RENATA DE PAULO ROCHA

PÓS-COLHEITA DE MAÇÃS TRATADAS TERMICAMENTE POR IMERSÃO EM ÁGUA QUENTE E ULTRAVIOLETA

LAVRAS - MG

2016

RENATA DE PAULO ROCHA

PÓS-COLHEITA DE MAÇÃS TRATADAS TERMICAMENTE POR IMERSÃO EM ÁGUA QUENTE E ULTRAVIOLETA

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, área de concentração em Ciência dos Alimentos, para obtenção do titulo de Doutor.

Prof. Dr. Luiz Carlos de Oliveira Lima

Orientador

Profa. Dra. Merete Edelenbos

Coorientadora

LAVRAS - MG

2016

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Rocha, Renata de Paulo.
Pós-colheita de maçãs tratadas termicamente por imersão em água quente e ultravioleta / Renata de Paulo Rocha. – Lavras : UFLA, 2016.
79 p. : il.

Tese (doutorado)–Universidade Federal de Lavras, 2016.
Orientador: Luiz Carlos de Oliveira Lima.
Bibliografia.

1. *Malus domestica.* 2. Hidrotermia. 3. Produção orgânica. 4. Podridão de armazenamento. I. Universidade Federal de Lavras. II. Título.

RENATA DE PAULO ROCHA

PÓS-COLHEITA DE MAÇÃS TRATADAS TERMICAMENTE POR IMERSÃO EM ÁGUA QUENTE E ULTRAVIOLETA

POST-HARVEST OF APPLES THERMALLY TREATED WITH IMMERSION IN HOT WATER AND ULTRAVIOLET

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, área de concentração em Ciência dos Alimentos, para obtenção do titulo de Doutor.

APROVADA EM 15 de setembro de 2016.

Dra. Alexandra Mara G. N. Mamede	EMBRAPA
Profa. Dra. Ana Carla Marques Pinheiro	UFLA
Prof. Dr. Antônio Decarlos Neto	UFLA
Prof. Dr. Jaime Vilela de Resende	UFLA

Prof. Dr. Luiz Carlos de Oliveira Lima
Orientador

Profa. Dra. Merete Edelenbos
Coorientadora

LAVRAS - MG

2016

Ao meu amado pai (in memoriam)
Dedico.

AGRADECIMENTOS

À Universidade Federal de Lavras, em especial ao Departamento de Ciência dos Alimentos pela oportunidade.

À Universidade de Aarhus e à professora Merete Edelenbos por ter me aceitado em seu grupo de pesquisa.

À CAPES pela concessão da bolsa de doutorado no Brasil e na Dinamarca através do Programa de Doutorado–Sanduíche no Exterior (PDSE).

Ao professor Luiz Carlos de Oliveira Lima, pelo seu conhecimento e orientação.

Ao professor Eduardo Valério de Barros Villas Boas e à professora Elisangela Nunes de Carvalho por sempre se fazerem disponíveis a ajudar os alunos em suas dúvidas.

Aos amigos que compreenderam minha distância e se fizeram sempre presentes.

Aos amigos de diferentes continentes que me acolheram em território europeu e aos que me acolheram e compartilharam bons momentos em terras mineiras.

Aos meus pais, Lindete e Valtair (*in memoriam*) por sempre terem me apoiado, demonstrando assim seu amor, à minha irmã Michele e ao meu sobrinho Guilherme que muitas vezes me fizeram esquecer, nem que por alguns momentos, as pressões da pós-graduação.

Muito obrigada!

RESUMO GERAL

A demanda por frutas livres de resíduos químicos vem aumentando com o passar dos anos. A adoção de leis que proíbem ou limitam o uso de agroquímicos nos pomares dinamarqueses e a elevada incidência de podridão de armazenamento em maçãs (*Malus domestica* Borkh) incentivaram o uso de medidas alternativas visando diminuir as podridões de armazenamento, resultando em frutos que atendam a qualidade desejada pelo consumidor final. No presente estudo, adotou-se a imersão de diferentes cultivares de maçãs (Ingrid Marie e Pinova) provenientes de pomar dinamarquês a diferentes temperaturas com o objetivo de avaliar as respostas fisiológicas no período pós-colheita e o grau de descontaminação das maçãs durante o armazenamento. Foram utilizados dois diferentes métodos de imersão: manual e automático. Depois de submetidas aos tratamentos, as maçãs imersas manualmente em água quente foram utilizadas para acompanhamento do processo de aquecimento interno das frutas. Durante o período de armazenamento, as maçãs foram avaliadas pela cor da superfície, taxa respiratória, perda de massa, firmeza, sólidos solúveis, desordens fisiológicas e incidência de podridão de armazenamento. Imagens multiespectrais e a avaliação de danos causados nas frutas pela imersão automática também foram avaliados. Verificou-se que para a cv. Ingrid Marie a faixa de temperatura entre 50 e 54 °C por 180s resultou em frutas com maior participação da cor vermelha (elevado valor de a*), já a cv. Pinova submetida a temperaturas de 50 °C mostrou maior participação da cor vermelha (maior valor de a*) e cores mais vívidas enquanto que no método automático as frutas submetidas à temperatura de 54 °C por 30s seguidas de aplicação de ultravioleta C resultaram em cores mais vívidas (maior valor de croma) e maior *hue* para a cv. Ingrid Marie. Em relação à taxa respiratória, a cv. Pinova mostrou menor respiração quando submetidas a 50 °C por 180s, já a cv. Ingrid Marie mostrou menor taxa respiratória quando submetidas a 52 °C por 180s. Frutas de ambas as cultivares Ingrid Marie e Pinova tratadas pelo método de imersão automático tiveram menor incidência de doenças quando submetidas à temperatura de 54 °C por 30s sem apresentarem danos fisiológicos e, para os frutos tratados manualmente a temperatura de 50 °C por 180s foi melhor tanto para a cv. Ingrid Marie quanto para cv. Pinova. A aplicação dos tratamentos térmicos na forma automatizada foi eficaz, mostrando-se como uma boa opção para o produtor.

Palavras-chave: Produção orgânica. Tratamento hidrotérmico. *Malus domestica*.

GENERAL ABSTRACT

The demand for fruit free of chemical residues have increased over the years. The adoption of laws that prohibit or limit the use of agrochemicals in the Danish orchards and the elevated incidence of rot in apple (*Malus domestica* Borkh) storage, encouraged the use of alternative measures aiming at reducing storage rot, resulting in fruits that meet the quality desired by the final consumer. In this study, we adopted the immersion of different apple cultivars (Ingrid Marie and Pinova), derived from a Danish orchard, in different temperatures, with the objective of evaluating the physiological responses in the post-harvest period and the degree of decontamination of the apples during storage. Two different immersion methods were used: manual and automatic. After submitted to the treatments, the apples manually immersed in hot water were used for accompanying the process of internal heating of the fruits. During the storage period, the apples were evaluated by surface color, respiratory rate, mass loss, firmness, soluble solids, physiological disorders and storage rot incidence. Multispectral images and the evaluation of damages caused to the fruits by automatic immersion were also evaluated. We verified that, for cv. Ingrid Marie, the range in temperature between 50 and 54^{o}C for 180 seconds resulted in fruits with higher participation of the color red (elevated a* value), while for cv. Pinova, submitted to the temperature of 50^{o}C, there was higher participation of the color red (higher a* value) and more vivid colors. In the automatic method, the fruits submitted to the temperatures of 54^{o}C for 30 seconds, followed by the application of ultraviolet C resulted in more vivid colors (higher croma value) and higher hue for cv. Ingrid Marie. In relation to the respiratory rate, cv. Pinova showed lower respiration when submitted to 50^{o}C for 180 seconds, while cv. Ingrid Marie showed lower respiration rate when submitted to 52^{o}C for 180 seconds. Fruits from both cultivars treated with the automatic immersion method had lower disease incidence when submitted to the temperature of 54^{o}C for 30 seconds without presenting physical damages and, for the fruits treated manually, the temperature of 50^{o}C for 180 seconds was best for cv. Ingrid Marie and Pinova. The application of the thermal treatments in the automated form was effective, presenting itself as a good option for the producer.

Keywords: Organic production. Hydrothermal treatment. *Malus domestica.*

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

Maçãs são frutas que se encontram disponíveis durante quase todo o ano para os mais diferentes mercados consumidores ao redor do mundo. Isso ocorre devido às diferentes épocas de amadurecimento da fruta nos hemisférios Norte e Sul. Porém, estas frutas estão sujeitas à contaminação, por diferentes tipos de fungos, ainda no campo e durante a colheita resultando em infecções fúngicas durante o armazenamento e, por consequência, perdas no período pós-colheita.

Doenças como mofo preto, cinza e azul, que são muito comuns em maçãs, são causadas principalmente por *Alternaria alternata*, *Botrytis cinerea* e *Penicillium expansum* respectivamente (ZHANG et al., 2010). A aplicação de fungicidas durante a pré e pós-colheita é necessária para o controle destas doenças permitindo a disponibilidade da fruta durante o ano (IPPOLITO; NIGRO, 2005). No entanto, alguns dos agentes mais efetivos contra esses patógenos são proibidos ou estão sujeitos à rigorosa legislação na Europa a Regulação 1107/2009 e Diretiva 2009/128 (EUROPEAN PARLIAMENT, 2009a, 2009b) e nos Estados Unidos da América a Food Quality Protection Ação de 1996 (WASGHINTON STATE UNIVERSITY, 2015). No Brasil, foi criado em 2013 o Programa Nacional de Redução de Uso de Agrotóxicos – PRONARA (BRASIL, 2014), este programa prevê a diminuição do uso de agrotóxicos nas lavouras por meio de incentivo à conversão dos sistemas de produção convencional para os sistemas orgânico e de base agroecológica. No entanto, ainda hoje, a existência de leis que limitam ou anulam o uso de agrotóxicos é inexistente.

Por esta razão, pesquisadores e produtores europeus e norte-americanos vêm se esforçando na elaboração de metodologias alternativas no combate a estes e demais patógenos, como técnicas de sanitização física, uso de compostos

fenólicos e leveduras antagônicas. Esforços para a redução das perdas pós-colheita também têm sido aplicados pelo uso de controle biológico, e métodos físicos como ultravioleta, tratamento com radiofrequência e tratamentos térmicos (BARKAI-GOLAN, 2001; NARAYANASAMY, 2006).

O uso de tratamentos térmicos aplicados a maçãs tem se mostrado promissor na redução do subsequente desenvolvimento de podridões de armazenamento. As respostas pós-colheita aos tratamentos térmicos em maçãs têm sido amplamente estudadas. A água quente pode melhorar a qualidade e a capacidade de armazenamento. No entanto, as técnicas de aquecimento expõem os frutos aos perigos das desordens fisiológicas e por isso o binômio tempo de exposição ao calor-temperatura deve ser levado em consideração, sendo este específico para cada fruta e para cada tipo de patógeno (WOOLF; FERGUSON, 2000).

Entretanto, o uso desta metodologia resulta em elevada demanda de energia. O emprego de temperaturas elevadas por um menor período de tempo pode ser uma alternativa eficaz e mais econômica para o produtor. A adoção de um sistema automatizado, que combine a aplicação do tratamento de forma rápida sob elevadas temperaturas sem a necessidade do manuseio direto dos frutos pode agilizar o processo, permitindo o tratamento de um maior número de frutas e maior rendimento.

O presente estudo avaliou a qualidade de maçãs (*Malus domestica* Borkh) submetidas à imersão em água quente sob diferentes temperaturas seguida de aplicação de ultravioleta C. Adotou-se um método de imersão manual e um automático e as respostas fisiológicas, assim como a incidência de podridão de armazenamento e os danos causados por aquecimento foram avaliados durante o período de armazenamento das frutas.

2 REFERENCIAL TEÓRICO

2.1 Tratamentos Térmicos

São conhecidos três métodos de aplicação de tratamentos térmicos em *commodities*: água quente, vapor de água e ar quente (LURIE, 1998), os frutos podem ser submetidos ao aquecimento por diferentes formas: imersão ou aspersão em água quente, vapor e ar quente úmido ou seco (FALLIK, 2004). A água quente foi primeiramente adotada para controle fúngico, mas tem sido estendida para desinfestação de insetos (LURIE, 1998). O aquecimento a vapor foi desenvolvido para o controle de insetos e o ar quente tem sido usado para ambos, controle de insetos e fungos, e para o estudo da resposta das *commodities* às altas temperaturas. Os dois últimos métodos (vapor e ar quente) apresentam subdivisões na qual algumas vezes o ar é relativamente estático e às vezes o fluxo de ar é bastante elevado (LURIE, 1998).

A água tem sido adotada como principal meio de aquecimento por ser mais eficiente na transferência de calor do que o ar (FALLIK, 2004). A aplicação de calor através do ar necessita de um maior tempo de aquecimento do que a água. A variação de temperatura e o tempo associado ao tratamento diferem de acordo com a cultivar e também com as condições ambientais de pré-colheita como, por exemplo, a exposição ao sol. Como já citado anteriormente (FALLIK et al., 2001), a aplicação de tratamentos térmicos em maçãs vem mostrando resultados promissores na redução das podridões de armazenamento. A incubação de maçãs em ar quente (72 horas a 40 °C) e a imersão em água quente por até 3 minutos mostrou elevada eficácia contra *Neofabraea* spp. e *Penicillium expansum* (AMIRI; BOMPEIX, 2011; FALLIK et al., 2001; MAXIN et al., 2005; TAHIR; JOHANSSON; OLSSON, 2009).

De acordo com Maxin et al. (2012b), a ação dos tratamentos com água quente depende de, ao menos, dois componentes no qual o primeiro seria uma

ação letal e direta do calor no inóculo fúngico como esporos e micélios instalados na superfície da fruta ou na primeira camada da casca. O segundo componente seria uma ação indireta do calor no hospedeiro, mediado por respostas do fruto induzido pelo estresse, ou seja, os tratamentos térmicos agem tanto diretamente no desenvolvimento do patógeno como pela indução da resistência natural no fruto (KLEIN; LURIE, 1992). Porém para Maxin et al. (2012b) e Maxin e Weber (2011), o fator primordial que determina a eficácia do tratamento com água quente contra podridões pós-colheita em maçãs pode ser uma característica do fruto ao invés do efeito direto do calor sob os fungos.

Embora os tratamentos realizados através de imersão em água quente venham mostrando bons resultados na redução de esporos e micélios, o uso de temperaturas elevadas nos tratamentos realizados a curto tempo pode resultar em tecidos danificados, no caso de exposição por um tempo que ultrapasse o tempo limite da fruta (KLEIN; LURIE, 1992). Por outro lado, diferente da imersão, a aplicação de ar quente pode ser realizada a baixas temperaturas por um tempo prolongado. Maçãs, abacates e tomates mantidos a 38 °C por três a quatro dias não apresentaram sinais de danos aos tecidos e tiveram menos de 5% de perda de massa (KLEIN; LURIE, 1992).

A administração de tratamentos térmicos deve ser sob medida para cada commodity, respeitando seus limites e os objetivos que desejam ser atingidos, e deve se levar em consideração as diferentes faixas de tolerância à temperatura apresentada por cada patógeno (FALLIK et al., 2001). Yun, Gao e Liu (2013) relataram a supressão do desenvolvimento de esporos de *Penicillium italicum* em casca de mandarim após imersão em água a 52 °C por dois minutos, e também a redução de injúria pelo *chilling* durante armazenamento. Podridão de armazenamento causado por *Neonectria galligena*, *Botrytis cinerea* e *Penicillium expansum* foram significativamente reduzidos em maçãs da cultivar Elstar, por imersão em água quente a 50 °C durante três minutos, enquanto

temperaturas acima de 52 °C causaram escaldadura na superfície das frutas (MAXIN et al., 2012a). Spadoni et al. (2013) constataram que o tratamento de peras por imersão em água à temperatura de 60 °C durante um minuto inibiu em até 78% a incidência de *brown rot*, causados por *Monilinia* (*M. laxa*, *M. fructicola* e *M. fructigena*) em testes comerciais.

