LAUANA PELLANDA DE SOUZA

OZÔNIO NA DEGRADAÇÃO DE RESÍDUOS DE AGROTÓXICOS E NA CONSERVAÇÃO PÓS-COLHEITA DE CENOURAS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Engenharia Agrícola, para obtenção do título de Doctor Scientiae.

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APROVADA: 20 de abril de 2017.

Maria Eliana Lopes Ribeiro de Queiroz

Marcio Arêdes Martins

Marco Aurélio Guerra Pimentel

Fernanda Fernandes Heleno
(Coorientadora)

Lêda Rita D'Antonino Faroni
(Orientadora)

DEDICO...

...aos meus queridos pais, familiares e amigo.

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BIOGRAFIA

Lauana Pellanda de Souza, filha de Rubens Castro de Souza e Clemiuda Pellanda de Souza, nasceu em 28 de novembro de 1987, no município de Alegre, Espírito Santo.

Em 2004 concluiu o curso de Técnico em Agropecuária pela antiga Escola Agrotécnica Federal de Alegre (EAFA), hoje Instituto Federal do Espírito Santo - Campos Alegre.

Em 2009 ingressou no serviço público federal como Técnica em Laboratório/Área: Agropecuária no Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo (UFES).

Em 2010 graduou-se no curso de Agronomia pela UFES.

Em agosto de 2011 iniciou o Mestrado no Programa de Pós-Graduação em Produção Vegetal, na área de Fitossanidade, pela UFES. Defendeu sua dissertação em agosto de 2013.

Em março de 2015 ingressou no curso de Doutorado no Programa de Pós-Graduação em Engenharia Agrícola, na área de concentração de Armazenamento e Processamento de Produtos Agrícolas, da Universidade Federal de Viçosa (UFV), sob orientação da Professora Dra. Lêda Rita D'Antonino Faroni, submetendo-se à defesa de tese em abril de 2017.

ÍNDICE

RESUMO

SOUZA, Lauana Pellanda de, D.Sc., Universidade Federal de Viçosa, abril de 2017. **Ozônio na degradação de resíduos de agrotóxicos e na conservação pós-colheita de cenouras.** Orientadora: Lêda Rita D'Antonino Faroni. Coorientadores: Fernada Fernandes Heleno e Frederico Garcia Pinto.

O uso indiscriminado de agrotóxicos e a não observância dos períodos de carência faz com que alguns ingredientes ativos sejam detectados em concentrações acima do limite máximo de resíduo (LMR) em diversos alimentos. Neste contexto, há uma necessidade de se estudar a dissipação de resíduos de agrotóxicos em alimentos, bem como desenvolver estratégias para remoção ou redução destes resíduos nos alimentos, após a colheita. Os tratamentos utilizando ozônio (O_3) vêm sendo estudados como alternativa para descontaminação de alimentos por apresentar uma série de vantagens como, por exemplo, seu alto potencial de oxidação mesmo em baixas concentrações. A presente pesquisa foi realizada em três etapas, as quais foram relatadas nos artigos que compõem esta tese. Na primeira etapa, investigou-se a dissipação de dois agrotóxicos, difenoconazol (fungicida) e linurom (herbicida) aplicados em culuturas de cenouras. Para tal, seguiu-se uma abordagem experimental de campo como cenário de exposição de cenouras a uma, duas e cinco vezes a dose recomendada destes agrotóxicos, e a formação de seus produtos de degradação natural. As tendências de degradação para as diferentes doses de difenoconazol e linurom na cenoura seguiram um modelo de pseudo-primeira ordem, porém com diferentes taxas de degradação. O difenoconazol foi o agrotóxico menos persistente, contudo os dois produtos mostraram persistência dependente da dose. Os tempos de degradação de cinquenta porcento dos resíduos (TD_{50}) dos agrotóxicos nas diferentes doses aplicadas variaram de 1,7 a 2,7 dias para difenoconazol e de 7,6 a 10,5 dias para linurom. Ao fim do período de carência, as cenouras tratadas com altas doses dos agrotóxicos foram consideradas impróprias para o consumo. Não foram encontrados produtos de degradação dos pesticidas estudados nas cenouras. Os resultados reforçam que o cumprimento das instruções de dosagem e período de carências estabelecidos para os agrotóxicos estudados na cultura da cenoura asseguram que os limites máximos de resíduos sejam respeitados. Na segunda etapa deste trabalho utilizou-se um planejamento fatorial do tipo composto central para estudar três variáveis que regem a eficácia dos tratamentos com ozônio: concentração de O_3, temperatura e tempo de tratamento. Este é o primeiro trabalho demonstrando a remoção de difenoconazol e linurom em cenoura pelos tratamentos com ozônio. A remoção dos agrotóxicos aumentou com a concentração de ozônio e com o tempo

de tratamento. Porém a temperatura não teve influência na remoção dos resíduos. Os maiores percentuais de remoção foram atingidos quando as raízes foram expostas durante 120 min a 5 e 10 mg L^{-1} de ozônio, respectivamente, nos tratamentos com O_3 em gás e dissolvido em água. Após cinco dias de armazenamento das cenouras, os valores máximos de remoção dos agrotóxicos pela exposição ao gás ozônio foram superiores a 98% para o difenoconazol e 95% para o linurom. Nos tratamentos com O_3 dissolvido em água, as remoções de difenoconazol e linurom atingiram valores de até 96,0 e 79,8%, respectivamente. Os produtos de degradação dos agrotóxicos pelo O_3 não foram encontrados. Na terceira etapa do estudo avaliou-se o efeito de tratamentos com ozônio em gás e dissolvidos em água na qualidade de cenouras. A exposição de cenouras ao ozônio em gás e dissolvido em água não alterou a porcentagem de perda de massa, firmeza e cor das cenouras. Os tratamentos com O_3 em gás também não afetaram o pH e os sólidos solúveis das cenouras. No entanto, em tratamentos com O_3 dissolvido em água, as concentrações de ozônio e sua interação com a temperatura afetaram temporariamente o pH das cenouras. Além disso, o O_3 em gás impediu o aumento acentuado do teor de sólidos solúveis durante o armazenamento, aumentando assim a vida de prateleira das cenouras. Portanto, pode-se concluir que o cumprimento das instruções de dosagem e período de carência estabelecidos para os agrotóxicos estudados na cultura da cenoura asseguram que os limites máximos de resíduos sejam respeitados e que o ozônio em gás e dissolvido em água pode ser utilizado para remoção de resíduos de difenoconazol e linurom em cenouras sem prejudicar a qualidade dos vegetais.

ABSTRACT

SOUZA, Lauana Pellanda de, D.Sc., Universidade Federal de Viçosa, April, 2017. **Ozone in pesticides waste degradation and post-harvest conservation of carrots**. Adviser: Lêda Rita D'Antonino Faroni. Co-advisers: Fernada Fernandes Heleno and Frederico Garcia Pinto.

The indiscriminate use of pesticides and non-compliance with the pre-harvest period causes some active ingredients are detected at concentrations above the maximum residue limit (MRL) in various foods. In this context, there is a need to study the dissipation of pesticide residues in food, as well as to develop strategies to remove or reduce these residues from food after harvesting. The treatments using ozone (O_3) have been studied as an alternative for food decontamination because it presents many advantages such as its high oxidation potential even at low concentrations. The present research was carried out in three stages, to which were reported in the articles that compose this thesis. In the first stage we had investigated the dissipation of two pesticides, difenoconazole (fungicide) and linuron (herbicide) applied in culuturas carrots. For this purpose, it was followed a field experimental approach as a scenario of carrots exposure to one, two and fivetimes the recommended dosage of the pesticides and the formation of their main natural degradation products. The degradation trends for differents doses of both difenoconazole and linuron in carrot followed a first-order model, but at different degradation rates. Difenoconazole was the least persistent pesticide, but for both pesticides the persistence dependedon the dosage. The degradation times of fifty percent of the waste (DT_{50}) of the pesticides at the different applied doses ranged from 1.7 to 2.7 days for difenoconazole and from 7.6 to 10.5 days for linuron. At the end of the pre-harvest period carrots treated with high doses of pesticides were considered unfit for consumption. No degradation products of the pesticides studied were found in the carrots. The results reinforce that comply with the dosing instructions and pre-harvest period established for the pesticides studied in the carrot field ensuring that maximum residue levels are respected. In the second stage of this work we employing a central composite design to study three important variables governing the efficacy of gas phase as well as liquid phase ozone treatments: O_3 concentration, temperature and treatment time. This is the first report demonstrating the difenoconazole and linuron removal from carrots by ozone treatment. The pesticides percentage of removal increases with the ozone concentration and treatment time. However, the temperature had no influence on the removal of residues. The highest percentages of pesticide removal were achieved when the roots were exposed for

approximately 120 min at 5 and 10 mg L^{-1} ozone, respectively in treatments with O_3 in gas and dissolved in water. After five days of carrots storage the maximum removal values of the pesticides by ozone in gas were greater than 98% for difenoconazole and 95% for linuron. In the treatments with O_3 dissolved in water, the removal percentages of difenoconazole and linuron reached values of up to 96.0 and 79.8%, respectively. The degradation products from the pesticides by O_3 were not found. In the third stage we evaluated the effect of treatments with ozone in gas and dissolved in water in carrots quality. The exposure of carrots to ozone in gas and dissolved in water did not alter the weight loss percentage, firmness and color of the carrots. The O_3 treatments in gas also did not affect the pH and soluble solids content of the carrots. However, in treatments with O_3 dissolved in water, the ozone concentrations and it interaction with temperature temporarily affected pH of carrots. In addition, O_3 in gas prevented the sharp increase of soluble solids content during storage, thus increasing the shelf-life of carrots. Therefore, it can be concluded that compliance with the dosage instructions and pre-harvest period established for the pesticides studied in the carrot crop ensure that maximum residue limits are respected and that ozone in gas is dissolved in water can be used for the removal of difenoconazole and linuron residues in carrots without impairing plant quality.

INTRODUÇÃO GERAL

Apesar dos elevados esforços globais para a implantação de programas de produção agrícola sustentáveis, a utilização de agrotóxicos vem aumentando em todo mundo (Schreinemachers & Tipraqsa, 2012; FAO, 2013; Ghimire & Woodward, 2013). Segundo Guedes et al. (2016), este fenômeno se justifica devido ao fato dos agrotóxicos serem indiscutivelmente a mais influente dentre as ferramentas de gerenciamento de pragas desde o início da sua utilização em larga escala, no inicio da década de 40.

Embora o uso dos agrotóxicos possua efeitos benéficos sobre a oferta mundial de alimentos e na maximização das atividades agrícolas, a sua utilização pode causar problemas ambientais como a contaminação do solo e da água, além de serem bioacumulados nos alimentos (Prestes, 2009). O consumo de alimentos com resíduos de agrotóxicos, mesmo em quantidades sub-letais, representa risco potencial à saúde do homem e dos animais. Os inseticidas organofosforados, por exemplo, quando ingeridos afetam o sistema nervoso, podendo provocar tremores, salivação excessiva, lacrimejamento, hipersecreção nasal, hipersensibilidade, distúrbios sensoriais cutâneos, irritação cutânea, cefaléia intensa, perda do apetite, fadiga, tonturas, perda da consciência, cãibras musculares e convulsões (Caldas, 2000).

A fim de controlar os níveis de resíduos de agrotóxicos nos alimentos, órgãos nacionais e internacionais estabelecem os limites máximos de resíduos (LMR) permitidos para cada agrotóxico em determinado produto alimentício. Internacionalmente esses limites são estabelecidos pelo Codex Alimentarius. No Brasil, a Agência Nacional de Vigilância Sanitária (ANVISA) é o órgão responsável pelo estabelecimento desses limites nos alimentos comercializados. Para monitorar a qualidade de alguns alimentos, foi criado pela ANVISA, em 2001, o Programa Nacional de Análise de Resíduos de Agrotóxicos em Alimentos (PARA). Com base neste programa, a ANVISA publica desde então, relatórios informativos sobre a qualidade de frutas, hortaliças e grãos consumidos no país.

Segundo os relatórios publicados pelo PARA, alguns alimentos têm apresentado resíduos de agrotóxicos proibidos para a cultura e/ou níveis de contaminação com agrotóxicos permitidos acima dos limites máximos estabelecidos pela legislação brasileira. Nos últimos anos, o pimentão vem ocupando o primeiro lugar no ranque dos alimentos mais contaminados, com 90% de suas amostras em situação irregular (ANVISA, 2011). Porém, o pimentão não é o único alimento preocupante quando se trata de contaminação por resíduos de agrotóxicos. A cenoura (*Daucus carota* L.), uma das hortaliças mais importantes devido ao

seu elevado consumo mundial, extensão da área cultivada, e grande envolvimento socioeconômico dos agricultores, também figura entre os alimentos com maior numero de amostras contaminadas por agrotóxicos, aparecendo sempre entre os seis alimentos com maior número de amostras irregulares, segundo os relatórios do PARA (ANVISA, 2010; 2011; 2012).

O consumo de cenoura é de suma importância para a saúde humana, uma vez que é fonte fundamental de carotenoides precursores da vitamina A (Luengo et al., 2000). A cenoura também está presente em diversos produtos alimentícios infantis (EMBRAPA, 2004). Estudos realizados pela University of Southern California comprovam que crianças com até um ano de vida que consomem alimentos convencionais, geralmente produzidos com o uso de agrotóxicos, têm quatro vezes mais chances de desenvolver problemas respiratórios, como a asma, que crianças que consomem alimentos orgânicos (Larramendi, 2003).

Para a cultura da cenoura, o uso intensivo e constante de agrotóxicos no controle de insetos-praga, doenças e plantas daninhas é necessário a fim de minimizar perdas na produtividade e qualidade do produto final (Caux et al., 1996; Carvalho et al., 2005; Liu et al., 2005). O difenoconazol (*cis-trans*-3-cloro-4-[4-metil-2-(1H-1,2,4-triazol-1-ilmetil)-1,3-dioxolan-2-il]fenil 4-clorofenil eter) é um fungicida sistêmico, do grupo químico dos triazois, muito empregado na cultura da cenoura para o controle da mancha-de-alternaria ou queima-das-folhas causada pelo fungo *Alternaria dauci* (Carvalho et al., 2005). No controle de diversas plantas daninhas na cultura, o herbicida linurom (3-(3,4-diclorofenil)-1-metoxi-1-metilurea), produto sistêmico do grupo químico das uréias, é um dos principais produtos utilizados (Andrei, 2017). O tipo de cultivo que este vegetal exige, ou seja, contato direto e imersão no solo, bem como sua anatomia, com raízes cobertas por uma fina epiderme, fazem com que a cenoura esteja facilmente em contato com os agrotóxicos usados naquele período de plantio, bem como com os resíduos deixados de outras colheitas (Souza et al., 2008).

Os potenciais riscos oferecidos pelos agrotóxicos à saúde humana, aliados ao risco de contaminação ambiental e a crescente preocupação do consumidor com a qualidade dos alimentos, têm evidenciado a necessidade de estudos sobre técnicas capazes de degradar os resíduos destes produtos nos alimentos antes do consumo ou processamento. As tecnologias atualmente adotadas com a finalidade de reduzir ou eliminar os resíduos de agrotóxicos em alimentos incluem o uso de compostos a base de cloro, radiação ultra-violeta (UV), peróxido de hidrogênio (H_2O_2), ultra-som, tratamentos térmicos e o gás ozônio (O_3) (Hwang et al., 2001; Lin et al., 2012; Al-Antary et al., 2015). Todavia, o uso do ozônio vem se destacando, graças ao elevado poder oxidativo e facilidade de obtenção deste gás.

O ozônio é um gás resultante do rearranjo de átomos de oxigênio e pode ser gerado por descargas elétricas ou pela incidência de radiação eletromagnética de alta energia (luz ultravioleta) no ar (Gabler et al., 2010). É uma molécula instável que decai rapidamente a oxigênio biatômico, liberando um átomo de oxigênio altamente reativo. Por essa característica, esse gás possui o segundo maior potencial de oxidação dentre os elementos químicos, sendo superado apenas pelo flúor (F_2) (Hill & Rice, 1982; Guzel-Seydim et al., 2004).

