coatings, but natural proteins, lipids or polysaccharides are common (BAI et al., 2003). The other technique, application of 1-methylcyclopropene (1-MCP), is also an alternative for delaying senescence of fruit. This compound reduces the ethylene action, since 1-MCP blocks the ethylene receptors and inhibit its hormonal action (WATKINS, 2002). As a result, physicochemical changes related to ripening delayed, extending shelf-life (BLANKENSHIP; DOLE, 2003). Considered safe for human, 1-MCP quickly diffuses from the plant tissue after the treatment (BLANKENSHIP; DOLE, 2003; WATKINS, 2006).

Although numerous researches have been performed on the irradiation or application of 1-MCP and edible coatings in vegetables, not much information is available about the effect of these postharvest treatments on antioxidants, particularly on profile of carotenoids and its isomers of fruits. Considering this issue, the present study aimed to evaluate the effect of gamma radiation, carnauba coating and 1-MCP on carotenoids, lycopene isomerization, phenolic compounds, ascorbic acid and antioxidant capacity of mini tomatoes cv. Sweet Grape during storage.

3.2 Material and Methods

3.2.1 Plant material

Mini tomatoes cv. “Sweet Grape” (Sakata Seed Sudamerica) were obtained from greenhouses in Crop Science Department of University of São Paulo (USP) in Piracicaba, SP, Brazil (22º42’30”S, 47º38’01”W). Fruits were harvested at the breaker stage of ripeness [Maturity stage 2 (according USDA Color Classification, USDA, 1991)]. After fruits selection (considering absence of physical defects, signs of rots and differences in size and color), they were washed with chlorinated water (200 ppm) for 2 min and left to dry at room temperature.

3.2.2 Postharvest treatments

Mini tomatoes were divided into four batches: control, gamma radiation, 1-methylcyclopropene (1-MCP) and carnauba coating, each one with approximately 5 kg. All treatments were performed within 24h after harvest and the analysis started at the same time for all treatments.

The fruits of irradiated group were packed on 300 g capacity commercial packages (polyethylene terephthalate, PET) commonly used for tomatoes and transported to Nuclear and Energy Research Institute (IPEN) in São Paulo, SP. The samples were irradiated in their own plastic package in a Compact Multiporpuse Irradiator (^{60}Co, C-188 model, MDS Nordion Canada). The applied radiation dosage was 0.6 kGy, which was established taking into account previous studies that suggested 0.6 kGy is within a range considered as effective to delay fruit ripening in tomatoes (ABREU; SOARES; JESUS, 1997; CASTRICINI et al., 2004; FABBRI et al., 2011; AKTER; KHAN, 2012; KUMAR et al., 2014). Dosimetric studies were performed using a gammachrome YR dosimeter to monitor the dose and estimate the dose rate (3.21 kGy h^{-1}). After irradiation, fruits were transported back and stored at room temperature (25±2°C) for 30 days.

1-MCP gas was prepared from SmartFresh (Agrofresh, Philadelphia) commercial powder (active ingredient 0.14%) at concentration of 500 nL L^{-1}. Predetermined amount of Smartfresh® were placed in flasks with lids and 5 mL of distilled water were added, flasks were shaken until complete dissolution. Then flasks were opened inside hermetic chambers containing the tomatoes. Fruit were treated for 12 h at room temperature (25±2°C). 1-MCP concentration is in accordance with recommendations for tomatoes of SmartFresh® and previous studies (GUILLEN et al., 2007; GUILLEN et al., 2006; CANTWELL et al., 2009). After treatment, fruits were packed as irradiated fruits and stored at room temperature (25±2°C) for 30 days.

The third group of tomatoes received carnauba coating treatment. Commercial carnauba coating Megh Wax ECF-124 (composed of carnauba wax emulsion, anionic surfactant, preservative and water) were provided by Megh Indústria e Comércio Ltda (São Paulo, Brazil). Carnauba coating was manually applied using brushes with the original concentration according to manufacturer's recommendations (1 L 1000 kg^{-1}). Previous studies support carnauba coating as an alternative to maintain postharvest quality in tomatoes (CHIUMARELLI; FERREIRA, 2006; DAVILA-AVIÑA et al., 2011). Before packed as irradiated and 1-MCP groups, fruit were dried at room temperature overnight. After packed, fruits were stored at room temperature (25±2°C) for 30 days.

Finally, the fourth group was control and received no treatment. Fruits were packed as other groups and maintained at room temperature (25±2°C) for 30 days.

During storage, fruits samples of each group were taken (approximately 100 g) in triplicate on days 0, 6, 12, 18, 24 and 30 after postharvest treatments, freeze-dried and stored at -18°C until required to analyze carotenoid profile, lycopene isomers, phenolic compounds, ascorbic acid and antioxidant capacity (H-TEAC and L-TEAC).

3.2.3 Carotenoids extraction

Carotenoids were extracted under subdued light to avoid photo degradation. For extraction, 0.15 g of lyophilized sample was dissolved in 5 mL MiliQ water for 5 min. Then, 35 mL of methanol/tetrahydrofuran (THF) (1/1, v/v) containing 0.1% BHT (to avoid oxidative degradation), 200 mg magnesium oxide, 200 mg sodium sulphate and 100 µL β-apo-8'-carotenal as the internal standard were added to dissolved sample (SEYBOLD et al., 2004). The mixture was homogenized on ice for 5 min using an ultra turrax at 10000 rpm (T25, IKA, Staufen, Germany). The supernatant was filtered under vacuum through filter paper no. 390 (Filtrak, Niederschlag, Germany) on a Büchner funnel. This extraction was repeated at least twice until the residue of the sample was colourless. The combined supernatants were concentrated in a rotary evaporator at reduced pressure and 30°C. The residue was redissolved in methanol/THF (1/1, v/v) containing 0.1% BHT using an ultrasonic bath, until the solution reached the defined volume of 5 mL. The solution was centrifuged for 5 min at 14000 rpm, and transferred into amber HPLC vials for analysis. Chromatographic analyses (carotenoids and lycopene isomers) were carried out directly after the extraction and 500 µL of the solution were injected into the HPLC system.

3.2.4 Analysis of carotenoids

Carotenoids were measured via high performance liquid chromatography with diode array detection at 450 nm (Merck Hitachi, Darmstadt, Germany). The chromatographic separation was performed at 13 ± 1°C on a Develosil RP-Aqueous (250 mm × 4.6 mm, 5 µm) C30-column (Phenomenex, Aschaffenburg, Germany). Mobile phase consisted of a gradient of MeOH (solvent A) and MtBE (solvent B): initial conditions 90% solvent A and 10% solvent B; 40 min linear gradient to 50% solvent B; 2 min linear gradient to 60% solvent B, 40% solvent A and 60% solvent B for 23 min; 5 min linear gradient to 10% solvent B; and 90% solvent A and 10% solvent B for 5 min. The flow rate was set at 1 mL min^{-1}. The concentrations of (all-*E*)-lutein, (all-*E*)-β-carotene, (9*Z*)-β-carotene and (all-*E*)-lycopene were

quantified by 5-point calibration curves of external standards. The concentrations of the stock solutions were checked periodically and were calculated using the specific extinction coefficients (BRITTON; LIAAEN-JENSEN; PFANDER, 2004).

3.2.5 Analysis of lycopene composition

Lycopene isomer composition as well as contents of lycopene were analyzed using an isocratic C_{30}-HPLC method using a Merck−Hitachi HPLC system (Darmstadt, Germany) and a Jetstream Plus column oven (JASCO, Groß-Umstadt, Germany). A C_{30} column (YMC Europe, Dinslaken, Germany) (250 mm × 4.6 mm, 5 μm), preceded by a C_{18} ProntoSil 120−5-C18 H (10 mm × 4.0 mm, 5 μm) column (Bischoff, Leonberg, Germany) was used. Mobile phase consisted of MtBE/MeOH/ethylacetate (50/45/5, v/v/v) and flow rate was set at 0.4 mL min^{-1}. Column temperature was 32±1°C and detection wavelength 470 nm. Lycopene contents were quantified by 5-point calibration curve of external standard. Retention time of (*Z*)-isomers in relation to that of (all-*E*)-lycopene was used to identify lycopene isomers, which are presented as ratios of (all-*E*)-lycopene/(*Z*)-isomer. Thus, exact contents of different lycopene isomers were not determined.

3.2.6 Total phenolic compounds

Total phenolic contents was determined based on the Folin-Ciocalteu method as described by Woisky and Salatino (1998), using gallic acid as standard for the calibration curve. Samples were mixed in 50-time volume of aqueous ethanol (80%) under subdued light in a shaker water bath at 40°C for 30 min. The homogenate was centrifuge at 5000 rpm for 15 minutes and supernatant was recovered. 0.5 mL of the extract was taken and added of 2.5 mL of Folin-Ciocalteu reagent (10%). After 5 minutes, 2 mL of sodium carbonate (4%) was added and the content was mixed thoroughly and let in the dark for 60 min. Absorbance was measured at 740 nm in a spectrophotometer (UNICO, model 2800 UV/Vis, Interprise, Brazil).

3.2.7 Ascorbic acid

Ascorbic acid was estimated by the method of AOAC (1984) modified by Benassi and Antunes (1988). Samples were homogeneized with 1% oxalic acid (1:10 m/v) and titrated against 2,6-dichlorophenol-indophenol dye. The ascorbic acid content in samples was determined from the standard ascorbic acid and the results were expressed in mg of ascorbic acid per 100 g of fresh weight.

3.2.8 Antioxidant capacity

For determination of antioxidant capacity, two versions (hydrophilic and lipophilic) of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay were conducted. This assay is based on the decolorization of the ABTS•+ (2,2'-azino-bis-(3-ethylbenzo-thiazoline-6- sulphonic acid) at approximately 730 nm to determine the antioxidant capacity (RE et al., 1999). The method was described by Miller et al. (1996) and was modified slightly by numerous researchers.

α-TEAC Assay

The lipophilic α-tocopherol (α-TE) antioxidant capacity (α-TEAC) assay was performed according to Müller, Theile and Böhm (2010) and calibrated with α-tocopherol instead of Trolox.

Sample preparation consisted of added 2 mL of *n*-hexane to the sample, shake for 30 s and centrifuge at 5000 rpm for 5 min. The supernatant was taken and this extraction process was repeated at least 5 times until the residue of the sample was colourless. The combined supernatants were concentrated in a rotary evaporator at reduced pressure and 30°C. The residue was redissolved in *n*-hexane using an ultrasonic bath, until the solution reached the final volume of 2 mL. The solution was centrifuged for 2 min at 13000 rpm.

The radical cation $ABTS^{\bullet+}$ was prepared by filtering an ABTS solution (tip of a spatula ABTS dissolved in PBS buffer) through a filter paper coated with manganese dioxide, followed by membrane filtration (0.2 µm). An $ABTS^{\bullet+}$ working solution was produced daily by diluting with 75 mM phosphate buffer (pH 7.4) to an absorbance of 0.70 ± 0.05 at 734 nm.

For the measurement, 100 µL of sample extract, or standard (ca. 4.5-125 µmol α-TE L^{-1}), or blank (*n*-hexane) and 1000 µL of adjusted $ABTS^{\bullet+}$ solution were vortexed for 30 s in reaction tubes. Following, the mixture was transferred into half micro-cuvettes and centrifuged for 30 s at 1,200 rpm to separate phases. Exactly 2 min after starting mixing, the absorbance of the lower phase was measured at 734 nm in a V-530 spectrophotometer (Jasco, Gross-Umstadt, Germany).

H-TEAC Assay

To analyse hydrophilic (H) trolox antioxidant capacity (H-TEAC) samples were prepared as follows. After a strong acidic hydrolysis with hydrochloric acid, a saponification with methanolic sodium hydroxide, and a precipitation of proteins with metaphosphoric acid (ARNOLD et al., 2013), antioxidants were extracted by 5 mL of ethanol/water (1/1, v/v),

vortexed for 30 s and centrifuged at 5000 rpm for 5 min. The supernatant was taken and the process (ethanol/water, vortex, centrifuge) was repeated twice. The stable radical cation $ABTS^{\bullet+}$ was performed by mixing 10 mL 7 mmol L^{-1} ABTS solution with 10 mL 2.45 mmol L^{-1} $K_2S_2O_8$ solution (RE et al., 1999). After 24 h at room temperature in the darkness, the $ABTS^{\bullet+}$ stock solution was ready to use. An $ABTS^{\bullet+}$ working solution was prepared daily by diluting the $ABTS^{\bullet+}$ stock solution with phosphate buffer (PBS, 75 mmol L^{-1}, pH 7.4) to an absorbance of 0.70±0.05 at 730 nm. To perform the assay, 20 µL of sample extract, or standard (ca. 12.5-250 µmol trolox L^{-1} or blank (water) were transferred into a 96-well microplate. After addition of 200 µL $ABTS^{\bullet+}$ working solution, absorbance was recorded after 1 min at 730 nm (MÜLLER; THEILE; BÖHM, 2010).

3.2.9 Statistical analysis

All data presented were arranged in completely randomized design, and values are given as means ± standard deviation (SD) of three replicates. Data were analyzed using SAS software version 9.0 (SAS Institute, Cary, NC, USA). Shapiro-Wilk test was applied to check on Gaussian distribution, and the Box-Cox test was used to evaluate the homogeneity of the variances. If the variances were not homogeneous, the values were transformed adequately before they were subjected to the tests. Analysis of variance (ANOVA) was performed to study the interactions between treatments (irradiation and control) and period of storage (0, 6, 12, 18, 24, 30 days). The means were compared by Tukey's test ($p<0.05$).

3.3 Results and Discussion

In the present study, we evaluated the effect of gamma radiation, 1-MCP and carnauba coating on tomato carotenoids, phenolic compounds, ascorbic acid and antioxidant capacity. High levels of these compounds and their relation to health benefits are considered an adjunctive quality parameter of tomatoes.

Carotenoids analysis of tomato extracts led to the typical chromatograms shown in Figures 1 and 2 detailing separation of carotenoids and lycopene isomers respectively. Detected carotenoids in mini tomatoes consisted of (all-*E*)-lutein, (all-*E*)-β-carotene and its isomer (9*Z*)-β-carotene, (all-*E*)-lycopene and its isomers (13*Z*)-lycopene, (9*Z*)-lycopene and (5*Z*)-lycopene. The major carotenoids in mini tomatoes were lycopene and β-carotene.

The changes in (all-*E*)-lycopene, (all-*E*)-β-carotene, (all-*E*)-lutein and (9*Z*)-β-carotene content of breaker tomatoes submitted to different postharvest treatments are presented in Table 1. The ANOVA analysis showed a significant influence of treatments and days of

storage ($P<0.05$) on content of β-carotene, lycopene and lutein, but the interaction of the two factors was not significant for (9*Z*)-β-carotene, only days of storage ($P<0.05$). All tomato treatment groups showed increasing trend in lycopene content during storage. As a normal physiological process of maturation, lycopene synthesis occurs during the off-vine ripening process (BRAVO et al., 2012). Tomatoes change from green to red color because chloroplasts transform into chromoplasts, chlorophyll is degraded and lycopene and β-carotene are synthetized (GRIERSON, 1985).

The initial lycopene content (day 0) was low for all treatments and no differences among them were observed. On the 6th day of storage, irradiated fruits showed the higher lycopene content, indicating the lycopene development, and consequently changes in peel color, occurring first in irradiated fruits (until 6th day), which is confirmed by color analysis that showed a more pronounced change in color of irradiated fruits at 6th day of storage (as shown in chapter 2). Similarly, but with a different dosage, Lee, McGlasson and Edwards (1968) reported that tomatoes harvested more immature and irradiated with 400 krad colored 2 or 3 days earlier than control tomatoes, which was not observed for tomatoes in more advanced mature stage.

In the following days, lycopene content in irradiated fruits did not differ from control until the 24th day of storage. However, by the end of storage, lycopene content in irradiated fruits did not increased as control and carnauba treatments, keeping lower values, differing only from 1-MCP treated fruits, which presented the lowest lycopene contents among all treatments, since day 6 of storage. Carnauba coating treatment also affected the lycopene accumulation. After 1-MCP treatment, fruits treated with carnauba coating had the lowest averages for lycopene on days 6, 12 and 18. From 24th day on, lycopene content of carnauba-treated fruits did not differ from control.

Studies have shown edible coatings treatments delay the ripening process in tomatoes by slowing down the respiration, decreasing fruit metabolism (ALI et al., 2010; DAVILA-AVIÑA et al., 2014). Similar effect was observed with the application of 1-MCP (WANG et al., 2008; SUN et al., 2012), which blocks the ethylene receptors and inhibit the hormonal action (WATKINS, 2002) delaying tomato ripening. Consequently, a slow development and lower content of lycopene could be attributed to the delaying in maturity process caused by these treatments during storage.

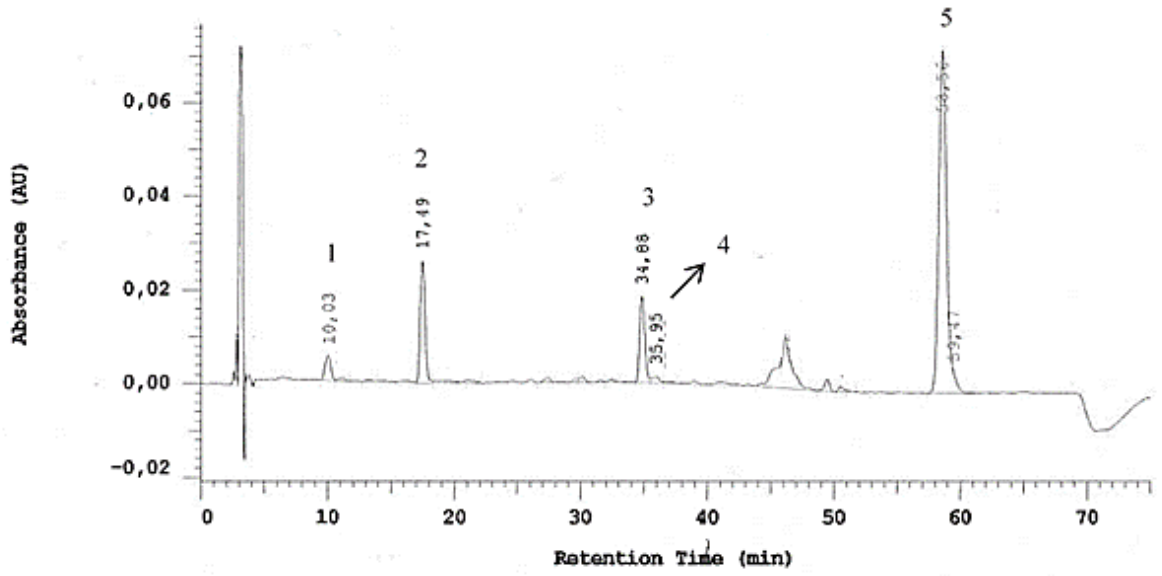

Figure 1 - Typical HPLC chromatogram (λ = 450 nm) of a tomato extract obtained using the conditions described herein. Major peaks corresponding to (all-*E*)-lutein (1), internal standard - β-apo-8'-carotenal (2), (all-*E*)-β-carotene (3), (9*Z*)-β-carotene (4), (all-*E*)-lycopene (5).