Os tratamentos térmicos estão associados com a indução de proteínas de choque térmico ou *heat shock proteins* (HSP's), que são responsáveis pela proteção das frutas contra injúrias do aquecimento (FLORISSEM et al., 1996). Estas proteínas atuam como chaperonas e protegem outras proteínas contra desnaturação ocasionada pela exposição da fruta a temperaturas elevadas (TAIZ; ZEIGER, 2004).

HSP's apresentam importante papel na aquisição de termotolerância pelo fruto. Pesquisas realizadas com cítrus (FALLIK, 2004; PORAT et al., 2000) indicaram que os tratamentos térmicos com água desencadeiam uma resposta fisiológica que envolve a rápida tradução e transcrição de HSP's. Estas devem então, assumir papeis fisiológicos em resposta aos fatores de estresse (SABEHAT; WEISS; LURIE, 1998). Um subgrupo das HSP's é conhecido por incluir proteínas relacionadas à patogênese, do inglês *pathogenesis-related proteins* (PRP's) como as quitinases ou □-1, 3-glucanases (PAVONCELLO et al., 2001). Essas proteínas exercem função sinérgica com os mecanismos de defesa da planta como, por exemplo, os sistemas antioxidantes e são capazes de auxiliar na proteção das plantas contra estresses oxidativos, um estresse secundário comum induzido por diversos estresses primários bióticos e abióticos em espécies de plantas susceptíveis (WANG et al., 2006).

Existem indicações da existência em maçãs de complexos de respostas ao calor similares ao verificado em cítrus, embora as evidências fisiológicas para isso sejam um tanto menos concretas do que em cítrus. Em células de maçã, a

produção de HSP's pode ser induzida até mesmo por suaves aquecimentos o que confere resistência a baixas temperaturas (WANG et al., 2001).

As principais vantagens dos tratamentos térmicos com água são sua alta reprodutibilidade e eficácia, assim como a falta de quaisquer restrições relacionadas ao seu uso. Esta técnica não gera resíduos e pode, de fato, até ajudar na redução de tais resíduos por minimizar a necessidade do uso de pesticidas ainda no campo (MAXIN; WILLIANS; WEBER, 2014).

2.2 Efeitos dos tratamentos térmicos sobre a qualidade e a fisiologia de frutas

Os tratamentos térmicos exercem grande influência na qualidade e na fisiológia das frutas, estando esses muito interligados. O nível de qualidade das frutas pode ser, além dos resultados de práticas pré-colheita, uma resposta aos parâmetros fisiológicos da fruta que podem ser inibidos ou estimulados pela exposição a elevadas temperaturas.

2.2.1 Qualidade

A qualidade de maças tem como base três principais parâmetros: firmeza do fruto, sólidos solúveis e amido. Tais parâmetros são no geral usados para monitorar o amadurecimento da fruta (DELONG; PRANGE; HARRISON, 1999; LAFER, 2010). A acidez titulável (ácido málico e ácido cítrico) também é rotineiramente determinada como um parâmetro de qualidade, baseando-se no fato de que a acidez exerce grande influência no sabor da fruta (DELONG; PRANGE; HARRISON, 1999). Outros parâmetros que indicam o processo de amadurecimento de maçãs são a emissão de etileno e CO_2, sendo o CO_2 um indicador da taxa respiratória, mudanças na coloração, formação de ceras cuticulares e síntese de compostos aromáticos que, de acordo com Taylor e Tucker (1993), parecem estar associados com o climatério.

O amadurecimento de frutos climatéricos é caracterizado pelo amaciamento da polpa, um aumento na razão açúcar:ácido, desenvolvimento acentuado da cor e aumento na taxa respiratória e na produção de etileno. A exposição de frutas a altas temperaturas pode resultar no abrandamento de alguns desses processos como também acentuar outros. Esta situação anômala resulta em frutos aquecidos com características de amadurecimento mais avançadas do que os frutos não tratados enquanto mantêm melhor qualidade na *shelf-life* (LURIE, 1998).

A taxa respiratória de frutas expostas a aquecimento sofre aumento inicial e após o tratamento térmico a taxa respiratória pode alcançar os mesmos níveis de frutas não tratadas (KLEIN; LURIE, 1990; PAULL; CHEN, 2000).

A exposição das frutas a elevadas temperaturas retarda o amadurecimento devido à ação do calor sobre a síntese de etileno. Quando expostos a temperaturas acima de 35 °C, maçãs e tomates demonstraram acúmulo endógeno de Ácido 1-Amino ciclopropano-1 Carboxílico (ACC) e menor síntese de etileno (ATTA-ALY, 1992; YU; ADAMS; YANG, 1980). A enzima ACC sintase limita a biossíntese de etileno nos tecidos vegetais e juntamente com a ACC oxidase atuam na regulação deste hormônio durante o processo de amadurecimento dos frutos climatéricos (CHITARRA; CHITARRA, 2005). Não apenas a inibição da síntese de etileno, mas também a diminuição da sensibilidade ao etileno exógeno são respostas das frutas ao aquecimento, sugerindo a perda ou a inativação de receptores de etileno ou algum tipo de barreira na transmissão dos sinais que levam ao amadurecimento (LURIE, 1998).

A produção de etileno em frutas submetidas aos tratamentos térmicos é cultivar dependente (SHAO et al., 2007). A síntese de etileno é inibida durante os tratamentos térmicos, mas é parcialmente restaurada de novo ao fim do período de aquecimento (BAI et al., 2006; SMITH; LAY-YEE, 2000; WANG et

al., 2006). A expressão de RNA mensageiro ligado aos processos de amadurecimento em tomates apresentaram diminuição durante o aquecimento a ar sob temperatura de 38 °C, mostrando recuperação após o tratamento. Isto inclui ACC oxidase, poligalacturonase e licopeno sintase (LURIE, 1998).

O impacto do tratamento térmico em relação aos voláteis pode variar com a espécie, temperatura e duração do tratamento (PAULL; CHEN, 2000). As altas temperaturas podem resultar em diminuição ou inibição da síntese de voláteis devido aos baixos níveis de ATP e NADPH resultantes da baixa taxa respiratória ocasionada pelo aquecimento, sendo isto um fator limitante para a disponibilidade do substrato e para a síntese de voláteis (BANGERTH et al., 1998). Os principais voláteis em maçãs são representados por ésteres (DIXON; HEWETT, 2000). O aquecimento de maçãs resultou em baixos níveis de hexanol, que serve como substrato para síntese de algumas cadeias de ésteres, e a submissão das frutas a aquecimento pode ter afetado alguma atividade metabólica de tal forma que reduziu a concentração de álcool e outros substratos para a síntese de ésteres (ESCALADA, 2006).

Assim como ocorre no processo respiratório e na síntese de etileno, a produção de compostos voláteis pode ser recuperada após a administração dos tratamentos térmicos. Fallik et al. (2001) verificaram aumento de voláteis de maçã quando as frutas foram expostas à temperatura de 38 °C, seguido de diminuição imediatamente após o período de tratamento e recuperação lenta durante a *shelf-life*, enquanto Escalada (2006) verificou redução considerável de compostos voláteis totais em maçãs termicamente tratadas após armazenamento.

No geral, frutos termicamente tratados apresentam maior retenção de firmeza mesmo depois de movidos para a *shelf-life* (LURIE, 1998; PAULL; CHEN, 2000). Maçãs expostas a aquecimento (38 °C por quatro dias) mostraram menor quantidade de pectina solúvel e maior de pectina insolúvel, assim como mamões tratados termicamente, que também apresentaram pequenas mudanças

nos teores de pectina solúvel (BEN-SHALOM et al., 1993; KLEIN; LURIE, 1990). Isso pode ser explicado pela diminuição do conteúdo de açúcares neutros durante o aquecimento, como arabinose e galactose, este fato pode ter levado à aproximação das cadeias pécticas o que, por sua vez, comprometeu a clivagem enzimática durante e após o armazenamento, mantendo os frutos firmes por mais tempo (BEN-SHALOM et al., 1993).

A acidez total titulável (AT) declinou em maçãs mantidas por três a quatro dias a temperatura de 38 °C enquanto o teor de sólidos solúveis totais (SST) não foi afetado (KLEIN; LURIE, 1990). Fruk et al. (2012) não relataram mudanças nos parâmetros de qualidade (SST, AT, SST:AT e pH) em nectarinas submetidas a diferentes tratamentos térmicos. O tratamento por aquecimento também não induziu efeitos significativos no conteúdo de sólidos solúveis e acidez em peras (SPADONI et al., 2013).

Os efeitos obtidos através dos tratamentos térmicos em relação à concentração de sólidos solúveis e principalmente à acidez titulável são variáveis, e dependem principalmente da temperatura e da duração do tratamento (PAULL; CHEN, 2000). No geral, uma melhora na razão SST:AT é obtida para os frutos devido à diminuição nos teores de acidez titulável (LURIE, 1998).

Os tratamentos térmicos aplicados em pós-colheita são não cancerígenos, não poluentes e têm potencial para reduzir doenças, controlar insetos, retardar o amadurecimento, aumentar a tolerância ao *chilling* e manter a qualidade em muitas frutas e vegetais (COUEY, 1989; LURIE, 1998; KLEIN; LURIE, 1992).

2.2.2 Fisiologia

A temperatura pode afetar muitos dos complexos processos fisiológicos, como respiração, fotossíntese, absorção de minerais e água, transpiração,

crescimento, amadurecimento, desenvolvimento e senescência. Não há apenas o efeito físico da temperatura nas taxas das reações químicas, como expressado pelo Q_{10}, mas também um efeito de temperaturas limite no tecido, como congelamento, *chilling*, transição dos estados das proteínas e membranas, choques de aquecimento e desnaturação térmica de proteínas (SALTVEIT, 2003).

As flutuações de temperatura fora do limite em que este amálgama de reações está envolvido para um funcionamento apropriado podem interromper o crescimento normal, desenvolvimento e comportamento pós-colheita da commodity (SALTVEIT, 2003). Também, a dinâmica da síntese proteica, como uma resposta ao estresse, deve determinar alguma resposta fisiológica ao aquecimento (FERGUSON et al., 2000). Diversos estudos (CONWAY et al., 1994; KLEIN; LURIE; BEN-AIRE, 1990; KLEIN; LURIE, 1990; PORRITT; LIDSTER, 1978; SAMS et al., 1993) têm mostrado que frutas que sofreram tratamentos térmicos mantiveram-se mais firmes do que os frutos não tratados mesmo após a *shelf-life*.

Conway et al. (1994), através de testes de compressão, viram que maçãs tratadas por aquecimento foram mais resistentes, enquanto Lurie e Nussinovitch (1996) verificaram que maçãs tratadas termicamente foram consideradas mais frescas do que as não aquecidas.

Maçãs submetidas a aquecimento apresentaram menor quantidade de cálcio ligado à pectina solúvel e uma maior quantidade deste elemento estava ligada à parede celular (LURIE; KLEIN, 1992). Isto pode ter sido o resultado da atividade da pectina esterase criando mais sítios de ligação para o cálcio, porém um estudo realizado com frutas aquecidas e não aquecidas mostrou grau de esterificação similar para ambos (KLEIN et al., 1995).

Um atraso no amaciamento da polpa de frutas foi observado em frutos expostos a tratamentos térmicos e armazenados em baixas temperaturas,

provavelmente devido à inativação de enzimas hidrolíticas da parede celular como poligalacturonase (LURIE, 1998), que é retomado quando o fruto é movido para *shelf-life*.

O tratamento térmico aplicado a maçãs diminuiu o conteúdo de arabinose e galactose, mas sem diminuição do teor de ácido galacturônico, (unidades que compõem as pectinas) o que seria um indicativo de síntese de parede celular (BEN-SHALOM et al., 1993; BOBBIO; BOBBIO, 1995). É possível que a perda destes açúcares das cadeias laterais das pectinas durante o tratamento térmico mude a configuração da parede celular, levando a uma vedação devido à aproximação das pectinas, dificultando a clivagem enzimática durante e após o armazenamento, resultando em frutos mais firmes (BEN-SHALOM et al., 1993).

Um dos aspectos mais interessantes dos tratamentos térmicos em pós-colheita é o efeito benéfico de redução do *chilling* em uma série de frutas durante subsequente armazenamento em baixas temperaturas (FERGUSON et al., 2000). Quando os produtos vegetais são armazenados abaixo da temperatura mínima de segurança, seu metabolismo é incapaz de se desenvolver normalmente e uma série de desordens fisiológicas e bioquímicas ocorre em resposta ao estresse (CHITARRA; CHITARRA, 2005). Os vegetais desenvolveram diferentes habilidades para responder a estresses bióticos e abióticos e a biossíntese de proteínas de choque térmico ou HSP's tem como objetivos manter a homeostase celular em condições de estresse (AGHDAM et al., 2013).

Existem diferentes condições que resultam na biossíntese e acumulação destas proteínas como amadurecimento da fruta, temperaturas elevadas ou baixas e estresses oxidativos (LAWRENCE; CLINE; MOORE, 1997; LOPEZ-MATAS et al., 2004; NETA-SHARIR et al., 2005). Zhang et al. (2005) verificaram que o tratamento térmico de uvas (38 °C por 10 horas) atenuou a

injúria por *chilling*. Os autores observaram que o tratamento com ar quente levou a um aumento da expressão do gene *HSP70* e acumulação da proteína HSP70, aumento na atividade de enzimas antioxidantes e houve uma ação sinérgica entre HSP70 e o sistema antioxidante da baga de uva, resultando na conservação da integridade da membrana e na indução de resistência ao *chilling*. Segundo Vierling (1991), a síntese das HSP's aumenta conforme o aumento da temperatura e a temperatura ótima de síntese está relacionada a cada espécie.

A redução da sensibilidade ao *chilling* nas frutas pode não ser apenas em razão da presença de HSP's. Tem sido pensado que o *chilling* inicia com dano à membrana (CHITARRA; CHITARRA, 2005; LYONS, 1973) e tratamentos térmicos entre 35 - 40 °C devem causar alterações na membrana. Altas temperaturas (35 - 40 °C) aumentam a dispersão da membrana (INABA; CHACHIN, 1988; LURIE; KLEIN, 1990), mas após remoção do estresse, o tecido se recupera retornando a níveis encontrados no tecido a 20 °C (LURIE; KLEIN, 1990).

Usando a dispersão da membrana como uma medição para o *chilling*, Saltveit (1991) relatou que o condicionamento de discos de tomate a 37 °C por quatro horas reduziu a sensibilidade do tecido ao *chilling* quando os discos foram armazenados em temperaturas propícias à injúria. Uma avaliação da composição lipídica do plasma de membrana de maçãs mostrou que após quatro dias de aquecimento a 38 °C em ar e armazenamento refrigerado a 0 °C por quatro meses existia mais fosfolipídeos e ácidos graxos insaturados em frutos aquecidos do que nos controles (LURIE; OTHMAN; BOROCHOV, 1995), resultando em membranas mais fluidas e reduzindo o risco de injúria por *chilling* (LYONS, 1973).