O gás ozônio foi classificado pela United States Food and Drug Administration, em 2001, como sanitizante seguro para aplicação em alimentos, já que o seu produto de degradação é o oxigênio e não deixa resíduos nos alimentos (Gabler et al., 2010). Na agricultura, a utilização do ozônio ainda é restrita, embora venha sendo apontada como uma estratégia potencial para o controle de insetos-praga, especialmente em grãos-armazenados (Kells et al., 2001; Pereira et al., 2007; Sousa et al., 2008; Sousa et al., 2016). Na indústria, o ozônio é utilizado para a descontaminação da superfície de frutas e legumes, auxiliando em sua preservação durante o armazenamento (Kim et al., 1999). Quando utilizado em tratamentos pós-colheita, durante o armazenamento ou processamento de alimentos, o alto poder oxidativo O_3 pode promover mudanças indesejáveis na qualidade dos alimentos. Frutas e verduras são os mais afetados pelos efeitos negativos do ozônio devido ao seu alto teor de umidade, enzimas e compostos fenólicos (Patil et al., 2010; Sandhu et al., 2011). Consequentemente, uma optimização das condições de tratamento deve ser estudada para cada alimento (Forney et al., 2007; Heleno et al., 2014; Heleno et al., 2015).

A utilização do ozônio na degradação de resíduos de agrotóxicos foi comprovada em diversos produtos agrícolas, tais como tomate, alface, uva, maçã, morango, mostarda, limão, laranja, toranja, milho, trigo, lichia, entre outros (Forney et al., 2007; Wu et al., 2007; Gabler et al., 2010; Alencar et al., 2013; Heleno et al., 2014; Heleno et al., 2015; Savi et al., 2016). Al-Antary et al. (2015) verificaram que o tratamento com água ozonizada (4 $\mu g\ L^{-1}$) em tomates destinados à fabricação de suco foi capaz de remover 100% do resíduo de carbosulfano no produto final. Heleno et al. (2014), estudando o efeito do gás ozônio na remoção de resíduos de difenoconazol, constataram que além do uso do gás reduzir de 5 mg kg^{-1} para 0,5 mg kg^{-1} a quantidade de resíduo em morangos, o ozônio foi capaz de impedir a redução de sólidos solúveis, açúcares totais e vitamina C nas frutas durante o armazenamento.

A oxidação de compostos orgânicos pelo gás O_3 pode ocorrer através de duas vias específica: reação direta da molécula de O_3 com a molécula do composto orgânico; e reação de radicais livres (-O), formados a partir da decomposição do ozônio, com os compostos

orgânicos. Em meio aquoso também pode ocorrer a oxidação dos compostos orgânicos pela ação dos radicais hidróxidos, formados a partir de átomos de oxigênio advindos da degradação do O_3, com átomos de hidrogênio livre presentes na solução (Chiron et al., 2000; Von Gunten, 2003). Os agrotóxicos difenoconazol e linurom apresentam em suas estruturas moleculares anéis aromáticos e radicais com duplas ligações. A ligação dupla é mais susceptível ao ataque do ozônio, conduzindo à formação de um ozonido primário que sendo instável se dissocia num composto estável e seu intermediário correspondente. Os intermediários podem sofrer uma decomposição adicional através de eliminação de átomo de oxigênio, canal de éster, canal de peróxido de hidrogénio ou podem ainda estabilizar-se após colisão com outro corpo (Al Rashidi et al., 2013).

Embora se conheça o potencial do ozônio como agente de degradação de resíduos de agrotóxicos, não há relatos sobre o uso deste gás para a degradação de resíduos de agrotóxicos em cenouras. Deste modo, a presente pesquisa foi desenvolvida com o objetivo de avaliar a eficiência do uso do ozônio na degradação de difenoconazol e linurom em cenouras, bem como avaliar seu efeito na conservação pós-colheita das raízes.

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ARTIGO 1

Cinética de dissipação de difenoconazol e linurom e seus produtos de degredação natural em cenouras

Resumo: A crescente preocupação com a segurança alimentar tem levado a um número crescente de estudos sobre o efeito dos resíduos de agrotóxicos em produtos agrícolas. Neste estudo investigou-se a dissipação de dois agrotóxicos, difenoconazol e linurom, seguindo uma abordagem experimental de campo como cenário de exposição de cenouras a uma, duas e cinco vezes a dose recomendada destes agrotóxicos, e a formação de seus produtos de degradação natural. As tendências de degradação para as diferentes doses de difenoconazol e linurom na cenoura seguiram um modelo cinético de pseudo-primeira ordem, porém com diferentes parâmetros cinéticos. O difenoconazol foi o agrotóxico menos persistente, contudo os dois produtos mostraram persistência dependente da dose. Os tempos de degradação de cinquenta porcento dos resíduos (TD_{50}) dos agrotóxicos nas diferentes doses aplicadas variaram de 1,7 a 2,7 dias para difenoconazol e de 7,6 a 10,5 dias para linurom. Ao fim do período de carência, as cenouras tratadas com altas doses dos agrotóxicos foram consideradas impróprias para o consumo. Não foram encontrados produtos de degradação dos agrotóxicos estudados nas cenouras. Os resultados reforçam que o cumprimento das instruções de dosagem e período de carência estabelecidos para os agrotóxicos estudados na cultura da cenoura asseguram que os limites máximos de resíduos sejam respeitados. Este é o primeiro estudo investigando a cinética de dissipação de difenoconazol e linurão em cenouras a campo.

Palavras-chave: *Daucus carota* L., agrotóxicos, tempo de degradação, extração solido-liquido com partição a baixa temperatura, cromatografia gasosa

Dissipation kinetics of difenoconazole and linuron and their natural degradation products in carrots in the field

Abstract: Rising concern about food safety has led to increasing number of studies on the effect of pesticide residues in agricultural products. We investigated the dissipation of two pesticides, difenoconazole and linuron, using a field experimental approach with carrots exposed to one-, two- and fivefold the recommended dose of the pesticides. The degradation kinetics for both pesticides in carrot followed a pseudo first-order model

but at different kinetic parameters. Difenoconazole was the least persistent pesticide, but for both pesticides, the persistence depended on the dose. The degradation time for fifty percent of the pesticide lost at the different doses ranged from 1.7 to 2.7 days for difenoconazole and from 7.6 to 10.5 days for linuron. At the end of the pre-harvest period, carrots treated with high doses of both pesticides were considered unfit for consumption. No degradation products of either pesticide were found in the carrots. No published data are currently available; therefore, this is the first study to provide data on the natural dissipation of difenoconazole and linuron in carrots in the field.
Keywords: *Daucus carota* L., pesticides, degradation times, solid-liquid extraction with low temperature partition, gas chromatography

Introduction

Carrot (*Daucus carota* L.) is one of the most important vegetables, with high worldwide consumption, extension of acreage, and great socioeconomic involvement of farmers. The consumption of carrots is critical for human health as a fundamental source of carotenoid precursors of vitamin A (Luengo et al., 2000). For the cultivation of carrots, intensive and constant use of pesticides is required to minimize losses and to increase productivity and maintain agricultural production quality (Carvalho et al., 2005; Liu et al., 2005; González-Rodríguez et al., 2011). Despite beneficial effects on the global food supply and in maximizing the economic gain from agricultural activities, the use of pesticides can cause environmental problems that include soil and water contamination and bioaccumulation in food (Prestes, 2009; Larramendi, 2003).

Increasing concerns about food safety and environmental impact has led to an increase in the number of studies investigating the effects of pesticide residues in agricultural products consumed by humans (Larramendi, 2003; Rozemeijer & Broers, 2007; Zhang et al., 2010). The intensive, indiscriminate and injudicious use of pesticides results in the contamination of food. Poor pesticide handling practices and the use of more toxic pesticides by farmers are associated with this contamination (APHA, 2005). Consequently, governments and international organizations have established maximum residue limits (MRLs) and pre-harvest intervals for fruits and vegetables. Pre-harvest intervals are defined as the time required to reduce the residue levels below the MRL and can be estimated from a residue dissipation curve (Ambrus & Lantos, 2002; Fenoll et al., 2009; Rahimi et al., 2015). The rate of dissipation depends on the

climatic conditions under which the pesticides are applied, among other factors. Hence, the dissipation of residues must be evaluated in each matrix as a function of time under the specific climatic conditions for each agricultural practice (Anastassiades & Lehotay, 2003).

Until this study, published data have not been available on the natural dissipation of the fungicide difenoconazole and the herbicide linuron in a carrot field. Therefore, in this research, the dissipation kinetics of difenoconazole and linuron were determined to evaluate the impact of using of those pesticides in carrot fields. The possible formation of natural degradation products of these pesticides was also determined. This research will provide information to develop strategies for the safe use of these pesticides in carrot fields.

Materials and Methods

Reagents and solutions

The solutions used in this study were prepared from the analytical standards of the fungicide difenoconazole 99.2% w/w (Sigma Aldrich, St. Louis, MO, USA) and the herbicide linuron 99.3% w/w (Sigma Aldrich, St. Louis, MO, USA) using acetonitrile as solvent, 99.9% v/v (Sigma Aldrich, St. Louis, MO, USA). Acetonitrile was also used as a solvent extractor. Stock solutions of 1000 mg L^{-1} of pesticides in acetonitrile were prepared, and subsequent dilutions of these solutions were prepared containing the pesticides in different concentrations according to the stage of the study. The solutions were stored at -20 °C. Commercial formulations of difenoconazole, Score 250EC, (Syngenta, Basel, Switzerland) with 25% a.i. and linuron, Afalon 450SC, (Adama, Airport, Israel) with 45% a.i. were applied to carrot fields.

Dissipation of pesticides in carrots in the field

The cultivation of carrots (Carandaí variety) was conducted at the Universidade Federal de Viçosa (UFV), Viçosa - MG, Brazil, in beds (1 x 2 m) previously prepared and fertilized according to a previous soil analysis. The cultural practices were conducted until the harvest according the recommendations of the Manual of Safety and Quality for Carrot Culture (EMBRAPA, 2004). No pesticides, other than those studied, were used in the cultivation of carrots.

Commercial formulations of the pesticides were applied individually in different areas of the planting to ensure that each batch of roots contained residues of a single product. Each product was used following the number and interval between applications recommended by the manufacturer in doses equivalent to one-, two- and fivefold the recommended dose in the product packaging. After the last application of pesticides, the carrots were harvested periodically until the end of the pre-harvest period to record the natural dissipation of the products in the field (Table 1.1).

Table 1.1. Pesticide commercial formulations, pre-harvest intervals, application times, days of harvest, and application doses.

Pesticide	Pre-harvest intervals (days)	Application time (days after planting)	Harvesting (days after planting)	Carrots bed	Dose (recommended dosage)*
Score (difenoconazole)	15	58 and 65	66, 69, 72, 76, and 80	A1	x1
				A2	x2
				A3	x5
Afalon (linuron)	60	41	42, 52, 70, 84, and 101	B1	x1
				B2	x2
				B3	x5

*The dosage of Score and Afalon recommended for the carrot culture are 600 and 2200 mL ha^{-1}, respectively.

After harvest, in the Postharvest Laboratory of the Agricultural Engineering Department of the UFV, roots were separated into samples of three roots each (replicates). The carrots were washed with tap water and submitted to the technique of pesticide extraction using solid-liquid extraction/low temperature partition (SLE/LTP). The extractions were performed in triplicate. The residue analyses were performed using a gas chromatograph equipped with an electron capture detector system (GC/ECD) and coupled to a mass spectrometer (GC/MS).

Pesticide residue analysis

Samples of carrot and SLE/LTP extraction

Difenoconazole and linuron residues were extracted from the carrot samples using SLE/LTP, adapted from Araújo et al. (2016). For the preparation of samples, 4.00 g of minced carrot was transferred to 22 mL vials, and 2 mL of deionized water (0.5 mS m^{-1}) and 4 mL of acetonitrile were added. The vials were agitated on an orbital shaker at 200 rpm for 10 min and then centrifuged at 3000 rpm for 3 min. These samples were stored in the freezer at -20 °C for 4 h. Then, the organic phase was separated, which does not freeze at -20 °C because the melting point is approximately -45 °C; thus, the matrix and aqueous phase was frozen, allowing the separation of the organic phase with pesticides. The organic phase was collected with a micropipette, transferred to 1.5 mL glass vials, and stored in a freezer at -20 °C for later chromatographic analysis.

Analysis by GC/ECD

The optimized conditions for the GC/ECD (Shimadzu GC-2014, Kyoto, Japan) analysis of the pesticides in carrot samples were as follow: injected sample volume 1.0 μL, drag gas flow (nitrogen at 1.3 mL min^{-1}), and initial column oven temperature at 100 °C (0.4 min), with heating pad at 25 °C min^{-1} up to 290 °C, maintained for 1 min. The injector temperature was fixed at 280 °C, and the detector was operated at 300 ºC. The separations were performed on a HP-5 capillary column, 30 m long, 0.25 mm inner diameter, and 0.10 μm film thickness (Agilent Technologies, Palo Alto, CA, USA), with the stationary phase consisting of phenyl 5% and dimethylsiloxane 95%. The total running time was 12 min.

Analysis by GC/MS

The presence of the degradation products of difenoconazole and linuron residues in carrot was analyzed on a GC/MS system composed of a 7820A gas chromatograph coupled to a 5977B mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The GC/MS was operated in full scan mode (mass acquisition range m/z 50-450) using ionization energy of 70 eV. The gas chromatograph was operated in splitless mode with injector temperature of 280 ºC. A capillary column HP-5m (30 m x 0.25 mm i.d. x 0.25 μm film thickness; stationary phase 5% diphenyl/95% dimethyl polysiloxane; Agilent Technologies) was used for the analysis. The initial column oven temperature was 100 °C (0.4 min), with heating pad at 25 °C min^{-1} up to 290 °C, maintained for 9 min.

Helium was used as carrier gas with a column flow rate of 1.2 mL min^{-1}. The initial solvent cut time was 2.9 min. The volume of injected samples was 1.0 μL, and the data acquisition time was 17 min. The MS spectrum was compared with the NIST mass spectra database.

SLE/LTP-GC/ECD method validation

Validation of the SLE/LTP-GC/ECD method was required to determine whether the methodology met the objective of its use, because the original method of Araújo et al. (2016) was changed. The validation assessed the following parameters of merit of the proposed method: selectivity, linearity, limits of detection (LOD) and quantification (LOQ), precision, and accuracy.

The selectivity of the analytical method was evaluated by comparing chromatograms of extracts from a pesticide-free array with those of extracts of the matrix fortified with the pesticides in concentrations equivalent to 1 x MRL (0.2 mg kg^{-1} for difenoconazole and 1 mg kg^{-1} for linuron) (ANVISA, 2012). The linearity of response of the method was determined using matrix-matched calibration by injecting extracts of fortified samples at ten pesticide concentrations: 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25 and 2.5 × MRL (ANVISA, 2012), subjected to the SLE/LTP technique. After the chromatographic analysis, calibration curves were constructed, linking the areas of analytes with the respective concentrations. The linearity was assessed by the correlation coefficient obtained by linear regression of these calibration curves. The LOD and LOQ were determined using a calculation based on 3.3- and 10-fold the ratio between the standard deviation of the intercept and the slope estimated from the calibration curve of the analytes (INMETRO, 2016). Based on repeatability, the precision of the method was determined on samples of carrot fortified with difenoconazole and linuron. The repeatability was verified by conducting injections of 0.5, 1.0, and 1.5 x MRL with six repetitions for the standard solution and subjected to the SLE/LTP method maintaining all the operational conditions constant. The accuracy is the systematic error of that measuring system and was evaluated by calculating the values of the coefficients of variation (CVs) of the results obtained (EURL, 2015; INMETRO, 2016).

Study of the dissipation of pesticides in carrot

Six kinetic models were tested to calculate pesticide dissipation kinetic parameters (Table 1.2). Describing the adequacy of a model can be subjective, because it depends on the study objectives and error-tolerance levels at which the model fit can be accepted. Several criteria were adopted in this study to determine the goodness-of-fit: visual graphical comparison, *r* value, and standard deviation of the residuals. The degradation times of 10%, 50% and 90% of the products (DT_{10}, DT_{50} and DT_{90}, respectively) were calculated from the selected kinetic degradation models. Parameters of the kinetic models and their standard errors were obtained by least square non-linear regression analysis using the statistical program Sigma Plot 12.5.

Table 1.2. Models tested to calculate pesticide dissipation kinetic parameters and their abbreviations and equations.

Dissipation model	Abbreviation	Equation*
Polynomial linear	POL	$C = C_0 + k*t$
Polynomial quadratic	POQ	$C = C_0 + k_1*t + k_2*t\text{^}2$
Polynomial cubic	POC	$C = C_0 + k_1*t + k_2*t\text{^}2 + k_3*t\text{^}3$
Exponential decay with 2 Parameter	ED2	$C = C_0 *exp(-k*t)$
Exponential decay with 3 parameter	ED3	$C = a + C_0 *exp(-k*t)$
Logarithm with 2 parameter	LO2	$C = if\ (t>0;\ a + C_0 *ln(abs(t));\ 0)$

*C = pesticide concentration; t = time after application; C_0 = initial concentration; a = final plateau concentration; k = degradation constant.