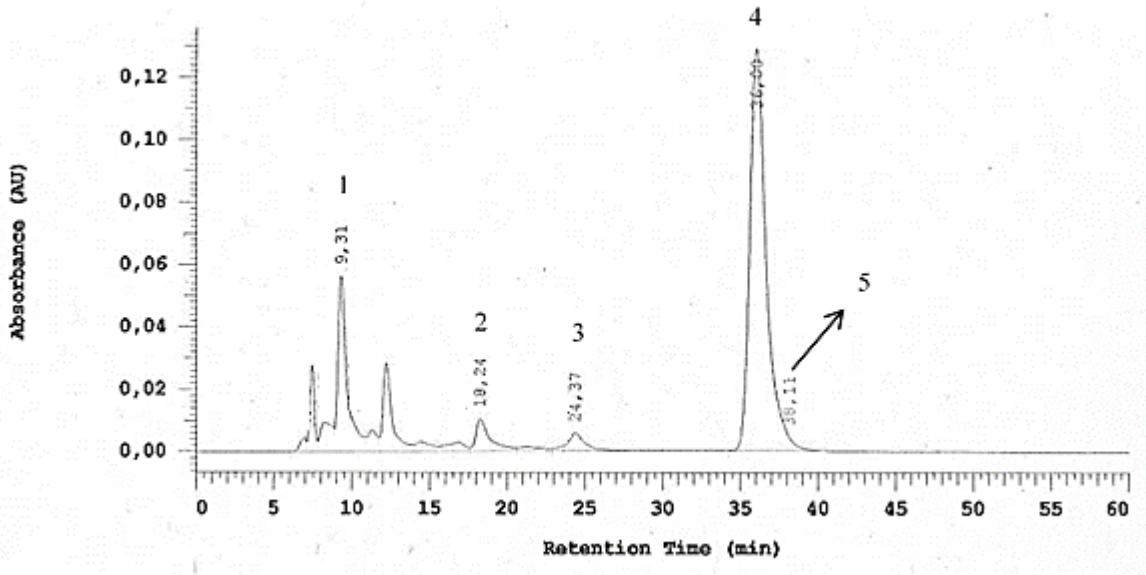

Figure 2 - Typical HPLC chromatogram (λ = 470 nm) of separation lycopene and lycopene isomers from a tomato extract obtained using the conditions described herein. Major peaks corresponding to internal standard - β-apo-8'-carotenal (1), (13*Z*)-lycopene (2), (9*Z*)-lycopene (3), (all-*E*)-lycopene (4) and (5*Z*)-lycopene (5).

As lycopene, β-carotene levels in tomato fruits increased significantly ($P<0.05$) during storage for all treatments (Table 1). Similarly, Bravo et al. (2012) and Kumar et al. (2014) found that β-carotene increased during storage in tomatoes harvest at breaker stage. In the

present study, control, carnauba coating and gamma radiation treatment increased β-carotene contents in fruits until the 24th day (control and carnauba) and 18th day (gamma radiation) of storage and then kept the levels by the end of storage time. However, for 1-MCP treated tomatoes, β-carotene content, although slower pathway, increased until the last day of storage, when reached the same levels of control tomatoes. Tomatoes treated with 1-MCP differed significantly from other treatments on the 12th and 18th days of storage, with the lower values for β-carotene. In addition, gamma radiation treatment affected the levels of β-carotene in the end of the storage period, decreasing the content of this compound ($P<0.05$) in comparison to control and carnauba coating.

The low final accumulation of (all-*E*)-β-carotene and (all-*E*)-lycopene (after 30 days of storage) in irradiated fruits probably is related to the gamma radiation treatment. According to Villegas et al. (1972) total carotenoid content is generally lower in irradiated fruits and the inhibition of carotenoid synthesis is dose dependent. They tested high doses of gamma radiation on tomato fruits (1, 3, 5, 7 and 10 kGy) and generally, the effect was more pronounced with high doses in the early stages of fruit maturation. Kumar et al. (2014) also reported that doses of 0.5 and 1 kGy decreased lycopene and β-carotene content on irradiated tomatoes.

The content of the isomer (9*Z*)-β-carotene in tomato fruits changed during the storage time (Table 1). The (9*Z*)-β-carotene content decreased from day 0 to day 6, and then increased on day 12, decreasing once more on day 18, remaining with a constant content until the end of storage. Thereby postharvest treatments appears to have no effect in producing (*Z*)-isomers of β-carotene, which is a positive point since (*Z*)-isomers of β-carotene (9*Z*, 13*Z*, and 15*Z*) possess lower pro-vitamin A activity and bioavailability compared to (*all*-E) (DEMING; BAKER; ERDMAN, 2002; DURING et al., 2002) and lower antioxidant capacity (BÖHM et al., 2002). It is reported high temperature treatments may increase contents of (*Z*)-isomers of β-carotene (IMSIC et al., 2010).

Table 1 - Carotenoids content (μg g^{-1} FW[1]) of mini tomatoes treated with gamma radiation, carnauba coating and 1-MCP during storage[2].

Treatment	Days of storage					
	0	6	12	18	24	30
			(all-E)-lycopene (μg g^{-1})			
Control	0.6 ± 0.05 ^{Ae}	2.8 ± 0.09 ^{Bd}	23.0 ± 1.40 ^{ABc}	27.2 ± 0.77 ^{Ab}	50.4 ± 2.69 ^{Aa}	56.2 ± 2.43 ^{Aa}
Gamma radiation	0.6 ± 0.04 ^{Ad}	3.8 ± 0.27 ^{Ac}	24.5 ± 3.18 ^{Ab}	27.2 ± 0.35 ^{Ab}	47.6 ± 2.48 ^{Aa}	45.0 ± 2.91 ^{Ba}
Carnauba coating	0.7 ± 0.08 ^{Ad}	2.2 ± 0.12 ^{Cc}	20.4 ± 1.68 ^{Cb}	20.1 ± 0.83 ^{Bb}	52.5 ± 1.06 ^{Aa}	57.7 ± 1.16 ^{Aa}
1-MCP	0.6 ± 0.05 ^{Ad}	1.6 ± 0.01 ^{Dd}	7.5 ± 1.18 ^{Dc}	9.0 ± 0.60 ^{Cc}	19.2 ± 0.79 ^{Bb}	34.3 ± 0.68 ^{Ca}
			(all-E)-β-carotene (μg g^{-1})			
Control	1.5 ± 0.02 ^{Ac}	1.5 ± 0.04 ^{Bc}	2.5 ± 0.02 ^{Ab}	3.1 ± 0.09 ^{Bb}	3.8 ± 0.15 ^{BCa}	4.0 ± 0.56 ^{Aa}
Gamma radiation	1.4 ± 0.07 ^{Ac}	1.7 ± 0.10 ^{ABc}	2.8 ± 0.48 ^{Ab}	3.8 ± 0.41 ^{Aa}	3.7 ± 0.20 ^{Ca}	3.3 ± 0.06 ^{Bab}
Carnauba coating	1.9 ± 0.09 ^{Ae}	2.0 ± 0.20 ^{Ade}	2.5 ± 0.12 ^{Acd}	2.9 ± 0.24 ^{Bc}	4.4 ± 0.01 ^{Aa}	3.9 ± 0.68 ^{ABa}
1-MCP	1.5 ± 0.23 ^{Ad}	1.3 ± 0.11 ^{Bcd}	1.7 ± 0.30 ^{Bcd}	2.0 ± 0.03 ^{Cb}	4.3 ± 0.20 ^{ABb}	4.3 ± 0.30 ^{Aa}
			(all-E)-lutein (μg g^{-1})			
Control	1.9 ± 0.18 ^{Aa}	1.4 ± 0.08 ^{ABb}	1.3 ± 0.08 ^{Bbc}	1.2 ± 0.01 ^{Acd}	0.9 ± 0.04 ^{Bd}	0.9 ± 0.09 ^{Ad}
Gamma radiation	1.8 ± 0.19 ^{Aa}	1.3 ± 0.01 ^{Bb}	1.4 ± 0.29 ^{Bb}	1.2 ± 0.02 ^{Abc}	1.3 ± 0.08 ^{Ab}	1.0 ± 0.03 ^{Ac}
Carnauba coating	1.9 ± 0.16 ^{Aa}	1.6 ± 0.03 ^{Ab}	1.7 ± 0.04 ^{Aab}	1.1 ± 0.02 ^{Acd}	1.1 ± 0.03 ^{ABc}	0.9 ± 0.09 ^{Ad}
1-MCP	1.9 ± 0.17 ^{Aa}	1.5 ± 0.02 ^{Ab}	1.9 ± 0.13 ^{Aa}	1.0 ± 0.05 ^{Ac}	1.0 ± 0.06 ^{Bc}	1.0 ± 0.05 ^{Ac}
			(9*Z*)-β-carotene (μg g^{-1})			
Control	0.20 ± 0.03	0.12 ± 0.02	0.22 ± 0.00	0.17 ± 0.03	0.18 ± 0.01	0.19 ± 0.07
Gamma radiation	0.15 ± 0.01	0.17 ± 0.03	0.29 ± 0.03	0.22 ± 0.02	0.19 ± 0.04	0.20 ± 0.02
Carnauba coating	0.20 ± 0.01	0.18 ± 0.04	0.24 ± 0.07	0.17 ± 0.00	0.18 ± 0.01	0.17 ± 0.13
1-MCP	0.17 ± 0.02	0.14 ± 0.02	0.21 ± 0.07	0.19 ± 0.05	0.18 ± 0.01	0.21 ± 0.00
Means	0.18 ± 0.02 ^{b}	0.15 ± 0.03 ^{c}	0.24 ± 0.03 ^{a}	0.19 ± 0.02 ^{ab}	0.18 ± 0.01 ^{b}	0.19 ± 0.02 ^{ab}

[1] Fresh weight.
[2] Data are means ± Standard Deviation (n=3). Means followed by same capital letter on column (within the same compound) and small letter on line were not significantly different by Tukey's test (P>0.05).

In addition to lycopene and β-carotene, lutein are also present in tomatoes but in a much smaller amounts (SHI; LE MAGUER, 2000). Even so, promote health benefits, particularly with zeaxanthin, because both selectively accumulate in the macula of the retina of the eye where they preserve eye health, protecting against the development of age-related macular degeneration (BONE; LANDRUM, 1992; GRANADO; OLMEDILLA; BLANCO, 2003). In the present study, lutein content reduced significantly ($P<0.05$) in tomatoes during storage, except for 1-MCP treated fruits whose content decreased until the 18th day of storage, remaining constant thereafter (Table 1). The differences among treatments occurred on day 6 and 12 when 1-MCP and carnauba treated tomatoes showed the high values, did not differing from control on day 6. In addition, on day 24 irradiated fruits showed high values for lutein content, but these levels were not different from carnauba coating treated fruits.

(*Z*)-isomers of lycopene identified in tomatoes were (13*Z*)-, (9*Z*)- and (5*Z*)- lycopene. As (all-*E*)-lycopene, (*Z*)-isomers increased significantly ($P<0.05$) in all tomato groups (control, gamma radiation, carnauba coating and 1-MCP) during storage, while the fruits were ripening (Table 2). Lycopene has 11 conjugated double bonds, and each of them could be either in an (*E*) or (*Z*)-configuration. It is known the most common geometrical isomer is (all-*E*)-lycopene in plants, which represents about 80−97% of total lycopene in tomatoes and related products (SHI; LE MAGUER, 2000), but food treatments and preparation may change the proportion of (*Z*)-isomers.

Immediately after postharvest treatments application (day 0) no (*Z*)-isomers were detected in breaker tomato fruits. From the 6th day on, (*Z*)-isomers appeared in tomatoes. We can observed 1-MCP and gamma radiation treated tomatoes showed lower ratios for (all-E)/(13*Z*) and (all-E)/(9*Z*) in most days of storage in comparison to control and carnauba coating groups. However, while 1-MCP treated fruits reached similar levels of control fruits ratio (all-E)/(13*Z*) by the end of storage time (day 30), gamma radiation presented lower ratio (all-E)/(13*Z*) also on day 30. In addition, 1-MCP treated fruits showed the highest ratio (all-*E*)/(9*Z*) on the 30th day of storage.

Ratios of (all-*E*)/(9Z)-lycopene were also lower for tomatoes treated with 1-MCP on days 6, 12 and 24, but were not different from control by the end of storage period (day 30). On the other hand, gamma radiation presented not so strong effect on (all-*E*)/(9Z)-lycopene ratio, once gamma radiation-treated tomatoes showed lower (all-*E*)/(9Z) ratio than control only on the 18th day of storage. Regarding carnauba coating treatment, in spite of low ratios on days 6 for (all-*E*)/(13*Z*) and days 12 and 18 for (all-*E*)/(5Z), there were no differences from control in the other days of storage.

Table 2 - Ratios of (all-*E*)-lycopene to the different (*Z*)-isomers of mini tomatoes treated with gamma radiation, carnauba coating and 1-MCP during storage[1].

Treatment	Days of storage					
	0	6	12	18	24	30
			Ratio (all-*E*)-lycopene/(13Z)-lycopene			
Control	0.0 ^{Ae}	7.6 ± 0.33 ^{Ad}	15.9 ± 0.87 ^{Ac}	17.8 ± 1.84 ^{Ac}	22.4 ± 1.13 ^{Ab}	28.8 ± 1.98 ^{Aa}
Gamma radiation	0.0 ^{Ac}	10.0 ± 1.14 ^{Ab}	9.5 ± 0.79 ^{Bb}	16.2 ± 0.47 ^{ABa}	18.2 ± 1.80 ^{Ba}	16.5 ± 2.93 ^{Ba}
Carnauba coating	0.0 ^{Ae}	4.3 ± 1.37 ^{Bd}	14.5 ± 1.39 ^{Ac}	17.2 ± 0.21 ^{Ac}	24.0 ± 1.27 ^{Ab}	30.4 ± 2.51 ^{Aa}
1-MCP	0.0 ^{Ac}	0.0 ^{Cc}	10.7 ± 1.39 ^{Bb}	13.4 ± 1.36 ^{Bb}	13.1 ± 2.60 ^{Cb}	29.5 ± 1.68 ^{Aa}
			Ratio (all-*E*)-lycopene/(9Z)-lycopene			
Control	0.0 ^{Ae}	6.5 ± 0.27 ^{Ad}	18.2 ± 1.20 ^{Ac}	25.5 ± 2.24 ^{Ab}	30.3 ± 1.46 ^{Aa}	28.2 ± 1.47 ^{Bab}
Gamma radiation	0.0 ^{Ac}	6.2 ± 0.29 ^{Ac}	10.2 ± 0.90 ^{Cc}	20.9 ± 1.74 ^{Bb}	23.3 ± 0.25 ^{Bab}	25.0 ± 1.13 ^{Ba}
Carnauba coating	0.0 ^{Ad}	6.5 ± 0.47 ^{Ad}	14.9 ± 0.88 ^{ABc}	26.0 ± 0.68 ^{Ab}	32.3 ± 1.59 ^{Aa}	27.3 ± 2.02 ^{Bb}
1-MCP	0.0 ^{Ac}	0.0 ^{Be}	11.2 ± 0.54 ^{BCd}	19.3 ± 1.18 ^{Bc}	24.9 ± 0.38 ^{Bb}	37.3 ± 3.83 ^{Aa}
			Ratio (all-*E*)-lycopene/(5Z)-lycopene			
Control	0.0 ^{Ac}	4.4 ± 0.34 ^{ABb}	5.8 ± 0.10 ^{Ab}	9.3 ± 0.59 ^{Aa}	9.1 ± 0.37 ^{Aa}	9.0 ± 0.29 ^{ABa}
Gamma radiation	0.0 ^{Ac}	4.8 ± 0.80 ^{Ab}	5.8 ± 0.29 ^{Ab}	7.8 ± 0.35 ^{Ba}	8.0 ± 0.32 ^{ABa}	7.9 ± 0.82 ^{Ba}
Carnauba coating	0.0 ^{Ad}	3.2 ± 0.93 ^{BCc}	3.2 ± 0.80 ^{Bc}	7.5 ± 0.97 ^{Bb}	7.9 ± 0.57 ^{ABb}	10.2 ± 1.41 ^{Aa}
1-MCP	0.0 ^{Ac}	2.0 ± 0.74 ^{Cb}	2.3 ± 0.50 ^{Bb}	8.0 ± 0.40 ^{ABa}	7.5 ± 0.84 ^{Ba}	8.1 ± 0.69 ^{Ba}

[1] Data are means ± Standard Deviation (n=3). Means followed by same capital letter on column (within the same compound) and small letter on line were not significantly different by Tukey's test (P>0.05).

Lower ratios indicate higher proportion of (*Z*)-isomers in fruits. Thus, our results indicate irradiated and 1-MCP fruits had more (*Z*)-isomers. Furthermore, both gamm radiation and 1-MCP treatments showed lesser lycopene contents, which could be a great loss. However, this finding may be interesting in view of some studies that indicated some (*Z*)-isomers showed a stronger in vitro antioxidant activity (BÖHM et al., 2002) and are more bioavailable than (all-*E*)-form (BOILEAU; BOILEAU; ERDMAN, 2002; SHI; LE MAGUER, 2000; STAHL; SIES, 1992; UNLU et al., 2007). Bioavailable is higher probably because (*Z*)-isomers are more soluble in bile acid micelles and may be preferentially incorporated into chylomicrons (BOILEAU et al., 1999). In addition, it has been reported (*Z*)-isomers of lycopene make up 50% of the total lycopene in human serum and tissues (FERRUZZI et al., 2001; STAHL; SIES, 1992). For these reasons lycopene (*Z*)-isomers are considered as having higher health benefits than the (all-*E*)-isomer (LAMBELET et al., 2009).

Tomatoes showed significant differences between the interaction of treatments and days of storage for ascorbic acid content ($P<0.05$). During the storage period (30 days), a significant increase ($P<0.05$) on the ascorbic acid contents in all group of tomatoes were observed (Figure 3a), probably due to the early maturation stage fruits were harvested (breaker stage), which continued their maturation process off-vine. According to Lee and Kader (2000) fruits accumulate ascorbic acid during ripening on or off the plant, however the accumulation is greater in those left on the plant. In addition, ascorbic acid of tomatoes harvested in advanced maturation stages tend to decrease during storage (CARON et al., 2013).

As shown in Figure 3a, at days 0, 6 and 12 the ascorbic acid content from control fruit was significantly higher than those from 1-MCP samples, but not different from carnauba and gamma radiation groups. On the 18th day of storage no differences among treatments were observed, the ascorbic acid contents were equated. However, from the 24th day on, differences reappeared. On the 24th day, 1-MCP treated fruits showed the lower values but did not differ from carnauba treatment, while the content in control and irradiated fruits did not differ significantly. In the last day of storage irradiated and 1-MCP fruit presented lower ascorbic acid contents in comparison to control, but did not differ from carnauba coating.

Lower ascorbic acid contents for 1-MCP treated fruits may occurred due to delayed ripening process caused by the inhibition of ethylene (WATKINS, 2002). As a consequence, ascorbic acid does not increase in fruits as fast as non-treated fruits. Sabir et al. (2012) found similar results studying the effects of 1-MCP in tomatoes. This can be related to the

phenomenon of slow down in ripening process reported by Wang et al. (2008) and Sun et al. (2012), which indicates 1-MCP treatment slowing down the increase or decrease in parameters related to maturation.