2.3 Aplicação de tratamentos térmicos combinados a outros tratamentos pós-colheita

Maçãs estão sujeitas a uma variedade de doenças que podem ser causadas por diferentes agentes como, por exemplo, fungos, bactérias, vírus e nematoides, mas existem também desordens induzidas por agentes desconhecidos. Muitas desordens resultam em perda total da produção. (AHMADI-AFZADI, 2012).

O sistema hortícola pós-colheita compreende múltiplas e interligadas atividades que abrangem etapas da colheita ao fim do armazenamento e do mercado até a mesa do consumidor. As perdas, em quantidade e qualidade podem ocorrer em qualquer passo durante a cadeia pós-colheita, com importante impacto econômico. Perdas pós-colheita de frutas e vegetais são difíceis de predizer (BARKAI-GOLAN, 2001).

O controle das doenças é o principal gasto anual para os produtores na maioria das áreas produtoras de maçãs. Os produtores precisam controlar as primeiras doenças da estação como *scab*, doenças do verão e também doenças de armazenamento. Uma abordagem bem integrada é comumente necessária para atingir um controle da doença como, por exemplo, a aplicação de fungicidas, pesticidas e bactericidas (o último normalmente não permitido na Europa), seleção de porta-enxertos resistentes ou tolerantes, controle biológico e a seleção de sítios adequados para o pomar (DEWASISH; AMAL, 2010; JÖNSSON, 2007).

Desde o início dos anos 60, tratamentos baseados em fungicidas químicos, como o principal método de redução de perdas pós-colheita, obteve resultados satisfatórios. No entanto, nos últimos anos, rigorosas políticas regulatórias começaram a ser impostas a seu uso (LIU et al., 2013).

Os tratamentos com água quente ou hidrotérmicos têm se mostrado muito efetivos no controle das podridões de armazenamento em maçãs e é um

método economicamente rentável para os produtores (MAXIN et al., 2012a, 2012b; MAXIN; KLOOP, 2004; SPADONI et al., 2013) Além do mais, o tratamento garante alta qualidade ao produto tanto na cadeia de vendas quanto nas casas de família. Mesmo assim, a perda de frutas como resultado direto de danos por aquecimento tem sido relatado, e estudos têm sido realizados para diminuir essas perdas. Desordens por altas temperaturas podem incluir falha no amadurecimento normal, menor vida útil, *browning*, e descoloração (SALTVEIT, 2003).

A aplicação de tratamentos térmicos em combinação com outros tratamentos durante a pós-colheita vem sendo realizada com o objetivo de se obter uma ação sinérgica, resultando em frutos que apresentem qualidade aceitável por um tempo prolongado devido às ações específicas de cada tratamento. A combinação de tratamentos térmicos com outros tipos de tratamentos pós-colheita é diversificada como a combinação com óleos essenciais (BAL, 2012), atmosfera controlada (TAHIR; JOHANSSON; OLSON, 2009), controle biológico e atmosfera controlada (WSZELAKI; MITCHAM, 2003). Até a combinação com agroquímicos pode ser aplicada com objetivo de acentuar a ação dos ingredientes ativos no controle das doenças e diminuir as doses químicas (SCHIRRA et al., 2011). A difusão do fungicida se dá pela cutícula, que age como uma barreira difusora. Com o aumento da temperatura a difusão do fungicida acontece de forma mais eficaz. Dessa forma, é possível reduzir as doses dos compostos químicos normalmente usados nos tratamentos convencionais sem comprometer a qualidade da fruta e a eficácia do tratamento em reduzir doenças (SCHIRRA et al., 2011).

Segundo Schirra et al. (2011), a combinação dos tratamentos térmicos com outros tratamentos seria essencial para acentuar sua eficiência. Tratamentos combinando a aplicação de aquecimento e solução de $CaCl_2$ (3%) em maçãs da cultivar Anna resultaram em frutos com melhor qualidade após armazenamento

do que os frutos controle (LURIE; KLEIN,1992). Os frutos mostraram-se mais firmes do que em qualquer outro tratamento aplicado sozinho, e o amarelamento da casca foi menos pronunciado (LURIE; KLEIN, 1992). O efeito positivo na combinação de tratamento hidrotérmico e armazenamento em atmosfera controlada relatado por Tahir, Johansson e Olsson (2009) em maçãs foi considerado original e inovador. O método melhorou o efeito erradicativo e aumentou a atividade residual do aquecimento e armazenamento em atmosfera controlada por ter resultado em uma diminuição significativa de doenças como *bitter rot* e *bull's eyes rot* quando comparados com frutas armazenadas em ar ou frutas não aquecidas armazenadas em atmosfera controlada (TAHIR; JOHANSSON; OLSSON, 2009). A imersão de cerejas em água quente combinada com óleo essencial (mentol e timol) foi um método efetivo no prolongamento do tempo de armazenamento de cerejas (BAL, 2012).

3 CONCLUSÃO

O presente estudo mostrou que as maçãs tratadas a 50 °C por 180s tiveram melhor resultado tanto para a cv. “Ingrid Marie” quanto para “Pinova” no método de imersão manual sem a presença de danos significativos. Para o método de imersão automática, ambas as cultivares responderam positivamente quando submetidas à temperatura de 54 °C por 30s. A adoção do processo de imersão automática mostrou-se promissora podendo agilizar o processo de tratamento térmico em casos de grande demanda.

REFERÊNCIAS

AGHDAM, M. S. et al. Heat shock proteins as biochemical markers for postharvest chilling stress in fruits and vegetables. **Scientia Horticulturae**, Amsterdam, v. 160, p. 54-64, Aug. 2013.

AHMADI-AFZADI, M. **Genetic and biochemical properties of apples that affect storability and nutritional value**. 2012. 40 p. Thesis (Doctor of Agricultural Science) - Swedish University of Agricultural Science, Uppsala, 2012.

AMIRI, A.; BOMPEIX, G. Control of Penicillium expansum with potassium phosphate and heat treatment. **Crop Protection**, Guildford, v. 30, n. 2, p. 22-227, Feb. 2011.

ATTA-ALY, M. A. Effect of high temperature on ethylene biosynthesis by tomato fruit. **Postharvest Biology and Technology**, Amsterdam v. 2, n. 1, p. 19-24, Aug. 1992.

BAI, J. et al. Effect of high-pressure hot-waterwashing treatment on fruit quality, insects, and disease in apples and pears. Part I: system description and the effect on fruit quality of 'd'Anjou' pears. **Postharvest Biology and Technology**, Amsterdam, v. 40, n. 3, p. 207-215, June 2006.

BAL, E. Effects of essential oil treatments combined with hot water treatment on improving postharvest life of sweet cherry. **Fruits**, Paris, v. 67, n. 4, p. 285-291, July/Aug. 2012.

BANGERTH, F. et al. A. Investigations into the physiology of volatile aroma production of apple fruits. In: INTERNATIONAL POSTHARVEST SCIENCE CONFERENCE PSTHARVEST, 96., 1998, Taupo. **Proceedings…** Bruxelas: ISH, 1998, v. 1, p. 189-194.

BARKAI-GOLAN, R. Novel approaches for enhancing host resistance. In: _____. **Postharvest diseases of fruits and vegetables**: development and control. Amsterdam: Elsevier, 2001. chap. 12, p. 252-266.

BEN-SHALOM, N. et al. A postharvest heat treatment inhibits cell wall degradation in apples during storage. **Phytochemistry**, New York, v. 34, n. 4, p. 955-958, Nov. 1993.

BOBBIO, O. F.; BOBBIO, A. P. **Introdução à química de alimentos**. 2. ed. São Paulo: Varela, 1995, 223 p.

BRASIL. Presidência da República. Comissão Nacional de Agroecologia e Produção Orgânica. **Proposta Pronara – Programa Nacional de Redução de Agrotóxicos Desenvolvido pelo GT Agrotóxicos/CNAPO**. Brasília, 2014. 37 p.

CHITARRA, M. I. F.; CHITARRA, A. B. **Pós-colheita de frutos e hortaliças**: fisiologia e manuseio. 2. ed. Lavras: Editora da UFLA, 2005. 785 p.

CONWAY, W. S. et al. Additive effects of postharvest calcium and heat treatment on reducing decay and maintaining quality in apples. **Journal of the American Society for Horticultural Science**, Alexandria, v. 119, n. 1, p. 49-53, Jan. 1994.

COUEY, H. M. Heat treatments for control of postharvest diseases and insect pests of fruits. **HortScience**, Alexandria, v. 24, n. 2, p. 198-202, 1989.

DELONG, J. M.; PRANGE, R. K.; HARRISON, P. A. Using the streif index as a final harvest window for controlled-atmosphere storage of apples. **HortScience**, Alexandria, v. 34, n. 7, p. 1251-1255, Dec. 1999.

DEWASISH, C.; AMAL, M. **Fruit crops**. Jaipur: Oxford Book, 2010. p. 278-290.

DIXON, J.; HEWETT, E. W. Factors affecting apple aroma/flavour volatile concentration: a review. **New Zealan**d **Journal of Crop and Horticultural Science**, Wellington, v. 28, n. 3, p. 155-173, Sept. 2000.

ESCALADA, V. S. **Interactions of AVG, MCP and heat treatment on apple fruit ripening and quality after harvest and cold storage**. 2006. 156 p. Doctoral Dissertation (Doctor of Philosophy) - College of Agricultural, University of Kentucky, Lexington, 2006.

EUROPEAN PARLIAMENT. Council of the European Union. Directive 2009/128/CE, of 21 of october 2009. It establishes a framework for action at EU level for the sustainable use of pesticides **Journal Official da Union Europea**n, Bruxelas, 21 out. 2009a. L. 309, p. 72-86.

EUROPEAN PARLIAMENT. Council of the European Union. Regulation (CE) nº 1107/2009, 21 of october of 2009. Concerning the placing of plant protection products on the market and repealing Directives 79/117 / EEC and 91/414 / CEC Council **Journal Official da Union Europea**n, Bruxelas, 21 out. 2009b. L. 309, p. 50-71.

FALLIK, E. et al. Ripening characterization and decay development of stored apples after a short pre-storage hot water rinsing and brushing. **Innovative Food Science and Emerging Technologies**, Amsterdam, v. 2, n. 2, p. 127-132, June 2001.

FALLIK, E. Prestorage hot water treatments (immersion, rinsing and brushing). **Postharvest Biology and Technology**, v. 32, n. 2, p. 125-134, May 2004.

FERGUSON, I. B. et al. Postharvest heat treatments: introduction and workshop summary. **Postharvest Biology and Technology**, Amsterdam, v. 21, n. 1, p. 1-6, Dec. 2000

FLORISSEN, P. et al. The effects of short heat-treatments on the induction of chilling injury in avocado fruit (Persea Americana Mill). **Postharvest Biology and Technology**, Amsterdam v. 8, n. 2, p. 129-141, May 1996.

FRUK, G. et al. Effects of different postharvest heat treatments on decreasing decay, deducing chilling injury and maintaining quality of nectarine fruit. **Agriculturae Conspectus Scientificus**, Zagreb, v. 77, n. 1, p. 27-30, Feb. 2012.

INABA, M.; CHACHIN, K. Influence of and recovery from high-temperature stress on harvested mature green tomatoes. **HortScience**, Alexandria, v. 23, n. 1, p. 190-192, 1998.

IPPOLITO, A.; NIGRO, F. Natural antimicrobials for preserving fresh fruit and vegetables. In: JONGEN, W. (Ed.). **Improving the safety of fresh fruit and vegetables**. Boca Raton: CRC Press, 2005. chap. 17, p. 513-555.

JÖNSSON, A, **Organic apple production in Sweden**: cultivation and cultivars. 2007. 34 p. Thesis (Doctor of Agricultural Science) - Swedish University of Agricultural Sciences, Uppsala, 2007.

KLEIN, J. D. et al. Pectin esterase activity and pectin methyl esterification in heated Golden Delicious apples. **Phytochemistry**, New York, v. 39, n. 3, p. 491-494, June 1995.

KLEIN, J. D.; LURIE, S.; BEN-ARIE, S. Quality and cell wall components of ‘Anna’ and ‘Granny Smith’ apples treated with heat, calcium and ethylene. **Journal of the American Society for Horticultural Science**, Alexandria, v. 115, n. 6, p. 954-958, Nov. 1990.

KLEIN, J. D.; LURIE, S. Prestorage heating of apple fruit for Enhanced Postharvest quality: interaction of time and temperature. **HortScience**, Alexandria, 27 n. 4, p. 326-328, Apr. 1992.

KLEIN, J. D.; LURIE, S. Prestorage heat treatment as a means of improving poststorage quality of apples. **Journal of the American Society for Horticultural Science**, Alexandria, v. 115, n. 32, p. 255-259, Mar. 1990.

LAFER, G. Storability and fruit quality of organically grown 'Topaz' apples as affected by harvest date and different storage conditions. **Acta Horticulturae**, Wageningen, v. 877, p. 795-798, Nov. 2010.

LAWRENCE, S. D.; CLINE, K.; MOORE, G. A. Chromoplast development in ripening tomato fruit: identification of cDNAs for chromoplast-targeted proteins and characterization of a cDNA encoding a plastid-localized low-molecular-weight heat shock protein. **Plant Molecular and Biology**, Dordrecht, v. 33, n. 3, p. 483-492, Feb. 1997.

LIU, J. et al. Utilization of antagonistic yeasts to manage postharvest fungal disease of fruit. **International Journal of Food Microbiology**, Amsterdam, v. 167, n. 2, p. 153-160, Sept. 2013.

LOPEZ-MATAS, M. A. et al. Protein cryoprotective activity of a cytosolic small heat shock protein that accumulates constitutively in chestnut stems and is up-regulated by low and high temperatures. **Plant Physiology**, Bethesda, v. 134, n. 4, p. 1708-1717, Apr. 2004.

LURIE, S.; KLEIN. J. D. Calcium and heated treatment to improve storability of “Anna” apples. **HortScience**, Alexandria, v. 27, n. 1, p. 36-39, Jan. 1992.

LURIE, S.; KLEIN, J. D. Heat treatment of ripening apples: differential effeets on physiology and biochemistry. **Physiologia Plantarum**, Copenhagen, v. 78, n. 2, p. 181-186, Jan. 1990.

LURIE, S.; NUSSINOVITCH, A. Compression characteristics, firmness and texture perception of heat treated and unheated apples. **International Journal of Food Science and Technology**, Oxford, v. 31, n. 1, p. 1-5, Feb. 1996.

LURIE, S.; OTHMAN, S.; BOROCHOV, A. Effects of heat treatment on plasma membrane of apple fruit. **Postharvest Biology and Technology**, Amsterdam, v. 5, n.1/2, p. 29-38, Jan. 1995.

LURIE, S. Postharvest heat treatments. **Postharvest Biology and Technology**, Amsterdam, v. 14, n. 3, p. 257-269, Nov. 1998.

LYONS, J. M. Chilling injury in plants. **Annual Reviews of Plant Physiology**, New York, v. 24, p. 445-466, June 1973.

MAXIN, P. et al. Control of a ride range of storage rots in naturally infected apple by hot water dipping and rising. **Postharvest Biology and Technology**, Amsterdam, v. 70, p. 25-31, Aug. 2012a.

MAXIN, P. et al. Control of postharvest decay in organic grown apples by hot water treatment. In: INTERNATIONAL POSTHARVEST SYMPOSIUM, 5., 2005, Verona. **Proceedings…** Bruxelas: ISH, 2005. p. 2153-2158.