Results and Discussion

SLE/LTP-GC/ECD method validation

The selectivity parameter was evaluated for the two pesticides by comparing the chromatogram of the extract of carrot pesticide-free matrix with that of the extract of matrix fortified with 1 × MRL of the monitored pesticides, prepared according to the method used. Based on the chromatograms obtained of extracts by SLE/LTP from carrot samples, the pesticides difenoconazole and linuron showed a retention time of 3.075 and 9.750 min, respectively (Fig. 1.1). The absence of any signal at the retention

times of difenoconazole and linuron indicated that there were no matrix compounds that could give a false positive signal. Furthermore, no peaks were found in the chromatograms of carrot without the pesticides at retention times corresponding to the difenoconazole and linuron peaks. These results demonstrated the validity of the method when used for the kinetic study of difenoconazole and linuron in carrot under field conditions.

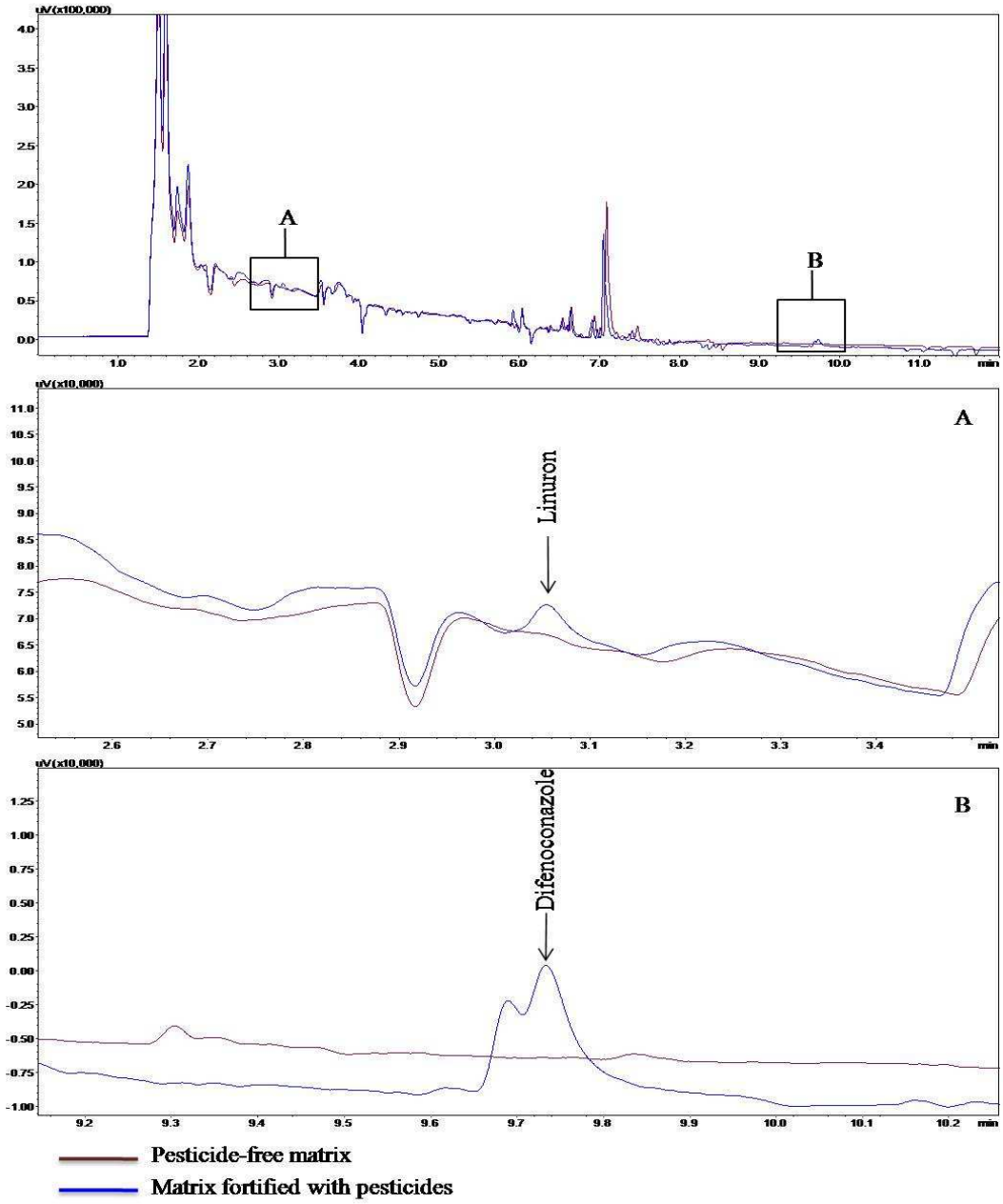

Fig. 1.1. Comparing the chromatograms of the extract of carrot pesticide-free matrix with the extract of matrix fortified with 1 × MRL of difenoconazole and linuron.

The calibration curves of difenoconazole and linuron showed good linearity and strong correlation between concentrations and peaks areas in the studied ranges. Analytical curves correlate the analyte areas and their concentrations, and the equations of the lines and the correlation coefficients (*r*) are shown in Table 1.3 The correlation coefficient of the calibration curve for difenoconazole was 0.994 and that for linuron was 0.990. Such values indicate the good linearity of the method in response to the two pesticides at concentrations close to the MRLs.

Table 1.3. Merit parameters for the determination of difenoconazole and linuron pesticides in carrots using SLE/LTP-GC/ECD.

Parameters	Difenoconazole	Linuron
Work range (mg kg^{-1})	0.05-0.5	0.5-2.5
LOD[1] (mg kg^{-1})	0.02	0.12
LOQ[2] (mg kg^{-1})	0.05	0.36
Analytical curve	y = 164349.78x + 3095.73	y = 7674.15x – 1179.03
Correlation coefficient (*r*)	0.994	0.990

[1] Limit of detection; [2] Limit of quantification;

The LOD is the lowest concentration of analyte detectable in the sample by an analytical method, and the LOQ is the lowest solute concentration that can be determined with an acceptable level of uncertainty (Abad et al., 2010; EURL, 2015). The LOD and LOQ values (Table 1.3) were acquired using the method based on analytical curve parameters, and the detection and quantification limit values were lower than the MRLs prescribed by regulatory agencies such as USEPA and ANVISA for difenoconazole and linuron in carrot (USEPA, 1996; ANVISA, 2012). Thus, for the two pesticides evaluated, the LOQs achieved were satisfactory to use the proposed method in the Brazilian and US markets. However, only the LOQ obtained for difenoconazole complied with European legislation, which established MRLs in carrots from 0.4 mg kg^{-1} for difenoconazole and 0.2 mg kg^{-1} for linuron (EFSA, 2015).

The precision and accuracy values obtained from the validation study are shown in Table 1.4. The average recovery percentages obtained for the three levels studied ranged from 93.4% to 116.6%, with the lowest recovery for difenoconazole and the highest recovery for linuron. For pesticide residue analysis, an analytical procedure should retrieve, at each level of fortification, an average from 70% to 120% (ANVISA, 2011). The accuracy related to chromatographic areas was expressed by the coefficients of variation (CV) and ranged from 3.5 to 10.4% (Table 1.4). According to Ribani et al.

(2004), CV values up to 20% are acceptable depending on the complexity of the sample.

Although similar to the method used in our study, the optimized SLE/LTP method that was validated per Araújo et al. (2016) for linuron pesticide extraction in carrot for analysis by GC/MS obtained an LOD of 0.20 mg kg^{-1} and LOQ of 0.61 mg kg^{-1}, which are values higher than those obtained by the SLE/LTP-GC/ECD method used in our study.

Table 1.4. The recovery percentages (precision) and coefficients of variation (accuracy) obtained by the analysis of extracts obtained from samples of carrot passed through SLE/LTP extraction and fortified with difenoconazole and linuron pesticides at three different concentrations (n = 6).

Pesticide	Concentration theoretical (mg kg^{-1})	Concentration obtained (mg kg^{-1})	Recovery (%)	CV (%)
Difenoconazole	0.1	0.093±0.010	93.4	10.4
	0.2	0.213±0.013	106.3	6.5
	0.3	0.306±0.010	101.9	3.5
Linuron	0.5	0.583±0.025	116.6	5.1
	1.0	0.981±0.088	98.1	8.8
	1.5	1.426±0.105	95.1	7.0

The results of the method validation demonstrated the SLE/LTP-GC/ECD approach had good performance and efficiency, with low consumption of sample and extractor solvent. Furthermore, this method has some advantages compared with traditional techniques, such as practicality and low number of steps, in addition to reliability and selectivity. Another advantage of this method is that the sample components are frozen with the aqueous phase, whereas pesticides are extracted in the organic phase, without the requirement to clean up one step (Costa et al., 2015).

Kinetic study of natural dissipation of pesticides during carrot cultivation

Estimation of the dissipation of pesticides in foods constitutes an important part of pesticide environmental risk assessment required for the authorization and placement on the market of plant protection products. We investigated the dissipation and the transformation of two pesticides using a field experimental approach as a scenario for carrot exposure to these pesticides and their primary transformation products. The dissipation kinetics of the three doses of difenoconazole and linuron in carrots in the

field were evaluated by plotting pesticide residues against time, and the equation of the best fitting curve was estimated, except for the x1 recommended dose of linuron (Fig.1.2). The dissipation kinetics of the x1 recommended dose of linuron could not be determined because from the third harvest (30 days after application), the levels of the herbicide residue were below the LOQ of the method used (0.36 mg kg^{-1}). For this treatment, at the first harvest (24 h after application), the average residue of linuron was 1.61 mg kg^{-1} of carrot, and at the second harvest (15 days after application), 77.3% of the residue had dissipated, with an average of 0.365 mg kg^{-1} of the herbicide remaining in the carrots.

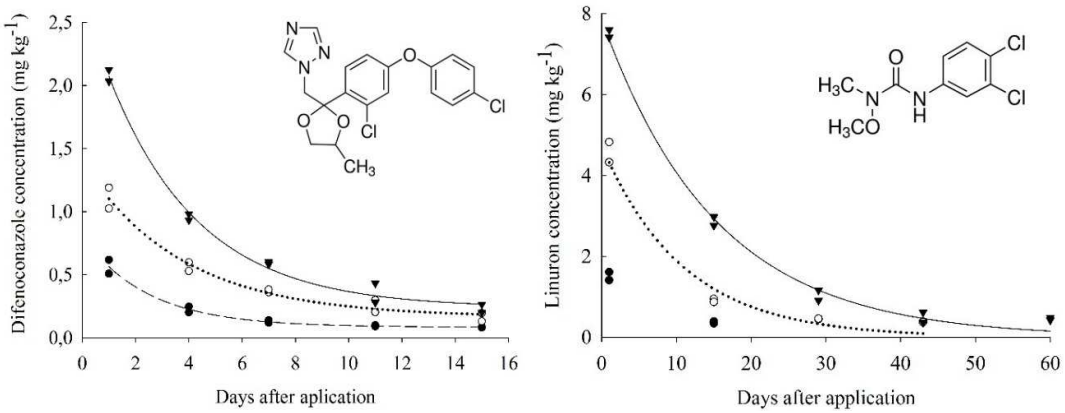

Fig. 1.2. The dissipation of difenoconazole and linuron in carrots in the field treated with x1 (●), x2 (○), and x5 (▼) the recommended dose of pesticide (600 mL ha^{-1} for Score and 2200 mL ha^{-1} for Afalon).

The residual concentrations of difenoconazole and linuron at all doses in carrots were well fitted ($r \geq 0.902$) by pseudo first-order kinetics. The exponential decay with three parameters model (ED3) was the best explanation for the data for difenoconazole, and the exponential decay with two parameters model (ED2) was the best explanation for the data for linuron (Table 1.5). In the ED3 and ED2 models, C_0 is the initial concentration of pesticide (mg kg^{-1}), C is the concentration of pesticide in the matrix at time t (mg kg^{-1}), t is the post application time (days), k is the degradation rate constant (day^{-1}) and a is the final plateau concentration (mg kg^{-1}).

In statistical descriptions of the dissipation of pesticides, the first-order model has been dominant for decades (Ntow et al., 2007; Juraske et al., 2011; Szpyrka & Walorczyk, 2013; Mohapatra, 2014; Zhang et al., 2015 However, a single first-order model may not provide the best description of the decay of a pesticide in vegetable fields for several reasons: both soil and water are complex environments in which

populations of degrading microorganisms vary considerably; many pesticides are degraded by different pathways that may involve both chemical and microbiological steps; and chemicals are distributed among soil, water and vegetables by complex adsorption/desorption mechanisms that influence the availability of the chemical to degradation (FOCUS, 2006). In our study, exponential decay models were chosen in which a rapid initial decrease in pesticide concentrations is followed by a very slow decline in concentration. The degradation trends of difenoconazole and linuron were similar (Fig. 1.2 However, the disappearance constant (*k*) of the pesticides was lower for linuron than for difenoconazole at all doses (Table 1.5). This difference was expected, because the preharvest interval of carrots established for linuron (60 days) is fourfold longer than that for difenoconazole (15 days).

Table 1.5. Dissipation kinetics parameters of difenoconazole and linuron in carrots by pseudo first-order kinetics based on models of exponential decay with three parameters (ED3) and exponential decay with two parameters (ED2).

Substance	Model	C_0 (mg kg^{-1})	a (mg kg^{-1})	k (day^{-1})	r^2	DT_{10} (day)	DT_{50} (day)	DT_{90} (day)
Lin x2	ED2	4.738 ±0.600	-	0.092 ±0.013	0.902	1.1	7.6	25.1
Lin x5	ED2	7.863 ±0.538	-	0.066 ±0.004	0.961	1.6	10.5	35.0
Dif x1	ED3	0.717 ±0.056	0.085 ±0.018	0.407 ±0.066	0.977	0.3	1.7	5.7
Dif x2	ED3	1.225 ±0.076	0.164 ±0.046	0.267 ±0.044	0.976	0.4	2.6	8.6
Dif x5	ED3	2.464 ±0.083	0.237 ±0.044	0.255 ±0.025	0.993	0.4	2.7	9.0

The degradation of 10%, 50%, and 90% of difenoconazole and linuron residues shown in Table 5 was obtained by DT_{10} = ln(0.9)/-k, DT_{50} = ln(0.5)/-k and DT_{90} = ln(0.1)/-k, respectively (Ma et al., 2004; Zhang et al., 2012; Zhang et al., 2014a; Yan et al., 2015; Zhang et al., 2015). The initial concentrations of difenoconazole in carrots were 0.718, 1.225 and 2.464 mg kg^{-1} for the x1, x2 and x5 recommended doses, respectively, whereas for linuron, the initial concentrations were 4.738 and 7.863 mg kg^{-1} for the x2 and x5 recommended doses, respectively. The DT_{10} value was less than 1

day for all doses of difenoconazole and less than 2 days for the x2 and x5 recommended doses of linuron (Table 1.5).

The DT_{50} values of both pesticides in carrots tended to increase with the increase in dose; 7.6 and 10.5 days for the x2 and x5 recommended doses of linuron and 1.7, 2.6 and 2.7 days for the x1, x2 and x5 recommended doses of difenoconazole, respectively (Table 1.5). The difenoconazole DT_{50} values were not similar to those of most previous literature reports. For example, the half-life of difenoconazole varies from 3.6 to 5.5 days in wheat straw (Zhang et al., 2015), 6.6 to 7.8 days in Chinese cabbage (Wang et al., 2008), 26.2 to 81.7 days in apples (Guo et al., 2010), 4.7-8.1 days in chili fruit (Mukhopadhyay et al., 2011), 4.4-5.1 days in grapes (Banerjee et al., 2008), and 1.4 to 2.6 days in rice plants (Wang et al., 2012). Our study is the first to report the DT_{50} of linuron in a carrot field. The study of degradation kinetics of this pesticide has been restricted to soils in controlled environments. Nevertheless, the values of DT_{50} for linuron in those soils fluctuate greatly, varying between 10.4 and 291.9 days (Usoroh & Hance, 1974; Walker, 1976; Walker & Thompson, 1977; Kempson-Jones & Hance, 1979; Hance & Haynes, 1981; Swarcewicz et al., 2013; Maín-Benito et al., 2014).