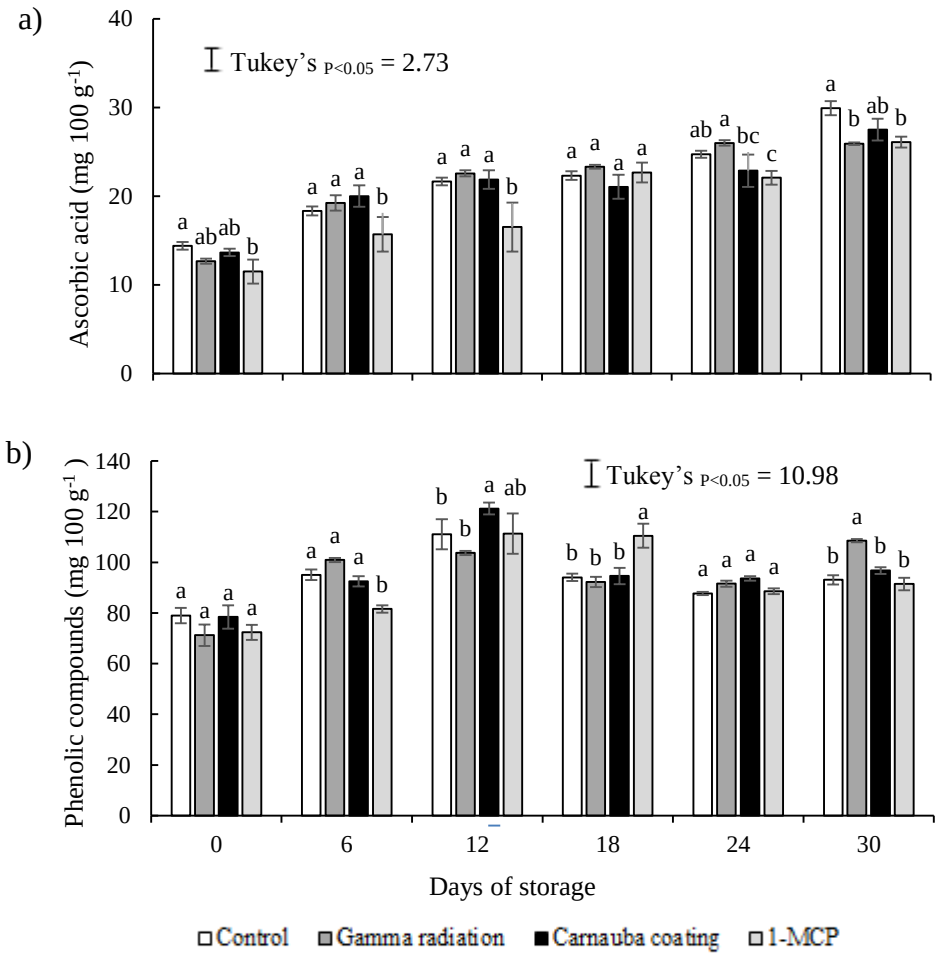

Figure 3 – Ascorbic acid content (mg 100 g^{-1} of fresh weight) (a) and total phenolic compounds (mg GAE 100 g^{-1} of fresh weight) (b) of mini tomatoes treated with gamma radiation, 1-MCP and carnauba coating during storage. Different letters indicate significant differences among treatments by Tukey's test ($P<0.05$). Vertical bars indicate least significant difference by Tukey's test ($P<0.05$) among days of storage. Each observation is a mean ± Standard Deviation (n=3).

Ascorbic acid content in tomatoes treated with gamma radiation and carnauba coating was not strongly affected by treatments. Gamma radiation treated fruits showed lower content of ascorbic acid compared to control only by the end of storage (day 30) while ascorbic acid contents of carnauba treated tomatoes did not differ significantly from control during all the period of storage. Regarding the irradiation treatment, studies suggested gamma radiation did not cause significant losses in ascorbic acid when the dosage is up to 1 kGy

(LEE; KADER, 2000; LACROIX et al., 1990; PATIL; VANAMALA; HALLMAN, 2004). Although, it has been reported a slight decreased in ascorbic acid content of irradiated fruits immediately after irradiation, but during the storage the content tends to equate with control (AHMAD et al., 1972).

The effects of postharvest treatments on total phenolic compounds of mini tomatoes expressed as mg equivalents of gallic acid 100 g^{-1} fresh weight are shown in Figure 3b. Significant differences between the interaction of treatments and days of storage for total phenolic contents were observed ($P<0.05$). The total phenolic compounds of tomatoes increased to a maximum at 18 days of storage for 1-MCP treated tomatoes and at 12 days of storage for control, gamma radiation and carnauba treated tomatoes and subsequently a declined (Figure 3b). Then, phenolic contents remained constant for 1-MCP, carnauba and control tomatoes by the end of storage. However, tomatoes treated with gamma radiation showed another increase in phenolic compounds content on the last day of storage, when the levels were significantly higher than other treatments. These results are consisted with those reported by Wang et al. (2008) and Kumar et al. (2014) who observed a trend of increasing in total phenolic compounds of tomatoes harvest at mature green stage, followed by a decline during storage. Furthermore, a delay observed in increasing of phenolic compounds of tomatoes treated with 1-MCP may be related to the slow down in fruit ripening process, as reported for ascorbic acid.

Regarding to the effects of gamma radiation on phenolic compounds by the end of storage, it has been reported the irradiation treatment may increase total phenolic compounds in tomatoes (KHALAF et al., 2014) or other fresh fruit (TAN; LAM, 1985; EISSA; SHAHEEN; BROTOS, 2014). This phenomenon probably occurred due to irradiation process increase the activity of phenylalanine ammonia lyase (PAL), the enzyme involved in phenolic compounds biosynthesis (REYES; CISNEROS-ZEVALLOS, 2007).

Phenolic compounds, such as flavonoids and hydroxycinamic acid derivatives, with ascorbic acid in its reduced form (ascorbic acid - AA) and its oxidized form (dehydroascorbic acid - DHA), represents the main water-soluble antioxidants in tomatoes and contribute to the antioxidant activity of the water-soluble fraction (RAFFO et al., 2002; MOCO et al., 2006; VALVERDU-QUERALT et al., 2011). In the present study, H-TEAC tended to increase during storage, except for gamma radiation treated fruits, whose H-TEAC increased until the 12^{th} day of storage and remained constant by the end of storage (Figure 4a). These results are consistent with those reported by Cano, Acosta e Arnao (2003) and Periago et al. (2009) which demonstrated increases in antioxidant capacity of hydrophilic fraction during tomato

ripening. Results also showed the differences among treatments occurred on the 12th and 24th days of storage. In these days, although carnauba-coating tomatoes showed higher values for H-TEAC there were no differences from control fruits. In addition, tomatoes treated with 1-MCP presented significant lower values than carnauba tomatoes in these days. At the beginning (days 0 and 6) and by the end of storage time (day 30), no differences among treatments were observed. Contrary to these results, Wang et al. (2008) showed 1-MCP treatment enhanced hydrophilic antioxidant capacity of tomatoes (WANG et al., 2008) measured by a different method (DPPH method). Whereas Ilic et al. (2013), using the ABTS method in mature green tomatoes stored at 12°C, detected no differences between the hydrophilic antioxidant capacity of 1-MCP treated tomatoes and control. Further, according to Ilic et al. (2013), the effects of 1-MCP on antioxidant capacity of tomatoes are still unclear, and need to be more precisely determined.

Concerning to the other treatments, it has been demonstrated edible coatings did not affect negatively the antioxidant capacity in tomatoes (DAVILA-AVIÑA et al., 2014) and might even preserve the antioxidant activity (ALI et al., 2013). However, radiation treatments might increase H-TEAC due to the effects on phenolic compounds through induction of PAL (DUBERY; VAN RENSBURG; SCHABORT, 1984; TAN; LAM, 1985).

The antioxidant capacity of lipophilic fraction, mainly represented by carotenoids and vitamin E (MARTÍNEZ-VALVERDE et al., 2002), changed according to the interaction of days of storage and treatments. Lipophilic antioxidant capacity results of tomatoes submitted to different postharvest treatments are presented in Figure 4b. α-TEAC sharply increased during storage for control tomatoes and for tomatoes treated with gamma radiation and carnauba coating, while the increase in α-TEAC for 1-MCP group was slower. Although α-TEAC values of tomatoes treated with 1-MCP were lower on days 0 and 6, they did not significantly differ from the other treatments, however on the 12th day of storage they were significantly lower. From the 18th day on, 1-MCP treated tomatoes showed lower α-TEAC content, only did not differing from gamma radiation fruits on the 18th day, which also presented lower contents. These results are consisted with lycopene trend observed in Table 1. As carotenoids are directly related to lipophilic antioxidant capacity, especially lycopene, the most potent antioxidant among carotenoids (DIMASCIO; KAISER; SIES, 1989; SHI et al., 2004), is expected that a rise in lycopene content, increase α-TEAC nearly the same proportion. In addition, some other possible factors such as the amount of other carotenoids and vitamin E also affect the antioxidant activity (DUMAS et al., 2003). Furthermore, higher

proportion of (*Z*)-isomers of lycopene in tomatoes treated with gamma radiation and 1-MCP seems not to affect the lipophilic antioxidant capacity in fruits.

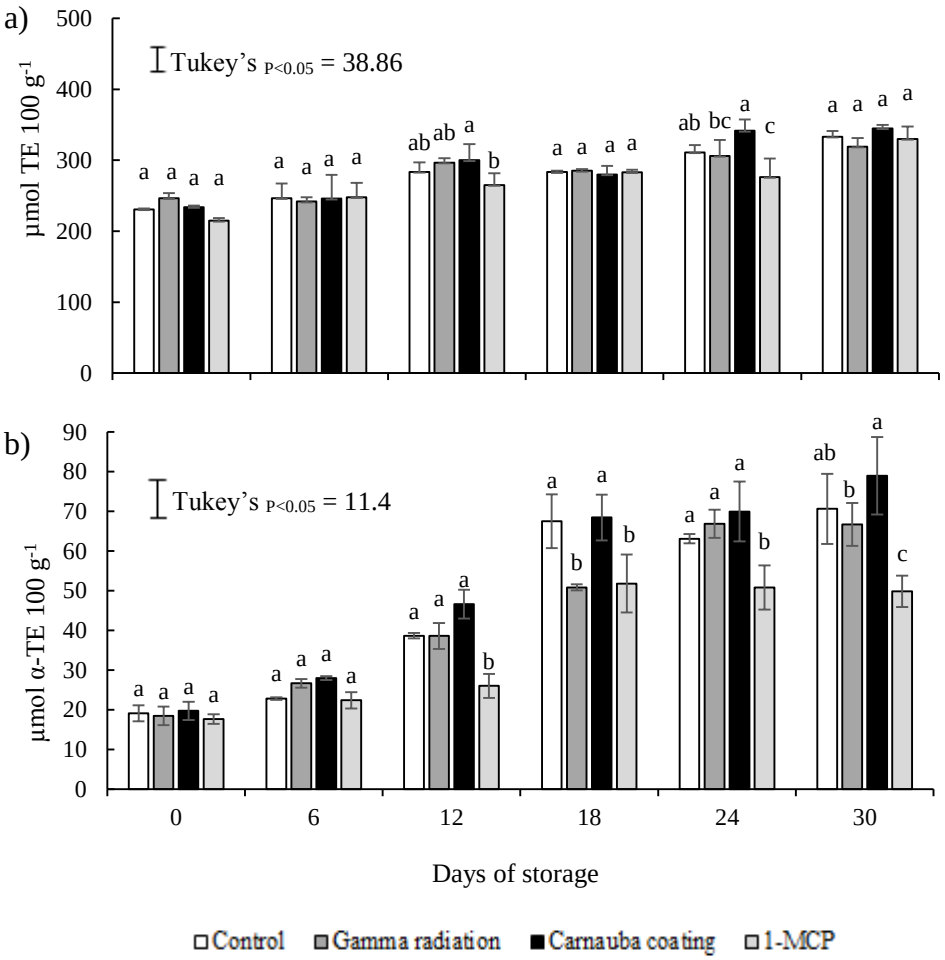

Figure 4 - Hydrophilic antioxidant capacity – H-TEAC (µmol TE 100 g^{-1} of fresh weight) (a) and lipophilic antioxidant capacity – α-TEAC (µmol α-TE 100 g^{-1} of fresh weight) (b) of mini tomatoes of mini tomatoes treated with gamma radiation, 1-MCP and carnauba coating during storage. Different letters indicate significant differences among treatments by Tukey's test (P<0.05). Vertical bars indicate least significant difference by Tukey's test (P<0.05) among days of storage. Each observation is a mean ± Standard Deviation (n=3).

3.4 Conclusions

The present study shows that gamma radiation and 1-MCP as a conservation process and during 30 days of storage induced a decrease in the final content of lycopene and produced more (*Z*)-isomers of lycopene. Final β-carotene content also decreased as an effect of gamma radiation and this same treatment increased phenolic compounds by the end of storage period. 1-MCP treatment promoted a slow down increase in ascorbic acid content, presenting the lower contents of this compound during storage. Antioxidant capacity of the hydrophilic fraction was not so dramatically affected by treatments and the lipophilic fraction was lower, especially for 1-MCP fruits, some days during storage. Carnauba coating seems to be the treatment that causes less change in bioactive compounds of breaker tomato fruits.

In addition, contents of β-carotene, lycopene, (*Z*)-isomers of lycopene, ascorbic acid and antioxidant capacity increased during the period of storage while contents of lutein and phenolic compounds tended to decrease.

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4 EFFECT OF GAMMA RADIATION, CARNAUBA COATING AND 1-MCP ON POSTHARVEST QUALITY OF MINI TOMATOES HARVEST AT LIGHT-RED STAGE

Abstract

The study aimed to evaluate the changes in physico chemical characteristics of mini tomatoes according to different postharvest treatments during storage. Mini tomatoes cv. Sweet Grape harvested at light-red stage were treated with gamma radiation (0.6 kGy), carnauba coating (1 L 1000 kg^{-1}) and 1-MCP (500 nL L^{-1}) and then stored at 25±2°C for 30 days with a control group tomatoes. Color modifications, fruit firmness, mass loss, soluble and total pectin, % of pectin solubilization, titratable acidity, soluble solids and SS/TA ratio were evaluated at days 0, 6, 12, 18, 24, 30 of storage. The most effective treatments for delaying fruit firmness and mass loss was carnauba and 1-MCP, while gamma radiation was the treatment with higher mass loss and the less firmness, which could be associated with the higher solubilization of pectins promoted by radiation treatment. Color (L^* and Hue) was mainly affected by 1-MCP treatment which delayed color development, however, by the end of storage, color development was not different from the other treatments. SS/TA ratio was lower for fruits treated with 1-MCP and TA was not so dramatically affected by treatments. In order to maintain postharvest quality of tomatoes harvested at light-red stage, carnauba and 1-MCP treatments may be indicated.

Keywords: *Solanum lycopersicum*, firmness, color, soluble pectin, pectin solubilization, soluble solids, titratable acidity

4.1 Introduction

Tomato (*Solanum lycopersicum*) is one of the most cultivated vegetable in the world and has a great popularity in today's maket, both as a processed ingredient or as a fresh fruit (PRAKASH et al., 2002). Its consumption has been associated to health benefits, because of the content of antioxidants such as lycopene, β-carotene, flavonoids, vitamin C and many essential nutrients (MARTINEZ-VALVERDE et al., 2002; GEORGE et al., 2004).

Among the several types of tomatoes, the small ones (mini-tomatoes) belongs to a group of cultivars for fresh consuption, which has been increasing its popularity and importance in the markets, probably due to small size and great versatility as well as dark red color of peel and pulp and high concentration of sugars (PICHA, 1986; JUNQUEIRA; PEETZ; ONODA, 2011). Whereas the common varieties of tomato has soluble solids content between 4 and 6 °Brix, varieties of mini tomatoes (cherry and grape) has concentrations

of sugars enough to reach values superior than 9 °Brix. (JUNQUEIRA; PEETZ; ONODA, 2011). In addition, as a product with high aggregated value, the market price of mini tomatoes could be 20-40% higher than traditional tomatoes (JUNQUEIRA; PEETZ; ONODA, 2011).

In order to be accepted by consumers who are willing to pay for a differentiated product, the tomato fruits should have high quality, which means acceptable firmness and flavor, uniformity and shiny color, good appearance, without signs of mechanical injuries or shriveling (SHAHNAWAZ et al., 2012) and also with optimal nutritional quality (BRAVO et al., 2012). However, to achieve these characteristics, harvest and postharvest handling are crucial and may limit the market period. That is why harvesting at early stages and use of conservation techniques are desirable to increase the marketing period (CARON et al., 2013) and maintain fruit quality.

During storage, a large number of chemical and physical processes takes place in vegetables, especially in tomatoes, which are climacteric fruits and continue to ripen after harvest (GHORBANI; POOZESH; KHORRAMDEL, 2012). During this process, modifications such as changes in color, texture, flavor, and chemical compositions are common (JAVANMARDI; KUBOTA, 2006). Furthermore, tomatoes are extremely pershability and susceptible to chilling injury, mechanical damage and the presence of microorganisms (PRAKASH et al., 2002). The rapid quality loss at relatively short period requires postharvest treatments to extend shelf life and maintain the quality longer (SHAHNAWAZ et al., 2012). Gamma radiation, 1-methylcyclopropene (1-MCP) and edible coatings can markedly extend the storage life of many fresh fruits and vegetables by different mechanisms of action, either by decreasing respiration rates or by inhibiting the action of ethylene (CHIUMARELLI; FERREIRA, 2006; DAVILA-AVIÑA et al., 2011; LARRIGAUDIÈRE et al, 1991; ASSI; HUBER; BRECHT, 1997; CASTRICINI et al., 2004; KUMAR et al., 2014; GUILLÉN et al., 2005; HURR; HUBER; LEE, 2005; PUSHPALATHA et al., 2006). In addition, safety and efficiency of these three techniques has been proven by several studies (WHO, 1981; WATKINS, 2006; DAVILA-AVIÑA et al., 2014).

However, as well as environmental (soil, temperature, weather) and genetic factors, postharvest conditions can cause severe effect on storage life and quality of tomatoes (CANO; ACOSTA; ARNAO, 2003; TOOR; SAVAGE; HEEB, 2006; HERNÁNDEZ; RODRÍGUEZ; DÍAZ, 2007). Therefore, postharvest treatments can also affect significantly the physico-chemical and sensory characteristics of tomatoes. Considering this issue and the economic value, perishability and quality expected by consumers, this study aimed to evaluate

the effect of gamma radiation, carnauba coating and 1-MCP treatments on tomato fruit quality during 30 days of storage.

4.2 Material and Methods

4.2.1 Plant material

Mini tomatoes (*Solanum lycopersicum*) cv. Sweet Grape (Sakata Seed Sudamerica) harvest at the light-red stage of ripening according to the USDA standard tomato color classification chart (USDA, 1991) were obtained from a comercial crop in Santa Isabel, SP, Brazil (23º18'56"S, 46º13'17"W). The fruit were visually selected for uniformity in size, color, absence of physical defects and rots, and transported to the laboratory in Piracicaba, SP. Before treatments application, fruit were washed with chlorinated water (200 ppm) for 2 min, and air-dried at room temperature.

4.2.2 Post harvest treatments

Mini tomatoes were divided into four groups of 5 kg each for the following treatments: 1. control; 2. gamma radiation; 3. carnauba coating and, 4. 1-MCP. The treatments were performed within 24h after harvest and the analysis started at the same time for all treatments.

For gamma radiation treatment tomatoes were transported to Nuclear and Energy Research Institute (IPEN) in São Paulo, SP after having been left at room temperature (25±2°C) overnight. Samples were irradiated in a Compact Multiporpuse Irradiator (^{60}Co, C-188 model, MDS Nordion Canada) at a dose of 0.6 kGy. The dosage was established taking into account previous studies that suggested 0.6 kGy is within a range considered as effective to delay fruit ripening in tomatoes (ABREU; SOARES; JESUS, 1997; CASTRICINI et al., 2004; FABBRI et al., 2011; AKTER; KHAN, 2012; KUMAR et al., 2014). Dosimetric studies were performed using a gammachrome YR dosimeter to monitor the dose and estimate the dose rate (3.21 kGy h^{-1}). After irradiation, fruits were transported back to the laboratory in Piracicaba, SP.