MAXIN, P. et al. Hot-water dipping of apples to control *Penicillium expansum*, *Neonectria galligena* and *Botrytis cinerea*: effects of temperature on spore germination and fruit rots. **European Journal of Horticultural Science**, Leuven, v. 77, n. 1, p. 1-9, Mar. 2012b.

MAXIN, P.; KLOPP, K. Economics of hot water dipping. In: INTERNATIONAL CONFERENCE ON CULTIVATION TECHNIQUE AND PHYTOPATHOLOGICAL PROBLEMS IN ORGANIC FRUIT-GROWING, 11., 2004, Weinsberg. **Proceedings…** Weinsberg: FÖKO, 2004. p. 75-78.

MAXIN, P.; WEBER, R. W. S. Control of phacidiopynis washingtonensis storage rot of apples by hot-water treatments without the ethylene inhibitor 1-MCP. **Journal of Plant Diseases and Protection**, Berlin, v. 118, n. 6, p. 222-224, Dec. 2011.

MAXIN, P.; WILLIANS, M.; WEBER, R. W. S. Control of fungal storage rots of apples by hot-water treatments: a northean european perspective. **Erwerbs-Obstbau**, Berna, v. 56, n. 1, p. 25-34, Mar. 2014.

NARAYANASAMY, P. (Ed.). **Postharvest pathogens and diseases management**. Hoboken: Wiley, 2006. 578 p.

NETA-SHARIR, I. et al. Dual role for tomato heat shock protein 21: protecting photosystem II from oxidative stress and promoting color changes during fruit maturation. **Plant Cell**, Rockville, v. 17, n. 6, p. 1829-1838, June 2005.

PAULL, R. E.; CHEN, N. J. Heat treatment and fruit ripening. **Postharvest Biology and Technology**, Amsterdam, v. 21, n. 1, p. 21-37, Dec. 2000.

PAVONCELLO, D. et al. A hot water treatment induces resistance to *Penicillium digitatum* and promotes the accumulation of heat shock and pathogenesis-related proteins in grapefruit flavedo. **Physiologia Plantarum**, Copenhagen, v. 111, n. 1, p. 17-22, Jan. 2001.

PORAT, R. et al. Induction of resistance to *Penicillium digitatum* and chilling injury in 'Star Ruby' grapefruit by a short hot-water rinse and brushing treatment. **Journal of Horticultural Science and Biotechnology**, Oxford, v. 75, n. 4, p. 428-432, July 2000.

PORRITT, S. W.; LIDSTER, P. D. The effect of prestorage heating on ripening and senescence of apples during cold storage. **Journal of the American Society and Horticultural Science**, Alexandria, v. 103, n. 5, p. 584-587, 1978.

SABEHAT, A.; WEISS, D.; LURIE, S. Heat-shock proteins and cross-tolerance in plants. **Physiologia Plantarum**, Copenhagen, v. 103, n. 3, p. 437-441, July 1998.

SALTVEIT, M. E. Prior temperature exposure affects subsequent chilling injury. **Physiologia Plantarum**, Copenhagen, v. 82, n. 4, p. 529-536, Aug. 1991.

SALTVEIT, M. E. Temperatures extremes. In: BARTZ, J. A.; BRECH, J. K. (Ed.). **Postharvest physiology and pathology of vegetables**. New York: M. Dekker, 2003. chap. 19, p. 457-483.

SAMS, C. E. et al. Firmness and decay of apples following postharvest pressure infiltration of calcium and heat treatment. **Journal of the American Society and Horticultural Science**, Alexandria, v. 118, n. 5, p. 623-627, Sept. 1993.

SCHIRRA, M. et al. Control of postharvest diseases of fruit by heat and fungicides: efficacy, residues levels, and residues persistence. A review. **Journal of Agricultural and Food Chemistry**, Easton, v. 59, n. 16, p. 8531-8542, Aug. 2011.

SHAO, X. F. et al. Effects of pre-storage heat treatment on fruit ripening and decay development in different apple cultivars. **Journal of Horticultural Science and Biotechnology**, Oxford, v. 82, n. 2, p. 297-303, 2007.

SMITH, K. J.; LAY-YEE, M. Response of 'Royal Gala' apples to hot water treatment for insect control. **Postharvest Biology and Technology**, Amsterdam, v. 19, n. 2, p. 111-122, June 2000.

SPADONI, A. et al. Control of *Monilinia* rots on fruit naturally infected by hot water treatment in semi-commercial trials. **Postharvest Biology and Technology**, Amsterdam, v. 86, p. 280-284, Dec. 2013.

TAHIR, I.; JOHANSSON, E.; OLSSON, M. E. Improvement of apple quality and storability by a combination of heat treatment and controlled atmosphere storage. **HortScience**, Alexandria, v. 44, n. 6, p. 1648-1654, Oct. 2009.

TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. 3. ed. Porto Alegre: Artemed, 2004. 719 p.

TAYLOR, J. E.; TUCKER, G. A. **Biochemistry of fruit ripening**. London: Chapman and hall, 1993. 454 p.

VIERLING, E. The roles of HSP in plants. **Annual Reviews Plant Physiology Plant Molecular Biology**, Palo Alto, v. 42, p. 579-620, June 1991.

WANG, C. Y. et al. Heat-induced protection against death of suspension-cultured apple fruit cells exposed to low temperature. **Plant, Cell and Environment**, Oxford, v. 24, n. 11,p. 1199-1207, Nov. 2001.

WANG, S. et al. Postharvest treatment to control codling moth in fresh apples using water assisted radio frequency heating. **Postharvest Biology and Technology**, Amsterdam, v. 40, n. 1, p. 89-96, Apr. 2006.

WASHINGTON STATE UNIVERSITY. Tree Fruit Research & Extension Center. Postharvest Information Network. **The food quality protection Act of 1996**. Washington, 2015. Disponível em: <http://www.postharvest.tfrec.wsu.edu/pages/PC98A>. Acesso em: 28 jul. 2015.

WOOLF, A. B.; FERGUSON, I. B. Postharvest responses to high fruit temperatures in the field. **Postharvest Biology and Technology**, Amsterdam, v. 21, n. 1, p. 7-20, Dec. 2000.

WSZELAKI, A. L.; MITCHAM, E. J. Effect of combinations of hot water dips, biological control and controlled atmospheres for control of gray mold on harvested strawberries **Postharvest Biology and Technology**, Amsterdam, v. 27, n. 3, p. 255-264, Mar. 2003.

YUN, Z.; GAO, H.; LIU, P. Comparative proteomic and metabolomics profiling of citrus fruit with enhancement of disease resistance by postharvest heat treatment. **BMC Plant Biology**, London, v. 13, n. 44, p. 1-16, Mar. 2013.

YU, Y. B.; ADAMS, D. O.; YANG, S. F. Inhibition of ethylene production by 2,4-dinitrophenol and high temperature. **Plant Physiology**, Bethesda, v. 66, n. 2, p. 286-290, Aug. 1980.

ZHANG, D. et al. Efficacy of the antagonist *Aureobasidium pullulans* PL5 against postharvest pathogens of peach, apple and plum and its modes of action. **Biological Control**, Orlando, v. 54, n. 3, p. 172-180, Sept. 2010.

ZHANG, J. et al. Improvement of chilling tolerance and accumulation of heat shock proteins in grape berries (Vitis vinifera cv. Jingxiu) by heat pretreatment. **Postharvest Biology and Technology**, Amsterdam, v. 38, n. 1, p. 80-90, Oct. 2005.

SEGUNDA PARTE-ARTIGO

ARTIGO 1 - EFFECT OF HOT WATER DIPPING (HWD) OF APPLES ON FRUIT TEMPERATURE, PHYSIOLOGICAL DISORDERS AND STORAGE ROT

Artigo a ser submetido à Revista Postharvest Biol. Tec. ISSN: 0925-5214, sendo apresentado segundo suas normas de publicação.

(VERSÃO PRELIMINAR)

Renata de P. Rocha*[a], Marianne Bertelsen[b], Martin Himmelboe[b], Luiz Carlos de O. Lima[a], Merete Edelenbos[b]

[a] Department of Food Science, Federal University of Lavras, 37200-000 Lavras, MG, Brazil

[b] Department of Food Science, Aarhus University, Kirstinebjergvej 10, 5792 Årslev, Denmark

*Corresponding author: + 55 21 959077306

e- mail address: re_p_rocha@yahoo.com.br

ABSTRACT

Heat treatments have been re-adopted as an alternative to implementing laws that prohibit or limit the use of agrochemicals and have shown promising results when applied to different apple cultivars. In this study, we adopted immersion techniques at various temperatures for different varieties of apples ('Ingrid Marie' and 'Pinova') from Danish orchards. Two different immersion methods were used: manual and automatic. The following analyses were carried out: Soluble Solids (SS), Firmness, respiration rate, skin color, mass loss, heat damage and storage rots. It was observed that for 'Ingrid Marie' cultivar that the temperature ranges between 50 °C and 54 °C for 180s, resulting in fruits with higher a* (more red color participation), while 'Pinova' cultivar subjected to a temperature of 50 °C showed a higher value for a*. The 'Pinova' cultivar when subjected to a temperature of 50 °C showed more vivid colors, whereas the treatment at 54 °C for 30s + ultraviolet C resulted in more vivid colors (highest chroma value) and the highest hue for 'Ingrid Marie' cultivar. Regarding the respiratory rate, 'Pinova' cultivar showed lower respiration rate when subjected to a temperature of 50 °C for 180s, while 'Ingrid Marie' presented a lower respiration rate when subjected to a temperature of 52 °C for 180s. There were no differences for firmness between treatments. Fruits from both cultivars, 'Ingrid Marie' and 'Pinova', had a low rate of disease when exposed to a temperature of 54 °C for 30s, showing no physiological damage. Fruits that were manually treated at 50 °C for 180s was suitable for both 'Ingrid Marie and 'Pinova' cultivars.

Keywords: Malus domestica, heat treatments, organic production, storage rot.

1. INTRODUCTION

Due to enforced laws that prohibit or limit the use of chemical products to control pests and diseases that affect fruits still in the field, as well as the increase in the demand for healthy and pesticide residue free food, studies are being developed to devise methods that are free of synthetic substances in the battle against pathogens and pests that result in high postharvest losses (Lurie, 1998; Tang et al., 2000). Special attention is being paid to heat treatments and alternative physical methods, which are based on inducing metabolic reactions to stress caused by exposure of the commodity to high temperatures.

According to Lurie (1998), the most common heat treatments used for fruits and vegetables are hot water, vapor heat and hot air. Hot water is used as a way to transfer the heat most effectively when compared to hot air and useful when contamination is found on the surface of the fruit, as is the case with fungal infections where pathogens are normally located on the surface or in the outer layers of the skin (Schirra et al., 2000). The binomial temperature-time of the commodity´s exposure to heat is established empirically and presents variations in agreement with the species, cultivar and method used. (Lurie, 1998; Lurie and Pedreschi, 2014).

The changes that take place on a molecular level as a reaction to heat are similar, regardless of the variation in the binominal temperature-time (Lurie and Pedreschi, 2014). These changes include synthesizing and accumulating Heat Shock Proteins (HSPs), antioxidant enzymes and phenolic compounds (Chen et al., 2015; Lurie and Pedreschi, 2014), which protect the tissue. HSPs play an important role for the fruit in the acquisition of thermo-tolerance. These proteins act as chaperones and protect other proteins against denaturing caused by fruit exposure to high temperatures. Considering this, the commodity can withstand

variations in temperatures without suffering physiological damage. (Fruk et al., 2012). Various authors have already reported the efficiency of different heat treatments to maintain fruit quality, extend shelf-life, retain firmness and control fungal disease (Chen et al., 2015; Fallik et al., 1995;Fruk et al., 2012; Lay-Yee et al., 1997).

Fungi is one of the major causes of fruit disease, resulting in high post-harvest losses. For instance, the main agents of fungal infections that affect apples (*Malus domestica* Borkh.), cultivated in the Northern region of Western Europe, still in the field or during harvesting are *Neofabraea* spp (*N. alba* and *N. perennans*), *Neonectria galligena*, *Monilinia fructigena* (Palm and Kruse, 2005 cited by Maxim et al., 2012a) and *Penicillium* spp and *Botrytis cinerea* to a lesser degree (Jijakli and Leproive, 2004 cited by Maximn et al., 2012a). Maxin and collaborators (2012) reported *Neofabraea* spp as the main fungus causing storage rot in cv. 'Ingrid Marie' and 'Pinova'. *N.alba* are the most abundant, followed by *N. perennans, N. galligena, M. fructigena, Cladosporium* spp. and *P. expansum.* Maxin and Weber (2011) reported that immersing apples cv. 'Elstar' in hot water, originating from Aarslev (DK) was very effective in eliminating *P. Washingtonensis* spores from naturally infected fruits. Furthermore, Spadoni et al (2013) proved inhibition of conidia of *Monilinia* in peaches after being immersed in hot water.

Research on combining heat treatments with other treatments has been recently used to obtain better results to eliminate various diseases that occur during storage, when conserving fruit quality and increasing storage time of various commodities. The combination of heat treatments and a controlled atmosphere was considered by Tahir et al (2009) as a promising strategy to control fungal diseases and increased resistance against bruising of the cultivars 'Aroma' and 'Ingrid Marie', providing good protection against bitter rot and

bull's eye rot. Bal (2012) established an increase in storage time of sweet cherries by combining Hot Water Dipping (HWD) with menthol and thymol essential oils.

The aim of this study was to assess the rate of disinfestations and quality of semi-organic apple cv. 'Ingrid Marie' and 'Pinova', resulting from hydrothermal treatments by adopting two different methods: one manual and the other automatic. In addition, attention was paid to the occurrence of physiological disorders and damage caused by the automatic method.

2. Material and Methods

2.1.Fruit for hot water dipping (HWD)

Apples cv. 'Ingrid Marie' and cv. 'Pinova' from the Årslev orchard (Aahrus university, DK) were harvested at the end of September (2014) and stored in a cold room at 2°C for 4 weeks to manual dipping method and 5 weeks for automatic dipping method until the beginning of the treatments. The fuits were stored during 4 weeks One week before starting the treatments, the fruits were selected according to size, weight, uniform color and presence of wounds. On the day before the treatment, the fruits were removed from cold storage and kept at room temperature. Uniform fruits were randomly selected according to size and no bruises and assigned to one of 10 batches of 460 fruits for 'cv. Pinova' and 500 fruits for cv. 'Ingrid Marie' for manual dipping and one of 18 batches of 1,190 fruits for cv. 'Pinova' and 20 batches of 1,014 fruits for cv. 'Ingrid Marie' for automatic dipping.

2.2. Hot water dipping and cold storage

Partly sorted first quality of apples cv. 'Ingrid Marie' and 'cv. Pinova' were transferred to room temperature 24 hours before starting the experiments to allow apples to reach room temperature at manual or automatic hot water dipping. Immediately after dipping, apples were transferred to drying at 24 °C for 25 minutes in a ventilated oven (Lytzen A/S, Herlev, Denmark) to remove free water from the surface. The total process from bringing apples into the water and then back to cold storage took approximately 51 minutes for each treatment or 6 hours in total for manual dipping, and for automatic dipping a total of approximately 8h. Apples were stored at 2°C for 15 weeks (manual dipping) and 16 weeks (automatic dipping).