The differences in the DT_{50} values of these two pesticides in foods, plants or soil samples in the literature can be explained by differences in crop varieties or matrices (Wang et al., 2012). Furthermore, under field conditions, the dissipation of pesticides is affected by a variety of factors, including volatilization, wash-off, photodegradation, stability of the pesticide, frequency and rate of pesticide application (initial concentration), weather (sunlight, temperature, humidity, and wind), and metabolism in plants or microorganisms (Fantke & Juraske, 2013; Lu et al., 2014; Zhang et al., 2014a; Zhang et al., 2014b). Some authors implicate volatilization as one of the primary routes of pesticide loss in the field (Kennedy et al., 2001; Ntow et al., 2007).

Concentrations of x2 and x5 the recommended dose of linuron were reduced more than 90% on days 26 and 36, respectively, and those of difenocanazol on days 6, 9 and 10 for the x1, x2 and x5 recommended doses, respectively. On the last day of analysis for the x1, x2 and x5 recommended doses of difenoconazole (15 days after application); the residue concentrations were 0.0847, 0.1636 and 0.2375 mg kg^{-1}, respectively. The final residue concentrations for the x2 and x5 recommended doses of linuron (60 days after application) were < 0.360 and 0.454 mg kg^{-1}, respectively. The residue content of the x2 and x5 recommended doses for difenoconazole and the x5 recommended dose for linuron in the carrots at harvest time exceeded the MRLs set for Brazil, the USA and European Union simultaneously (USEPA, 1996; ANVISA, 2012,

EFSA, 2015). Thus, the results in our study reinforce the importance of proper use of pesticides. Furthermore, compliance with the dosage instructions and with the pre-harvest periods established for the use of difenoconazole and linuron in carrot crops ensures that the residue limits set for these pesticides are not exceeded.

Degradation products were also monitored but were not found. Metabolism of a given pesticide in plants is a multi-step process. The first step in the metabolism of a pesticide generally involves hydrolysis or oxidation of the parent molecule, leading to functional groups that are subject to secondary enzymatic conjugation. This process increases the water solubility of the molecules, facilitating the translocation and consequent elimination of these products from the plant (Van Eerd et al., 2003). Moreover, low concentrations of pesticide degradation products in plants are difficult to identify by analytical methods.

Conclusion

The patterns of degradation were similar for different doses of both difenoconazole and linuron in carrots, following a pseudo first-order model but with different kinetic parameters. Both pesticides increased in persistence at increased doses. At the end of the pre-harvest period, carrots treated with high doses of both pesticides were considered unfit for consumption. The primary factors for the dissipation of pesticide residues in crops are application times, doses and pre-harvest intervals. From the results of this study, the advantages of the application of pesticides in agriculture in producing better crops must be clearly weighed against the possible health hazards that come with toxic pesticide residues in food.

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ARTIGO 2

Tratamentos com ozônio para remoção de agrotóxicos de cenouras: Otimização por metodologia de superfície de resposta

Resumo: Os riscos potenciais dos agrotóxicos à saúde e a crescente preocupação dos consumidores com a qualidade dos alimentos evidenciam a necessidade de estudos sobre técnicas capazes de remover esses resíduos nos alimentos. Os tratamentos com ozônio para a descontaminação alimentar foram estudados nos últimos anos devido ao elevado potencial de oxidação deste gás mesmo em baixas concentrações. Com o presente trabalho objetivou-se otimizar os tratamentos com ozônio em gás e dissolvido na água para remoção de difenoconazol e linurom em cenouras. Utilizou-se um planejamento fatorial do tipo composto central para estudar três variáveis que regem a eficácia dos tratamentos com ozônio: concentração de O_3, temperatura e tempo de tratamento. A temperatura não teve efeito significativo sobre a eficácia dos tratamentos com ozônio. A remoção dos agrotóxicos aumentou com o incremento da concentração de ozônio e do tempo de tratamento. Reduções de mais de 80% dos resíduos dos agrotóxicos foram observadas por ambas as formas de aplicação de O_3. Os maiores percentuais de remoção foram atingidos quando as raízes foram expostas durante 120 min a 5 e 10 mg L^{-1} de ozônio, respectivamente nos tratamentos com O_3 em gás e dissolvidos em água. Após cinco dias de armazenamento das cenouras, os valores máximos de remoção dos agrotóxicos pela exposição ao gás ozônio foram superiores a 98% para o difenoconazol e 95% para o linurom. Nos tratamentos com O_3 dissolvido em água, as remoções de difenoconazol e linurom atingiram valores, respectivamente, de até 96 e 79,8% ao fim do armazenamento. Os produtos de degradação dos agrotóxicos pelo O_3 também foram monitorados, mas não foram encontrados. Este é o primeiro trabalho demonstrando a remoção de difenoconazol e linurom de cenoura pelos tratamentos com ozônio.

Palavras-chave: *Daucus carota* L., difenoconazol, linurom, armazenamento, extração solido-liquido com partição a baixa temperatura, cromatografia gasosa

Ozone treatment for pesticide removal from carrots: Optimization by response surface methodology

Abstract: The potential risks by pesticides to health and the growing consumer concern about food quality have evidenced need for studies about techniques capable to degrading these

residues in food. Treatments using ozone as an alternative for food decontamination have been studied in recent years due to ozone's high oxidation potential even at low concentrations. The present study aimed to optimize ozone (O_3) treatments, as gas and dissolved in water, to remove difenoconazole and linuron in carrots. We employed a central composite design to study three variables governing the efficacy of ozone treatments: O_3 concentration, temperature and treatment time. The temperature did not influence the efficacy of treatments. The removal percentage of pesticides increases with increases in ozone concentration and the time of treatment. O_3 application promoted the removal of more than 80% of pesticides when the roots were exposed for approximately 120 min at 5 and 10 mg L^{-1} ozone, respectively, in treatments with O_3 as gas and dissolved in water. After storage, pesticide removal was higher than 98% for difenoconazole and 95% for linuron. The degradation products from the pesticides resulting from O_3 treatment were monitored, but none were found. This is the first report demonstrating the removal of difenoconazole and linuron from carrots by ozone.

Keywords: *Daucus carota* L., difenoconazole, linuron, storage, solid-liquid extraction with low temperature partition, gas chromatography

Introduction

Carrots (*Daucus carota* L.) are widely consumed all over the world. With a total production of 38.8 million tons, carrots are an economically important crop for the producing countries (FAO, 2014). The intensive use of pesticides for controlling insects, diseases and invasive plants is necessary for carrot cultivation to minimize losses in productivity and maintain the quality of the final product (Carvalho et al., 2005; Liu et al., 2005 Carrots are in direct contact with soil, and their roots, covered by a thin permeable film, expose them to contamination by pesticides used in the crop cycle and the residues left in the soil by prior cultures (Souza et al., 2008).

The potential risks of pesticides to health and the growing consumer concern about food quality have evidenced the need to study techniques capable of degrading these residues in food. Technologies currently adopted to reduce or eliminate pesticide residues in foods include the use of chlorine, hydrogen compounds, ultraviolet radiation, ultrasound, heat treatments and ozone gas (O_3) (Hwang et al., 2001; Lin et al., 2012; Al-Antary et al., 2015). However, the use of ozone has been highlighted due to its high oxidative power and easy

availability (Santos et al., 2016; Laureano et al., 2016). Ozone is formed from the rearrangement of oxygen atoms and can be generated by electric discharges or the incidence of high-energy electromagnetic radiation in the air. Moreover, O_3 is an unstable molecule that rapidly decays to diatomic oxygen and therefore leaves no residue in food (Gabler et al., 2010). The oxidation of organic compounds by O_3 can occur through the reaction of the O_3 molecule with organic compounds and the reaction of the free radicals formed by the O_3 decomposition with organic compounds (Chiron et al., 2000; Von Gunten, 2003).

In the food industry, ozone is used for the decontamination of fruits and vegetables to preserve food during storage without modifying its physical-chemical and organoleptic characteristics. Al-Antary et al. (2015) found that the use of O_3 dissolved in water (4 $\mu g\ L^{-1}$) to treat juice-producing tomatoes removed 100% of the carbosulfan residue in the final product. Moreover, Heleno et al. (2014) studied the effect of ozone gas on difenoconazole removal and found that O_3 treatment reduced the pesticide residue in strawberries from 5 to 0.5 mg kg^{-1}. The use of ozone in pesticide removal has been demonstrated in other agricultural products such as lettuce, grape, apple, mustard, lemon, orange, grapefruit, corn, wheat, and lychee (Forney et al., 2007; Wu et al., 2007; Gabler et al., 2010; Heleno et al., 2015; Lozowicka et al., 2016). Although the potential for ozone in the removal of pesticide residues is known for several foods, there are no reports on the use of this gas for the removal of the pesticide residue in carrots.

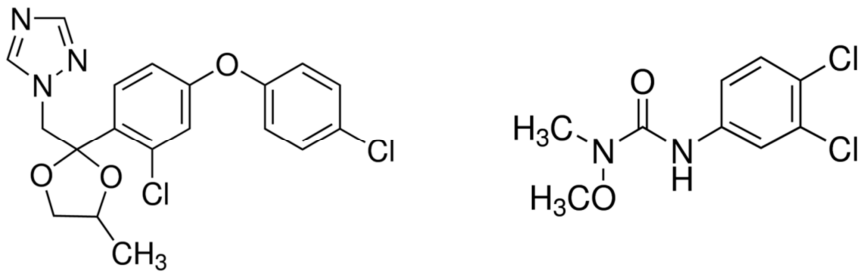
The effectiveness of ozone applied as gas or dissolved in water depends on factors such as the time of exposure, temperature and chemical composition of food (Misra, 2015). Therefore, the application parameters of ozone cannot be generalized, and specific studies are necessary for obtaining information about the ozonation process of each food. Thus, this study aimed to optimize the use of ozone in gaseous form and dissolved in water as an immediate and long-term degradation agent of difenoconazole and linuron in carrots. The pesticide degradation products in the carrots were also evaluated.

Materials and Methods

Reagents and solutions

The solutions employed in this study were prepared from the analytical standard of fungicide difenoconazole 99.2% w/w and the herbicide linuron 99.3% w/w using acetonitrile

99.9% w/w as solvent, all from the Sigma-Aldrich brand (St. Louis, MO, USA) (Fig. 2.1). Acetonitrile was also used as an extraction solvent. Stock solutions of 1000 mg L^{-1} of pesticides in acetonitrile were prepared and subsequently diluted to obtain different concentrations according to the stages of the study. Commercial formulations with 25% difenoconazole fungicide (Score 250EC, Syngenta, Basel, Switzerland) and 45% linuron herbicide (Afalon 450SC, Adama, Airport, Israel) were applied on carrot fields.



Difenoconazole Linuron

Fig. 2.1. Chemical structure of difenoconazole and linuron.

Carrot field cultivation

Carrots (Carandaí variety) were grown at the Universidade Federal de Viçosa (UFV), Viçosa - MG, Brazil, in beds (1 m x 10 m) previously prepared and fertilized according to the soil analysis. The cultural practices carried out until the harvest followed the recommendations of the Manual of Safety and Quality for Carrot Culture (EMBRAPA, 2004). No pesticides, other than those studied, were used in the cultivation of carrots. Each pesticide was applied individually in different areas of the planting to ensure that each batch of roots possessed the residue of a single product. Each pesticide was applied 80 days after planting in doses equivalent to five times the recommended dose in the product packaging (totalizing 3 L ha^{-1} of Score 250EC and 11 L ha^{-1} of Afalon 450SC). Three days after pesticide application, the carrots were harvested and taken to the Postharvest Laboratory of the Agricultural Engineering Department of the UFV. The carrots were washed with tap water and submitted to solid-liquid extraction/low-temperature partition (SLE/LTP) for pesticide analysis. Pesticide extractions were performed in triplicate. The analyses were performed by a gas chromatograph equipped with an electron capture detector system (GC/ECD) and a gas chromatograph coupled to a mass spectrometer (GC/MS).

Pesticide residue analysis

Samples of carrot and SLE/LTP extraction

The method SLE/LTP, adapted from Araújo et al. (2016), was used to extract the difenoconazole and linuron residues from the carrot samples. For the preparation of samples, 4.00 g of minced carrot was transferred to 22 mL vials, and 2 mL of deionized water (0.5 mS m^{-1}) and 4 mL of acetonitrile were added. The vials were subjected to agitation on an orbital shaker at 200 rpm for 10 min and were later subjected to centrifugation at 3000 rpm for 3 min. The samples were stored in a freezer at -20 °C for 4 h. After this time, the matrix and aqueous phase freeze, allowing the extraction of the organic phase with pesticides. The organic phase was collected with a micropipette and transferred to 1.5 mL glass vials for later chromatographic analysis.

Analysis by GC/ECD

The optimized conditions for the GC/ECD (Shimadzu GC-2014, Kyoto, Japan) analysis of the pesticides in carrot samples were as follows: the injector temperature was fixed at 280 °C, the detector temperature was operated at 300 °C, the injected sample volume was 1.0 μL, drag gas flow was applied (nitrogen at 1.3 mL min^{-1}), the initial column oven temperature was 100 °C (0.4 min), with a heating pad at 25 °C min^{-1} up to 290 °C, and this temperature was fixed for 1 min. The total running time was 12 min. The separations were performed on an HP-5 capillary column (Agilent Technologies, Palo Alto, CA, USA), 30 m, 0.25-mm inner diameter, and 0.10 μm film thickness, with the stationary phase consisting of phenyl 5% and dimethylsiloxane 95%.

Analysis by GC/MS

The presence of the degradation products from difenoconazole and linuron residues in carrot was analyzed on a GC/MS system composed of a 7820A gas chromatograph coupled to a 5977B mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The GC/MS was operated in full scan mode (mass acquisition range m/z 50-450) using an ionization energy of 70 eV. The gas chromatograph was operated in splitless mode with an injector temperature of 280 °C. The initial column oven temperature was 100 °C (0.4 min), with a heating pad at 25 °C min^{-1} up to 290 °C, which was maintained for 9 minutes. Helium was used as the carrier gas with a column flow rate of 1.2 mL min^{-1}. The initial solvent cut time was 2.9 min. The injected sample volume was 1.0 μL, and the data acquisition time was 17 min. A capillary

column HP-5ms (Agilent Technologies, Palo Alto, CA, USA) 30 m x 0.25 mm i.d. x 0.25 µm film thickness with stationary phase 5% diphenyl/95% dimethyl polysiloxane was used for analysis. The MS spectrum was compared with the NIST mass spectra database.

Optimization of ozone treatment conditions for pesticide removal

Two experiments were carried out separately, one for the optimization of the use of O_3 as gas and the other with O_3 dissolved in water. In both experiments, ozone was obtained through the ozone generator O&L3.ORM (Ozone & Life, São José dos Campos, SP, Brasil). The ozone generator used an oxygen flow of 2 L min^{-1} from the Mark 5 Plus Concentrator Oxygen Concentrator (Nidek Medical Products, Birmingham, AL, EUA). The ozone concentrations in the gas and dissolved in water were quantified before and after the passage through the treatment chambers using the iodometric method by indirect titration (Eaton & Franson, 2005; Gottschalk et al., 2010). After the passage through the entire system, the residual ozone was directed to a catalyst filter (Ozone & Life, São José dos Campos, SP, Brasil) with the function of transforming ozone into oxygen. Eighteen carrots (≅ 2000 g) were used in each treatment of both experiments.

Experiment 1 - O_3 in gas: The carrot treatment with ozone gas was carried out in a 0.075 m³ acrylic chamber (0.32 x 0.53 x 0.44 m), with perforated shelves that allowed the gas flow of O_3 inside the chamber. The chamber was fitted with an inlet at the top coupled to the ozone generator. The gas outlet, connected to a catalyst filter, was inserted in the lower part of the chamber.

Experiment 2 - The treatment of the carrots with ozone dissolved in water was carried out in a circular chamber of PVC, 50 x 80 cm (diameter x height), containing 10 L of deionized water (0.5 mS m^{-1}). The ozone gas inlet was an aperture in the medial portion of the chamber, and it was coupled to a perforated spiral that ran through the water column until it was concentrated in the bottom of the chamber. A perforated metal plenum was placed over a 10-cm layer of glass beads (2 cm) above the spiral located at the bottom of the chamber to provide support for the carrots and a better distribution of the ozone gas in the water. The outlet of the remaining gas was an aperture in the top of the chamber, and it was connected to a catalyst filter. After each treatment, the carrots were withdrawn from the water and allowed to dry at room temperature (23 °C) for 30 min.

In both experiments, to control the temperature throughout the treatment, the chambers were inserted into a climatic chamber that allowed a variation of ± 1 °C. After ozonation, the

samples of three carrots were analyzed for pesticide residues by SLE/LTP-GC/ECD. The analysis of the pesticide residues was carried out in triplicate.