The application of 1-MCP was performed in the Laboratory of Physiology and Biochemistry Postharvest of "Luiz de Queiroz" College of Agriculture (ESALQ/USP) in Piracicaba, SP. 1-MCP gas was prepared from SmartFresh (Agrofresh, Philadelphia) commercial powder (active ingredient 0.14%) at concentration of 500 nL L^{-1}. Predetermined amount of Smartfresh® were placed in flasks with lids and 5 mL of distilled water were added, flasks were shaken until complete dissolution. Then flasks were opened inside hermetic chambers containing the tomatoes. Fruit were treated for 12 h at room temperature

(25±2°C). 1-MCP concentration is in accordance with recommendations for tomatoes of SmartFresh® and previous studies (GUILLEN et al., 2007; GUILLEN et al., 2006; CANTWELL et al., 2009).

Commercial carnauba coating Megh Wax ECF-124 (composed of carnauba wax emulsion, anionic surfactant, preservative and water) was provided by Megh Indústria e Comércio Ltda (SP, Brazil). Carnauba coating was manually applied using brushes with the original concentration according to manufacturer's recommendations (1 L 1000 kg^{-1}) and tomatoes were air-dried at room temperature overnight. Previous studies support carnauba coating as an alternative to maintain postharvest quality in tomatoes (CHIUMARELLI; FERREIRA, 2006; DAVILA-AVIÑA et al., 2011). The procedure was realized in the Laboratory of Human Nutrition and Bromatology, in Piracicaba, SP.

Control group received no treatment and was maintained at room temperature until the other treatments were performed (within 24 hours after harvest).

Subsequently to treatments, tomato samples were packed on 300 g capacity commercial packages (polyethylene terephthalate, PET) commonly used for tomatoes, except for gamma radiation treated tomatoes that were package before treatment, and stored at room temperature (25±2°C) for 30 days. During storage, fruits samples of each group were taken on days 0, 6, 12, 18, 24 and 30 after postharvest treatments to analyze fruit firmness, color, soluble solids and titratable acidity.

To analyze mass loss tomato fruits from each treatment were separated in different packages, in order to asses de same samples during storage.

4.2.3 Mass loss

Mass loss was determined by calculating the difference between the initial mass of fresh fruits and the mass at the time of each assessment, measured by semi-analytical scales. The results were reported as mass loss percentage. Four replications with ten fruits were used per treatment.

4.2.4 Fruit firmness

In order to assess firmness, four replications with five fruits were sampled per treatment per day of assessment. Firmness was determined by the flattening method proposed by Calbo and Nery (1995), with fruits being evaluated over a 30-day period, at six-day intervals. In a horizontal flattener, fruit receive pressure from a test point of 0.902 kg. In the test point basis, a small acrylic plate horizontally acts directly on the surface of the fruit,

always at the same point previously marked in the equatorial region, where it remain for 15 seconds. The direct pressure on the fruit promotes the formation of a contact surface with ellipsoidal shape. By a digital caliper the smaller (a) and larger diameter (b) of the ellipsoid delineated were measured and the surface area was calculated by the expression A = a x b x 0.7854. The firmness was then determined by dividing the test point and flat area (A). The results of this relationship were expressed in N m^{-2}.

4.2.5 Total and soluble pectin

Pectic substances were extracted from tomato fruits following the technique described by McReady and McComb (1952) and total and soluble pectin were determined colorimetrically according to the method of Bitter and Muir (1962). The results were expressed in mg of galacturonic acid per 100 g of pulp ± standard deviations of three replications. The percentage of pectin solubilization was obtained using the following equation: % solubilization = [(soluble pectin content/total pectin content) x 100].

4.2.6 Color

Three readings were made on top, equatorial region and bottom of each fruit with a Minolta colorimeter, model CR-400 (Minolta Co., Japan), using CIELAB scale (L*, a*, b*). (L) lightness (0 = black and 100 = white), a^* ranging from green (a-) to red (a+), b^* ranging from blue (b-) to yellow (b+). Hue angle (°Hue) was calculated by the equation: °Hue = arctg (b*/a*). The results are means ± standard deviation of 16 fruits per treatment per day of evaluation.

4.2.7 Soluble solids and titratable acidity

Ten tomatoes from each treatment were ground in a blender in triplicate (n=3) and the grounded pulp was used to determine the soluble solids (SS) concentration and titratable acidity (TA). Total Soluble Solid (SS) content of tomato fruits was determined by using an Abbe refractrometer (Gehaka, Brazil) by placing a drop of filtered pulp solution on its prism. The TSS was obtained from direct reading of the refractrometer and temperature correction was calculated as described by Rangana (1979). Results were expressed in percentage.

Titratable acidity (TA) was determined by potentiometric titration with 0.1 mol L^{-1} NaOH up to pH 8.1, using 10 g of diluted pulp in 100 mL distilled water (AOAC, 2000). The results were expressed in percentage of citric acid in the pulp. The ratio between SS and TA was also calculated.

4.2.8 Statistical analysis

Statistical analysis was performed using SAS software version 9.0 (SAS Institute, Cary, NC, USA). The data were submitted to the Shapiro-Wilk and Box-Cox tests to verify the normality and homogeneity of variance among the treatments. Then analysis of variance (ANOVA) was carried out by the F test ($P<0.05$ and $P<0.01$) in order to study the factors - treatments and periods of storage - as well as the interaction between them. According to the significance, the means were compared by the Tukey test ($P<0.05$). When appropriate the means of the quantitative data were submitted to regression analysis ($P<0.05$). A Pearson correlation was carried out to study the relationship between soluble pectin content and fruit firmness and between pectin solubilization and fruit firmness. The values were recorded as means ± standard deviations.

4.3 Results and Discussion

The mass loss (%) of tomatoes during storage is shown in Figure 1. The interaction between treatments and days of storage was significant ($P<0.05$) and the mass loss of tomatoes increased linearly for all treatments with the storage period. On days 0 and 6 the mass loss of postharvest treated tomatoes did not differ from untreated ones, but from the 12^{th} day until the end of storage 1-MCP and carnauba reduced mass loss in tomatoes. Gamma radiation treatment differed from control only by the end of storage (day 30) when showed the highest percentage of mass loss among treatments. Whereas control and gamma radiation treated fruits lost 11.9% and 12.9% of mass by the end of storage, respectively, carnauba coated fruits lost 8.9% and 1-MCP fruits 9.9% by the end of the 30 days.

Tomatoes are living tissues and continue to respire and transpire during storage (RAMASWAMY, 2014); therefore, as a normal process, increasing in mass loss is observed. On the other hand, treatments like carnauba and 1-MCP had the effect of delay mass loss in tomato fruits, probably due to both treatments decreased the respiratory rate. In addition, carnauba coating promotes a physical barrier against water loss (MARTÍNEZ-ROMERO et al., 2006). Similarly results were observed by Zhuang and Huang (2003), Chiumarelli and Ferreira (2006) and Davila-Aviña et al. (2011) for tomatoes treated with wax coating and by Guillen et al. (2007) for tomatoes treated with 1-MCP (0.5 $\mu L\ L^{-1}$).

However, gamma radiation treatment increased mass loss in tomatoes by the end of storage. This phenomenon might be associated to the maturation stage of tomatoes at the time of irradiation treatment, once in the present study the fruits were irradiated in an advanced maturation stage (light-red). Depending of ripening stage, climacteric fruits submitted to

gamma radiation may respond either a delay of ripening (AKAMINE; MOY, 1983; URBAIN, 1986; THOMAS, 1988) or an advance (MAXIE et al., 1966). Studies that present mass loss results for tomatoes treated with gamma radiation in more advanced stages of maturation are limited. Castricini et al. (2004), observerd gamma radiation at the doses 0.25, 0.5 and 1 kGy presented no differences in mass loss of tomatoes (conventional size) harvest at ripe stage and stored at room temperature.

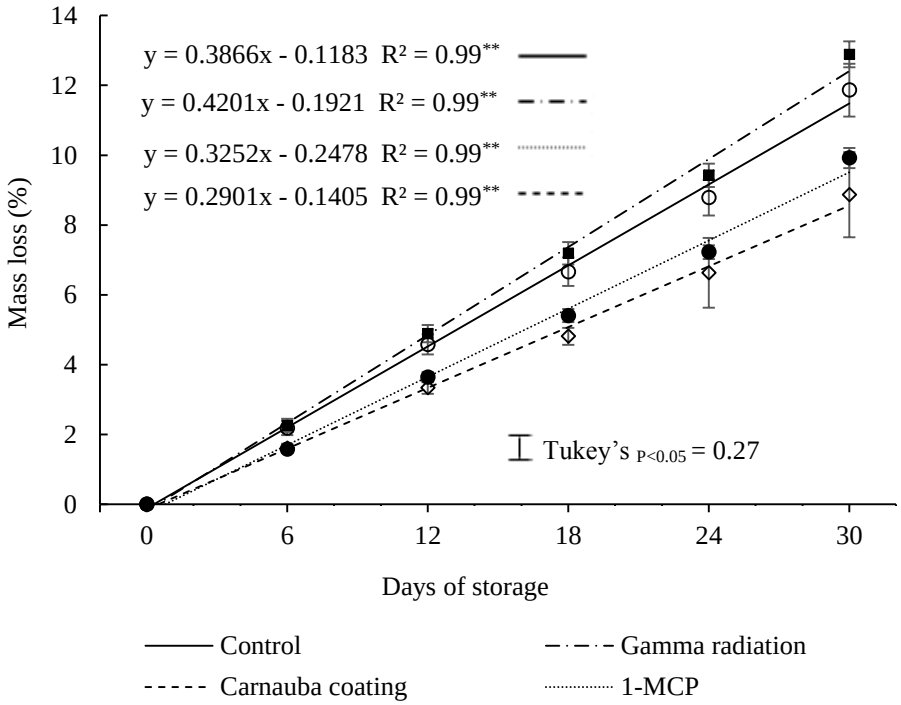

Figure 1 – Mass loss (%) of mini tomatoes treated with gamma radiation, 1-MCP and carnauba coating during storage. Vertical bar indicate least significant difference by Tukey's test ($P<0.05$) among treatments. Each observation is mean ± Standard Deviation (n=4).

Treatments and storage time both impacted firmness (Figure 2), but there was no significant interaction between these two factors. Tomatoes treated with gamma radiation were significantly ($P<0.05$) softer or less firm when compared to the other groups, while carnauba and 1-MCP treatments delayed fruit firmness loss (Figure 2a). Additionaly, fruit firmness tended to decrease during storage for all treatments (Figure 2b). Corroborating with our results Guillen et al. (2007), Zhang et al. (2009) and Guillen et al. (2006) verified higher fruit firmness for tomatoes treated with 1-MCP in comparison to control. Ali et al. (2010) and Chiumarelli and Ferreira (2006) also reported a delaying in loss of fruit firmness for tomatoes treated with edible coatings (ALI et al., 2010; CHIUMARELLI; FERREIRA, 2006).

The conversion of insoluble pectin to soluble pectin by pectinolytic enzymes during ripening is one of the most ethylene-sensitive processes, which promotes fruit softening (LELIEVRE et al., 1997). As an inhibitor of ethylene, 1-MCP treatment reduced the activity of pectinolytic enzymes, thus decreasing the loss of firmness in tomatoes. Edible coatings also delay fruit firmness due to the limitation of pectinolytic enzymes by the reduction of respiration rates promoted by the coating barrier, which decline levels of O_2, and CO_2 (SALUNKHE et al., 1991). In addition, the coated decrease the water vapor transmission rate, which prevents firmness reduction by preserving the cell turgor (PEREZ-GAGO; GONZALEZ-AGUILAR; OLIVAS, 2010).

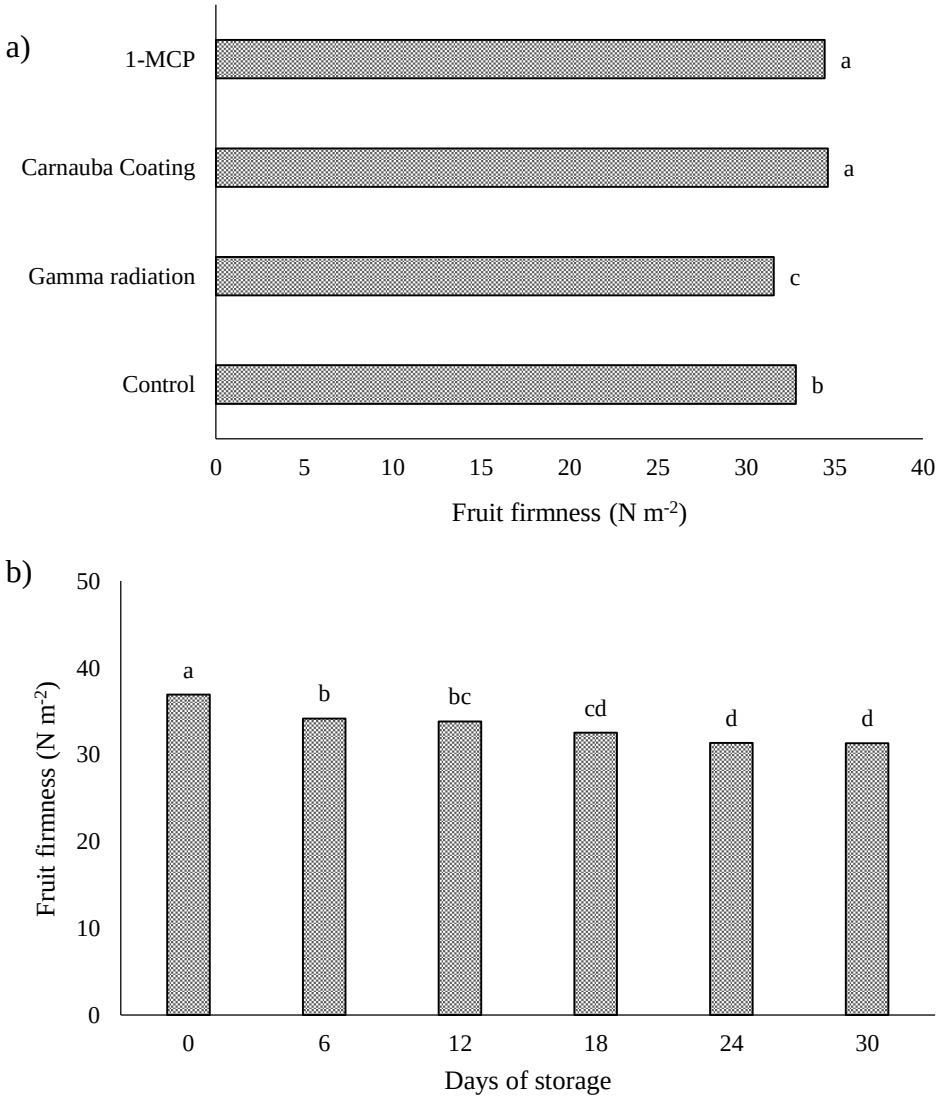

Figure 2 – Fruit firmness means (N m^{-2}) of diferent treatments (a) and of diferent days of storage (b) of mini tomatoes treated with gamma radiation, 1-MCP and carnauba coating during storage. Each observation is mean ± Standard Deviation (n=4).

It is clear in literature gamma radiation softening effects in fresh fruits is dose dependent (YASIA; CHACHIN; IWATA, 1987; ASSI; HUBER; BRECHT, 1997; PRAKASH et al., 2002; AKTER; KHAN, 2012), i.e., higher doses promote greater firmness losses. However, cultivar and maturation stage are also related (BRAMLAGE; LIPTON, 1965; ABDEL-KADER; MORRIS; MAXIE, 1968). Bramlage and Lipton (1965) observed riper tomatoes lost firmness more immediately following irradiation than mature green or breaker fruits. Furthermore Adam et al. (2014) showed gamma radiation (0.25, 0.5 and 1 kGy) treatment in two cultivars of tomatoes harvested at breaker stage delayed fruit firmness, independent of the dose. Nevetheless, Assi, Huber and Brecht (1997) reported the effect of gamma radiation on pink tomatoes was in smaller proportions than for mature green fruit. They observed softening effects of irradiation on pink tomatoes were more pronounced in the initial days of storage, but did not persist by the end of storage, while for mature green fruits the effects on decrease firmness were persistent. Different from the present study, they used higher doses of gamma radiation (0.72, 0.73, 1.41 and 2.21 kGy) and tomatoes were harvested at pink stage, a stage before of light-red. Probably in the present study, due to the advanced stage of fruit maturation, gamma radiation treatment stimulated ethylene synthesis instead of delay ripening, which is one of the effects of irradiation (LARRIGAUDIÈRE et al., 1991).

Losses in fruit firmness induced by irradiation have been associated with changes in cell wall components, mainly by an accelerated breakdown of pectin and other structural polysaccharides (PRAKASH et al., 2002; McDONALD et al., 2012), which may be confirmed in the presente study. Total and soluble pectin and % of pectin solubilization are shown in Figure 3. Significant interaction between treatments and days of storage was observed for total and soluble pectin as well as pectin solubilization ($P<0.05$). Tomatoes treated with gamma radiation showed higher values of soluble pectin on the most days of storage (Figure 3b) as well as the % of solubilization was significantly higher for irradiated fruits (Figure 3c). Studies have reported there is a link between changes in soluble pectin and softening in tomatoes and other fruits (PRAKASH et al., 2002; GUNES; HOTCHKISS; WATKINS, 2001). Althoug there was no significant correlation between soluble pectin and fruit firmness, our results demonstrated a significant inverse correlation (r = -0.5848, $P<0.05$) between fruit firmness and pectin solubilization for gamma radiation treated tomatoes. According to Costa (2000), soluble pectin seems to be a major cause of tissue softening in tomatoes treated with gamma radiation, although changes in other cell wall components such as cellulose, hemicellulose, pectin enzymes, and osmotic equilibrium could also contribute to loss of firmness.