2.2.1. Manual dipping

Apples (10 x 7.9 Kg) cv. 'Pinova' and (10 x 8.0 Kg) cv. 'Ingrid Marie' were manually dipped in water at 50, 52, 54, and 56 °C for 180 s in a 350L water bath using a methodology adapted from Maxin, (2012). The control group was dipped in water at 20 °C for 180 s. The box containing the fruit was immerged in water and the top of each box was covered with a water gallon containing a 5 kg weight thereby ensuring that all the fruits remained entirely submerged throughout the HWD period, being 3 plastic box each 180 seconds, and to each change of treatments, the water from water bath was changed. The water temperature during dipping was monitored using an analogue mercury thermometer (Carl Roth, Karlsruhe, Germany). Samples for fruit quality measurement were taken after 1, 9 and 15 weeks of storage at 2°C . For the respiration rate and mass loss, samples were taken after 1, 3, 5, 7, 9, 11 and 15 weeks of storage. Rot evaluation samples were taken after 16 weeks of storage and multispectral analysis after 15 weeks of storage.

2.2.2. Automatic dipping

For automatic dipping apples (18 x 10.5 Kg) cv. 'Pinova' and (20 x 9.0 Kg) cv. 'Ingrid Marie' were dipped in 54 °C for 180 s, 54 °C for 30 s and 54 °C for 30 s followed by UVC light source application for 30 s. The control group was dipped in water at 20 °C for 180 s. The water temperature was monitored using an analogue mercury thermometer (Carl Roth, Karlsruhe, Germany). Regarding automatic dipping, the machine water was not changed. Automatic dipping was done using a machine prototype under development from a company called Innhoteque and the machine water capacity was 220 L. The water temperature inside the machine was controlled by a temperature probe that controls a thermostat. The holding time in the machine is controlled by a stepless system with a frequency converter and the internal material of the machine is a food approved plastic and stainless steel.

Samples for fruit quality measurements were taken after 1, 6 and 16 weeks of storage at 2 °C. For respiration rate and mass loss, samples were taken after 1, 3, 5, 7, 9, 11 and 13 weeks of storage, for rot evaluation after 14 weeks of storage and, for multispectral analysis after 13 weeks of storage.

2.3. Temperature measurement by thermography

Thermography is an image processing technique, which transforms thermal radiation, recorded by a camera, into a thermographic image or thermogram. A thermogram is a representation of the specific temperature distribution on the object´s surface. In the manual dipping method thermograms of apples were obtained before HWD, immediately afer HWD, after drying, 3 hours after HWD and 7 hours after cold storage by using an InfraRed (IR) camera (Testo IR T870-2). The Measurement range of IR camera is -20 to 280 °C Accuracy Information valid for specified measurement range + tolerance ±2 °C, ±2 % of reading (higher value applies). Software testo IRsoft 3.3 was used to

obtain a thermogram. The emissivity of the fruits was set to 0.95 (Veraverbeke et al., 2006)

A portable camera (Testo IR T870-2) was used for the manual experiment and photos were taken of the fruits placed on a plastic support. Photos of whole fruits and half fruits were taken. The thermograms were created according to the temperature emitted by the product and that is why thermal images with the same color do not necessarily have the same temperature.

The process of internal heating of both cultivars was similar, then only cv. 'Pinova' was used in the present work to demonstrate the results of temperature measurements.

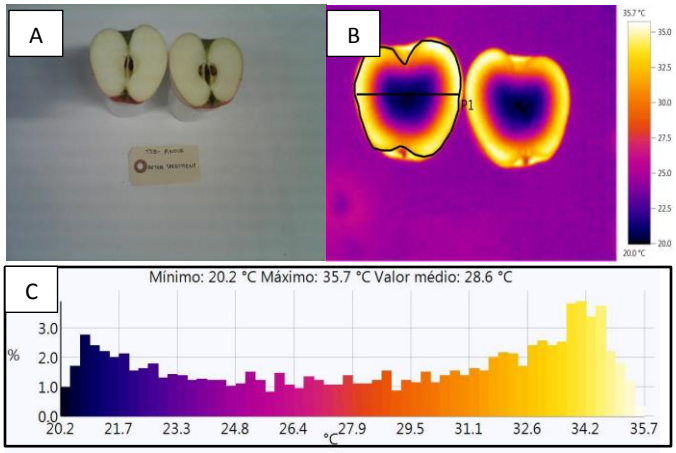


Fig.1. Example of a real image (A) a corresponding thermogram (B) and histogram (C) from selected area on fruit.

2.4. Fruit quality

The initial fruit quality was measured at the time of HWD and after 1, 9 and 15 weeks of storage of the manually dipped (3 fruits per replicate) and after 1, 6 and 16 weeks of storage for the automatically dipped fruits (5 fruits per replicate). Some quality parameters were only taken at HWD (fruit weight, dry matter content, starch) while others were taken throughout the experiment (firmness and soluble solid contents (SSC)). Fruit weight and then the firmness were recorded on a bench-mounted penetrometer (GS20 Fruit Texture Analyser, Güss Ltd., South Africa). For firmness, a portion of the skin was removed from two opposite sides of the fruits, a 11 mm metal probe was pressed into the fruit flesh and readings were recorded in kg/cm^2. The starch index was determined on a cross-section of the remaining flesh (~3 cm from the calyx) by dipping the tissue for 30 s into a standard iodine solution containing 10 g potassium iodide and 3 g iodine in 1 L of distilled water. After 2 min, the index was determined by visual ranking of the dipped tissue on a scale from 1 (black tissue ~ starch present) to 10 (white tissue ~ no starch present) using the EUROFRU CTIFL (*Centre Technique Interprofessionnel des Fruits et Légumes*) starch index scale (Planton, G. 1994) The SSC was determined in juice from two wedges taken near the core of the fruit using a temperature compensated digital refractometer (RFM712, Bellingham Stanley Ltd., Basingstoke, Hants, United Kingdom). For dry matter analysis, another two wedges were taken and freeze-dried (Gamma 1-20, Martin Christ, Osterode, Germany). The dry matter content was determined by weighing a total of 200 g of sample, including skin, before and after drying for 48 h.

2.5. Mass loss and respiration rate determination

Repeated mass loss and respiration rate determinations were taken during cold storage to evaluate if HWD resulted in differences between treatments. The static headspace method described by Fonseca et al. (2002) was used for respiration measurements. Five apples per replicate were incubated in a glass gallon jar and closed with an airtight lid mounted with a septum for gas measurements. For mass loss determinations, the initial weights of jars with lids with and without apples were recorded on a METTLER PJ3000 balance (Mettler-Toledo GmbH, Giessen, Germany). The weight of the jars containing the apples was recorded every other week throughout storage on a METTLER PJ3000 balance (Mattler-Toledo GmbH Laboratory & Weighing, Switzerland Technologies) and from this reading, the fruit mass was calculated. The mass loss was expressed as a percentage loss of the original mass and expressed as a cumulative mass loss.

$$Cumulative\ mass\ loss\ (\%) = \frac{(m_i - m_f)}{m_i} \times 100 \quad \text{(Equation 1)}$$

where m_i = initial mass and m_f = measured mass

The respiration rate was also measured every other week since the first week of storage until the end of the storage period by a gas analyzer (Checkmate 9900, PBI Dansensor, Ringsted, Denmark) in 24 hour intervals during 2 weeks and expressed as mL. gas. Kg^{-1}. h^{-1} ..

2.6. Determination of physiological disorders

Physiological disorders were evaluated using standard methods, such as expert evaluation and colorimetry and multispectral imaging analysis.

2.6.1. Expert evaluation

The evaluation of heat damage was done prior to destructive analysis using a grade scale of 5 points where 1= no visual color changes; 2= slight color changes (due to treatments or not); 3= color change affecting less than 50% of the fruit skin area (skin color turns more brownish red than clear red); 4= severe discoloring/ damage; 5= fruit totally damaged (turned brown). Using a total of 3 evaluators. Damage caused by the automatic dipping method due to the hot water machine or the UVC machine were evaluated using a scale of 2 points where 1= no damage , 2= damage. Regarding storage rots was stipulated that number 1 determined fruits without rots and number 2 determined unspecified rots on fruits.

2.6.2. Colorimetry

Two skin colour readings were taken on 2 opposite points using images registered by videometer Lab (Videometer A/S, Hørsholm, Denmark) in the measurement of L*, a*, b*, Chroma and hue angle (h). L*a*b* is a 3-D co-ordinate system containing three axes, L*, a* and b*, where the vertical axis, L* is lightness (black=0 and white=100) and on the horizontal axes, a* is the trend from red to green and b* is the trend from blue to yellow. Chroma is degree of departure from gray toward pure chromatic color and is calculated as $(a^{*2} + b^{*2})^{0,5}$. Hue is the angle between the hypotenuse and 0^{o} on the a* axis. It is calculated by the arc tangent of b*/a* and is expressed in degrees where 0^{o}=red, 90^{o}= yellow, 180^{o}=green and 280^{o}= blue (McGuire, 1992).

2.6.3. Multispectral imaging analysis

To determine some information of fruit quality the multispectral image acquisition was done using Videometer Lab (Videometer A/S, Hørsholm,

Denmark), which acquires multi-spectral images at 18 different wavelengths in the ultraviolet A (UVA), visual (VIS) and NIR region: 405, 435, 450, 470, 505, 525, 570, 590, 630, 645, 660, 700, 780, 850, 870, 890, 940, and 970 nm. The acquisition system records surface reflections using a standard monochrome charged coupled device chip, nested in a Point Grey Scorpion camera (Dissing et al, 2011). The object of interest is placed inside an integrating sphere containing a matte white coating. The coating and the curvature of the sphere ensure a homogeneous diffusion of light around the whole sphere area. At the border of the sphere light emitting diodes (LED) positioned side by side distributes in a uniform way the LED's from each wavelength. The homogeneous diffusion of light and the calibration steps ensure an optimized dynamic range minimizing the shadow effects, as well as specular effects and gloss-related effects (Dissing et al, 2011).

2.7. Storage rots

Storage rots and fungi associated to it were identified by the appearance of microscopic symptoms, sporulating structures and microscopy analysis of spores produced (Maxin et al., 2012a).

2.8. Data analysis

The experiment data were analyzed by analysis of variance (ANOVA) to evaluate the main effect of the treatment and storage weeks. For diseases, the least significance difference (LSD) test at $P \leq 0.05$ was used, and for all the others the Tukey's test at $P \leq 0.05$ was carried out. The data of treatments were assessed in separate for each method R statistics software ver. 3.2.2 with R-package "ExpDes.pt" ver. 1.1.2 was used

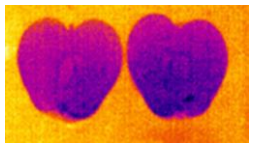
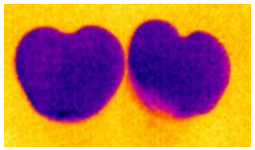
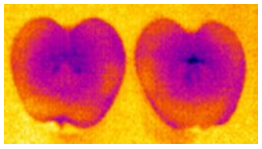
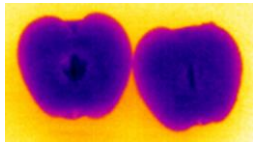
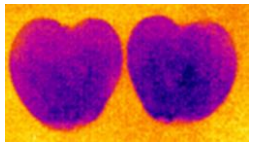
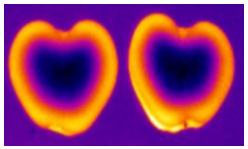
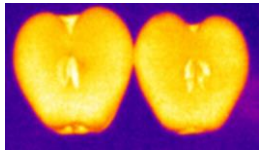
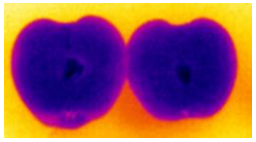
3. Results and discussion

3.1. Effect of HWD on fruit temperature

The temperature of cv. Pinova changed during handling if the fruits were HWD. Table 1 shows the temperature profile before HWD, after HWD at 20°C and 54°C for 180 s, after drying and after 7 h in cold storage. The highest temperature was recorded near the outer side of the apples HWD at 54°C where the heat penetrated and the lowest temperature was recorded in the inner part (apple core), which resulted in high ΔT values (Table 1). From the readings, it was observed that a maximum temperature of 34.1 °C was recorded and that this temperature was below the 54°C of the water which was used. It should be mentioned that as the Testo IR was used at a fixed setting, apples were removed from the HWD facilities to the camera setting within 15 s and the outside temperature was measured. Then the apple was cut and the inside temperature measured on the same apple. During this period the outside temperature decreased somewhat which explains why no temperature readings were similar to those of the water temperature.

Interestingly, the immersion temperature gradient at 54°C decereased during the 25 min of ventilation air. In these apples, ΔT was reduced to 6.8 °C. Even after 7 hours after cold storage, the fruits did not show a homogeneous temperature either in fruits dipped at 20 °C or fruits dipped at 54 °C.

Table 1. Processing of cv. Pinova using HWD. Temperature is taken on the total area of one half of apple in two fruits and average values used.

Treatment	Before HWD	After HWD	After drying	After 7 h at 2 °C
20 °C 180 s				
Min T [1]	17.7 °C	19.8 °C	20.2 °C	1.3 °C
Max T [2]	19.6 °C	21.9 °C	22.7 °C	8.5 °C
Δ T [3]	1.9 °C	2.1 °C	2.5 °C	7.2 °C
54 °C 180 s				
Min T [1]	19.9 °C	19.6 °C	25.3 °C	2.5 °C
Max T [2]	21.7 °C	34.1 °C	32.1 °C	9.05 °C
Δ T [3]	1.8 °C	14.5 °C	6.8 ° C	6.5 °C

[1]Min T= minimum temperature;
[2] Max T= maximum temperature;
[3]ΔT= temperature range

The outside and average inside temperatures of cv. 'Pinova' treated with 20 °C and 54 °C for 180 s before HWD, after HWD, after drying and after 7 h at 2 °C are shown in Fig. 2. There were small variations in the outer and inner temperatures of apples treated with 20 °C warm water for 180 s. The outside temperature did not change much from 20.1 °C to 20 °C reaching 23.1°C in a short period of 25 min to 24°C, and then finally decreasing to 4.25 °C over the following 7 h in cold storage. During the same period, the inside average temperature varied from 18.5 °C to 21.2 °C after drying reached 3.4 °C. On the other hand, fruits dipped at 54 °C had the highest variation either on the surface or for pulp (Fig.2). The pulp of the fruit showed a linear increase in temperature from 20.6 °C rising to 30.4 °C after drying reaching 4.1°C in cold storage (Fig.2).