Factorial planning

The ozone treatments were optimized for the removal of the pesticides difenoconazole and linuron in carrots employing a central composite design, with five replicates in the central point (Table 2.1). Three variables were studied: ozone concentration, treatment time and temperature. The variables were studied at two levels, and the analyses were performed in triplicate. The effects of each variable and the interactions between the variables in the difenoconazole and linuron removal from carrots were calculated using the Statistica 13.0 software (Statsoft Inc., Tulsa, OK, USA). The data were presented in graphs made by SigmaPlot 12.5 software (Systat Software, Inc., San Jose, CA, USA).

Table 2.1. Central composite design, with five replicates at the central point (C), to investigate the effects of the concentration of O_3 as gas (experiment 1) and dissolved in water (experiment 2), temperature and treatment time on the removal of difenoconazole and linuron residue in carrots.

Treatment	Levels of coded variables			Levels of real variables for experiment 1			Levels of real variables for experiment 2		
	X_1	X_2	X_3	O_3 concentration (mg L^{-1})	Time (min)	Temperature (ºC)	O_3 concentration (mg L^{-1})	Time (min)	Temperature (ºC)
1	-1	-1	-1	1.0	30.0	8.0	2.0	30.0	8.0
2	-1	-1	+1	1.0	30.0	20.0	2.0	30.0	20.0
3	-1	+1	-1	1.0	90.0	8.0	2.0	90.0	8.0
4	-1	+1	+1	1.0	90.0	20.0	2.0	90.0	20.0
5	+1	-1	-1	4.0	30.0	8.0	8.0	30.0	8.0
6	+1	-1	+1	4.0	30.0	20.0	8.0	30.0	20.0
7	+1	+1	-1	4.0	90.0	8.0	8.0	90.0	8.0
8	+1	+1	+1	4.0	90.0	20.0	8.0	90.0	20.0
9	-α	0	0	0.0	60.0	14.0	0.0	60.0	14.0
10	+α	0	0	5.0	60.0	14.0	10.0	60.0	14.0
11	0	-α	0	2.5	9.5	14.0	5.0	9.5	14.0
12	0	+α	0	2.5	110.5	14.0	5.0	110.5	14.0
13	0	0	-α	2.5	60.0	3.9	5.0	60.0	3.9
14	0	0	+α	2.5	60.0	24.1	5.0	60.0	24.1
15 (C)	0	0	0	2.5	60.0	14.0	5.0	60.0	14.0
16 (C)	0	0	0	2.5	60.0	14.0	5.0	60.0	14.0
17 (C)	0	0	0	2.5	60.0	14.0	5.0	60.0	14.0
18 (C)	0	0	0	2.5	60.0	14.0	5.0	60.0	14.0
19 (C)	0	0	0	2.5	60.0	14.0	5.0	60.0	14.0

Removal of pesticides in carrots stored after ozonations

Carrots submitted to treatments 9, 10 and 15 (Table 2.1) were used to study the long-term effects of ozone on pesticide removal. These treatments were selected because they were conducted at the same temperature (14 °C) and treatment time (60 min). Thus, it was possible to evaluate the isolated ozone effect. The carrots submitted to these treatments for both methods of ozone application were stored for up to five days in a climate controlled room (18 ± 2 °C, 80 ± 5% RH). A daily sample of three carrots from each treatment were taken for the verification of the long-term effect of ozone on pesticide residues. The analysis of pesticides residues was carried out in triplicate. The percentages of pesticide removal were subjected to a variance analysis, and the means were compared by the Tukey test at the 5% significance level. Data were analyzed with SAS 9.0 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Method validation

The extraction technique assessed the following parameters of merit of the proposed method: selectivity, linearity, limit of detection (LOD) and quantification (LOQ), precision, and accuracy. The selectivity of the analytical method was evaluated by comparing the chromatograms of the extracts from a pesticide-free array with the chromatograms of the extracts of the matrix fortified with pesticides studied in concentrations equivalent to the Maximum Residue Limit (MRL) (0.2 mg kg^{-1} for difenoconazole and 1 mg kg^{-1} for linuron) (ANVISA, 2012). The chromatogram of extracts from carrot samples (obtained by SLE/LTP) containing the pesticides difenoconazole and linuron showed retention times of 3.075 and 9.750 min, respectively. The absence of any signal at the retention time of difenoconazole and linuron indicated that no matrix compounds were present that could give a false positive signal. This demonstrated the validity of the method when used in the study of difenoconazole and linuron in carrot.

The linearity of the response of the method was determined by using matrix-matched calibration by injecting extracts of samples fortified at ten pesticide concentrations (0.25 - 2.5 × MRL) subjected to the SLE/LTP technique. After the chromatographic analysis, analytical curves were constructed, linking the areas of the analytes with the concentrations mentioned. Analytical curves relate the ratio of the analyte areas and their concentrations, thus obtaining the linear equations and the correlation coefficient. The calibration curves of difenoconazole

(y = 164349.78x + 3095.73) and linuron (y = 7674.15x – 1179.03) showed a good linearity and a strong correlation between the concentrations and peak area in the studied range. The correlation coefficients of the calibration curves were 0.994 for difenoconazole and 0.990 for linuron. Such values indicate the good linearity of the method in response to the two pesticides at concentrations close to the MRL.

LOD is the lowest concentration of the analyte detectable in the sample by any analytical method, while LOQ is the lowest solute concentration that can be determined with an acceptable level of uncertainty (Abad et al., 2010; EURL, 2015). LOD and LOQ were determined with a calculation based on 3.3 and 10 times the ratio between the standard deviation of the intercept and the slope estimated from the calibration curve of the analytes (INMETRO, 2016). The LOD and LOQ values were 0.020 and 0.050 mg kg^{-1} for difenoconazole and 0.120 and 0.360 mg kg^{-1} for linuron, respectively. These values were acquired using the method based on analytical curve parameters with a working range of 0.05-0.5 mg kg^{-1} for difenoconazole and 0.5-2.5 mg kg^{-1} for linuron. The detection and quantification limit values were lower than the MRL prescribed by regulatory agencies such as USEPA (0.5 mg kg^{-1} for difenoconazole and 1 mg kg^{-1} for linuron) and ANVISA (0.2 mg kg^{-1} for difenoconazole and 1 mg kg^{-1} for linuron) for both pesticides in carrot (USEPA, 1996; ANVISA, 2012).

The precision of the method in terms of repeatability was determined by carrot samples fortified with difenoconazole and linuron. The repeatability was verified by conducting injections of 0.5, 1.0, and 1.5 x MRL subjected to the SLE/LTP method, with six repetitions for the standard solution, maintaining all operational conditions constant. The precision values obtained in the three studied levels ranged from 93.4 to 116.6%, the lowest recovery being for difenoconazole and the highest for linuron. For the pesticide residue analysis, the analytical procedure should be able to retrieve an average of 70 to 120% (ANVISA, 2011) residue at each level of fortification.

The accuracy is the systematic error of the measuring system and was calculated by evaluating the values of the coefficients of variation (CVs) of the results obtained (EURL, 2015; INMETRO, 2016). The accuracy related to chromatographic areas ranged from 3.5 to 10.4%. According to Ribani, Botolli, Collins, Jardim and Melo (2004), CV values up to 20% are acceptable depending on the complexity of the sample. These results demonstrate the good performance of the method.

Optimization of ozone treatments for pesticide removal

Ozone treatment for pesticide removal is a typical example of gas absorption in a chemical reaction, which affects the mass transfer. Therefore, all process variables affecting these two phenomena will govern the efficacy of the ozone treatment. We studied three important variables (O_3 concentration, temperature and treatment time) governing the efficacy of ozone treatment in gaseous and liquid phase. Temperature variation in the range of 4-24 °C did not have a significant effect ($P > 0.0972$) on the efficacy of ozone treatments. For the same inlet O_3 concentrations, the O_3 concentrations in the chamber were similar even at different temperatures. This indicates that carrots treated with the same inlet O_3 concentration and same treatment time were exposed to the same O_3 concentration inside the chamber, even with different environmental temperatures (Table 2.2).

Table 2.2. Average ± SD of O_3 concentration (mg L^{-1}) in the inlet, in the chamber, and dissolved in water at different temperatures (°C) and treatment times (min).

Treat.	Time (min)	Temp. (°C)	O_3 concentration			
			Experiment 1		Experiment 2	
			Inlet (mg L^{-1})	In chamber (mg L^{-1})	Inlet (mg L^{-1})	Dissolved in water (mg L^{-1})
1	30.0	8.0	1.0	0.354 ± 0.100	2.0	0.689 ± 0.073
2	30.0	20.0	1.0	0.366 ± 0.072	2.0	0.513 ± 0.054
3	90.0	8.0	1.0	0.488 ± 0.143	2.0	0.379 ± 0.062
4	90.0	20.0	1.0	0.610 ± 0.132	2.0	0.243 ± 0.022
5	30.0	8.0	4.0	1.952 ± 0.133	8.0	0.689 ± 0.073
6	30.0	20.0	4.0	2.440 ± 0.231	8.0	0.513 ± 0.054
7	90.0	8.0	4.0	2.928 ± 0.132	8.0	0.629 ± 0.021
8	90.0	20.0	4.0	2.806 ± 0.120	8.0	0.533 ± 0.056
9	60.0	14.0	0.0	0.000 ± 0.000	0.0	0.000 ± 0.000
10	60.0	14.0	5.0	2.930 ± 0.291	10.0	0.683 ± 0.002
11	9.5	14.0	2.5	1.464 ± 0.112	5.0	0.229 ± 0.033
12	110.5	14.0	2.5	1.579 ± 0.103	5.0	0.397 ± 0.042
13	60.0	3.9	2.5	1.708 ± 0.193	5.0	0.454 ± 0.032
14	60.0	24.1	2.5	1.532 ± 0.086	5.0	0.234 ± 0.022
15 - 19	60.0	14.0	2.5	1.590 ± 0.193	5.0	0.337 ± 0.032

Unlike the gaseous O_3 treatments, the concentration of the dissolved ozone in the water decreased with an increase in temperature (Table 2.2). However, this variation was not sufficient for the temperature to be a significant factor in the removal of pesticides by O_3 treatments. The solubility of ozone decreases with increasing temperature (Achen & Yousef, 2001). Moreover, ozone decomposes in water to yield hydroxyl radicals (OH). However, the reaction rate of O_3 decomposition is much faster when the water temperature is high; and the relative contribution of these factors in pesticide removal from carrots may compensate each other (Ikeura et al., 2011).

The average concentration of pesticides found in carrots before submission to treatments was 2.500 mg kg^{-1} for difenoconazole and 7.200 mg kg^{-1} for linuron. Both the exposure time ($0.018 < P < 0.048$) and the ozone concentration ($P < 0.001$) were found to be significant in terms of pesticide removal. Three-dimensional response surface graphs were generated to demonstrate the effects of exposure time and ozone concentration on the removal of difenoconazole and linuron from carrots (Fig. 2.2 Moreover, the percentage of pesticide removal increases with increases in ozone concentration and treatment time. Both forms of O_3 application caused a significant reduction (over 80%) in pesticide residue, whereas non-O_3 treatments removed less than 20 and 45% of the pesticides in the gaseous and liquid treatments, respectively. The highest percentages of pesticide removal were achieved when the roots were exposed to ozone for approximately 120 min at 5 and 10 mg L^{-1} ozone, respectively, in the gaseous state and dissolved in water.

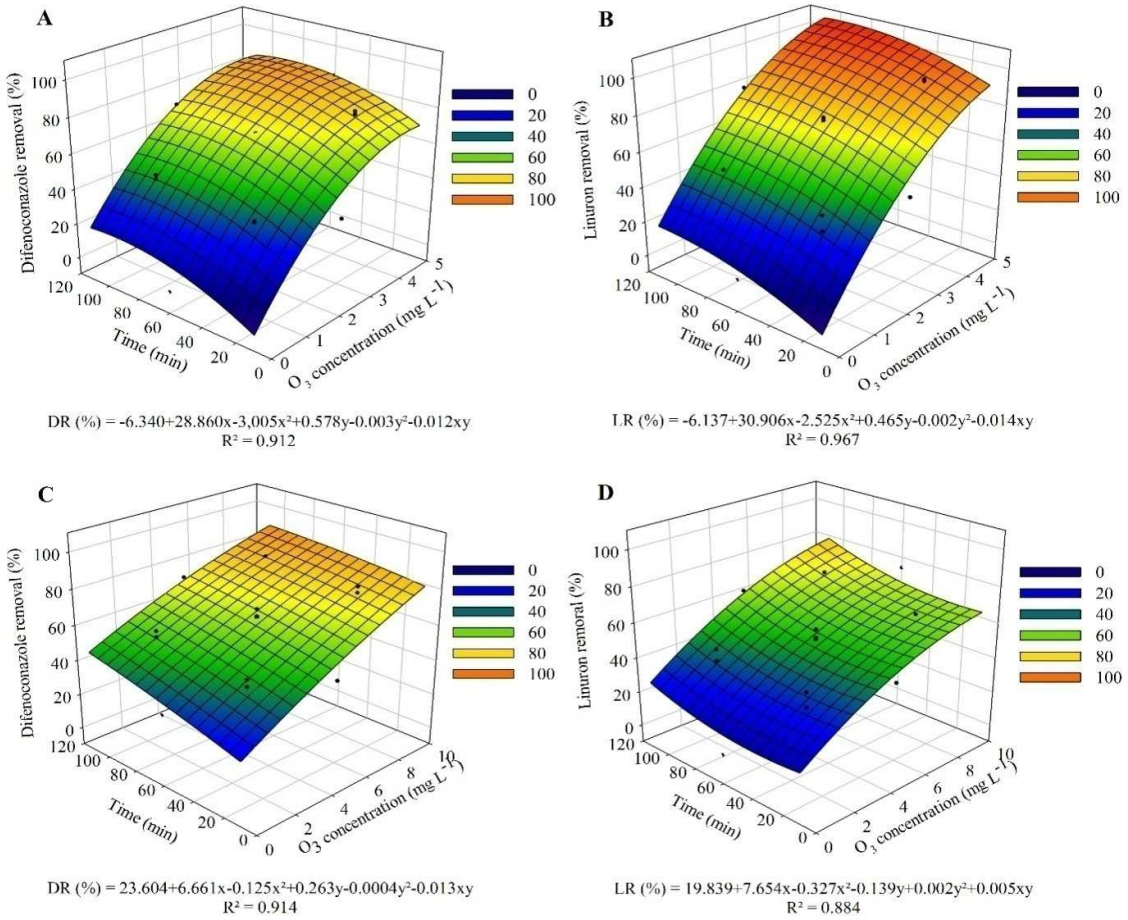


Fig. 2.2. Response surface showing the effect of $x = O_3$ concentration as gas (A and B) and dissolved in water (C and D) and y = treatment time on the removal of difenoconazole (DR) and linuron (LR) residue in carrots.

Ozone is a well-known gaseous chemical agent capable of oxidizing a variety of organic and inorganic compounds in the gaseous phase, as solid substrates and in aqueous solutions, either by direct attack or through a radical-mediated mechanism involving the hydroxyl radical (Segat et al., 2014). Recently, several studies have reported effective removal of pesticide residues from fruits and vegetables by ozone (Gabler et al., 2010; Heleno et al., 2015; Lozowicka et al., 2016; Heleno et al., 2016). Although the use of ozone in pesticide removal has been proven for many agricultural products, its effect on the removal of difenoconazole and linuron from fruits and vegetable has not been well studied. Heleno et al. (2014) observed that the concentration of difenoconazole in strawberries reduced drastically as the concentration of ozone dissolved in water increased. After 1 h of exposure to ozone at a

concentration of 0.800 mg L^{-1}, the fungicide residue showed a 95% reduction compared with the fungicide concentration before ozone treatment.

The oxidation of organic compounds by O_3 can occur through two specific reactions: the reaction of the O_3 molecule with the molecules of the organic compounds and the reaction of the free radicals (-O) formed by O_3 decomposition, with organic compounds. In aqueous solution, the organic compounds are oxidized by the hydroxyl radical action formed from the oxygen atoms resulting from O_3 degradation with free hydrogen atoms in solution (Chiron et al., 2000; Von Gunten, 2003).

The pesticides difenoconazole and linuron have aromatic rings and double bonds in their molecular structure. The double bond is most susceptible to ozone attack, leading to the formation of a primary ozonide, which, being unstable, dissociates into a stable compound and a corresponding intermediate. The intermediates undergo further decomposition via O-atom elimination, the ester channel, or the hydrogen peroxide channel or might stabilize after collision with another body (Al Rashidi et al., 2013).