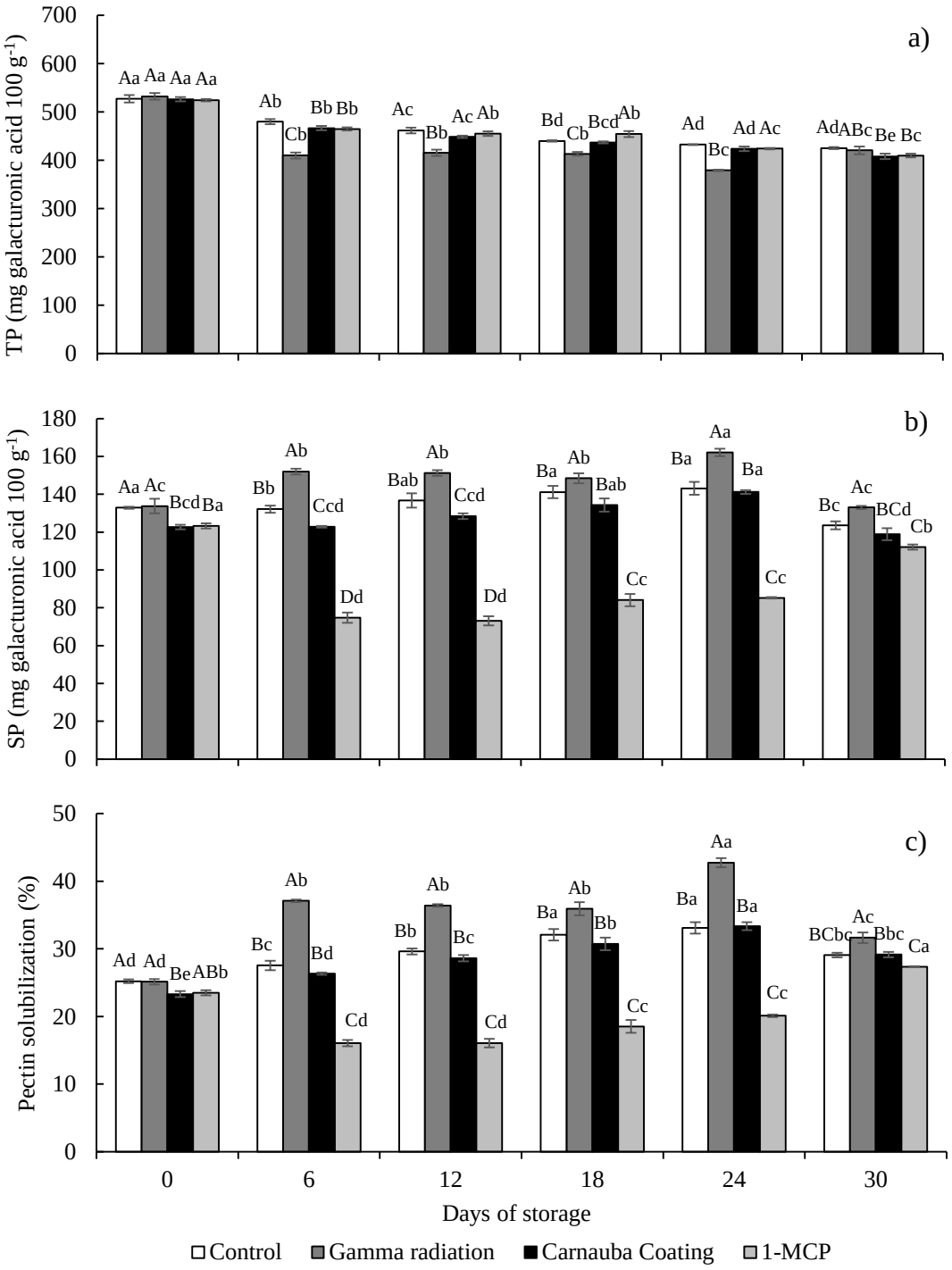

Figure 3 – Total pectin content (TP) (mg galacturonic acid 100 g^{-1} of fresh weight) (a), soluble pectin (SP) (mg galacturonic acid 100 g^{-1} of fresh weight) (b) and % of pectin solubilization (c) of mini tomatoes treated with gamma radiation, 1-MCP and carnauba coating during storage. Different capital letters indicate significant differences among treatments and small letters indicate significant diferences among days of storage by Tukey's test ($P<0.05$). Each observation is a mean ± Standard Deviation (n=3).

Total pectin tended to decrease for all treatments during storage (Figure 3a). This phenomeon is normal during ripening process and occurred, mainly, due to pectinametylsterase and poligalacturonase enzymes, which promote pectin degradation, resulting in softening of tissues (FISCHER; BENNETT, 1991). The initial content of total pectin (day 0) has no difference among treatments, however from the 6th day of storage to the 24th day tomatoes treated with gamma radiation showed lower total pectin content.

Different from total pectin, soluble pectin (Figure 3b) and the % of pectin solubilization (Figure 3c) tended to increase over 24 days of storage, except for 1-MCP whose fruits presented a decrease in soluble pectin as well as % of solubilization from day 0 to the 6th day of storage remaining with lower values. Then, soluble pectin content increased from the 18th day by the end of storage. Reduction in soluble pectin contents was aslo observed for papaya treated with 1-MCP (ASMAR et al., 2010). For the other treatments from the 24th day to the 30th day of storage, soluble pectin and % of solubilization decreased. Fruit receiving 1-MCP and carnauba treatment retained fruit firmness longer, therefore soluble pectin and % of solubilization for these treatments had lower values (when treated with 1-MCP) or intermediate values (for carnauba treated tomatoes - day 0, 6, and 12), since this treatments delayed ripening process as well as the solubilization of pectins. Correlation between fruit firmness and soluble pectin or % of solubilization were not significant ($P>0.05$) for tomatoes treated with carnauba and 1-MCP or untreated fruits.

During ripening, chlorophyll, the green pigment is degraded and there is accumulation of carotenoids, particularly lycopene giving the red color to ripe tomatoes (KHUDAIRI, 1972). In the present study, tomatoes did not change from green to red because they were harvested in advanced mature stage, but they changed from light-red to dark or deep red. Figure 4 shows the effects of postharvest treatments on the color attributes (L^* and hue angle) of tomato fruits stored at 25±2°C. Significant interaction ($P<0.05$) between treatments and storage time on the L^* and Hue angle values of tomato fruits was observed. A decrease in L^* values of tomato fruits treated with gamma radiation, carnauba coating and control was observed from day 0 to day 6, remaining constant thereafter. L^* values of 1-MCP treated tomatoes decreased from day 0 to day 12 and then remained constant by the end of storage (Figure 4a). On the first day of storage (day 0) carnauba and 1-MCP fruits showed higher L^* values than control fruits, but 1-MCP did not differ from gamma radiation values. This indicate a delaying in color development following the application of 1-MCP and carnauba treatments, once high values of L^* represents lighter colors (ARIAS et al., 2000). The delaying persisted over 24 days of storage only for 1-MCP treated fruits, while tomatoes

coated with carnauba accompanied the changes just as the other treatmets. No differences among L^* values of carnauba, gamma radiation and control fruits were observed from day 6 until the end of storage and, on the 30th day L^* values of 1-MCP fruits equated to the other treatments.

Hue angle was higher for carnauba and 1-MCP treated tomatoes at day 0, although 1-MCP did not differ from control (Figure 4b). This suggests a delay in devolopment of red color followed by treatments application, since hue of 180° represents pure green and a hue of 0°, pure red (SHEWFELT; THAI; DAVIS, 1988). Similarly to L^* results, the dalaying in red color development persisted to 1-MCP fruits until the 18th day of storage, while Hue angle of carnauba tomatoes equated to the other treatments since the 6th day of storage. However, at 24 and 30 days of storage no differences among treatments were observed and the hue angle reached around 42° by the end of storage for all treatments.

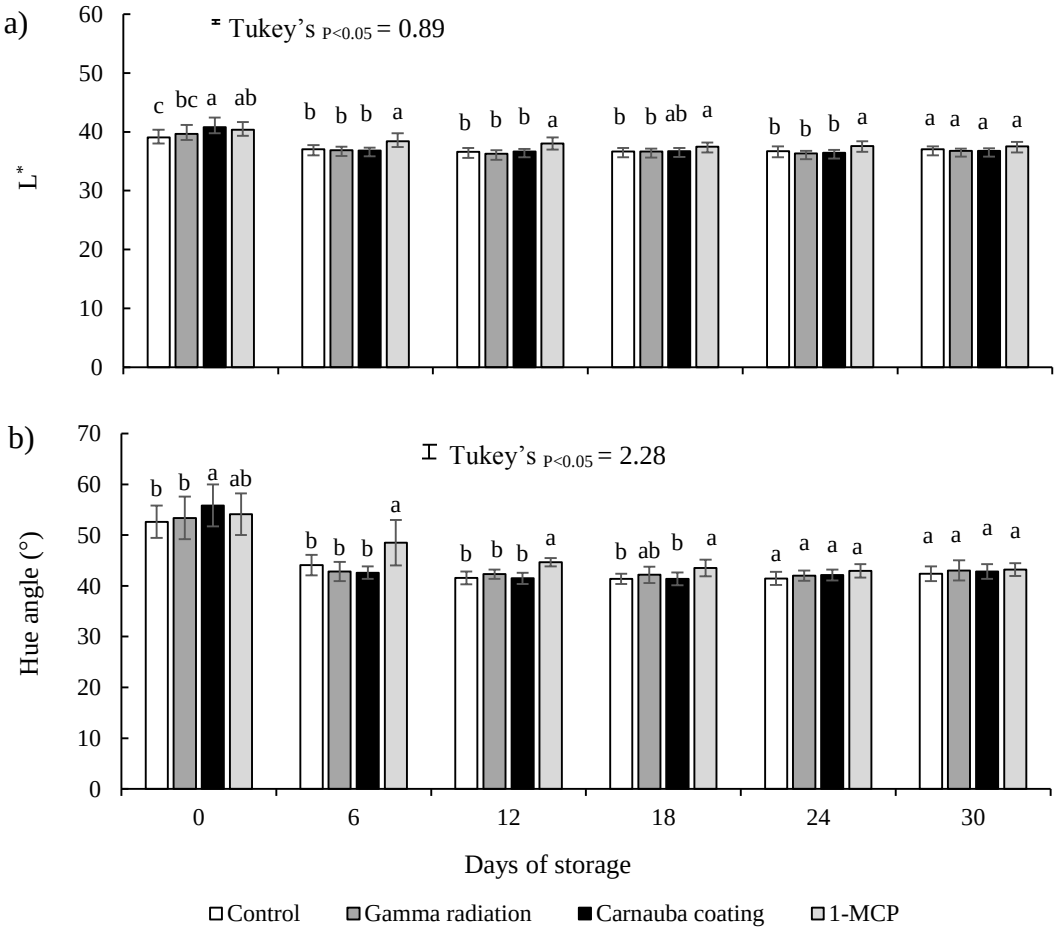

Figure 4 – Lightness (L^*) (a) and Hue angle (°) (b) of mini tomatoes treated with gamma radiation, 1-MCP and carnauba coating during storage. Different letters indicate significant differences among days of storage by Tukey's test (P<0.05). Vertical bars indicate least significant difference by Tukey's test (P<0.05) among treatments. Each observation is mean ± Standard Deviation (n=16).

As 1-MCP blocks the ethylene action, a delaying in normal ripening process is observed, resulting in retarding of color development. Postponing in color development in tomatoes harvest in more advanced stages and treated with 1-MCP have also been reported by Cantwell et al. (2009), Guillen et al. (2006) and Ilic et al. (2013). However, despite the delaing color development, 1-MCP treatment did not affect the final color of tomatoes, which were similar to control and other treatments. Probably, this fact occurred due to maturation stage of tomatoes at harvested. When 1-MCP is applied at early stages of maturity pigment synthesis and expression is more strongly delayed (MORETTI et al., 2001), but when applied in avanced maturity stages, color is less affected by 1-MCP treatment (ERGUN; SARGENT; HUBER, 2006). This is in agreement with our results for breaker tomatoes treated with 1-MCP (chapter 2) that even in the end of storage presented higher Hue angle in comparison to the other treatments.

Similar to 1-MCP, carnauba coating tomatoes presented a delaying in color development as reported by Ali et al. (2010), however this effect was less pronounced and occurred immediately after treatment, equating to control thereafter.

The changes in soluble solids (SS), titratable acidity (TA) and ratio SS/TA of light-red tomatoes submitted to different postharvest treatments are shown in Table 1. The interaction between two factors (treatments and days of storage) was significant ($P<0.05$) for the three characteristics.

In general, tomato SS content ranged from 6.83 to 5.57 °Brix. There was a slight decrease in SS content for control, carnauba and gamma radiation tomatoes from day 0 to the 6^{th} day of storage, remaining constant by the complete storage period for gamma radiation and control, because in the last day of storage carnauba treated tomatoes showed another slight decrease. SS of 1-MCP treated tomatoes decreased from 6^{th} day to the 12^{th} day of storage, remaining constant until the 18^{th} day when decreased again (Table 1). Regarding differences among treatments, the initial content (day 0) was similar to all treatments. In this day forward, SS contents presented some fluctuations and vary widely. 1-MCP maintained higher SS values in tomatoes than control on the 6^{th} and 12^{th} day of storage, although did not differ from SS content of carnauba and gamma radiation tomatoes. At the 18^{th} day of storage, no differences for SS content were observed among treatments. On the 24^{th} day of storage, SS of 1-MCP treated tomatoes decreased and was lower than the content of irradiated and coated fruits, but did not differ from control. By the end of storage, gamma radiation treatment showed the higher SS content (6.27°Brix) in comparison to other treatments, this value was constant since the 12^{th} day of storage.

Table 1 – Soluble solids content (°Brix), titratable acidity (g citric acid 100 g^{-1}) and SS/AT ratio of mini tomatoes treated with gamma radiation, carnauba coating and 1-MCP during storage[1].

Treatment	Days of storage					
	0	6	12	18	24	30
			Soluble Solids (°Brix)			
Control	6.83 ± 0.06 ^{Aa}	6.03 ± 0.12 ^{Cb}	6.10 ± 0.10 ^{Bb}	6.10 ± 0.00 ^{Ab}	5.93 ± 0.06 ^{BCb}	5.77 ± 0.06 ^{Bb}
Irradiated	6.73 ± 0.06 ^{Aa}	6.50 ± 0.10 ^{Bab}	6.27 ± 0.15 ^{ABb}	6.27 ± 0.15 ^{Ab}	6.27 ± 0.25 ^{Ab}	6.27 ± 0.06 ^{Ab}
Carnauba coating	6.73 ± 0.06 ^{Aa}	6.27 ± 0.12 ^{BCb}	6.43 ± 0.23 ^{Aab}	6.17 ± 0.06 ^{Ab}	6.10 ± 0.00 ^{ABb}	5.57 ± 0.06 ^{Bc}
1-MCP	6.70 ± 0.10 ^{Aa}	6.83 ± 0.12 ^{Aa}	6.27 ± 0.25 ^{ABb}	6.13 ± 0.25 ^{Ab}	5.77 ± 0.29 ^{Cc}	5.60 ± 0.10 ^{Bc}
			Titratable Acidity (g citric acid 100 g^{-1})			
Control	0.62 ± 0.00 ^{Aa}	0.54 ± 0.01 ^{Bb}	0.49 ± 0.01 ^{Ab}	0.47 ± 0.00 ^{Ab}	0.40 ± 0.00 ^{ABc}	0.37 ± 0.01 ^{Ac}
Irradiated	0.62 ± 0.01 ^{Aa}	0.52 ± 0.00 ^{Bb}	0.50 ± 0.05 ^{Ab}	0.44 ± 0.02 ^{Bc}	0.36 ± 0.01 ^{Bd}	0.37 ± 0.01 ^{Acd}
Carauba coating	0.64 ± 0.02 ^{Aa}	0.54 ± 0.01 ^{Bb}	0.48 ± 0.01 ^{Ab}	0.44 ± 0.01 ^{Bc}	0.40 ± 0.01 ^{ABc}	0.38 ± 0.01 ^{Ac}
1-MCP	0.64 ± 0.00 ^{Aa}	0.62 ± 0.01 ^{Aa}	0.54 ± 0.00 ^{Ab}	0.49 ± 0.01 ^{Ab}	0.44 ± 0.02 ^{Ac}	0.39 ± 0.01 ^{Ac}
			Ratio SS/TA			
Control	11.11 ± 0.14 ^{Ac}	11.27 ± 0.36 ^{Bc}	12.57 ± 0.24 ^{Ab}	12.96 ± 0.04 ^{BCd}	14.77 ± 0.32 ^{Ba}	15.60 ± 0.37 ^{Ba}
Irradiated	10.90 ± 0.15 ^{Ad}	12.55 ± 0.17 ^{ABc}	12.52 ± 0.63 ^{ABc}	14.22 ± 1.02 ^{Ab}	17.61 ± 0.36 ^{Aa}	16.95 ± 0.67 ^{Aa}
Carauba coating	10.57 ± 0.37 ^{Ad}	11.70 ± 0.35 ^{ABd}	13.32 ± 0.21 ^{Ac}	13.91 ± 0.38 ^{ABbc}	15.36 ± 0.56 ^{Ba}	14.62 ± 0.46 ^{BCab}
1-MCP	10.39 ± 0.19 ^{Ad}	10.94 ± 0.28 ^{Bd}	11.54 ± 0.46 ^{Bcd}	12.40 ± 0.36 ^{Cbc}	13.06 ± 1.22 ^{Cb}	14.31 ± 0.11 ^{Ca}

[1] Data are means ± Standard Deviation (n=3). Means followed by same capital letter on column (within the same compound) and small letter on line were not significantly different by Tukey's test (P>0.05).

Studies have suggested that application of 1-MCP may prevent quickly changes in SS in tomatoes due to the delaying in ripening process (ERGUN; SARGENT; HUBER, 2006; GUILLEN et al., 2007), while gamma radiation (doses until 3 kGy) and edible coatings treatments may not significantly change SS from the untreated fruit (SHURONG et al., 2005; PRAKASH et al., 2002; AKTER; KHAN, 2012; MEJIA-TORRES et al., 2009), which was not observed in the present study for irradiated fruits.

TA of tomatoes decreased during storage for all treatments (Table 1). Decreasing in TA is a normal process related to organic acids reduction during fruit ripening by the oxidation process in order to produce energy (CHITARRA; CHITARRA, 2005). Despite some variations during storage, on the 0, 12th, 24th and 30th day of storage there were no differences among TA of treatments and the TA of control fruits. These results suggested the postharvest treatments (carnauba, 1-MCP and gamma radiation) applied in light-red tomatoes did not strongly influenced titratable acidity. Corroborating with our results Prakash et al. (2002), Patil, Vanamala and Hallman (2004), Zhang et al. (2014) showed no differences for titratable acidity between irradiated and non-irradiated tomatoes, grapefruit and citrus, respectively. Ergun, Sargent and Huber (2006) also demonstrated light-red tomatoes treated with 1-MCP showed no differences in TA when compared to control fruits. Finally, tomatoes harvested at pink-stage and coated with carnauba and mineral oil presented no differences from control in relation to TA content (DAVILA-AVIÑA et al., 2011).

SS/TA ratio increased during storage for all treatments. However, the increased was lower for 1-MCP fruits from the 12th day by the end of storage when compared to control fruits, mainly due to low contents of SS, since TA of 1-MCP fruits was not dramatically affected. On the contrary, for gamma radiation treated fruits the SS/TA ratio was higher than control from the 18th until the end of storage, while the SS/TA ratio of carnauba tomatoes did not significantly differ from control during all the period.

4.4 Conclusions

In conclusion, mini tomatoes harvest at light-red stage and stored for 30 days showed changes in all evaluated characteristics. The most effective treatments for delaying fruit firmness and mass loss was carnauba and 1-MCP, while gamma radiation was the treatment with higher mass loss and the less firmness. This result could be associated with the higher solubilization of pectins promoted by gamma radiation treatment in light red tomatoes. 1-MCP fruits presented lower contents of soluble pectins as well as lower % of pectin solubilization. Color (L^* and Hue) was mainly affected by 1-MCP treatment which delayed

color development, however, by the end of storage, color development was equated to the other treatments. SS/TA ratio was lower for fruits treated with 1-MCP and TA was not so dramatically affected by treatments.

Considering the better SS/TA ratio, which is related to quality, it is very indicated tomatoes are harvested in advanced stages of maturation. Further, in order to maintain this postharvest quality, among the tested treatments, carnauba and 1-MCP seems to be the better choice.

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5 EFFECTS OF POSTHARVEST TREATMENTS ON BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF MINI TOMATOES DURING STORAGE

Abstract

This study aimed to investigate the effect of gamma irradiation, carnauba coating and 1-methylcyclopropene (1-MCP) on the carotenoids profile, lycopene isomerization and antioxidant capacity of mini tomatoes cv. Sweet Grape. Fruits were harvested in light-red stage of maturation and treated with gamma radiation (0.6 kGy), carnauba coating (1 L 1000 kg^{-1}) and 1-MCP (500 nL L^{-1}) and then stored at 25±2°C for 30 days. Carotenoids profile, lycopene isomers, phenolic compounds, ascorbic acid and antioxidant capacity were evaluated in mini tomatoes, on days 0, 6, 12, 18, 24, 30 post treatment by a C_{30}-high performance liquid chromatography (carotenoids) and ABTS method (antioxidant capacity). Results demonstrated irradiation induced changes in the final content of lycopene, increasing it, and formed less (13*Z*)-lycopene, while 1-MCP and carnauba coating slow down the increase in lycopene and slown down decrease of ascorbic acid and phenolic compounds. Antioxidant capacity of lipophilic fraction was not affected by treatments and hydrophilic fraction was lower for irradiated fruits only on day 0 as well as phenolic compounds. In the other days of storage, no diferences among treatments were observed for hydrophilic antioxidant capacity.