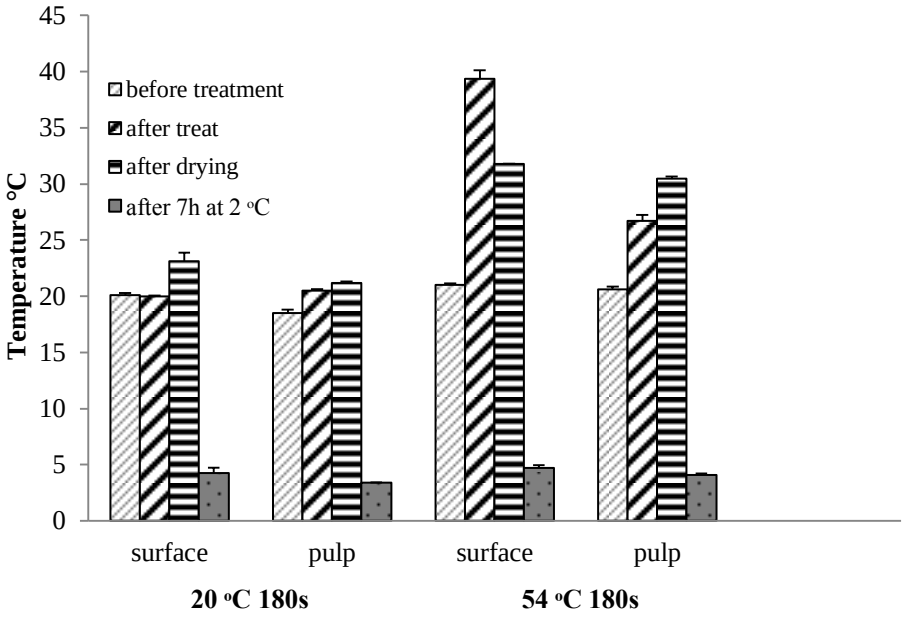


Fig. 2. Temperature variation of apple cv. Pinova treated by manual dipping method at 20 °C and 54 °C for 180 s. The bars correspond to average between medium value registered by testo software + standard deviation

The surface temperature did not show the same temperature of the water used in the treatments due to temperature compensation between the apples and the enviroment (Varith et al.,2003). As reported by Dincer (1994, 1995), the cooling rate of spherical fruits increased with air velocity and the core temperature showed a lagging decrease. The same was observed in this study, when after 25 minutes at 24 °C in a ventilation oven, the temperature inside the fruit showed a drop in temperature for fruits dipped at 54°C for 180 s concerning the maximum temperature, while for minimum temperatures there was an increase due to the oven temperature being higher than the minimum temperatures recorded by the fruits (Table 1). Considering this, the average values of the inside of the fruits were slightly higher than before drying, as can be seen in Fig. 2.

Thermal energy reaches the fruit core by conduction. This phenomenon occurs slowly in fruits due to the low relative value of thermal conductivity (K ≈ 0.5 W/ m °C), (Tang et al.,2000). Food thermal conductivity is largely influenced by various factors connected to the nature of food (i.e.: water content and amount of air between cells), as well as the temperature and environment pressure. A reduction in the water content decreased the thermal conductivity (Fellows, 2006) and has an influence on specific heat. Fresh fruits generally have a high water content, but thermal conductivity of water is around 0.6 W/m °C (Fellows, 2006) and it is a factor that may respond to the low heating rates shown for cv. 'Pinova'.

Hansen, (1992) also reported the low heating rate of fruits and Tang et al (2000) demonstrated that the heating time for an apple core to reach 50 °C when submitted to water dipping at 52 °C was 42 minutes. According to Wang et al (2001) thermal conductivity, density and specific heat are thermal properties that

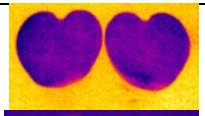
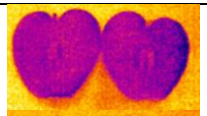
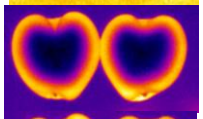
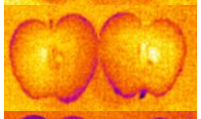
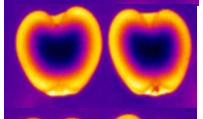
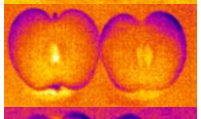
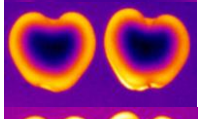
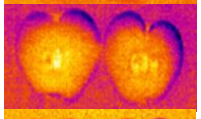
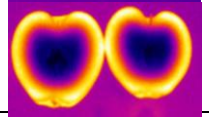
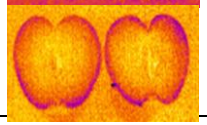
influence heating rates of fruit. The most relevant parameters found in a simulation model of fruit heat transfer were fruit size, heating medium and the heating medium speed.

In our study, the main point to be considered is the heating medium speed because the water was static inside the water bath. If the water inside the bath had been circulating, the pulp would have. reached the highest temperatures during 180 seconds of dipping. It could have kept the inner temperature slightly high for a long time, influencing the fruit quality. Thus, it should be mentioned that the heating period might influence some fruit ripening processes.

3.2. Effect of water temperature and treatment duration

The information concerning temperatures inherent to fruits immediately after HWD in the manual method and 3 hours after cold storage are shown in Table 2. The ΔT was high for fruits dipped at 56 °C and it showed a large variation between the maximum and minimum temperatures in the apple pulp. After 3 hours of cold storage, the ΔT decreased considerably showing that there was a temperature homogenization in the inner region mainly in the core that usually presents the lowest temperature after treatment. As explained before, the time of treatment has an important role in the HWD process. The longer the time, the higher the inner temperature and, it is directly proportional to the temperature applied. In our study, the time of different treatments was 180 seconds for all of them, however it can be observed that immediately after the treatment, the maximum temperature was higher for apples dipped at 56 °C (Table 2). On the other hand, the minimum temperature (recorded in the core) did not show significant differences among the treatments. This probably happened because the length of the treatment was not sufficient to reach the core. This can be very important for pathogen cases located in the apple core.

Table 2. Inside average temperature of HWD cv. ‘Pinova’ in the manual dipping method.

Treatment	After treatment	Avg[1]	Min[2]	Max[3]	**ΔT**[4]	After 3 h at 2°C	Avg	Min	Max	**ΔT**
		(°C)					(°C)			
20 °C 180 s		20.5	19.8	22.0	2.2		20.0	19.5	21.2	1.7
50 °C 180 s		26.3	20.6	33.1	12.5		23.1	22.1	24.0	1.9
52 °C 180 s		26.7	19.7	34.0	14.3		23.8	22.7	25.0	2.3
54 °C 180 s		26.7	19.6	34.1	14.1		24.2	22.6	25.4	2.6
56 °C 180 s		28.1	19.7	36	16.3		23.1	21.7	24	2.3

[1]Avg= average temperature;
[2] Min= minimum temperature;
[3]Max= maximum temperature;
[4]ΔT= temperature range

3.3 Initial quality of apple

Table 3 shows that there were statistical differences (P≤0.05) for dry matter, soluble solids, firmness and starch index for fruits before both manual and automatic dipping treatments. Dry matter is an important quality metric for apples (Palmer et al, 2010) and it can vary from fruit to fruit and also orchard sample to orchard sample (Palmer et al, 2010). A variation of dry matter between cultivars shows that cv. 'Pinova' is sweeter than cv. 'Ingrid Marie' and/or has more cell wall material and a higher osmotic potential and due to that greater flash firmness.

Table 3.Initial quality of apples for hot water treatment

Cultivar	Ingrid Marie		Pinova	
Method	MD	AD	MD	AD
weeks in storage before treatment	4	5	4	5
Fruit weight (g)	150.7 ±6.3a	140.7 ±6.5a	171.9 ±36.2a	144 ±13.3a
Dry matter (g.100g FW)	15.0 ±0.6b	15.0 ±0.7b	17.1. ±0.6a	16.6 ±0.6ab
Soluble solids (%)	12.8 ±0.4b	13.4 ±0.4b	14.4. ±0.3a	14.6 ±0.3a
Firmness (kg)	6.0 ±0.3b	4.2 ±0.1c	8.0 ±0.6a	8.0 ±0.9a
Starch index	6.4 ±0.7b	7.9 ±0.7ab	8.3 ±0.5ab	9.3 ±1.5a

Means with different letters within rows are significantly different according to Tukey's test at P≤0.05 MD= Manual dipping, AD= Automatic dipping.

The starch index was also higher for cv. 'Pinova' than cv. 'Ingrid Marie' showing that the starch content in cv. 'Pinova' is less than cv. 'Ingrid Marie', but this index should not be used to compare the maturity between different cultivars due to differences in the characteristics of each cultivar. cv. 'Pinova' can reach

9.5 Kg at harvest to 6.5 Kg at the end of storage (Fisher and Fisher, 2002), while cv. 'Ingrid Marie' has a soft flesh, medium texture and strong sweetness (Korsgaard et al, 2009). This was the tendency shown by the cultivars in this study. Martinez Vega et al (2014) reported that cv. 'Ingrid Marie' flesh tended to soften after harvesting, as we also observed in apples automatic dipped probably due to more days in storage before treatment.

The cv. 'Ingrid Marie' was less firm than cv. 'Pinova' at HWD treatments because their characteristics differ from cv. 'Pinova'. Ng et al (2013) identified differences between apple cultivars in microstructure and cell wall properties that could influence the rate of fruit softening during ripening.

3.4 Quality parameters of heat-treated apple fruit

3.4.1 soluble solids of apple

The results showed significant differences ($P \leq 0.05$) in soluble solids between cv. 'Ingrid Marie' and cv. 'Pinova' throughout storage irrespective of the HWT method (data not shown), but there were no significant differences ($P \geq 0.05$) among treatments within manual or automatic dipping. The cultivar 'Ingrid Marie' showed less soluble solids than cv. 'Pinova' throughout storage. These differences are not a response of heat treatments, but they may happen due to the characteristic of the fruits and the ripening process of them. Tahir et al. (2009) and Fan et al.,(2011) also observed that there is no relation between heat treatments and soluble solid content for apples.

3.4.2 Fruit firmness

Significant differences ($P \leq 0.05$) in firmness were detected between the cultivars for both manual and automatic dipping (Table 4) and storage time (Table 4) and there were no differences ($P \geq 0.05$) among treatments either for

manual dipping or automatic dipping. Despite there being no differences, it was observed that at the end of the storage time, automatically and manually dipped cv. 'Pinova' showed more firmness retention compared to the control (Fig.3). This often observed in fruits after heat treatments.

Table 4. The effect of storage time on the firmness of cv. Ingrid Marie and cv. Pinova. The values are the average of 3 fruits to manual dipping and 5 fruits to to automatic dipping.

Method	Storage weeks	Firmness(kg)	
		cv. Ingrid Marie	cv. Pinova
MD	1	5.21bA	7.80aA
	9	3.34bB	7.18aB
	15	3.23bB	6.80aB
AD	1	4.38bA	7.68aA
	6	3.68bB	7.15aB
	16	3.11bC	6.78aB

Means with different small letters within lines for manual or automatic dipping and different capital letters within columns are significantly different according to Tukey's test ($P \leq 0.05$). The data evaluation were done separatelly for each method.

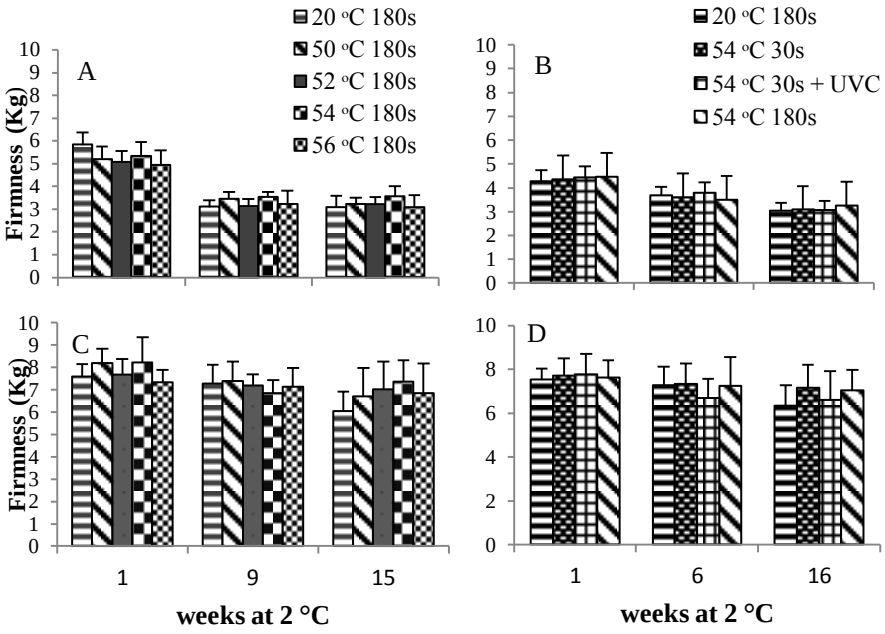


Fig. 3. Effect of manual and automatic dipping on fruit firmness. Cv Ingrid Marie manual dipping (A), cv. Ingrid Marie automatic dipping (B), cv. Pinova manual dipping (C) and cv. Pinova automatic dipping (D). The bars represents the average values + standard deviation

Vincente (2005) observed that strawberries heated in air in an oven at 45°C for 3 hours had lower Poligalacturonase (PG) activity compared to non-treated fruits. After 1 day at 20°C, heated treated fruits still showed lower PG activity than the control fruits.

Pectin methylesterase (EC 3.1.1.11) (PME) and polygalacturonase (EC 3.2.1.15) (PG) are hydrolases and they act on the pectin fraction in the cell wall and most of the time are responsible for loss firmness on plant tissues (Chitarra and Chitarra,2005; Wei et al., 2010). PME attacks ester bonds causing pectin demethylation, increasing the susceptibility of walls to degradation by PG (Wei et al., 2010). Temperatures around 55-80°C should stimulate PME action and

maintain the firmness of the tissue promoting adhesion between the cell wall and the middle lamella (Parkin et al., 2010). PME activity results in the appearance of chemical groups with negative charges ($-COO^-$) over the pectin chains increasing the affinity of Ca^{+2} bonds, keeping the cell wall structure and hindering PG action, increasing the firmness. (Kluge et al., 2002).

Spadoni (2014) observed a low genetic expression of PME and PG after heat treatment in peaches dipped in water at 60°C for 20 seconds. Regarding PME, 3 hours after treatment, the gene expression was 0.4-fold lower than non-treated fruits. For PG, the expression was 0.4-fold lower than the control after 15 minutes of treatment.

Different authors reported similar results for apples (Klein and Lurie,1992; Lurie and Klein,1990; Lurie and Nussinovitch, 1996), nectarines (Fruk et al ,2012) strawberries (Vincente et al, 2005), bananas (Amnuaysin et al,. 2012). Despite numerous reports about the increase in the firmness of fruits treated by different types of heat treatments, the explanation for this phenomenon is not clear. Enzymatic inactivation due to heating could be a possible explanation (Conway et al, 1994).

3.4.3. Colorimetric analysis by videometer Lab

The results of color show differences ($P \leq 0.05$) between the cultivar in the automatic and manual dipping methods (Table 5). As expected, L*, b* and Chroma were higher for cv. 'Pinova' in the manual dipping method and only b* and chroma for the automatic dipping method (Table 5). Cultivar ‘Pinova’ presented higher L* in the manual dipping method. As L* means luminosity, high L* represents a bright color for cv. 'Pinova', which is its characteristic, compared to cv. 'Ingrid Marie'. High temperatures in the manual dipping method

showed less a* for cv. 'Ingrid Marie' (Table 5). This could be due to a decrease in chlorophyll degrading enzyme activity. Kawsuksaeng et al (2015) observed a decrease in chlorophyll degradation in Thai lime treated at 50°C for 5 minutes. They also observed that HWD suppressed activities of chlorophyll degrading enzymes such as chlorophylase, chlorophyll degrading peroxidase and Mg-dechelation. Lurie and Klein (1990) showed an increase in chlorophyll degradation at 38 °C for 4 days when applied to apple cv. 'Anna'. Results show that the HWD temperature can influence the red color of apples, decreasing the red shades or keeping the chlorophyll degrading enzymes activity limited.