In some cases, the disappearance of the pesticide residue does not indicate safe treatment because the degraded products may be as toxic as the parent compounds (Wu et al., 2007). The intermediates from the degradation of difenoconazole and linuron by O_3 were also monitored, but none were found in this study. Ten intermediates from the degradation of linuron by O_3 were identified by Rao and Chu (2009). The authors suggested that N-terminus oxidation should be the major mechanism of linuron decay by ozonation, and all the intermediates should be possibly susceptible to the ozonation process. However, the intermediates may not accumulate to an appreciable level due to fast decays. Our study corroborated the results of Rao and Chu (2009), who did not found intermediates in the solution when 99% linuron degradation was achieved.

Removal of pesticides from carrots stored after ozonation

In addition to the immediate effect on pesticide removal, our research also evaluated the possible long-term effect of ozone treatment on carrots. As seen in Table 2.3, in all treatments, at 14 °C for 60 min, the removal percentages of pesticides increased up to the fifth day of storage. However, the removal percentages reached higher values in the ozone treatments.

Table 2.3. Effect of O_3 concentration on the removal of difenoconazole and linuron residue (%) from carrots in storage.

Pesticide	O_3 concentration (mg L^{-1})	Storage time (days)		
		1	3	5
Ozone in gas				
Difenoconazole	0	18.1 b	53.1 b	53.2
	2.5	94.9 a	95.7 a	> 98.0*
	5	95.3 a	95.7 a	> 98.0*
	P value	*0.0007*	*0.0001*	-
Linuron	0	12.1 b	19.4 b	22.9 b
	2.5	73.9 a	74.2 a	78.1 a
	5	> 95.0*	> 95.0*	> 95.0*
	P value	*0.0021*	*0.0101*	*0.0027*
Ozone dissolved in water				
Difenoconazole	0	33.1 c	40.3 b	44.8 b
	5	70.3 b	79.0 a	88.8 a
	10	88.7 a	91.1 a	96.0 a
	P value	*0.0015*	*0.0014*	*0.0020*
Linuron	0	7.4 c	13.0 c	15.8 b
	5	50.9 b	57.7 b	63.2 a
	10	75.7 a	78.2 a	79.8 a
	P value	*0.0003*	*<0.0001*	*0.0034*

* Removal percentages higher than 98% for difenoconazole and 95% for linuron could not be quantified because they correspond to values lower than LOQ of the analytical method used (0.05 and 0.36 mg kg^{-1}, respectively). Values followed by the same letters in the column are not significantly different by the Tukey test at 5% significance level ($P< 0.05$).

The removal percentages of difenoconazole remained the same statistically ($P < 0.0014$) in all treatments with O_3 as gas and dissolved in water from the first and third storage days, respectively. The same behavior was observed for linuron on the fifth day of storage after treatment with O_3 dissolved in water ($P = 0.0034$). Toward the end of the carrots' storage period, the maximum removal values of the pesticides by ozone as gas were greater than 98% for difenoconazole and 95% for linuron. In the treatments with O_3 dissolved in water, the removal percentages of difenoconazole and linuron reached values of up to 96 and 79.8%, respectively.

In our experiments, approximately 92% of the difenoconazole residue and 86% of the linuron residue needed to be removed so that the MRLs set for Brazil and USA were respected simultaneously (USEPA, 1996; ANVISA, 2012). Values equal to or lower than the MRLs were reached on the first day of storage of the carrots after 60 min of treatment with O_3 as gas at a concentration of 2.5 mg L^{-1} for difenoconazole and 5 mg L^{-1} for linuron. However,

in treatments with O_3 dissolved in water, the MRL was only reached for difenoconazole on the fifth day of storage of carrots treated with at least 2.5 mg L^{-1}.

The ability to remove difenoconazole and linuron from carrots without the formation of toxic intermediates strengthens the GRAS (Generally Recognized as Safe) status for the treatment, storage and processing of food and water recognized by the United States Food and Drug Administration (FDA, 2001). O_3 is environmentally friendly to produce and does not leave any residues in the food when it dissociates into oxygen; thus, it has a distinct advantage over other oxidants.

Conclusion

This is the first study to investigate the effect of ozone in the removal of difenoconazole and linuron in carrots. Ozone treatments in gaseous form and dissolved in water were effective methods for postharvest removal of the pesticides residues without the formation of toxic intermediates in carrots. Removal percentages increased with increases in ozone concentration and treatment time. In addition to being a strong oxidant, ozone also has the advantage of not leaving a decontaminant residue in treated foods because it quickly decomposes to oxygen.

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ARTIGO 3

Tratamentos com ozônio em cenouras: Impactos na qualidade pós-colheita

Resumo: O ozônio, um poderoso oxidante, é utilizado no tratamento de água, desinfestação por pragas e na remoção de agrotóxicos, micotoxinas e outros contaminantes de frutas e hortaliças. O *status* de tecnologia verde e a multifuncionalidade do ozônio o tornam um promissor agente no processamento de alimentos. No entanto, as condições de tratamento devem ser especificamente determinadas para cada tipo de produto para o uso eficaz e seguro do ozônio. O objetivo deste estudo foi avaliar o efeito de tratamentos com ozônio em gás (0 - 5 mg L^{-1}) e dissolvidos em água (0 - 10 mg L^{-1}) na qualidade de cenouras. A exposição de cenouras ao ozônio em gás e dissolvido em água não alteraram a porcentagem de perda de massa, firmeza e cor das cenouras. Os tratamentos com O_3 em gás também não afetaram o pH das cenouras. No entanto, em tratamentos com O_3 dissolvido em água, as concentrações de ozônio e sua interação com a temperatura afetaram temporariamente o pH das cenouras. Além disso, o O_3 em gás impediu o aumento acentuado de sólidos solúveis durante o armazenamento, aumentando assim a vida de prateleira das cenouras. Portanto, pode-se concluir que o ozônio em gás e dissolvido em água pode ser usado em cenouras sem prejudicar a qualidade das raízes.

Palavras-chave: *Daucus carota* L., armazenamento, ozonização, sólidos solúveis

Ozone treatments on carrots: Impact on post harvested quality

Abstract: Ozone is a powerful oxidant and is used in water treatment, pest disinfection and the removal of pesticides, mycotoxins and other contaminants from fruits and vegetables. However, the treatment conditions should be specifically determined for all types of products for the effective and safe use of ozone. The aim of this study was to evaluate the effect of ozone applied as gas (0 - 5 mg L^{-1}) and dissolved in water (0 - 10 mg L^{-1}) on the quality of carrots. The exposure of carrots to ozone as gas and dissolved in water did not alter the weight loss percentage, firmness and the color of the vegetable. The O_3 treatments as gas also did not affect the pH of the carrots. However, in treatments with O_3 dissolved in water, the ozone concentrations and its interaction with temperature temporarily affected the pH of carrots. Moreover, O_3 as gas prevented the sharp increase in soluble solids during storage, therey

increasing the shelf-life of carrots. Therefore, ozone as gas and dissolved in water can be used in carrots without affecting the quality of roots.
Keywords: *Daucus carota* L., storage, ozonation, soluble solids

Introduction

The fresh produce industry is constantly growing, due to increasing the consumer demand (Alothman et al., 2010). The shelf-life of the fresh produce, however, is limited and determined by its initial quality at harvest and subsequent storage conditions (Zhang et al., 2007; Nunes et al., 2009). Thus, techniques for reducing undesired microbial contamination, spoilage and decay and for maintaining the visual, textural and nutritional quality of the product are required at all steps of the production and distribution chain.

Sanitizing agents have widespread applications for assuring safety and quality in the food industry. However, certain agents, such as chlorine, can react to form trihalomethanes, which are of concern for both human dietary safety and as environmental pollutants (Charisiadis et al., 2014). Therefore, the food industry is searching for technologies that effectively inactivate pathogens and remove contaminants, limit the loss in product quality and ensure food freshness, are adaptable to food processes and economically feasible, and are environment-friendly (Pandiselvam et al., 2016). For meeting these criteria, the use of ozone in the food industry has been studied in the disinfection of microorganisms and controlling pests and mycotoxins, pesticide decontamination and preservation of food quality (Alencar et al., 2013; Ali et al., 2014; Karaca & Velioglu, 2014; Freitas et al., 2016; Heleno et al., 2016; Sousa et al., 2016; Tabakoglu & Karaca, 2015).

Ozone (O_3) was discovered and named by Schoenbein in 1840, but its applications for food treatment were not developed until considerably later (Rice, 1986; Gabler et al., 2010). Ozone is the tri-atomic oxygen formed by the addition of a free radical of oxygen to the molecular oxygen (Tiwari et al., 2008). Ozone exists in the gaseous state at room temperature and is partially soluble in water. In both cases, ozone is unstable with a short half-life (Cullen et al., 2009). Ozone decomposes to form oxygen; therefore, food products treated with ozone are free of disinfectant residue (Tiwari et al., 2008). Thus, according to the United States Department of Agriculture, food can be treated with ozone and can still be classified as “100% organic” or “organic”, depending on the O_3 usage (USDA, 2011).

When O_3 is used in post-harvest treatments, during storage or food processing, its high oxidation power may promote undesirable changes in the food quality. Fruits and vegetables are the most affected by the negative effects of ozone due their high moisture content, enzymes and phenolic compounds (Patil et al., 2010; Gabler et al., 2010; Sandhu et al., 2011). Consequently, an optimization of the conditions for treatment must be studied for each food. The aim of this study was to evaluate the effect of treatments with ozone as gas and dissolved in water in on carrots (*Daucus carota* L.). Thus, the immediate impact and the effect throughout storage of combinations of O_3 concentration, temperature and treatment time on weight loss, color, soluble solids concentration, pH and firmness of fresh carrots was assessed.

Material and Methods

Carrots field

The carrot cultivation (Carandaí variety) was performed in the Universidade Federal de Viçosa (UFV), Viçosa - MG, Brazil in four beds (1 x 10 m) previously prepared and fertilized according to the soil analysis. The cultural practices were carried out until harvest following the recommendations of the Manual of Safety and Quality for Carrot Culture (EMBRAPA, 2004). After harvest (80 days after the planting), the roots were taken to the Postharvest Laboratory of the Agricultural Engineering Department of the UFV and washed with tap water. Next, the carrots were separated into samples with three roots each, which constituted the replicates.

Optimization of ozone treatments conditions

Two experiments were performed separately, one for the optimization of the treatment conditions of O_3 as gas and the other one O_3 dissolved in water. In both experiments, ozone was produced by an ozone generator O&L3.ORM (Ozone & Life, São José dos Campos, SP, Brasil). The ozone generator used an oxygen flow of 2 L min^{-1} from the Mark 5 Plus Concentrator Oxygen Concentrator (Nidek Medical Products, Birmingham, AL, EUA). The ozone concentration in the gas and dissolved in water was quantified before and after the passage through the treatment chambers using the iodometric method by indirect titration (Eaton & Franson, 2005; Gottschalk et al., 2010). After the passage through the entire system, the residual ozone was directed to a catalyst filter (Ozone & Life, São José dos Campos, SP,

Brasil) to degrade ozone to oxygen. Eighteen carrots (≅ 2000 g) were used in each treatment of both experiments.

Experiment 1 – O_3 as gas: The carrots were treated with ozone as gas in an acrylic chamber of 0.075 m³ (0.32 x 0.53 x 0.44 m) with perforated shelves that enabled the free flow of O_3 gas inside the chamber. The chamber was fitted with an inlet at the top coupled to the ozone generator. The gas outlet connected to catalyst filter was inserted in the lower part of the chamber.

Experiment 2 – O_3 dissolved in water: The treatment of the carrots with ozone dissolved in water was carried out in a circular chamber of PVC, 50 x 80 cm (diameter x height) containing 10 L of deionized water (0.5 mS m^{-1}). The ozone gas inlet occurred through an aperture in the medial portion of the chamber and was coupled to a perforated spiral that ran through the water column until it was concentrated in the inferring part of the chamber. A perforated metal plenum was placed over a 10 cm layer of glass beads (2 cm) above the spiral located at the bottom of the chamber to provide support for the carrots and a better distribution of the ozone gas in water. The outlet of the remaining gas was through an aperture at the top part of the chamber, and it was connected to a catalyst filter. After ozonation, the carrots were withdrawn from the water and allowed to dry at room temperature (23 °C) for 30 min.

In both experiments, for the temperature to remain constant throughout the treatment, the chambers were inserted into a climatic chamber that allowed the variation of ± 1 °C. The carrots were submitted to quality analysis before and after the ozonations.

Factorial planning

The ozone treatments were optimized to maintain the quality of the carrots employing a central composite design with five replicates at the central point (Table 3.1). Three variables were studied: ozone concentration, treatment time, and temperature. The variables were studied at two levels, and the analyses were performed in triplicate. The effects of each variable and the interactions between the variables in the carrots quality were calculated using the Statistica 13.5 software (Statsoft Inc., Tulsa, OK, USA). The data were presented in graphs generated using the SigmaPlot 12.5 software (Systat Software, Inc., San Jose, CA, USA).

Table 3.1. Central composite design with five replicates at the central point (C) to investigate the effect of the O_3 concentration as gas (experiment 1) and dissolved in water (experiment 2), temperature and treatment time on the carrots' quality.

Treatment	O_3 concentration on the experiment 1 (mg L^{-1})	O_3 concentration on the experiment 2 (mg L^{-1})	Time (min)	Temperature (ºC)
1	1.0 (-1)	2.0 (-1)	30.0 (-1)	8.0 (-1)
2	1.0 (-1)	2.0 (-1)	30.0 (-1)	20.0 (+1)
3	1.0 (-1)	2.0 (-1)	90.0 (+1)	8.0 (-1)
4	1.0 (-1)	2.0 (-1)	90.0 (+1)	20.0 (+1)
5	4.0 (+1)	8.0 (+1)	30.0 (-1)	8.0 (-1)
6	4.0 (+1)	8.0 (+1)	30.0 (-1)	20.0 (+1)
7	4.0 (+1)	8.0 (+1)	90.0 (+1)	8.0 (-1)
8	4.0 (+1)	8.0 (+1)	90.0 (+1)	20.0 (0)
9	0.0 (-α)	0.0 (-α)	60.0 (0)	14.0 (0)
10	5.0 (+α)	10.0 (+α)	60.0 (0)	14.0 (0)
11	2.5 (0)	5.0 (0)	9.5 (-α)	14.0 (0)
12	2.5 (0)	5.0 (0)	110.5 (+α)	14.0 (0)
13	2.5 (0)	5.0 (0)	60.0 (0)	3.9 (-α)
14	2.5 (0)	5.0 (0)	60.0 (0)	24.1 (+α)
15 (C)	2.5 (0)	5.0 (0)	60.0 (0)	14.0 (0)
16 (C)	2.5 (0)	5.0 (0)	60.0 (0)	14.0 (0)
17 (C)	2.5 (0)	5.0 (0)	60.0 (0)	14.0 (0)
18 (C)	2.5 (0)	5.0 (0)	60.0 (0)	14.0 (0)
19 (C)	2.5 (0)	5.0 (0)	60.0 (0)	14.0 (0)

Storage of carrots after ozonation

Carrots submitted to treatments 9, 10 and 15 (Table 3.1) were used to study the long-term effects of ozone on the quality of carrots. These treatments were chosen because they had the same temperature (14 °C) and treatment time (60 min). Thus, it was possible to evaluate the ozone effect in isolation. The carrots submitted to these treatments for both methods of ozone application were stored for up to five days in a climatic-controlled room (18

± 2 °C, 80 ± 5% RH). A sample of three carrots from each treatment was taken daily for the verification of the long-term effect of ozone on soluble solids, pH and firmness of carrots. A single sample with three carrots was maintained throughout the storage for analysis of weight loss and color. The quality parameters data were subjected to descriptive statistics and presented in graphs generated using the SigmaPlot 12.5 software (Systat Software, Inc., San Jose, CA, USA).

Quality analysis

Carrot quality analysis was performed immediately before and after treatments and was conducted daily over five days of storage. The effects of ozone on carrot quality were investigated by measuring firmness, weight loss, concentration of soluble solids (SS), pH and color according to the methods described by Instituto Adolfo Lutz (IAL, 2008).

Firmness

The firmness of roots was determined with a PTR 300 digital penetrometer (Sail Control, Austin, Texas, USA). A thin layer of the epidermis was excised from the epidermis, 5 cm from the top of the carrot, for measurement. For each replication, the carrots were compressed using the 6 mm diameter probe. The compression force measured at the maximum peak of the recorded force was expressed in Newtons (N).