Keywords: gamma radiation, carnauba coating, 1-methylcyclopropene, lycopene, β-carotene, lycopene isomers

5.1 Introduction

Tomatoes (*Solanum lycopersicum*) are one of the most popular and widely used vegetables in the world (GRANDILLO; ZAMIR; TANKSLEY, 1999; STAJCIC et al., 2015). Either as a fresh fruit or processed products, tomatoes provide a large variety of nutrients and health benefits (GIOVANUCCI, 1999; MOCO et al., 2006), therefore has assumed the status of functional food (ALSHATWI et al., 2010). Epidemiologic studies suggest dietary intake of tomato and tomato-based products reduces risk of certain types of cancer (NGUYEN; SCHWARTZ, 1999; GIOVANNUCCI, 1999; GIOVANUCCI et al., 2002), and cardiovascular diseases (WILLCOX; CATIGNANI; LAZARUS, 2003).

The beneficial role of tomato consumption has been attributed to antioxidant components such as ascorbic acid, phenolic compounds, tocopherols and carotenoids (particularly lycopene and β-carotene), besides the synergistic interaction among them (MARTINEZ-VALVERDE et al., 2002; GEORGE et al., 2004). Carotenoids are responsible for the final red color of tomatoes and in addition to lycopene and β-carotene, α-carotene, lutein, zeaxanthin, α-cryptoxanthin and β-cryptoxanthin, are other carotenoids commonly

reported in tomato and tomato products (FRASER et al., 1994; KHACHIK et al., 2002; BURNS et al., 2003). Among these, lycopene constitutes about 80 to 90% of total carotenoid content of red ripe tomatoes (SHI; LE MAGUER, 2000) and is the most efficient antioxidant among carotenoids, while β-carotene accounts for around 7% of tomato carotenoids (NGUYEN; SCHWARTZ, 1999) and is a dietary precursor of vitamin A (BURNS et al., 2003). Naturally, carotenoids occur predominantly as all-*trans* configuration, which is thermodynamically the more stable isomer. However, food processing may increase the formation of *cis*-isomers, which possess different biological properties (SCHIEBER; CARLE, 2005).

Aside from food processing, bioactive compounds in tomato are influenced by several factors and varies considerably according the genetic potential of cultivars, ripening stage, growing conditions (SAHLIN; SAVAGE; LISTER, 2004; HERNÁNDEZ; RODRÍGUEZ; DÍAZ, 2007; NOUR; TRANDAFIR; IONICA, 2014), postharvest handling and treatments (TOOR; SAVAGE; HEEB, 2006; HERNÁNDEZ; RODRÍGUEZ; DÍAZ, 2007). Relatively short postharvest life and the perishable nature of tomatoes lead to great losses (KUMAR et al., 2014), often requiring postharvest treatments to delay ripening and senescence and maintain fruit quality during storage. Therefore, different techniques have been developed to extend shelf life of fresh fruit like refrigeration, disinfection, ethylene absorbers, gamma radiation, edible coatings, chemical dipping, controlled/modified atmosphere, etc. (BICO et al., 2010). For the present study, we will discuss three of these important techniques, edible coatings, gamma radiation and use of 1-methylcyclopropene (1-MCP).

Edible coatings commonly based on natural proteins, lipids or polysaccharides (BAI et al., 2003), have been used to preserve whole or fresh-cut fruit. They act generating a modified atmosphere by creating a barrier against water loss, oxygen and carbon dioxide, reducing respiration and oxidation reaction rates (MARTÍNEZ-ROMERO et al., 2006). In tomatoes, the application of edible coatings is safe and a low cost alternative, which contributes to reduce the fresh mass loss and decrease the number of discarded fruit due to mechanical injury and diseases (CHIUMARELLI; FERREIRA, 2006; DAVILA-AVIÑA et al., 2014).

Another postharvest technique widely used is gamma radiation, which has shown satisfactory results in relation to the extension of shelf life and delay ripening in tomatoes (LARRIGAUDIÈRE et al., 1991; ASSI; HUBER; BRECHT, 1997; CASTRICINI et al., 2004; KUMAR et al., 2014). The safety, economic viability and benefits of radiation treatment has been extensively studied and proven worldwide. According

to the World Health Organization (WHO, 1981) the irradiation of food up to the dose of 10 kGy presents no health risks.

Synthetic compound, 1-MCP is a potential regulator of the ripening of many climacteric fruits including tomatoes (WATKINS, 2008; HUBER, 2008). This compound acts as an inhibitor of ethylene-binding receptors, delaying fruit ripening and senescence (FAN; MATTHEIS, 2000; WILLS; KU, 2002; WATKINS, 2006). Considered safe for human, 1-MCP quickly diffuses from the plant tissue after the treatment (BLANKENSHIP; DOLE, 2003; WATKINS, 2006). Studies have shown 1-MCP prolong the shelf-life of development tomatoes through retaining firmness, delaying lycopene accumulation and external color (KRAMMES et al., 2003; GUILLÉN et al., 2005; HURR; HUBER; LEE, 2005; PUSHPALATHA; SINGH; SRIVASTANA, 2006).

The technological effects of these postharvest treatments on tomatoes have been widely examined. However, little is known about the effects of these treatments on antioxidants, particularly on profile of carotenoids and its isomers. Thus the present study aimed to analyze the effect of gamma radiation, carnauba coating and 1-MCP on the carotenoids profile, lycopene isomerization, phenolic compounds, ascorbic acid and antioxidant capacity.

5.2 Materials and Methods

5.2.1 Plant material

Mini tomatoes cv. “Sweet Grape” (Sakata Seed Sudamerica) at the light-red stage of ripening according to the USDA standard tomato color classification chart (USDA, 1991) were obtained from a commercial crop in Santa Isabel, SP, Brazil (23º18’56”S, 46º13’17”W). Fruits were harvested and transported to the laboratory in Piracicaba, SP, Brazil. Samples were selected based on uniformity in size, color, absence of physical defects and signs of rots. Before postharvest treatments, fruit were washed with chlorinated water (200 ppm) for 2 min and then left to dry at room temperature.

5.2.2 Postharvest treatments

Mini tomatoes were divided into four batches: control (C), irradiated (I), 1-methylcicloprope ne (1-MCP) and carnauba coating (CC). All treatments were performed within 24 h after harvest and the analysis started at the same time for all treatments.

The irradiated group was packed in commercial packages (polyethylene terephthalate, PET) commonly used for tomatoes and transported to Nuclear and Energy Research Institute

(IPEN) in São Paulo, SP. The samples were irradiated in their own plastic package in a Compact Multiporpuse Irradiator (^{60}Co, C-188 model, MDS Nordion Canadá). The applied radiation dosage was 0.6 kGy, which was established taking into account previous studies that suggested 0.6 kGy is within a range considered as effective to delay fruit ripening in tomatoes (ABREU; SOARES; JESUS, 1997; CASTRICINI et al., 2004; FABBRI et al., 2011; AKTER; KHAN, 2012; KUMAR et al., 2014). Dosimetric studies were performed using a gammachrome YR dosimeter to monitor the dose and estimate the dose rate (3.21 kGy h^{-1}). After irradiation, fruits were transported back and stored at room temperature (25±2°C) for 30 days.

1-MCP gas was prepared from SmartFresh (Agrofresh, Philadelphia) commercial powder (active ingredient 0.14%) at concentration of 500 nL L^{-1}. Predetermined amount of Smartfresh® were placed in flasks with lids and 5 mL of distilled water were added, flasks were shaken until complete dissolution. Then flasks were opened inside hermetic chambers containing the tomatoes. Fruit were treated for 12 h at room temperature (25±2°C). 1-MCP concentration is in accordance with recommendations for tomatoes of SmartFresh® and previous studies (GUILLEN et al., 2007; GUILLEN et al., 2006; CANTWELL et al., 2009). After treatment, fruits were packed as irradiated fruits and stored at room temperature (25±2°C) for 30 days.

The third group of tomatoes received carnauba coating treatment. Commercial carnauba coating Megh Wax ECF-124 (composed of carnauba wax emulsion, anionic surfactant, preservative and water) was provided by Megh Indústria e Comércio Ltda (São Paulo, Brazil). Carnauba coating was manually applied using brushes with the original concentration according to manufacturer's recommendations (1 L 1000 kg^{-1}). Previous studies support carnauba coating as an alternative to maintain postharvest quality in tomatoes (CHIUMARELLI; FERREIRA, 2006; DAVILA-AVIÑA et al., 2011). Before packed as irradiated and 1-MCP groups, fruit were dried at room temperature overnight. After packed, fruits were stored at room temperature (25±2°C) for 30 days.

Finally, the fourth group was control and received no treatment. Fruits were packed as other groups and maintained at room temperature (25±2°C) for 30 days.

During storage, fruits samples of each group were taken on days 0, 6, 12, 18, 24 and 30 after postharvest treatments, freeze-dried and stored at -18°C until required to analyze carotenoid profile, lycopene isomers, phenolic compounds, ascorbic acid and antioxidant capacity (H-TEAC and L-TEAC).

5.2.3 Carotenoids extraction

Carotenoids were extracted under subdued light to avoid photo degradation. For extraction, 0.15 g of lyophilized sample was dissolved in 5 mL MiliQ water for 5 min. Then, 35 mL of methanol/tetrahydrofuran (THF) (1/1, v/v) containing 0.1% BHT (to avoid oxidative degradation), 200 mg magnesium oxide, 200 mg sodium sulphate and 100 µL β-apo-8'-carotenal as the internal standard were added to dissolved sample (SEYBOLD et al., 2004). The mixture was homogenized on ice for 5 min using an ultra turrax at 10000 rpm (T25, IKA, Staufen, Germany). The supernatant was filtered under vacuum through filter paper no. 390 (Filtrak, Niederschlag, Germany) on a Büchner funnel. This extraction was repeated at least twice until the residue of the sample was colourless. The combined supernatants were concentrated in a rotary evaporator at reduced pressure and 30°C. The residue was redissolved in methanol/THF (1/1, v/v) containing 0.1% BHT using an ultrasonic bath, until the solution reached the defined volume of 5 mL. The solution was centrifuged for 5 min at 14,000 rpm, and transferred into amber HPLC vials for analysis. Chromatographic analyses (carotenoids and lycopene isomers) were carried out directly after the extraction and 500 µL of the solution were injected into the HPLC system.

5.2.4 Analysis of carotenoids

Carotenoids were measured via high performance liquid chromatography with diode array detection at 450 nm (Merck Hitachi, Darmstadt, Germany). The chromatographic separation was performed at 13 ± 1°C on a Develosil RP-Aqueous (250 mm × 4.6 mm, 5 µm) C30-column (Phenomenex, Aschaffenburg, Germany). Mobile phase consisted of a gradient of MeOH (solvent A) and MtBE (solvent B): initial conditions 90% solvent A and 10% solvent B; 40 min linear gradient to 50% solvent B; 2 min linear gradient to 60% solvent B, 40% solvent A and 60% solvent B for 23 min; 5 min linear gradient to 10% solvent B; and 90% solvent A and 10% solvent B for 5 min. The flow rate was set at 1 mL min^{-1}. The concentrations of (all-*E*)-lutein, (all-*E*)-β-carotene, (13*Z*)-β-carotene and (all-*E*)-lycopene were quantified by 5-point calibration curves of external standards. The concentrations of the stock solutions were checked periodically and were calculated using the specific extinction coefficients (BRITTON; LIAAEN-JENSEN; PFANDER, 2004).

5.2.5 Analysis of lycopene composition

Lycopene isomer composition as well as contents of lycopene were analyzed using an isocratic C_{30}-HPLC method using a Merck−Hitachi HPLC system (Darmstadt, Germany) and

a Jetstream Plus column oven (JASCO, Groß-Umstadt, Germany). A C_{30} column (YMC Europe, Dinslaken, Germany) (250 mm × 4.6 mm, 5 μm), preceded by a C_{18} ProntoSil 120−5-C18 H (10 mm × 4.0 mm, 5 μm) column (Bischoff, Leonberg, Germany) was used. Mobile phase consisted of MtBE/MeOH/ethylacetate (50/45/5, v/v/v) and flow rate was set at 0.4 mL min^{-1}. Column temperature was 32±1°C and detection wavelength 470 nm. Lycopene contents were quantified by 5-point calibration curve of external standard. Retention time of (*Z*)-isomers in relation to that of (all-*E*)-lycopene was used to identify lycopene isomers, which are presented as ratios of (all-*E*)-lycopene/(*Z*)-isomer. Thus, exact contents of different lycopene isomers were not determined.

5.2.6 Total phenolic compounds

Total phenolic contents was determined based on the Folin-Ciocalteu method as described by Woisky and Salatino (1998), using gallic acid as standard for the calibration curve. Samples were mixed in 50-time volume of aqueous ethanol (80%) under subdued light in a shaker water bath at 40°C for 30 min. The homogenate was centrifuge at 5000 rpm for 15 minutes and supernatant was recovered. 0.5 mL of the extract was taken and added of 2.5 mL of Folin-Ciocalteu reagent (10%). After 5 minutes, 2 mL of sodium carbonate (4%) was added and the content was mixed thoroughly and let in the dark for 60 min. Absorbance was measured at 740 nm in a spectrophotometer (UNICO, model 2800 UV/Vis, Interprise, Brazil).

5.2.7 Ascorbic acid

Ascorbic acid was estimated by the method of AOAC (1984) modified by Benassi and Antunes (1988). Samples were homogeneized with 1% oxalic acid (1:10 m/v) and titrated against 2,6-dichlorophenol-indophenol dye. The ascorbic acid content in samples was determined from the standard ascorbic acid and the results were expressed in mg of ascorbic acid per 100 g of fresh weight.

5.2.8 Antioxidant capacity

For determination of antioxidant capacity, two versions (hydrophilic and lipophilic) of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay were conducted. This assay is based on the decolorization of the ABTS•+ (2,2'-azino-bis-(3-ethylbenzo-thiazoline-6- sulphonic acid) at approximately 730 nm to determine the antioxidant capacity (RE et al., 1999). The method was described by Miller et al. (1996) and was modified slightly by numerous researchers.

α-TEAC Assay

The lipophilic α-tocopherol (α-TE) antioxidant capacity (α-TEAC) assay was performed according to Müller, Theile and Böhm (2010) and calibrated with α-tocopherol instead of Trolox.

Sample preparation consisted of added 2 mL of *n*-hexane to the sample, shake for 30 s and centrifuge at 5000 rpm for 5 min. The supernatant was taken and this extraction process was repeated at least 5 times until the residue of the sample was colourless. The combined supernatants were concentrated in a rotary evaporator at reduced pressure and 30°C. The residue was redissolved in *n*-hexane using an ultrasonic bath, until the solution reached the final volume of 2 mL. The solution was centrifuged for 2 min at 13000 rpm.

The radical cation $ABTS^{\bullet+}$ was prepared by filtering an ABTS solution (tip of a spatula ABTS dissolved in PBS buffer) through a filter paper coated with manganese dioxide, followed by membrane filtration (0.2 µm). An $ABTS^{\bullet+}$ working solution was produced daily by diluting with 75 mM phosphate buffer (pH 7.4) to an absorbance of 0.70 ± 0.05 at 734 nm.

For the measurement, 100 µL of sample extract, or standard (ca. 4.5-125 µmol α-TE L^{-1}), or blank (*n*-hexane) and 1000 µL of adjusted $ABTS^{\bullet+}$ solution were vortexed for 30 s in reaction tubes. Following, the mixture was transferred into half micro-cuvettes and centrifuged for 30 s at 1200 rpm to separate phases. Exactly 2 min after starting mixing, the absorbance of the lower phase was measured at 734 nm in a V-530 spectrophotometer (Jasco, Gross-Umstadt, Germany).

H-TEAC Assay

To analyse hydrophilic (H) trolox antioxidant capacity (H-TEAC) samples were prepared as follows. After a strong acidic hydrolysis with hydrochloric acid, a saponification with methanolic sodium hydroxide, and a precipitation of proteins with metaphosphoric acid (ARNOLD et al., 2013), antioxidants were extracted by 5 mL of ethanol/water (1/1, v/v), vortexed for 30 s and centrifuged at 5000 rpm for 5 min. The supernatant was taken and the process (ethanol/water, vortex, centrifuge) was repeated twice. The stable radical cation $ABTS^{\bullet+}$ was performed by mixing 10 mL 7 mmol L^{-1} ABTS solution with 10 mL 2.45 mmol L^{-1} $K_2S_2O_8$ solution (RE et al., 1999). After 24 h at room temperature in the darkness, the $ABTS^{\bullet+}$ stock solution was ready to use. An $ABTS^{\bullet+}$ working solution was prepared daily by diluting the $ABTS^{\bullet+}$ stock solution with phosphate buffer (PBS, 75 mmol L^{-1}, pH 7.4) to an absorbance of 0.70±0.05 at 730 nm. To perform the assay, 20 µL of sample extract, or standard (ca. 12.5-250 µmol trolox L^{-1} or blank (water) were transferred into a 96-well

microplate. After addition of 200 µL $ABTS^{\bullet+}$ working solution, absorbance was recorded after 1 min at 730 nm (MÜLLER; THEILE; BÖHM, 2010).

5.2.9 Statistical analysis

The experiment was conducted using a completely randomized design, and values are given as means ± standard deviation (SD) of three replicates. Statistical procedures were performed using SAS software version 9.0 (SAS Institute, Cary, NC, USA). Shapiro-Wilk test was applied to check on gaussian distribution, and the Box-Cox test was used to evaluate the homogeneity of the variances. If the variances were not homogeneous, the values were transformed adequately before they were subjected to the tests. All data were subject to analysis of variance (ANOVA), and means were compared using Tukey's test ($P<0.05$).

5.3 Results and Discussion

Carotenoids detected in mini tomatoes consisted of (all-E)-lutein, (all-E)-β-carotene and its isomer (13*Z*)-β-carotene, (all-E)-lycopene and its isomers (13*Z*)-lycopene, (9*Z*)-lycopene and (5*Z*)-lycopene. Figures 1 and 2 shows a typical chromatogram from samples, detailing separation of carotenoids and lycopene isomers respectively. As expected, lycopene was the major carotenoid compound found in red tomatoes.

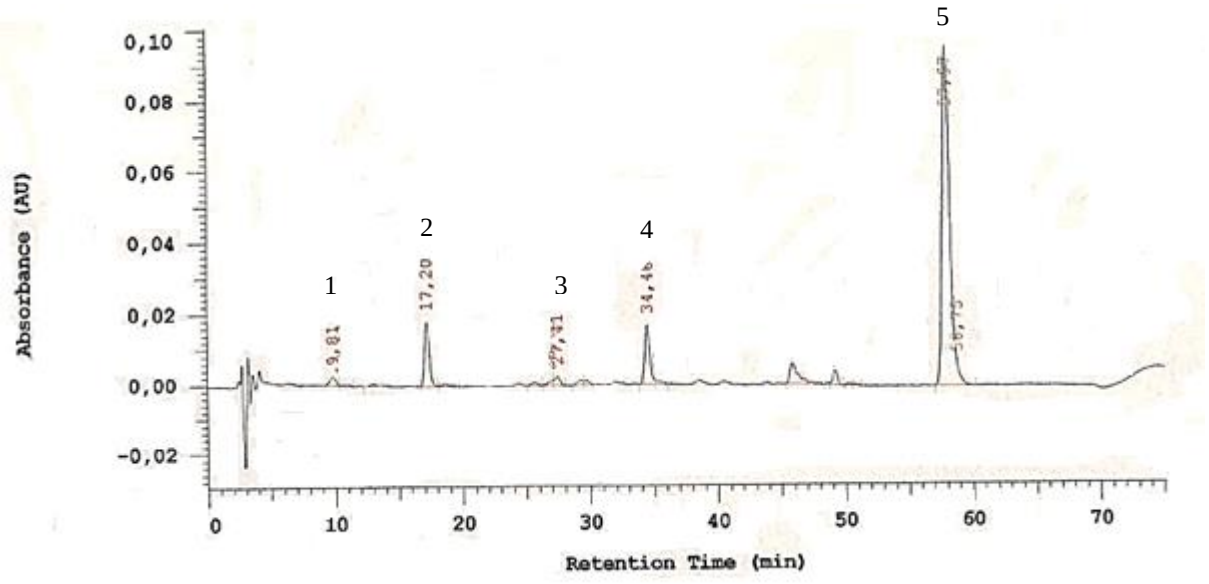

Figure 1 - Typical HPLC chromatogram (λ = 450 nm) of a tomato extract obtained using the conditions described herein. Major peaks corresponding to (all-*E*)-lutein (1), internal standard - β-apo-8'-carotenal (2), (13*Z*)-β-carotene (3), (all-*E*)-β-carotene (4) and (all-*E*)-lycopene (5).