Cultivar 'Pinova' has more yellow shades than cv. 'Ingrid Marie' and this is clearly shown in Table 5. Only cv. 'Ingrid Marie' presented differences for b* among the treatments for the manual and automatic dipping methods. In manual dipping, fruits treated at 56°C showed high b* compared to fruits at 20°C (Table 5). Automatic dipping treatments at 54°C for 30 s and 54 °C at 180s presented lower b* than apples treated at 54 °C for 30s + UVC (Table 5). High b* shows a trend to yellow that could be due to an exposition of carotenoids, that act as a secondary pigment linked to chlorophyll (Damodaram et al.,2010). Furthermore, the exposition of fruits to UV-C light can stimulate the phenylalanina amonia-liase enzyme (EC 4.3.1.5.)- PAL. This enzyme is a key enzyme in the phenylpropanoid and phenol synthesis (Ryalls et al., 1996), and is also correlated to quercetin and anthocyanins synthesis, proving to be a light-dependent process (Bakhshi and Arakawa, 2006).

In the manual and automatic dipping methods, significant differences ($P \leq 0.05$) were observed between cultivars for Chroma (Table 5). In manual dipping, only cv. 'Pinova' showed differences between treatments, and in automatic dipping only cv. 'Ingrid Marie' showed differences among the

treatments (Table 5). The increase in temperature resulted in a decrease of Chroma for cv. 'Pinova' in manual dipping (Table 5). It was also observed in automatic dipping that the highest temperature for more time resulted in lower values of chroma for cv. 'Ingrid Marie' (Table 5).

Regarding the hue°, differences ($P \leq 0.05$) were observed between the treatments concerning cv. 'Ingrid Marie' in the automatically dipped method. Apples heated at 54 °C 30 s + UVC showed a high hue, which may not be good for this cultivar showing a brown-yellow color, appearing burned.

Comparing the results to the same temperature (20°C and 54°C for 180 s) in both the automatic and manual methods in general, cv. 'Pinova' showed higher L* than cv. 'Ingrid Marie' mainly for fruits dipped automatically. The chroma was higher for cv. 'Pinova' in the automatic dipping method and the manual dipping method showing vivid colors.

The changes in these color parameters indicate that temperatures induced a degradation of chlorophyll and exposition of carotenoids or anthocyanins, which are found in the cells. The results agree with Wang et al (2006), who observed that chroma values in apples treated by radio frequency were significantly lower than the control, and partially agree with Moscetti et al (2013) who observed a decrease in both the hue and chroma. Our results showed a decrease in the hue° in the case of cv. 'Pinova', despite no significant differences.

Table 5. Color measurements and respiration rate (mL. O_2. $Kg.^{-1}h^{-1}$ and mL. $CO_2.Kg^{-1}.h^{-1}$) of apples treated by HWD by manual and automatic dipping method. Color measured was done by videometer Lab witch 2 measures for surface to each fruit (n= 6 to MD and 15 to AD) and to respiration rate n= 15 .

Method	Treatment	L*		a*		b*		Chroma		hue°		O_2		CO_2	
		IM	Pinova	IM	Pinova	IM	Pinova	IM	Pinova	IM	Pinova	IM	Pinova	IM	Pinova
MD	20 °C 180 s	46.2b	65.4a	33.1A	27.8AB	22.7bB	45.1a	43.1b	56.9aA	32.5	53.0	2.87AB	2.60A	3.01A	2.45A
	50 °C 180 s	53.2b	60.7a	23.7bAB	32.5aA	29.3bAB	42.4a	42.6b	56.9aA	44.4	54.4	2.66ABC	1.94C	2.58B	2.00B
	52 °C 180 s	53.1b	61.3a	25.4AB	28.8AB	30.3bAB	43.3a	42.8b	54.6aAB	48.0	48.6	2.41C	1.98BC	2.60B	2.00B
	54 °C 180 s	46.7b	59.6a	25.8AB	19.8B	25.4bAB	44.3a	40.2b	50.5aBC	33.7	54.1	2.97A	2.53A	3.22A	2.63A
	56 °C 180 s	55.8	57.9	19.5B	22.0AB	33.0bA	41.9a	41.6b	48.8aC	47.3	46.8	3.04A	2.50A	3.18A	2.52A
AD	20 °C	53.6	67.2	21.1	22.0	32.6bAB	47.0a	48.0bA	55.5a	34.0bB	56.4a	2.76	1.62C	2.78	1.83C
	54 °C 30s	47.1	68.5	27.9	20.7	25.6bB	49.0a	40.8bBC	56.8a	39.2bAB	66.4a	2.80	1.98B	2.86	2.07B
	54 °C 30s +UVC	56.7	67.6	19.4	23.8	35.5bA	46.2a	44.3bAB	55.8b	55.0A	51.5	2.66	2.23A	2.85	2.41A
	54 °C 180s	49.3	65.1	22.2	18.6	26.2bAB	49.4a	39.0bC	55.6a	43.7AB	58.2	2.74	2.01B	2.77	2.12B

Means with different small letters within line and different case letters within column indicate significant differences between cultivars and treatments respectively within each method according to Tukey's test ($P \leq 0.05$)**.** Averages without letters means no statistical differences.

3.5 Respiration Rate

The respiration rate between the cultivars Ingrid Marie and Pinova showed a significant difference in manual dipping and automatic dipping ($P \leq 0.05$). In the manual dipping method cv. 'Ingrid Marie' had an average of 2.92 mL CO_2. Kg^{-1}. h^{-1} while cv. 'Pinova' presented an average of 2.32 mL CO_2. Kg^{-1}. h^{-1} and for O_2, there was an average consumption of 2.79 mL O_2. Kg^{-1}. h^{-1} for cv. 'Ingrid Marie' and 2.31 mL O_2. Kg^{-1}. h^{-1} for cv. 'Pinova'. In the automatic dipping method, cv. 'Ingrid Marie' showed an average of 2.82 mL CO_2. Kg^{-1}. h^{-1} and 2.74 mL O_2. Kg^{-1}. h^{-1}, cv. 'Pinova' showed an average of 2.11 mL CO_2. Kg^{-1}. h^{-1} and 1.96 mL CO_2. Kg^{-1}. h^{-1}. It shows that cv. 'Ingrid Marie' may present less storage life compared to cv. 'Pinova'.

The results shown in Table 5 suggest that for cv. 'Pinova' in the manual dipping method, the treatment of 50 °C for 180 seconds is the most recommended, showing low O_2 consumption and low CO_2 production and for cv. 'Ingrid Marie', treatment 52 °C for 180 seconds is suggested for the same reason related to cv. 'Pinova'. Concerning the automatic dipping method, there was no differences ($P \geq 0.05$) to treatments to cv.'Ingrid Marie' while to cv.'Pinova' fruits heat-treated at 54 °C for 30 s followed by UVC radiation for 30 s showed high respiration rate (table 5). Possible damage caused in the cell tissue due to UVC application and inappropriate doses could explain this increase in the respiration (Escalona et al., 2010). A slighty higher CO_2 production in watermelons, which received the highest doses of UVC, was related to Artés-Hernandez et al (2010). In baby spinach, a high respiration rate was also observed for all samples treated by UVC regardless of the doses used (Escalona et al., 2010).

Fallik et al (2001) reported a low respiration rate in apples cv. 'Golden Delicious' treated at 55 °C suggesting that this may partially happen due to some kind of inhibition of some ripening processes. However, in this study, apples treated at 54 °C and 56 °C presented a respiration rate statistically similar or higher than the control fruits and apples heated at 50 °C and 52 °C for 180 s had a better response in the manual dipping method. Smith and Lay-Yee (2000) did not report any differences between heat treatments and control fruits for cv. Royal Gala, and we also did not observe any differences for cv. 'Ingrid Marie' in the automatic dipping method. Lurie and Klein (1990, 1991) reported that heat-treated fruits had a high respiration rate during the treatment period and decreased thereafter, remaining low during shelf-life, but there is still a lack of data in the literature concerning the respiration rate for fruits treated in short periods at high temperatures.

According to Chitarra and Chitarra (2005), controlling the respiration rate is essential for maintaining the quality and prolonging the shelf-life of fruits and vegetables and the intensity of its biochemistry and physiology reactions can quickly lead to senescence. In this study, results show that fruits automatically dipped had a low respiration rate, considering the same temperature of treatments (54 °C for 180 s) of both methods.

3.6 Mass loss

During cold storage, apples lost very little mass, less than 0.5 % of the initial content. Mass loss was higher in cv. 'Ingrid Marie' than cv. 'Pinova' and higher in the manually dipped than in the automatically dipped apples (Fig. 5). There were no differences ($P \geq 0.05$) in the treatments for cv. 'Ingrid Marie' and 'Pinova' in the automatic dipping method.

There was significant interaction (treatments x storage week) for cv. 'Ingrid Marie' and 'Pinova' in the manual dipping method (Fig. 5A and 5C). Treatment at 20 °C for 180 s showed a difference compared to other treatments from the seventh week of storage, maintaining this difference until the end of storage, where treatment at 20 °C for 180 s had less mass loss and the treatment at 56 °C for 180s had a higher mass loss (Fig. 5A). All the treatments had high R^2 and this shows that the storage time contributed significantly to the mass loss of heat-treated apples.

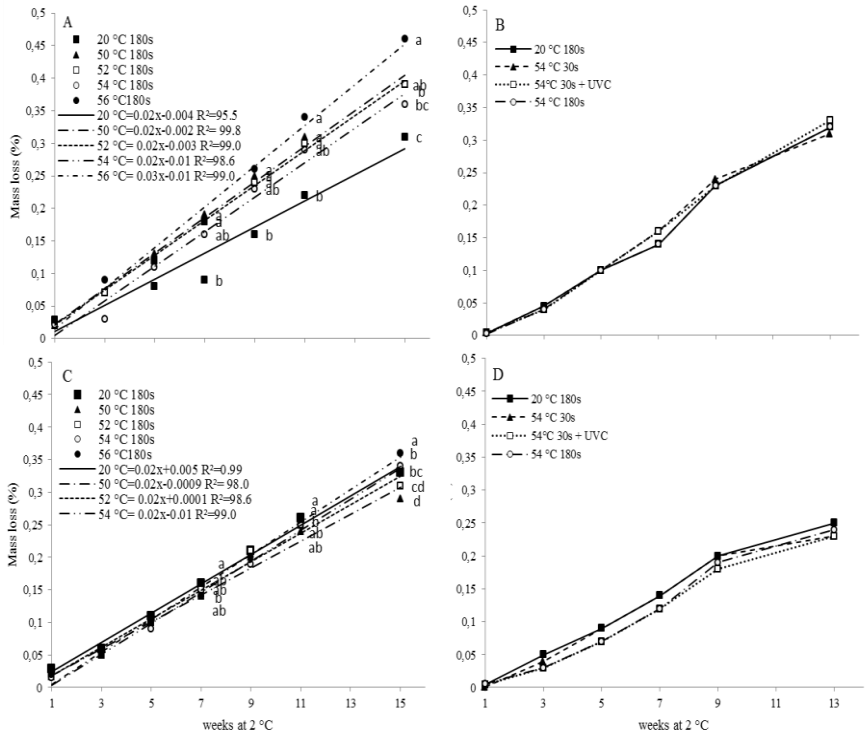


Fig. 5. Effect of HWD on mass loss of apples storage at 2 °C during 15 weeks (manual dipping method) and 13 weeks (automatic dipping). Manual dipping (A and C) and automatic dipping (B and D) and cv. Ingrid Marie (A and B) and cv. Pinova (C and D). The results were expressed by average of 9 fruits distributed in 3 glass jars per treatment. In the figure (A) and (C) different small letters into storage weeks indicates difference among treatments according Tukey's test ($P \leq 0.05$).

In the case of cv. 'Pinova', the differences among treatments were observed in the seventh, eleventh and last weeks. In the seventh week, all treatments showed less mass loss than the control fruits, while in the eleventh storage week, the control and the apples treated at 56 °C for 180 s showed the higher mass loss. In the last week, apples treated at 56°C for 180 s showed higher mass loss than the other treatments (Fig. 5C). These results are in agreement with the respiration rate reported in the current study, where the apples heat-treated at high temperatures showed a high respiration rate.

The respiration rate of fruit has an influence in mass loss because there are carbon losses, but most of the mass loss (80-85%) is due to water vapour loss and apple water permeance (Miguire et al., 2000; Link et al., 2004; Brackmann et al.,2014). According to Schönherr et al (1979), and Montero et al (2010) cuticles and cuticular waxes act as barriers to water transport and reduction of cuticular transpiration. This process is influenced by the composition of soluble cuticular lipids on cuticular membranes (Schönherr et al., 1979).

An increase in temperatures led to irreversible structural changes in membranes resulting in an increase of water permeability (Schönherr et al., 1979). This was reported by Schönherr et al (1979) by heating isolated membranes to different temperatures and they observed that temperatures above 45°C caused irreversible changes to the membranes. On the other hand, application of different heat treatments can lead to the melting of wax from cuticular membranes sealing the fissures found in the fruits´ cuticles (Montero et al., 2010; Schirra et al., 2000). It is necessary to consider each cultivar from each species because the cuticle presents distinct characteristics even between cultivars of the same species. Montero et al (2010) found a distinct pattern of cuticles in two different apple cultivars leading to a different effect of coating by melting wax.

The results presented for cv. 'Ingrid Marie' and 'Pinova' in the manual dipped method shows that a high mass loss of treatment at 56°C for 180s could be due to the irreversible structural changes, as those observed by Schönherr et al (1979) in isolated membranes.

3.7 Rot and damage incidence

The percentage of non- affected fruits reached 89 % and 83 % for cv. 'Pinova' and cv. 'Ingrid Marie' respectively at 50 °C for 180 s in the manual dipping method , while for the automatic dipping method both cv. 'Pinova' and cv. 'Ingrid Marie' showed less affected fruits in the treatments at 54 °C for 30 s and 54 °C for 30 s + UVC reached 77 % of fruits without rots (Fig.6). These treatments had less primary rot than control fruits (data not shown). Treatments 54 °C 30s and 54 °C for 30s +UVC showed a better response in percentage of rots for cv. 'Pinova' as well as cv. 'Ingrid Marie'. Moreover, the presence of *P. expansum* was not identified in fruits heated at 54°C for 30 s in cv. 'Pinova' and cv. 'Ingrid Marie' (data not shown). The incidence of *P.expansum* in apples treated at 54°C for 30 s+UVC and 54°C for 180 s was 7% and 61% respectively for cv. 'Ingrid Marie' and 7% and 30% for cv. 'Pinova' (data not shown). Apples manually dipped showed a high incidence of *P. expansum* for fruits heated at 54 and 56°C for 180s, 68% and 73% respectively to cv. 'Ingrid Marie' and 68% and 64% respectively to cv. 'Pinova' (data not shown).

In this study, we observed a high infection incidence for treatments carried out at high temperatures showing higher rates than the control for fruits manually dipped (Fig.6A).