Weight loss

Weight loss of the carrots was monitored using an analytical balance (Shimadzu Corp., Kyoto, Japan) with the precision of 1 x 10^{-4} g. The percentage of weight loss was determined according to Equation 1:

$$WL_t(\%) = \frac{W_0 - W_t}{W_0} \; x \; 100 \qquad (1)$$

Where WL_t is the percent weight loss at time t; W_0 is the initial sample mass and W_t is the sample mass at time t.

pH

The pH measurement was performed by immersing an electrode in a solution containing 10 g of the ground roots in 100 mL of distilled water using the digital benchtop pH meter MA-522 (Marconi Equip. para Laboratórios Ltda, Piracicaba, SP, Brazil).

Soluble solids

Soluble solids concentration (SS) was determined by a PAL-3 digital refractometer (Atago Co., Ltd., Tokyo, Japan) calibrated with deionized water (0.5 mS m^{-1}) before taking readings. The apparatus uses a drop of crushed carrot extract filtered through a muslin cloth to perform the measurement in °Brix.

Color

The color was always evaluated at a single point in the carrots with a CR-400 colorimeter (Konica Minolta, Tokyo, Japan) with direct reading of the reflectance of the L* (luminosity: black - white axis), a* (green - red axis) and b* (blue - yellow axis) coordinates. Using the values of these coordinates, it was possible to calculate the parameters related to color saturation: chroma (Equation 2) and the total color variation (ΔE) (Equation 3).

$$Chroma = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$\Delta E = \sqrt{(\Delta L^{*})^{2} + (\Delta a^{*})^{2} + (\Delta b^{*})^{2}} \quad (3)$$

Results

Optimization of ozone treatment conditions

In both approaches, treatment with ozone in gas and dissolved in water and such variables as ozone concentration, treatment time (10-120 min) and temperature (4-24 °C) were optimized to maintain the quality of the carrots. In tests with ozone in gas to (0-5 mg L^{-1}), these variables did not affect the weight loss percentage, L*, a*, b*, chroma, Δ*E*, firmness, SS and pH of the carrots immediately after treatment. (Fig. 3.1).

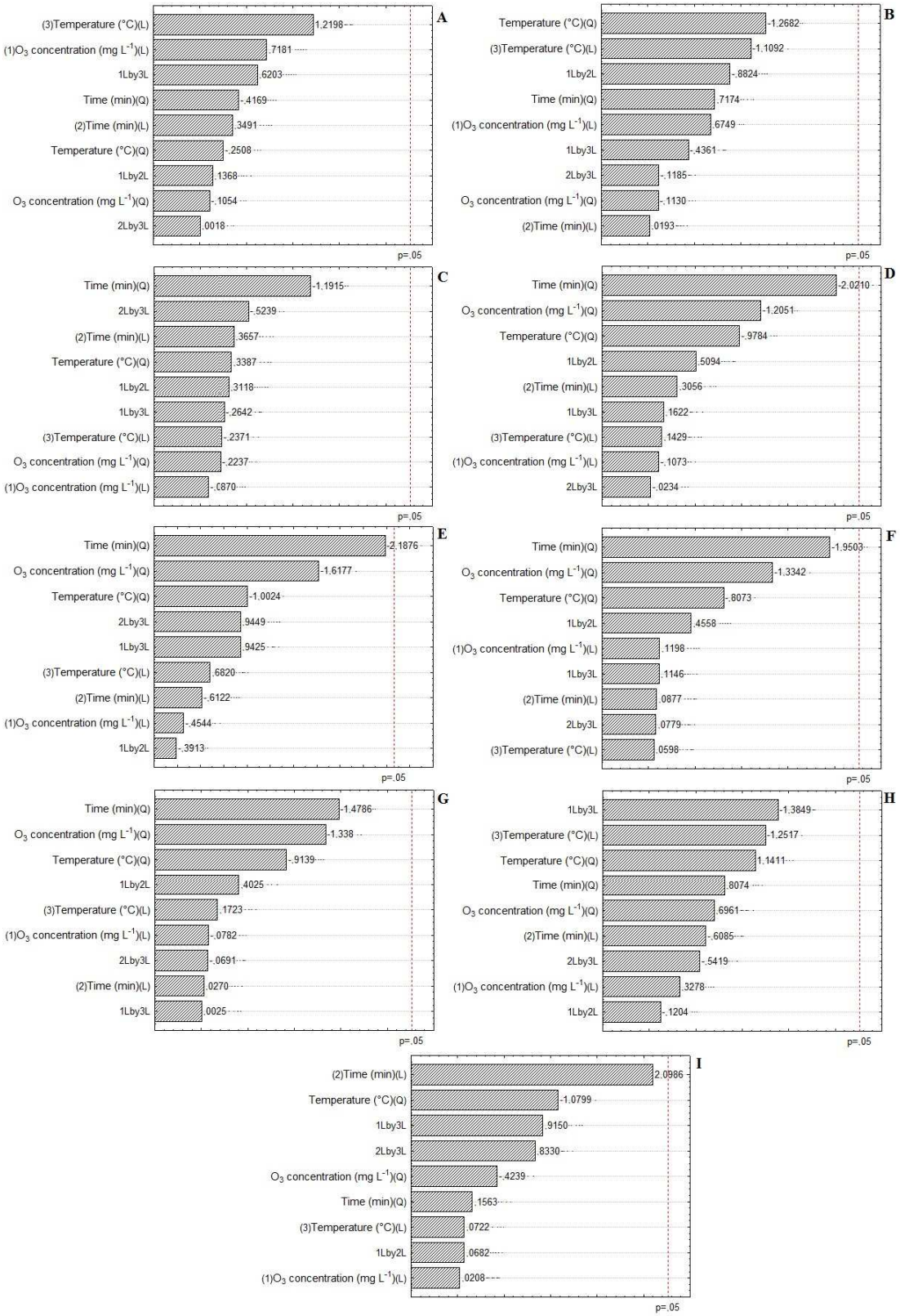


Fig. 3.1. Pareto diagrams of the effects of O_3 concentration in gas, treatment time and temperature on weight loss percentage (A), firmness (B), L* (C), a* (D), b* (E), chroma (F), ΔE (G), SS (H) and pH (I) of the carrots.

Similarly, in the treatments with ozone (0-10 mg L^{-1}) dissolved in water, the variables did not influence the values of weight loss percentage, L*, a*, b*, chroma, Δ*E*, firmness and SS of the carrots immediately after treatment. (Fig. 3.2).

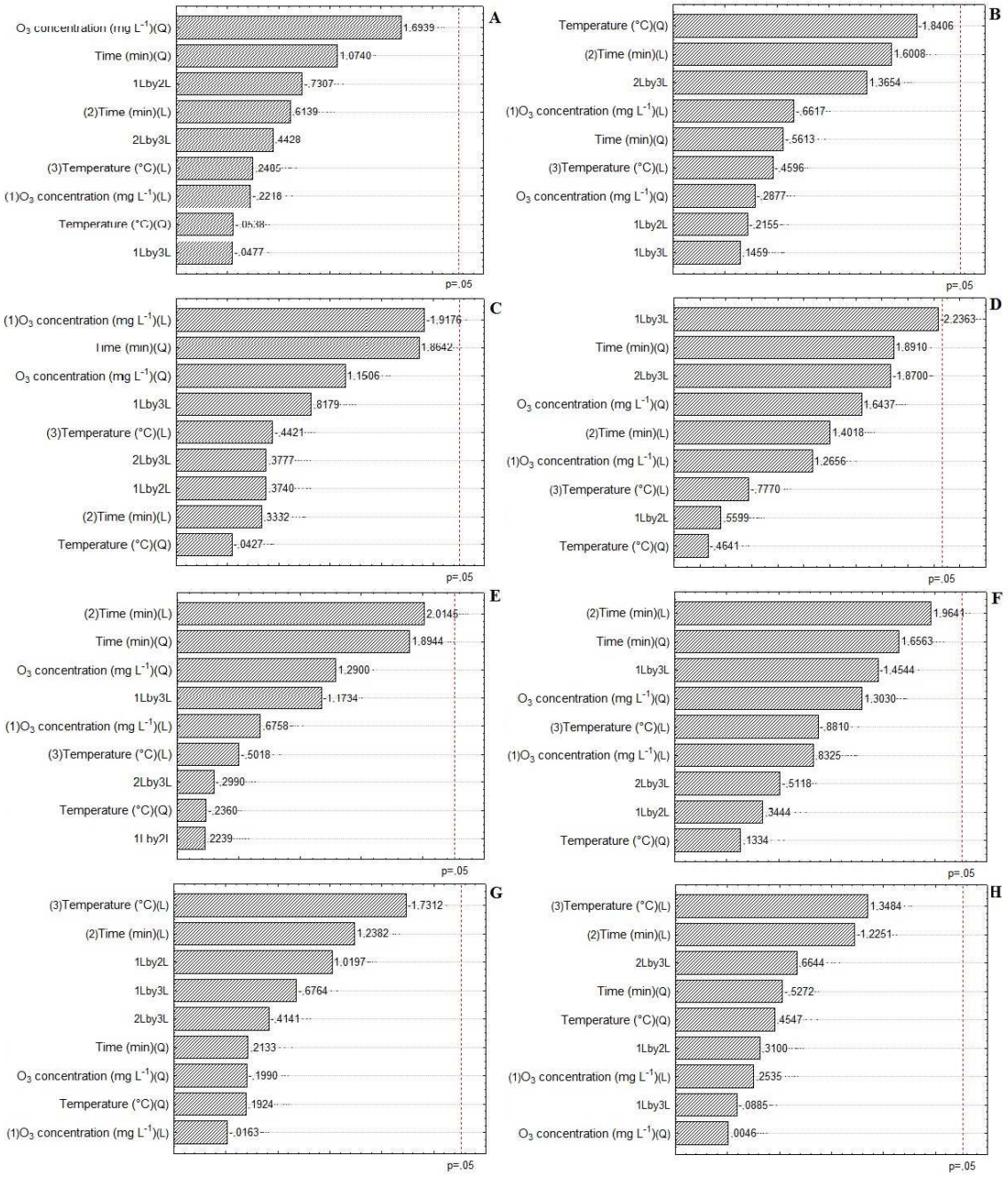
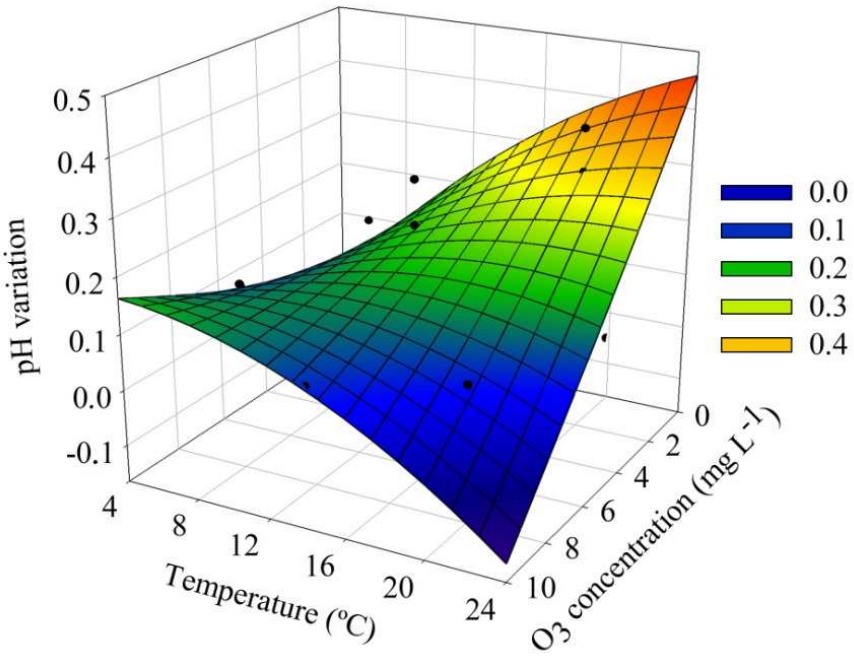


Fig. 3.2. Pareto diagrams of the effects of O_3 concentration dissolved in water, treatment time and temperature on weight loss percentage (A), firmness (B), L* (C), a* (D), b* (E), chroma (F), Δ*E* (G) and SS (H) of the carrots.

However, the O_3 concentration (P = 0.019) and the interaction between O_3 concentration and temperature (P = 0.032) for treatment in water was significant for the pH of carrots, and their effect was expressed in Fig. 3.3. According to the shape of the surface response plots, the highest pH variations in carrots were induced by the increase in temperature and low O_3 concentrations.



$$\Delta pH = - 0.182 + 0.028x + 0.0004x^2 + 0.042y - 0.0006y^2 - 0.004xy$$

$$R^2 = 0.674$$

Fig. 3.3. Response surface showing the effect of the x = O_3 concentration dissolved in water and y = temperature on the pH variation (ΔpH) of carrots.

Carrots storage after ozonation

During the five days of storage, the average quality parameters for carrots treated with ozone as gas and dissolved in water for 60 min at 14 °C were compared through descriptive statistics. There was a slight increase in the percentage of carrot weight loss in all treatments with no differences between treatments with and without ozone (Fig. 3.4). At the end of the fifth day of storage, the average weight loss of the carrots treated with gas was 20%, whereas that of the carrots treated with water was 14%. The average firmness of the carrots (140 N)

was also influenced neither by different treatments nor by the storage time for up to five days (Fig. 3.4).

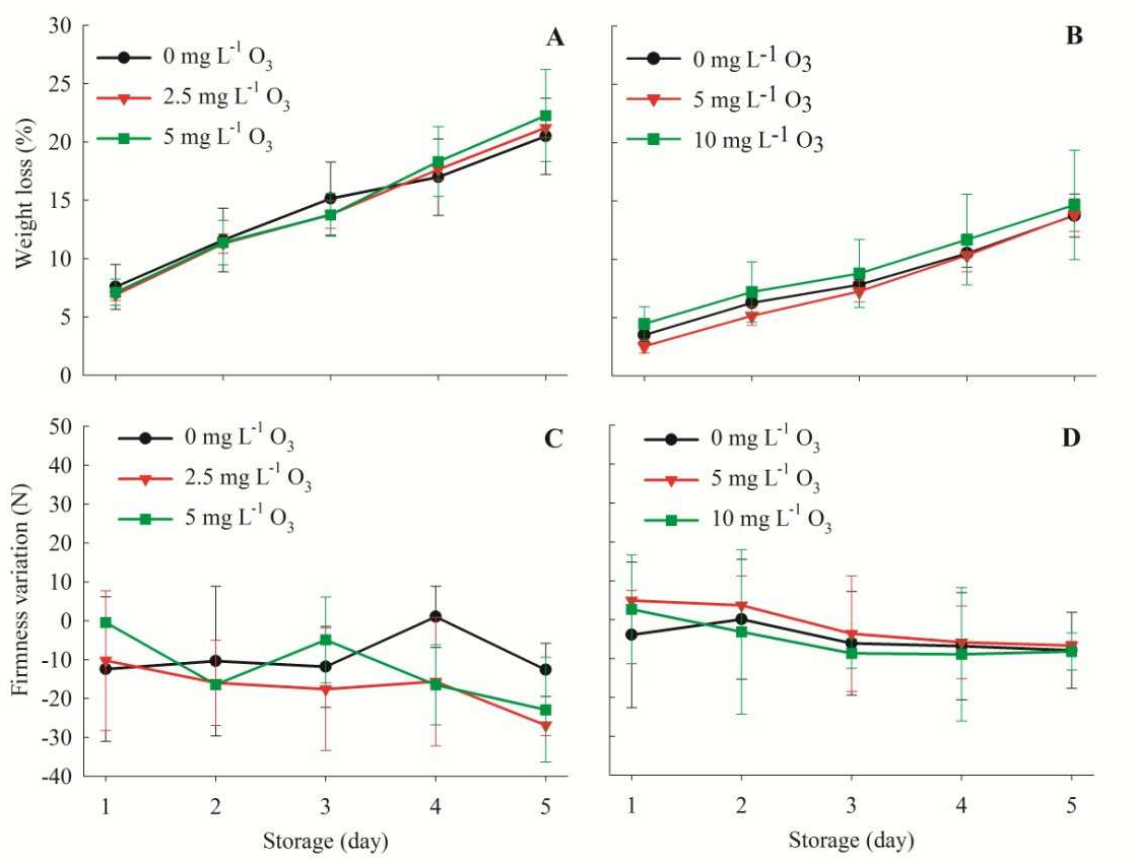


Fig. 3.4. Effect of ozone treatments as gas (A and C) and dissolved in water (B and D) on weight loss and firmness of stored carrots. The bars show standard deviation values.