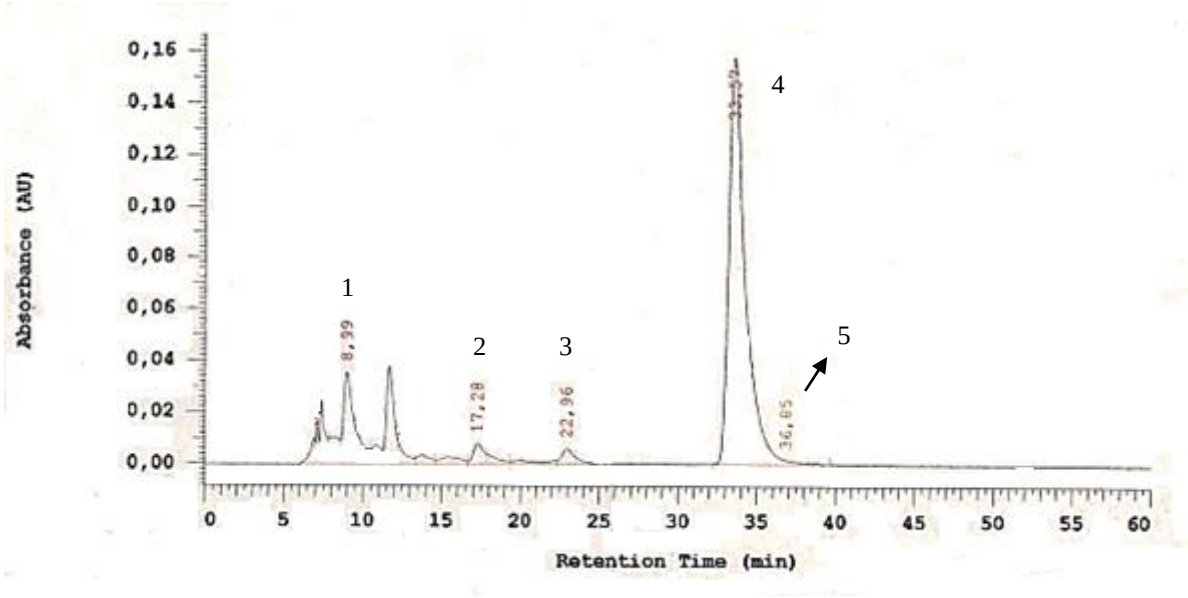

Figure 2 - Typical HPLC chromatogram (λ = 470 nm) of separation lycopene and lycopene isomers from a tomato extract obtained using the conditions described herein. Major peaks corresponding to internal standard - β-apo-8'-carotenal (1), (13*Z*)-lycopene (2), (9*Z*)-lycopene (3), (all-*E*)-lycopene (4) and (5*Z*)-lycopene (5).

Table 1 shows the changes in carotenoids content of tomato fruit treated with gamma radiation, carnauba coating and 1-MCP during 30 days of storage at room temperature (25±2° C). Postharvest treatments and storage time factors were significant ($P<0.05$) for the variables (all-*E*)-β-carotene, (all-*E*)-lycopene and (all-*E*)-lutein, as well as the interaction between the two factors. Lycopene content increased for all treatments during the storage time, the content ranged from 14.08 to 56.55 μg g^{-1} (Table 1). As a climacteric fruit, tomato continues maturing during postharvest, thus is a normal process of maturation, tomatoes change from green to red color because chloroplasts transform into chromoplasts, chlorophyll is degraded and lycopene and β-carotene are synthetized (GRIERSON, 1985). It has been extensively demonstrated there is an increase in carotenoids content during tomato ripening (JAVANMARDI; KUBOTA, 2006; ILAHY et al., 2011; NOUR; TRANDAFIR; IONICA; 2014).

In the first day of storage, there were no differences for lycopene content among treatments (Table 1). Application of 1-MCP had the most pronounced effect on lycopene on days 6, 12, 18 and 24 because the higher inhibition of the pigment accumulation in comparison to control and gamma radiation treatment; however, it was similar to the lycopene

content of tomatoes treated with carnauba coating on the 18th day. Carnauba coating treatment had also an inhibition effect on lycopene accumulation in mini tomatoes after 6 days and until 24 days of storage. Interestingly, in the last day of storage (day 30) lycopene content of carnauba an 1-MCP treated fruits were no different from control. In contrast, gamma radiation treatment increases the amount of lycopene in mini tomatoes, which was higher than control and other treatments in the end of storage period (days 24 and 30 of storage).

Different studies have shown that applying edible wax reduces tomato metabolism (ALI et al., 2010; DAVILA-AVIÑA et al., 2014) as well as the application of 1-MCP (WANG et al., 2008; SUN et al., 2012). Therefore, lower lycopene content could be attributed to the delaying in maturity process caused by these treatments during storage. However, gamma radiation effects in carotenoids, especially lycopene, of tomatoes harvested in red/mature stages are not clear. It would appear that ripening process post irradiation is a function of physiological age at time of irradiation (LEE et al., 1968) as well as a function of the applied dose (VILLEGAS et al., 1972). Studies conducted with mature green or breaker tomatoes irradiated with low doses of gamma radiation (up to1 kGy) showed a decreased in lycopene content (KUMAR et al., 2014; our study in chapter 3), but generally, the effect was more pronounced with higher doses (1 to 10 kGy) in the early stages of fruit maturation (VILLEGAS et al., 1972). Depending of ripening stage, climacteric fruits submitted to gamma radiation may respond either a delay of ripening (AKAMINE; MOY, 1983; URBAIN, 1986; THOMAS, 1988) or an advance (MAXIE et al., 1966). In the present study, probably an acceleration of the ripening process had occurred.

The β-carotene contents of all tomatoes (untreated, gamma radiation, 1-MCP and carnauba coating) did not change significantly during the first 6 days of storage (Table 1). Between days 6 and 12, β-carotene levels in 1-MCP, carnauba coating and untreated tomatoes slight decreased, maintaining the contents until the 30th day of storage. The results were similar to those observed by Thiagu, Onwuzulu and Ramana (1993) and Liu et al. (2009), who found that β-carotene increased up to the light-pink stage and decreased in subsequently ripening stages. However, the β-carotene contents of gamma radiation-treated tomatoes did not change significantly during 30 days of storage. Because of this, at 24 and 30 days of storage, β-carotene levels of irradiated tomatoes was higher compared to the other treatments, except on day 24 which carnauba coating treatment did not differ from irradiated treatment. Villegas et al. (1972) describe β-carotene synthesis is not extensively changed by gamma radiation as lycopene.

Table 1 - Carotenoids content (µg g^{-1} FW[1]) of mini tomatoes treated with gamma radiation, carnauba coating and 1-MCP during storage[2].

Treatment	Days of storage					
	0	6	12	18	24	30
			(all-*E*)-lycopene (µg g^{-1})			
Control	15.85 ± 1.39 ^{Ad}	25.03 ± 0.40 ^{Ac}	34.87 ± 0.57 ^{Ab}	38.75 ± 1.16 ^{Ab}	52.59 ± 0.89 ^{Ba}	51.39 ± 1.56 ^{Ba}
Gamma radiation	16.03 ± 0.29 ^{Ad}	25.91 ± 0.67 ^{Ac}	38.55 ± 1.36 ^{Ab}	41.49 ± 2.06 ^{Ab}	60.10 ± 1.04 ^{Aa}	57.55 ± 1.04 ^{Aa}
Carnauba coating	16.35 ± 1.52 ^{Ad}	28.12 ± 1.09 ^{Ac}	26.18 ± 1.10 ^{Bc}	34.43 ± 1.90 ^{Bb}	52.34 ± 3.45 ^{Ba}	50.53 ± 1.05 ^{Ba}
1-MCP	14.08 ± 0.66 ^{Ae}	16.74 ± 0.51 ^{Bd}	17.61 ± 1.52 ^{Cd}	32.72 ± 3.95 ^{Bc}	43.02 ± 1.43 ^{Cb}	49.92 ± 2.01 ^{Ba}
			(all-*E*)-β-carotene (µg g^{-1})			
Control	13.91 ± 0.43 ^{Aa}	13.60 ± 0.03 ^{Aa}	11.44 ± 0.26 ^{Ab}	11.48 ± 0.09 ^{Ab}	11.43 ± 0.27 ^{Bb}	11.14 ± 1.23 ^{Bb}
Gamma radiation	13.05 ± 0.47 ^{Aa}	13.20 ± 0.44 ^{Aa}	11.89 ± 0.83 ^{Aa}	11.83 ± 0.40 ^{Aa}	12.96 ± 0.80 ^{Aa}	13.14 ± 1.01 ^{Aa}
Carnauba coating	14.12 ± 0.54 ^{Aa}	13.55 ± 0.12 ^{Aa}	10.97 ± 0.15 ^{Ab}	10.87 ± 0.06 ^{Ab}	11.99 ± 0.16 ^{ABb}	10.91 ± 1.16 ^{Bb}
1-MCP	13.43 ± 0.47 ^{Aa}	13.69 ± 0.06 ^{Aa}	11.16 ± 0.25 ^{Ab}	11.26 ± 0.15 ^{Ab}	11.08 ± 0.21 ^{Bb}	10.84 ± 1.30 ^{Bb}
			(all-*E*)-lutein (µg g^{-1})			
Control	1.19 ± 0.06 ^{Aa}	0.92 ± 0.03 ^{Ab}	0.80 ± 0.08 ^{Abc}	0.76 ± 0.06 ^{Abc}	0.71 ± 0.02 ^{Bc}	0.67 ± 0.04 ^{ABc}
Gamma radiation	1.09 ± 0.03 ^{Aa}	0.90 ± 0.06 ^{Ab}	0.92 ± 0.10 ^{Aab}	0.79 ± 0.03 ^{Ab}	0.88 ± 0.03 ^{Ab}	0.81 ± 0.11 ^{Ab}
Carnauba coating	1.18 ± 0.02 ^{Aa}	1.06 ± 0.06 ^{Aa}	0.79 ± 0.09 ^{Ab}	0.71 ± 0.12 ^{Ab}	0.68 ± 0.01 ^{Bb}	0.68 ± 0.06 ^{Ab}
1-MCP	1.20 ± 0.09 ^{Aa}	1.03 ± 0.04 ^{Aa}	0.81 ± 0.07 ^{Ab}	0.71 ± 0.08 ^{Abc}	0.62 ± 0.12 ^{Bc}	0.54 ± 0.09 ^{Bc}
			(13*Z*)-β-carotene (µg g^{-1})			
Control	0.34 ± 0.05	0.56 ± 0.10	0.43 ± 0.04	0.40 ± 0.02	0.58 ± 0.15	0.67 ± 0.04
Gamma radiation	0.46 ± 0.03	0.56 ± 0.02	0.41 ± 0.08	0.51 ± 0.11	0.61 ± 0.10	0.72 ± 0.05
Carnauba coating	0.43 ± 0.10	0.62 ± 0.07	0.35 ± 0.04	0.27 ± 0.08	0.62 ± 0.18	0.64 ± 0.04
1-MCP	0.27 ± 0.05	0.57 ± 0.05	0.35 ± 0.06	0.37 ± 0.08	0.49 ± 0.16	0.70 ± 0.16
Means	0.37 ± 0.09 ^{b}	0.58 ± 0.03 ^{a}	0.38 ± 0.04 ^{b}	0.39 ± 0.10 ^{b}	0.58 ± 0.06 ^{a}	0.68 ± 0.04 ^{a}

[1] Fresh weight.
[2] Data are means ± Standard Deviation (n=3). Means followed by same capital letter on column (within the same compound) and small letter on line were not significantly different by Tukey's test (P>0.05).

For the isomer (13*Z*)-β-carotene, no significant effect of the treatments was observed. This indicates that the postharvest treatments applied in light-red mini tomatoes (gamma radiation, carnauba coating and 1-MCP) have no effect in producing (*Z*)-isomers of β-carotene. This fact may be considered a positive point since it is known the isomers of β-carotene (9*Z*, 13*Z*, and 15*Z*) possess lower pro-vitamin A activity and bioavailability compared to (*all*-E)-β-carotene (DEMING; BAKER; ERDMAN, 2002, DURING et al., 2002) and lower antioxidant capacity (BÖHM et al., 2002). In the present study, treatments had no effect on (13*Z*)-β-carotene levels in mini tomatoes, but there were significant differences for the factor days of storage. This carotenoid isomer increases from day 0 to day 6 ($P<0.05$) and then decreased in the next day of storage evaluation (day 12), remaining constant until day 18, to increase once more on the 24th day of storage ($P<0.05$).

Lutein is another carotenoid detected in mini tomatoes. This compound is one of the most widely found carotenoid xanthophyll pigments in fruits and vegetables normally consumed (PERRY; RASMUSSEN; JOHNSON, 2009). In tomatoes, lutein content is lower than other carotenoids, even so has health benefits such as preserving eye health in association with zeaxanthin (GRANADO; OLMEDILLA; BLANCO, 2003). In the present study, lutein content in mini tomatoes ranged from 1.19 to 0.67, from 1.09 to 0.79, from 1.18 to 0.68 and from1.20 to 0.54 μg g^{-1} of fresh weight for control, gamma radiatiom, carnauba coating and 1-MCP tomatoes (Table 1). D'Evoli, Lombardi-Boccia and Lucarini (2013) reported similar lutein amounts in raw cherry tomatoes. During storage, the content of lutein decreased from day 0 until 24th day of storage for 1-MCP treated tomatoes and the control group. For irradiated tomatoes and carnauba coating treatment lutein levels decreased until 18th day and 12th day respectively. For all treatments, afterwards to decline lutein levels remained constant up to 30 days of storage. Tomatoes treated with gamma radiation showed the higher content of lutein on day 24 of storage ($P<0.05$) and 1-MCP-treated tomatoes were different from gamma radiation and carnauba coating groups on the 30th day of storage because of the lower content of lutein, which did not differ from control tomatoes.

Most common geometrical isomer in plants is (all-*E*)-lycopene, which represents about 80−97% of total lycopene in tomatoes and related products (SHI; LE MAGUER, 2000), but food treatments and preparation may change the proportion of (*Z*)-isomers. Table 2 shows the changes in lycopene isomers, expressed as ratios (all-*E*)-lycopene/*Z*-isomer, of mini tomatoes treated with gamma radiation, carnauba coating and 1-MCP and storage for 30 days. Treatment methods and storage time were significant ($P<0.05$) for the ratios (all-*E*)-lycopene/(13*Z*)-lycopene and (all-*E*)-lycopene/(9*Z*)-lycopene, as well as the interaction

between the two factors. The initial ratio of (all-*E*)-lycopene/(13*Z*)-lycopene (day 0) was 19.36, 14.53, 14.12 and 14.49 for control, gamma radiation, carnauba coating and 1-MCP groups respectively and there was no differences among treatments (P>0.05). This value tended to increase during storage, reaching a peak on the 12th day of storage in control fruits and fruits treated with carnauba and gamma radiation. After 12th day of storage (all-*E*)-lycopene/(13*Z*)-lycopene ratio decreased for these treatment and, increased again in the 30th day. Nevertheless, for 1-MCP treated tomatoes, the ratio (all-E)/(13Z) increased during the storage, reaching the maximum in the 30th day. Probably it happened because 1-MCP treatment was more efficient in delaying ripening and the accumulation of lycopene as well as its isomers than the other treatments. Same performance was observed when breaker tomatoes were treated by 1-MCP, the ratio (all-*E*)/(13*Z*) isomer increased during storage (data shown in the 3th chapter). In relation to the treatments effects, gamma radiation showed higher ratios of (all-*E*)/(13*Z*) in days 6, 12, 24 and 30 of storage, not significantly different from control and carnauba treated tomatoes on 24th day. These results indicate irradiated fruits had less (13*Z*)-isomers of lycopene compared to fruits treated with carnauba coating and 1-MCP or untreated tomatoes, different from results obtained when breaker mini tomatoes were irradiated with the same dose (data shown in the 3th chapter). (all-*E*)-lycopene content was also higher for fruits treated with gamma radiation, which might be a reason of the higher ratio.

For control and irradiated tomatoes (all-*E*)/(9Z)-lycopene ratios had no changes during the storage period (P>0.05), ranging from 30.41 to 32.31 for untreated fruits and from 31.96 to 33.6 for gamma radiated tomatoes. However, mini tomatoes treated with carnauba coating increased the (all-*E*)/(9Z) ratio from 24.64 and 24.33 on days 0 and 6 to 30.87 on day 12, remaining constant since then. Initial (all-*E*)/(9Z) ratios (days 0 and 6) were lower for tomatoes treated with carnauba and 1-MCP (P<0.05) in comparison to control and gamma radiation groups of tomatoes. Furthermore, for 1-MCP-treated fruits (all-*E*)/(9Z) ratio increased during storage period differing from the other treatments in the last day of evaluation (day 30), when had the highest ratio (37.75).

Application of the postharvest treatments had no effect on ratio between (all-*E*)-lycopene and (5*Z*)-lycopene of mini tomatoes, but significant differences among days of storage were observed; day 0 had the lowest ratio compared to the other days that did not differ among them. Some studies indicate (*Z*)-isomers have a stronger in vitro antioxidant capacity (BÖHM et al., 2002) and are more bioavailable than the (all-*E*)-form (BOILEAU; BOILEAU; ERDMAN, 2002; SHI; LE MAGUER, 2000; STAHL; SIES, 1992; UNLU et al., 2007). In addition, it has been reported (*Z*)-isomers of lycopene make up 50% of the total

lycopene in human serum and tissues (FERRUZZI et al., 2001; STAHL; SIES, 1992). For these reasons lycopene (*Z*)-isomers are considered as having higher health benefits than the (all-*E*)-isomer (LAMBELET et al., 2009). In the present study none postharvest treatment dramatically increased *Z*-isomers, on the contrary decreased, as for irradiated fruits.

The ascorbic acid content of tomatoes increased to a maximum at 12 days of storage for carnauba treated tomatoes and at 24 days of storage for control, gamma radiation and 1-MCP treated tomatoes and subsequently declined (Figure 3a). These results are consisted with those reported by Wang et al. (2008) and Tigist, Workneh and Woldetsadik (2013) who observed a general trend of increase in ascorbic acid content of pink tomatoes, followed by a decline during the full ripening stage. Once tomatoes were not harvested in full ripening stage, the increase in ascorbic acid content is in accordance with the increase in other parameters associated with ripening (ALI et al., 2010), such as lycopene. In tomatoes, ascorbic acid content increases with fruit ripening (MATHOOKO, 2003), however after fruits reach the full ripening stage, ascorbic acid content starts to decline (AOAC, 1984).

The highest levels of ascorbic acid were observed in 1-MCP treated fruits at 12, 18, 24 and 30 days of storage. At 12 days, values for 1-MCP treated tomatoes did not differ from carnauba-coated tomatoes, at 18 days did not differ from the control and at 24 days did not differ from irradiated fruits. As previous describe by Wang et al. (2008) these results indicate 1-MCP treatment retard the decrease of ascorbic acid content, because despite ascorbic acid increased and declined as well to other treatments, with the 1-MCP treatment fruits maintained high levels of ascorbic acid even on the 30th day of storage.

After 12 days of storage ascorbic acid contents of tomatoes treated with carnauba coating seems to slow down, different from the other treatments that simply decreased. Ali et al. (2010) and Davila-Aviña et al. (2014) reported similar effects for edible coatings in tomatoes. They suggested that the coating slowed down the increase in ascorbic acid content, but did not prevent the synthesis of ascorbic acid during ripening.

Table 2 - Ratios of (all-*E*)-lycopene to the different (*Z*)-isomers of mini tomatoes treated with gamma radiation, carnauba coating and 1-MCP during storage[1].

Treatment	Days of storage					
	0	6	12	18	24	30
	(all-*E*)-lycopene/(13*Z*)-lycopene					
Control	19.36 ± 1.08 ^{Ad}	25.42 ± 4.57 ^{Babc}	30.25 ± 0.32 ^{Bab}	24.41 ± 0.55 ^{Acd}	24.76 ± 4.30 ^{ABcd}	31.95 ± 1.73 ^{Ba}
Gamma radiation	14.53 ± 0.59 ^{Ac}	37.99 ± 3.92 ^{Aa}	40.76 ± 2.30 ^{Aa}	26.67 ± 0.26 ^{Ab}	29.25 ± 0.90 ^{Ab}	44.58 ± 1.28 ^{Aa}
Carnauba coating	14.12 ± 3.86 ^{Ac}	22.21 ± 1.27 ^{BCab}	28.41 ± 3.83 ^{Ba}	22.10 ± 3.82 ^{Aab}	25.57 ± 5.03 ^{ABa}	29.73 ± 2.45 ^{Bbc}
1-MCP	14.49 ± 3.92 ^{Ac}	17.22 ± 1.13 ^{Cbc}	17.65 ± 0.70 ^{Cbc}	20.42 ± 3.42 ^{Abc}	22.26 ± 4.58 ^{Bb}	33.90 ± 2.21 ^{Ba}
	(all-*E*)-lycopene/(9*Z*)-lycopene					
Control	31.66 ± 0.31 ^{Aa}	31.26 ± 1.74 ^{Aa}	32.29 ± 3.14 ^{Aa}	30.41 ± 0.05 ^{Aa}	32.31 ± 1.37 ^{Aa}	32.29 ± 1.66 ^{Ba}
Gamma radiation	33.60 ± 1.18 ^{Aa}	32.19 ± 0.98 ^{Aa}	31.96 ± 1.33 ^{Aa}	32.25 ± 0.96 ^{Aa}	33.29 ± 0.91 ^{Aa}	32.61 ± 0.86 ^{Ba}
Carnauba coating	24.64 ± 1.19 ^{Bc}	24.33 ± 1.49 ^{Bc}	30.87 ± 3.00 ^{Aab}	28.05 ± 2.16 ^{Abc}	34.25 ± 1.29 ^{Aa}	32.14 ± 3.11 ^{Bab}
1-MCP	24.84 ± 3.31 ^{Bc}	29.13 ± 1.72 ^{Abc}	29.92 ± 3.12 ^{Ab}	28.99 ± 1.85 ^{Abc}	30.67 ± 0.96 ^{Ab}	37.75 ± 2.74 ^{Aa}
	(all-*E*)-lycopene/(5*Z*)-lycopene					
Control	22.74 ± 3.38	28.67 ± 4.05	31.20 ± 4.30	27.41 ± 2.70	27.33 ± 1.00	30.60 ± 2.75
Gamma radiation	20.82 ± 0.78	32.12 ± 1.35	27.84 ± 0.31	30.12 ± 0.45	31.11 ± 3.33	33.16 ± 2.36
Carnauba coating	22.03 ± 3.89	25.02 ± 3.34	32.61 ± 4.05	27.78 ± 4.37	27.78 ± 0.79	30.43 ± 1.76
1-MCP	22.57 ± 3.48	28.89 ± 3.54	32.22 ± 4.70	27.09 ± 3.06	24.66 ± 1.85	29.58 ± 2.40
Means	22.04 ± 0.87 ^{b}	28.67 ± 2.90 ^{a}	30.97 ± 2.17 ^{a}	28.10 ± 1.38 ^{a}	27.72 ± 2.65 ^{a}	30.94 ± 1.54 ^{a}

[1] Data are means ± Standard Deviation (n=3). Means followed by same capital letter on column (within the same compound) and small letter on line were not significantly different by Tukey's test (P>0.05).

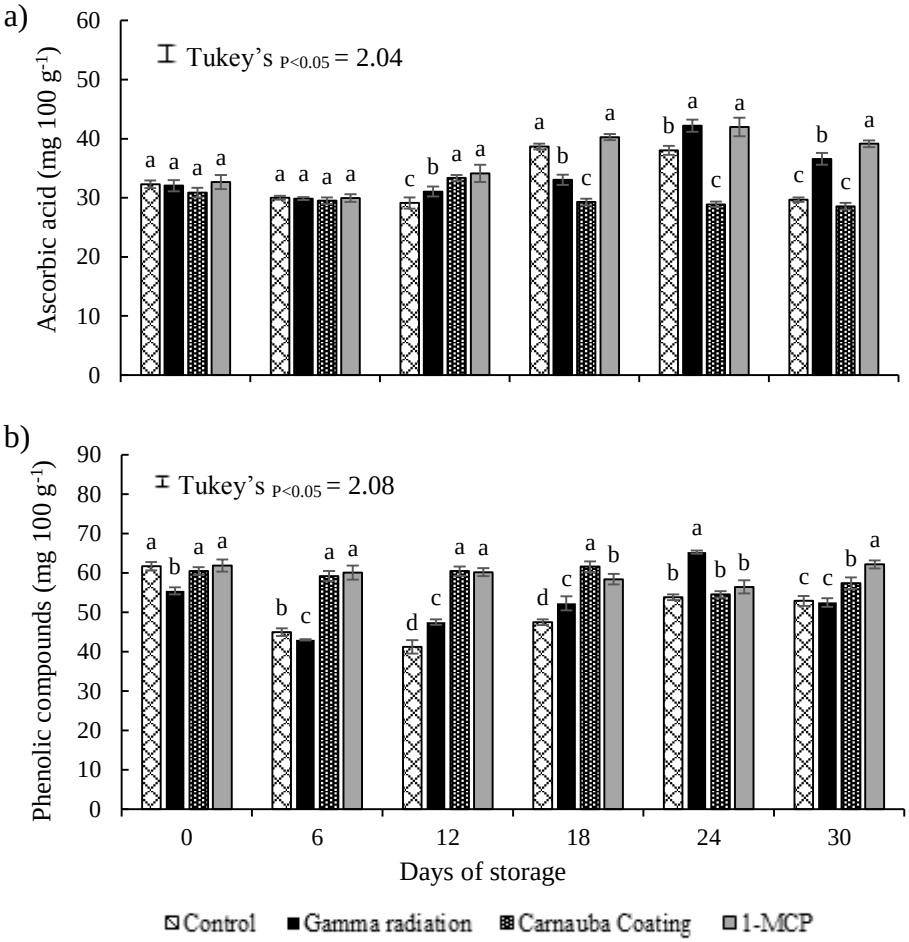

Figure 3 – Ascorbic acid content (mg 100 g^{-1} of fresh weight) (a) and total phenolic compounds (mg GAE 100 g^{-1} of fresh weight) (b) of mini tomatoes treated with gamma radiation, 1-MCP and carnauba coating during storage. Different letters indicate significant differences among treatments by Tukey's test ($P<0.05$). Vertical bars indicate least significant difference by Tukey's test ($P<0.05$) among days of storage. Each observation is a mean ± Standard Deviation (n=3).

Effects of postharvest treatments on the concentration of total phenolic compounds of mini tomatoes expressed as mg equivalents of gallic acid 100 g^{-1} fresh weight are reported in Figure 3b. Tomatoes showed significant differences between the interaction of treatments and days of storage for total phenolic contents ($P<0.05$). The initial content (day 0 after treatments) of total phenolic compounds was significant lower for irradiated fruits, indicating gamma radiation negatively impact phenolic compounds (KUMAR et al., 2014) immediately after the treatment. However, this content increased during storage reaching the maximum at 24 days of storage for irradiated tomatoes. Increase in phenolic compounds contents in irradiated tomatoes (KHALAF et al., 2014) and other fresh fruit have also been reported (TAN; LAM, 1985; EISSA; SHAHEEN; BROTOS, 2014). The increase in total phenolic compounds of irradiated tomatoes could be attributed to a slight increase in the activity of

phenylalanine ammonia lyase (PAL), an enzyme involved in phenolic compounds biosynthesis (REYES; CISNEROS-ZEVALLOS, 2007), as suggested by Dubery, Van Rensburg and Schabort (1984) and Tan and Lam (1985) who found the ionizing radiation increased phenolic compounds in citrus fruits and mangos through induction of PAL.

Mini tomatoes treated with carnauba coating and 1-MCP had the highest values for phenolic compounds contents in the most evaluated days of storage (days 6, 12, 18 and 30) compared to control and gamma radiation groups (Figure 3b). Applications of these treatments possibly slow down the increase or decrease of phenolic compounds during storage as pointed in different studies (WANG et al., 2008; SUN et al., 2012). In addition, the changes in total phenolic compounds of carnauba and 1-MCP treated tomatoes during storage were not so expressively as for gamma radiation and control tomatoes.

The antioxidant capacity of hydrophilic fraction, mainly represented by phenolic compounds and ascorbic acid (RAFFO et al., 2002; MOCO et al., 2006; VALVERDU-QUERALT et al., 2011), changed according to the interaction of days of storage and treatments ($P<0.05$). Differences among treatments only occurred on day 0 as shown in Figure 4a. Fruits treated with gamma radiation had the lower value for H-TEAC (320.02 µmol TE 100 g^{-1}), however did not differ from 1-MCP treated fruits. No differences were observed among control, carnauba coating and 1-MCP groups on day 0. As mentioned (Figure 3b), phenolic compounds had also lower values for irradiated tomatoes in day 0, and this probably decreased the hydrophilic antioxidant capacity of fruits. Gamma radiation interferes in food composition through direct or indirect mechanisms. In case of indirect mechanism, radiolysis of water results in the production of free radicals (FAN; MASTOVSKA, 2006), and then perhaps as an initial response of the radiation dose, content of phenolic compounds decreased as showed in Figure 3b and, consequently, decreased the hydrophilic antioxidant capacity. This effect was only observed in day 0, immediately after postharvest treatment.

In addition, gamma radiation-treated fruits had a constant value of H-TEAC until day 24, when the antioxidant capacity increased to 429.13 µmol TE 100 g^{-1}, decreasing in the following day of evaluation (day 30). For control, carnauba coating and 1-MCP groups of tomatoes, H-TEAC decreased from day 0 to day 6, and increased on 24th day of storage did not differing from 30th of storage. Specific studies have shown 1-MCP treatment enhanced hydrophilic antioxidant activity of tomatoes (WANG et al., 2008) and other fruits (JIANG et al., 2004; WANG et al., 2006; MACLEAN et al., 2003), which are not consistent with our results, once antioxidant capacity of 1-MCP treated tomatoes did not differ from control, even though high values for phenolic compounds.

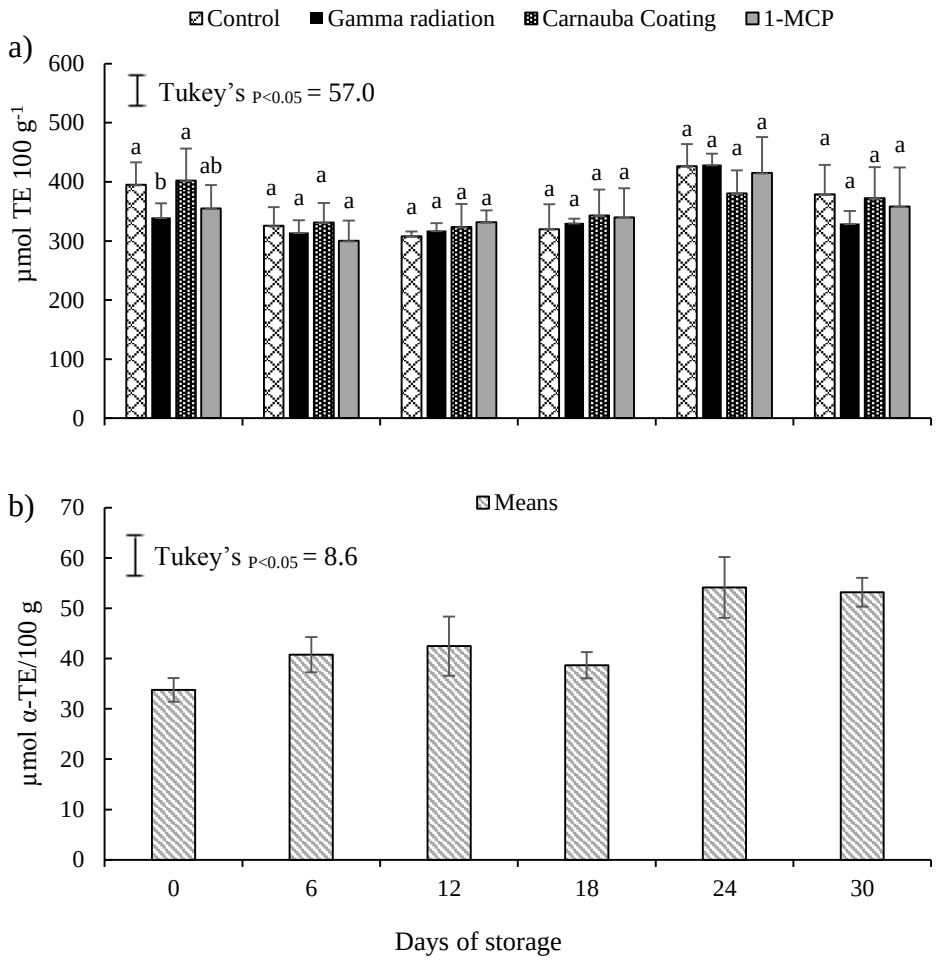

Figure 4 - Hydrophilic antioxidant capacity – H-TEAC (µmol TE 100 g^{-1} of fresh weight) (a) and lipophilic antioxidant capacity – α-TEAC (µmol α-TE 100 g^{-1} of fresh weight) (b) of mini tomatoes of mini tomatoes treated with gamma radiation, 1-MCP and carnauba coating during storage. Different letters indicate significant differences among treatments by Tukey's test (P<0.05). Vertical bars indicate least significant difference by Tukey's test (P<0.05) among days of storage. Each observation is a mean ± Standard Deviation (n=3).

Studies reported that carotenoids and vitamin E, represents the main lipophilic-soluble antioxidants in tomatoes and contribute to the antioxidant activity of the lipophilic-soluble fraction (MARTÍNEZ-VALVERDE et al., 2002). In the present study, L-TEAC of mini tomatoes ranged between 35.85 and 56.01 µmol α-TE 100 g^{-1}, from 30.44 to 59.50 µmol α-TE 100 g^{-1}, from 34.96 to 49.17 µmol α-TE 100 g^{-1} and from 33.83 to 58.81 µmol α-TE 100 g^{-1}, for the treatments control, gamma radiation, carnauba and 1-MCP, respectively. No differences among treatments were observed (P>0.05), however L-TEAC values had a significant difference among days of storage, and lipophilic antioxidant capacity increased during storage time (Figure 4b), corroborating with the results of lycopene, which

increased during storage. Lycopene is the most potent reactive oxygen species scavenger among carotenoids and other antioxidants, including vitamin E (DIMASCIO; KAISER; SIES, 1989; SHI et al., 2004), thus has a great antioxidant capacity, indicating that an increase in lycopene may be correlated with a rise in L-TEAC.

5.4 Conclusions

In conclusion, our data demonstrate the use of gamma radiation, carnauba coating and 1-MCP as postharvest treatments induced changes in bioactive compounds of mini tomatoes harvest at light-red stage during storage. 1-MCP and carnauba coating promoted a slow down increase on lycopene and a slow down decrease in ascorbic acid and phenolic compounds of treated fruits, while gamma radiation increases the final content of lycopene and had high peaks of ascorbic acid and phenolic compounds in the end of storage. β-carotene and lutein were not dramatically affected by treatments, but the radiation treatment maintained β-carotene values constant during all the period of storage. Postharvest treatments did not increase (Z)-isomers of lycopene, however gamma radiation decreased (13*Z*) isomer during storage and 5(*Z*)-isomer was not affected by postharvest treatments. Antioxidant capacity of the lipophilic fraction was not affected by treatments and the hydrophilic fraction was lower for irradiated fruits only on day 0 of storage. Other treatments presented no diferences for H-TEAC during storage.

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6 GENERAL CONCLUSIONS

This study showed physical and chemical characteristics of mini tomatoes, as well as the bioactive compounds changed according to the postharvest treatment applied and during the storage period. Furthermore, these changes are different according to the maturation stage of fruit at harvest.

Fruits harvested at breaker stage did not presented acceptable values for SS and SS/TA ratio, which compromise the flavor of fruits. In addition, bioactive compounds were more affected by treatments, especially by gamma radiation and 1-MCP, for fruits harvested at breaker stage, because there were a decrease in (all-*E*)-lycopene and more formation of (*Z*)-isomers of lycopene, while for fruits at light-red stage the contrary was observed; gamma radiation treatment promoted an increase in (all-*E*)-lycopene and lower formation of (*Z*)-isomers of lycopene. Gamma radiation also induced a decreased in β-carotene and an increased in phenolic compounds by the end of storage period in breaker tomatoes and 1-MCP treatment promoted a slow down increase/decrease in ascorbic acid content during storage in both breaker and light-red tomatoes. Carnauba coating was the treatment that did not affect negatively bioactive compounds: the effects were slow down the increase in lycopene and slown down the decrease of ascorbic acid and phenolic compounds.

Interestingly that antioxidant capacity of the hydrophilic fraction was not dramatically affected by treatments independent of breaker or light-red fruits, however the lipophilic fraction was affected only for 1-MCP treatment in breaker fruits.

Regarding to physical quality it is clear that carnauba coating was the treatment which showed better results either for breaker or light-red tomatoes, because delay mass loss and fruit firmness, maintained good values of SS/TA and color. Furthermore, 1-MCP treatment could be a good choice for mini tomatoes harvest at light-red stage, because retained fruit firmness, delay mass loss and presented acceptable color, which did not occured when this treatment was applied in breaker tomatoes. On the other hand, gamma radiation was not a good treatment for fruits in light-red stage, because promotes mass loss and solubilization of pectins which leds to loss in fruit firmness. On the contrary, gamma radiation has positive effects in breaker tomatoes due to not affect fruit firmness, maitain fruit firmness and promote earlier homogeneous color in tomato fruits in comparison to control and other treatments.

Therefore, as a suggestion, more studies should be conducted with other postharvest treatments in different fruits and vegetables to identify, particularly the changes in bioactive compounds, especially the formation of (*Z*) isomers by different treatments, something not much investigated, but important for human health and for the consumers who became even more health conscious.