The changes in the color of the carrots were evaluated in all treatments, and the results are shown in Fig. 3.5. There were no significant changes in the luminosity (L*), redness (a*) and yellowness (b*) of the samples. Consequently, ΔE and chroma, which showed a degree of saturation, purity or intensity of color, were also not altered by treatments or storage time.

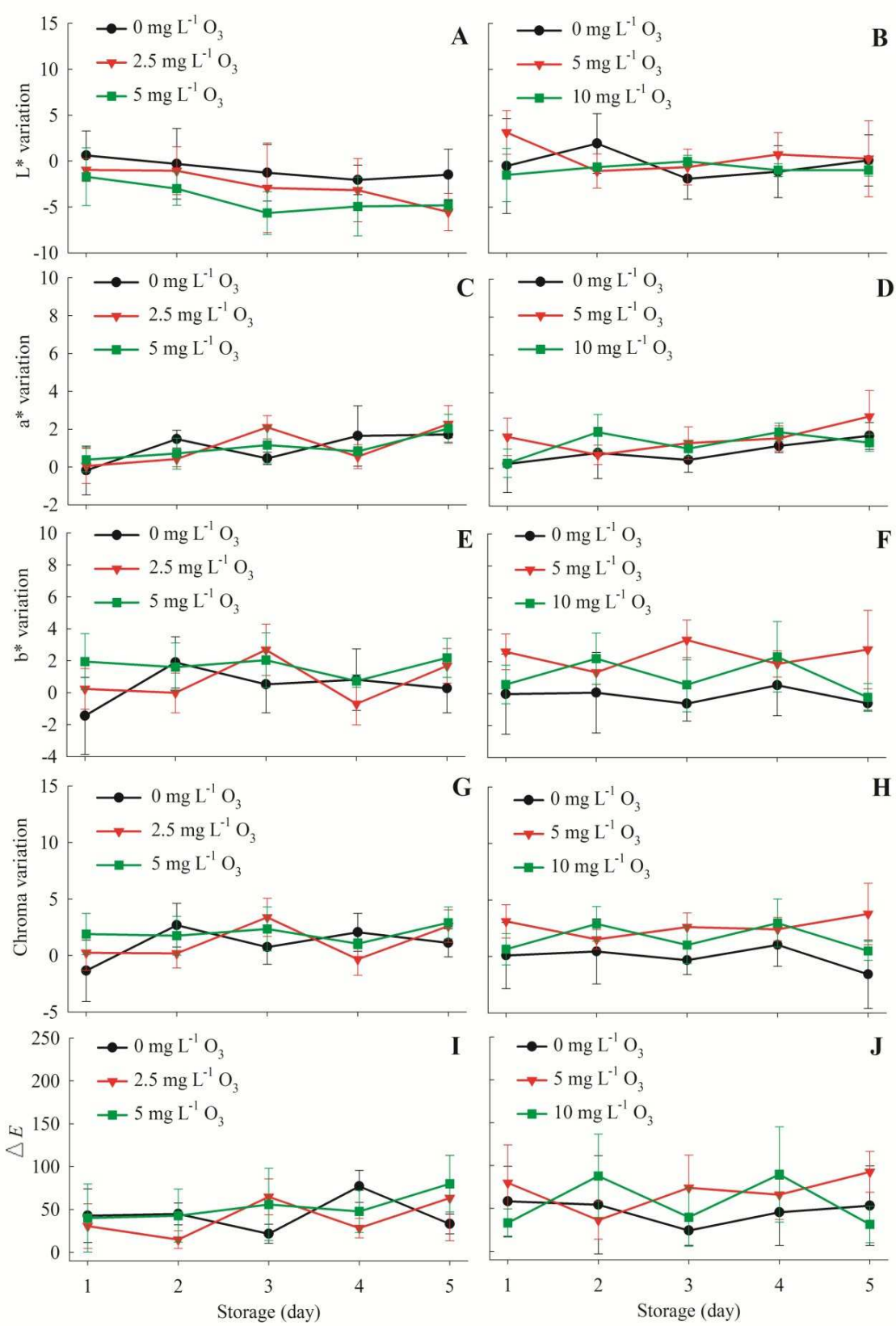


Fig. 3.5. Effect of ozone treatments on gas (A, C, E, G and I) and dissolved in water (B, D, F, H and J) on the color of stored carrots. The bars show standard deviation values.

The results of pH and SS are shown in Fig. 3.6. The pH values remained approximately 6.0 during the storage of the carrots treated in water; however, for treatment in gas a slight pH slight drop was observed after the second day of storage. In general, the SS values oscillated within the range of 7 to 8 °Brix up to the third day of storage for all treatments. From the fourth day of storage, an increase in the SS content was observed in all ozone treatments (gas and water), but the treatment with no O_3 in gas induced superior SS values in carrots compared with other treatments.

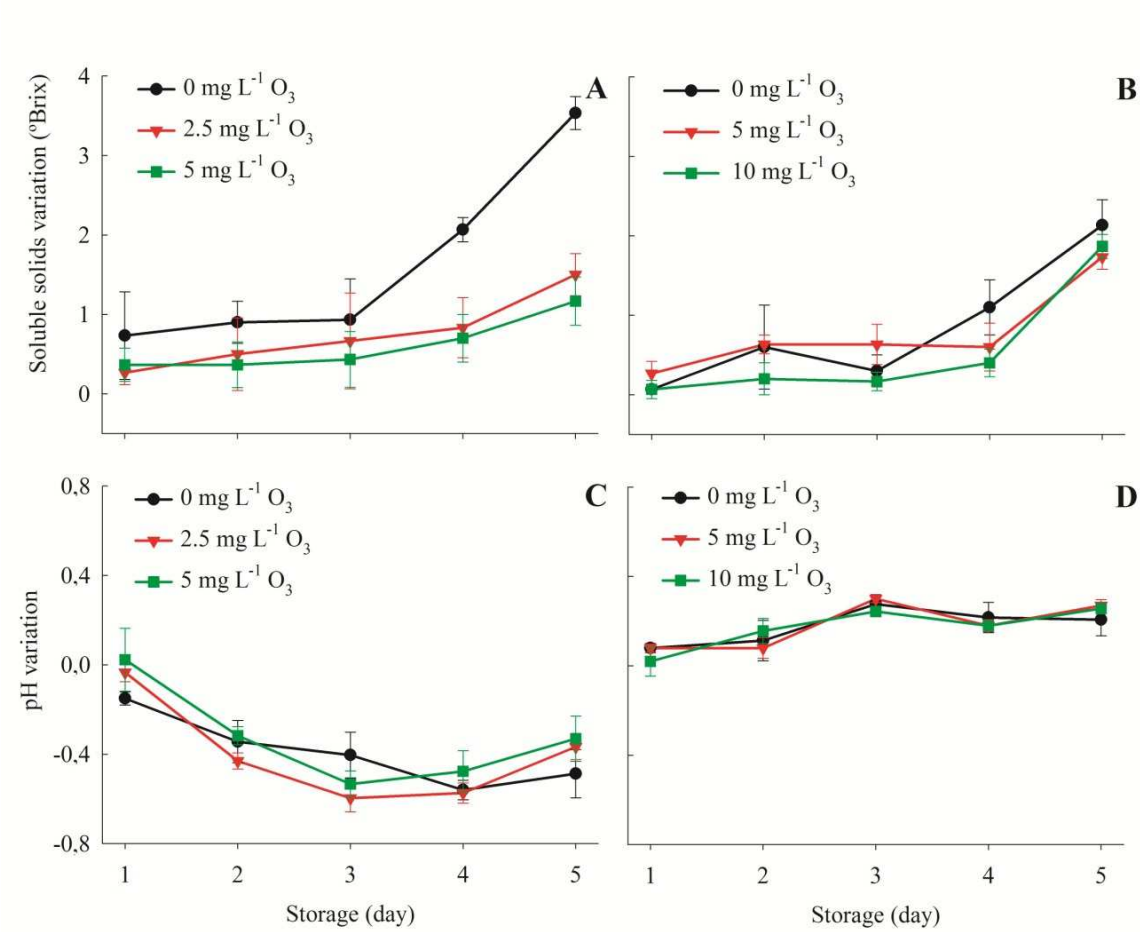


Fig. 3.6. Effect of ozone treatments as gas (A and C) and dissolved in water (B and D) on the soluble solids and pH of stored carrots. The bars show standard deviation values.

Discussion

The effect of ozone on quality parameters, such as weight loss percentage, was studied in several fruits and vegetables with the most diverse results. Weight loss was reduced in kiwi continuously exposed to ozone at 0.8 µg L^{-1} (Minas et al., 2012), in papaya exposed to ozone

at 2.8 - 9.3 µg L^{-1} for 4 days (Ali et al., 2014) and strawberries exposed to ozone at 3 µg L^{-1} for 3 days (Nadas et al., 2003). It was shown in several other studies that the weight loss was unaffected, for example, in carrots treated with ozone at 0.6 - 2 µg L^{-1} for up to 4 days (Forney et al., 2007) or continuously exposed to ozone at 0.1 µg L^{-1} for 6 months (Hildebrand et al., 2008); in peppers (Horvitz & Cantalejo, 2012) treated with ozone at 2 µg L^{-1} for 1 - 5 min, and in tomatoes (Rodoni et al., 2010) treated with ozone at 20 µg L^{-1} for 10 min when the fresh produce was exposed to relatively low concentration of ozone. In the present study, we observed that treatments with up to 5 mg L^{-1} of ozone as gas and up to 10 mg L^{-1} of ozone dissolved in water did not affect the weight loss of carrots for up to five days of storage (Fig. 3.4). These findings suggest that for each commodity, there is a threshold in ozone concentration above which the exposure may cause damage to the produce.

Firmness, associated with weight loss, is also an important rheological property pertinent to fresh fruits and vegetables. Fruits and vegetables with a firm texture are highly desirable because consumers associate these textural attributes with freshness and wholesomeness (Rico et al., 2007). Many studies showed that ozone did not have any effect on the change in firmness of apples, grapes, pears, peppers and rocket leaves (Skog & Chu, 2001; Martinez-Sanchez et al., 2008; Sharpe et al., 2009; Cayuela et al., 2009; Horvitz & Cantalejo, 2012). Our study (Fig. 3.4) corroborated these studies. However, several studies showed that there was better firmness retention in kiwi, papaya, strawberries and tomatoes (Tzortzakis et al., 2011; Alexandre et al., 2012; Minas et al., 2012; Ali et al., 2014; Kying & Ali, 2016) in response to ozone treatment. Ozone treatment delayed the tissue toughening in carrot sticks (Forney et al., 2007; Chauhan et al., 2011). These changes were associated with changes in cellulose, hemicellulose and lignin content, namely, due to the reduced lignification of the cell walls (An et al., 2007). However, cuticle thickness and the composition of each fruit and vegetable often depend on the cultivar and maturity, thereby making it even more difficult to select an optimum dose of ozone.

The visual quality of the product is important because any color alteration might be recognized as a symptom of senescence (Nunes et al., 2009). The commonly used parameters of color in the three-dimensional color space are lightness (L* = from black to white), greenness/redness (a*) and yellowness (b* = from blue to yellow) values (IAL, 2008). Another variable related to color is saturation (chroma), which is related to the variation of color intensity during storage. These parameters have been used to assess color changes by ozone treatment during the storage of various products. Zambre et al. (2010) observed delayed development of red color during the storage of tomatoes treated with gaseous ozone at 38 to

95 µg L^{-1} for 10 min. Similar findings were reported by Ali et al. (2014), who observed that change in the peel color of the papaya fruit was affected by exposure to gaseous ozone at 4.5 µg L^{-1} for 96 h. According to Sandhu et al. (2011), ozone may react with the conjugated double bonds in the carotenoids, thereby decreasing the yellowness. In carrots treated with ozone at concentrations from 10 to 115 µg L^{-1} (Bermudez-Aguirre & Barbosa-Canovas, 2013), the lightness value increased significantly, suggesting that the typical orange-red color was bleached by the treatment. Conversely, ozone treatment at 0.8 µg L^{-1} had no effect on the color of carrots (Sharpe et al., 2009). Ozone treatment had no effect on the change in color of the fruit in papaya (Kying & Ali, 2016), apples (Sharpe et al., 2009), tangerine (Boonkorn et al., 2012) and tomatoes (Bermudez-Aguirre & Barbosa-Canovas, 2013; Ikeura et al., 2013) during storage, which was corroborated by our study (Fig. 3.5). Our results show that the treatment with ozone does not prejudice the original quality of the vegetable with respect to the color characteristics.

Although a number of storage studies have implicated ozone in oxidation and the loss of quality of fruits and vegetables (Alexandre et al., 2012; Minas et al., 2012; Ali et al., 2014), this statement may not be appropriate, since such variables as temperature may also affect the product quality. In our study, it was verified that the temperature associated with the O_3 concentration could affect the pH of carrots. Apparently, increasing the ozone concentration to above 5 mg L^{-1}, prevented immediate changes in the pH of carrots when they were exposed to treatment with ozonated water at temperatures above 14 °C (Fig. 3.3). However, throughout the storage, the pH of the carrots did not change due to the elevation of O_3 concentrations (Fig. 3.6). This finding suggested that pH alteration may have been a temporary effect of the treatment. Moreover, pH values of samples were similar throughout the storage time, being close to 6.0. These values are the expected values for carrots in storage (Lima et al., 2001; Mastromatteo et al., 2012).

The treatments with O_3 (2.5 and 5 mg L^{-1}) as gas also prevented the SS concentration of the carrots from increasing from the fourth day of storage (Fig. 3.6). According to Chitarra and Chitarra (2005), the SS is related to flavor and is indicative of the fruit and vegetable ripeness level. In the case of such vegetables as carrots, a high level of ripeness of the product is considered a type of deterioration and is not well-accepted by the consumer. Thus, the ozone as gas could prevent the deterioration of the product and increase its shelf-life.

Conclusion

The exposure of carrots to ozone as gas and dissolved in water did not change its characteristics, including weight loss percentage, firmness and color. However, treatments with O_3 dissolved in water temporarily affected the pH value. Moreover, O_3 as gas prevented the sharp increase in soluble solids during storage, thereby increasing the shelf-life of carrots. Therefore, it can be concluded that ozone as gas and dissolved in water can be used in carrots without harming the quality of vegetables.

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CONCLUSÕES GERAIS

A presença de resíduos de agrotóxicos em alimentos vem sendo constatada há vários anos pela ANVISA, através do Programa de Análise de Resíduos de Agrotóxicos (PARA). A grande preocupação com a presença desses compostos em alimentos levou os pesquisadores a investigarem sobre o comportamento desses nos produtos agrícolas nos períodos de pré e pós-colheita. Esses estudos abrangem monitoramento, dissipação e estratégias de remoção. Neste trabalho foi investigada a cinética de dissipação natural de difenoconazol e linurom e a remoção desses agrotóxicos de cenouras empregando o ozônio como estratégia de remoção.

Este é o primeiro estudo que investiga a cinética de dissipação de difenoconazol e linurom em cenouras a campo. As tendências de degradação para diferentes dosagens de difenoconazol e linurom possuem o mesmo padrão, seguindo um modelo cinético de primeira ordem, porém com diferentes taxas de degradação. Ao fim do período pré-colheita, as cenouras tratadas com altas dosagem dos agrotóxicos foram consideradas impróprias para consumo.

Os tratamentos com ozônio em gás e dissolvida em água foram métodos eficazes para a remoção pós-colheita dos resíduos de difenoconazol e linurom sem a formação de intermediários tóxicos nas cenouras. As percentagens de remoção aumentaram com concentração de ozônio e do tempo de tratamento. Além disso, a exposição de cenouras ao ozônio em gás e dissolvido em água não alterou a porcentagem de perda de massa, firmeza e cor dos vegetais. No entanto, os tratamentos com O_3 dissolvido em água afetaram temporariamente o pH das cenouras e os tratamentos com O_3 em gás impediram o aumento acentuado de sólidos solúveis durante o armazenamento, prolongando assim a vida de prateleira das cenouras.

A partir dos resultados desta pesquisa, é possível concluir que as vantagens da utilização de agrotóxicos a fim de melhorar a produção agrícola devem ser ponderadas com os possíveis riscos para a saúde, decorrentes de seus resíduos deixados nos alimentos. Além disso, o ozônio mostrou-se uma ferramenta eficiente na remoção de resíduos de agrotóxicos em cenouras sem prejudicar a qualidade e nem deixar resíduos nos vegetais.