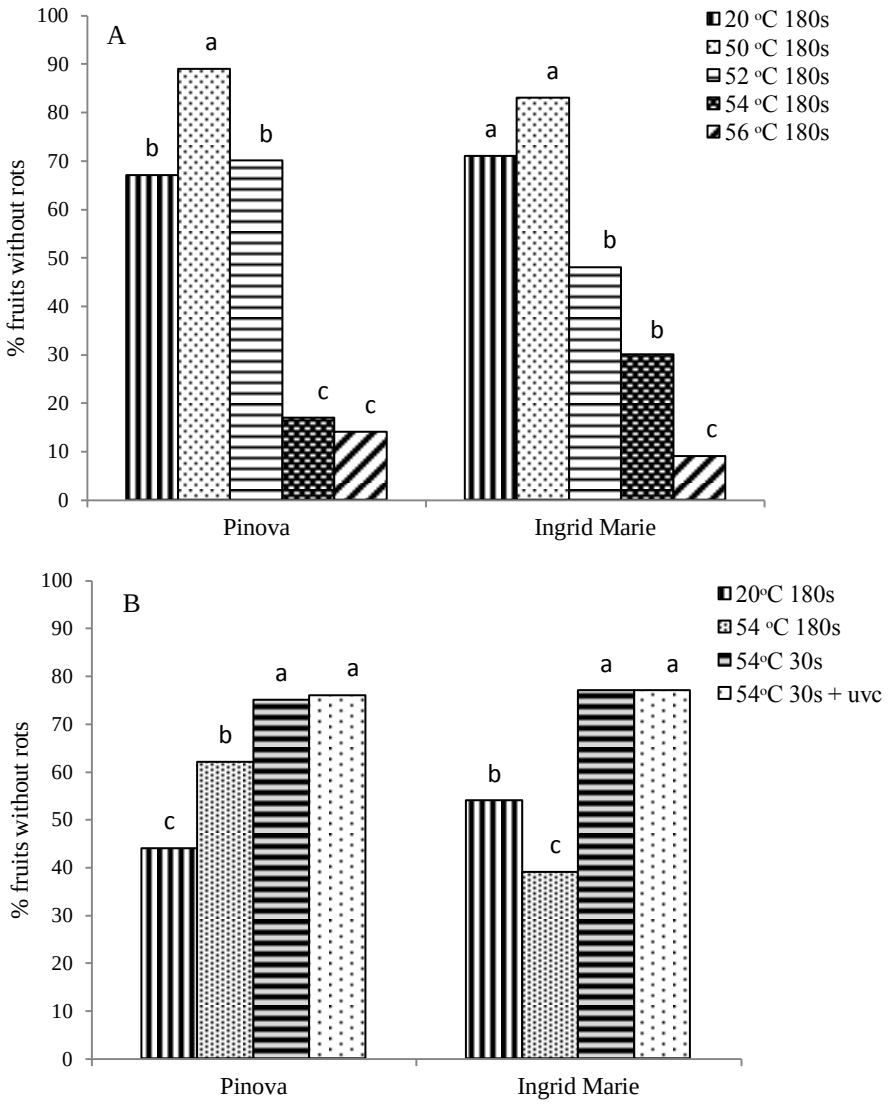


Fig. 6. Effect of HWD on rot occurrence for fruits treated by manual dipping method (A) and automatic dipping method (B). The assessment was carried out after cold storage period. The bars shows the average of 45 fruits per treatment to manual dipping method and 150 fruits per treatment to cv. Ingrid Marie and 300 fruits per treatment to cv. Pinova at automatic dipping method. Different letters in each cultivar indicates difference among treatments according to an LSD test ($P \leq 0.05$)

Fruits treated using the automatic dipping method were analyzed in order to verify specific damage caused by the HWD machine. It was observed that cv. 'Ingrid Marie' had 2.2 % of affected fruits and cv. 'Pinova' had 7.3 % of affected fruits.

Regarding skin damage in manually dipped fruits, cv. 'Ingrid Marie' showed minor and noticeable color changes as a response to treatments at 52 °C for 180 s and treatments at 54 and 56 °C for 180 s showed 100 % of severe color changes (Table 6). In cv. 'Pinova', the treatment at 50 °C for 180 s had a low color change and the treatment at 52 °C for 180s showed more changes in the color due to temperatures which were mostly affected by noticeable changes (Table 6) and apples heated at 54 and 56 °C 180 s showed 100 % of severe changes of color (Table 6). For fruits dipped automatically, severe noticeable changes were observed in fruits heated at 54 °C for 30 s + UVC and 54 °C for 180 s for cv. 'Ingrid Marie', and for cv. 'Pinova' only apples heated at 54 °C for 180 s had severe changes (Table 6).

The action of heat could be directly on the fungi spores and mycelium or by inducing the host natural resistance (Maxin et al., 2012a; Klein and Lurie, 1992). Melting epicuticular wax and its capacity to close or seal the cracks found on the fruit surface, as well as the encapsulation of spores and mycelin plays an important role in increasing natural resistance (Schirra et al., 2000; Porat et al, 2000).

An increase in temperture can affect the plant´s metabolism in different ways such as increasing the accumulation of heat shock proteins (HSPs) that protect cell integrity and provide thermo-tolerance.(Pavoncello et al., 2001; Lurie and Padreschi, 2014; Spadoni et al., 2015). Another important point that should be considered is the pathogen thermo-tolerance and its development

phase. It may be different according to the species and its different fungal growth stages (spores, mycelium and survival structures) (Barkai-Golan and Phillips, 1991).

A positive correlation between high temperatures and an increase of rot incidence caused by *P. expansum* was also reported by Maxin et al., (2012a, 2012b). The temperatures range between 47 – 52 °C showed a suppression in the development of blue mold caused by *P. expansum,* but higher temperatures (53°C, 54°C and 56°C for 3 minutes) gave rise to high levels of diseases that reached the same levels of the control fruits, and this increased with storage time (Maxin et al., 2012b). Contamination of apples by *Penicillium* spp. occurs mainly in storage (Amiri and Bompeix, 2005) and due to the possibility of damage that could be a result of dipping fruits at high temperatures, pathogens that have high tolerance to heat are able to grow on fruit tissue damaged by HWD at high temperatures.

UVC light is known as an efficient germicide that acts causing damage to the nucleic acid, affecting the multiplication of microoganisms (Artés-Hernández, 2010). This treatment in inappropriate doses can cause damage to the cell tissue (Artés et al., 2007; Nimitkeatkai and Kulthip, 2016), enabling fungi to accumulate. This would explain the percentage of P. expansum found in apples treated at 54 ºC for 30 s followed by UVC application.

According to Terão (data not published), there is always an ideal combination between time and temperature to control each fungi, fruit species and even the cultivar. Considering treatments not in this specific combination, the control is low and it is likely that in some cases, there is an increase in the severity of the disease. This happens due to selecting sensitive pathogens, and those that have a high heat tolerance are able to develop throughout storage.

Moreover, above this ideal combination, tissue damage can occur, which is an open door for fungi, increasing the incidence and severity of rots.

Table 6. Damage after HWD on cv. 'Ingrid Marie' and cv. 'Pinova' that has been storaged at 2 °C until 03.05.2015 for both methods. To cv. Ingrid Marie and cv. Pinova AD method a total of 15 fruits per treatments were analyzed. To MD method a total of 9 fruits per treatment were examined

Method	Treatment	Cv. Ingrid Marie			Cv. Pinova		
		% fruit with minor color changes	% fruit with noticeable color changes	% fruit with severe color changes*	% fruit with minor color changes	% fruit with noticeable color changes	% fruit with severe color changes*
AD	20 °C180s	0	0	0	0	0	0
	54°C 30s	0	0	0	0	0	0
	54°C30s+uvc	0	3,1	5	0	0	0
	54 °C 180 s	0	6,6	29	0	0	56
	LSD 0.05	-	ns	11.3	-	-	10
MD	20 °C 180s	0	0	0	0	0	0
	50 °C 180s	0	0	0	23	0	0
	52 °C 180s	33	14	0	0	62	38
	54 °C 180s	0	0	100	0	0	100
	56 °C 180s	0	0	100	0	0	100
	LSD 0.05	19	ns	5	ns	34	34

Heat damage should occur when the commodity is subjected to improper treatments, which means excessive time and/or high temperatures. Some of the symptoms of these injuries are skin browning, surface pitting, flash darkening and water loss (Lurie, 2008). Treatments at 54°C and 56 °C for 180s for cv. 'Ingrid Mari'e and 52°C, 54°C and 56°C for 180 s for cv. 'Pinova' resulted in severe heat injuries in fruits manually dipped. Moreover, cv. 'Pinova' had almost no damage after treatments compared to cv. 'Ingrid' Marie when automatically dipped. These results agree with Fallik et al., 2001; Bompeix and Coureau, 2007; Maxin et al., 2012a .

3.8 Multispectral image analysis

In this study, no differences ($P \geq 0.05$) were observed for both cv. 'Ingrid Marie' and cv. 'Pinova' heat-treated using the automatic dipping method after 16 weeks of storage (Fig.7B and D) and for fruits manually dipped only cv. 'Pinova' showed differences ($p<0.05$) among treatments after 15 weeks of storage at 2°C (Fig.7C). In Fig. 7C, cv. 'Pinova' heated at 54°C and 56°C for 180 s showed lower reflectance compared to the control and other treatments in the VIS region (400-760 nm) and NIR region (780-1000 nm). These treatments differed statistically from others in the region of 405-470 nm and 630-870 nm. The lower reflectance of treatments (Fig. 7C) indicates a high content of compounds due to the absorption of light, which will not be reflected.

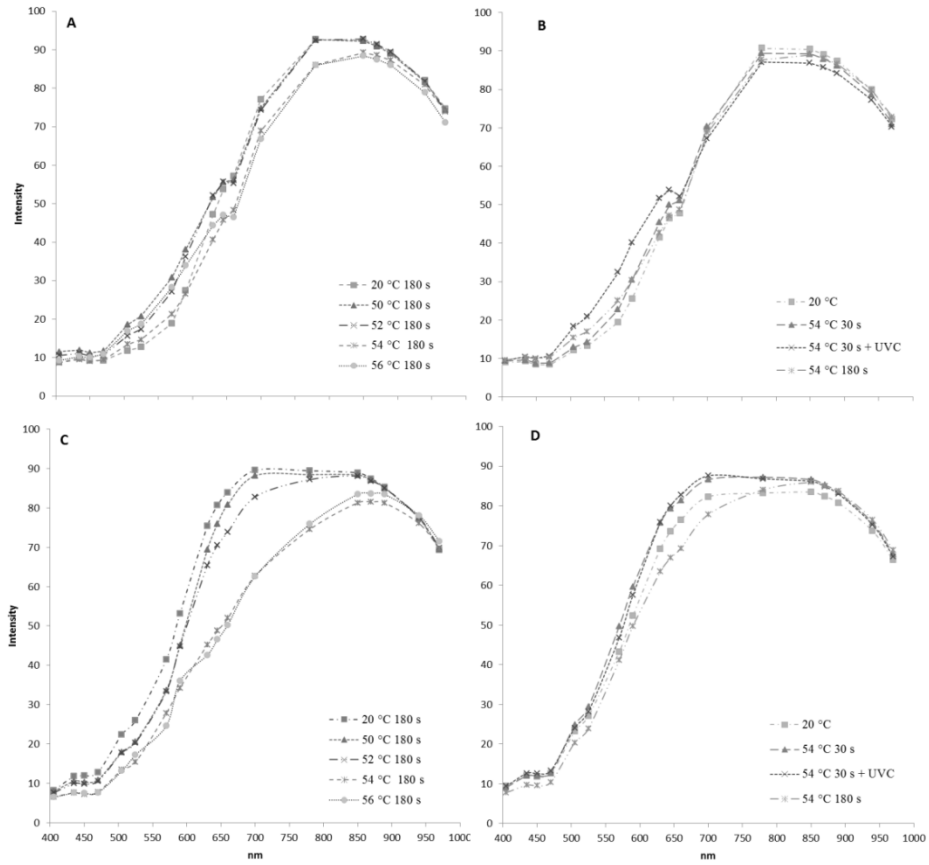


Fig. 7. Reflectance observed on apples cv. Ingrid Marie (A and B) and cv. Pinova (C and D) manually dipped (A and C) and automatically dipped (B and D) stored at 2 °C. The assessement was carried out in the last week of storage.

The region of 405-470 nm that showed differences assumes a higher carotenoid content (Lokke at al., 2013) for these fruits, which agrees with results shown in this study in Section *3.2.3* about colorimetric analysis, and may be correlated with chlorophyll degrading enzymes such as chlorophylase, chlorophyll degrading peroxidase and Mg-dechelation (Kawsuksaeng et al., 2015) as a response to high temperatures. The region of 630-870 nm also showed differences for cv. 'Pinova' and the wavelengths at 780 and 850nm are

absorption points of bruises (Huang et al., 2015) and the region at 810 and 970 was reported as effective in terms of detecting rotting caused by *Pencillium* (Zhang et al., 2015). The data reported in this study regarding cv. 'Pinova' are in agreement with the above study cited. Despite no differences shown for others cultivars studied in the current work, the results shown by videometer Lab are in agreement with the other results related here.

Multispectral imaging has been used over the last years as a rapid and efficient alternative tool able to predict bruises, contamination, distribution of bioactive compounds, quality attributes, ripeness stages and changes in color and texture of different material during storage in a non-destructive way (Liu et al, 2014; Liu et al 2015; Lu 2003; Huang, 2015, Lokke et al, 2013).

4. Conclusion

Based on the data presented, it can be concluded that:

- Due to the low thermal conductivity of apples, the heating and cooling times and how they are used can influence the final quality of fruit;
- The temperature at 54°C for 30 s showed better results for cv. 'Pinova' and 'Ingrid Marie' in automatic dipping method;
- Concerning the manual dipping method, the treatment at 50 °C for 180 s was better for cv. 'Ingrid Marie' and cv. 'Pinova'
- Automatic method proved to be an efficient system, and further studies and adaptations to the machine are needed to minimize damage occurrence in fruits.

Acknowledgements

The authors would like acknowledge the Coordination for the Improvement of Higher Level of Education Personnel (Capes) process number 99999.003072/2014-02 and the ProtecFruit project from Aarhus Universitet for the financial support.

References

Amir-Shapira, D., Goldschmidt, E. & Altman, A. 1986. Autolysis of chlorophyll in aqueous and detergent suspensions of chlorophyll fragments. Plant Sci. 43: 201-206.

Amiri, A and Bompeix, G. 2005. Diversity and population dynamics of *Penicillium* spp. on apples in pre- and postharvest environments: consequences for decay development, Plant Pathol. 54, 74–81

Amnuaysin, N., Jones, M.L., Seraypheap, K., 2012. Changes in activities and geneexpression of enzymes associated with cell wall modification in peels of hotwater treated bananas. Sci. Hortic. 142, 98–104

Artés-Hernández, F., Robles, P.A., Gómez, P.A.,Tomás-Collejas, A., Artés, F. 2010. Low UV-C illumination for keeping overall quality of fresh-cut watermelon. Postharvest Biol Tec. 55,114-120.

Artés, F., Goméz, P., Artéz-Hernández, F., Aguayo, E. and Escalona, V. 2007. Improved strategies for keeping overall quality of fresh-cut produce. Acta Hortic. 746, 245-258 .

Bakhshi, D. and Arakawa,O. 2006. Effects of UV-B irradiation in phenolic compound accumulation and antioxidant activity in "jonathan" apple influenced by bagging, temperature and maturation. J. Food Agric. Environ. 4,75-79.

Bal, E. 2012 Effects of essential oil treatments combined with hot water treatment on improving postharvest life of sweet cherry. Fruits, 67 (4), 285-291

Barkai-Golan, R., Phillips, D.J., 1991. Postharvest heat treatment of fresh fruits and vegetables for decay control. Plant Dis. 75, 1085–1089.

Bompeix, G., Coureau, C., 2007. Practical use of thermotherapy against apple parasitic disorders. Proceeding of the International Congress Novel Approaches for the Control of Postharvest Diseases and Disorders. Bologna, Italy, 3–5 May 149–155.

Brackmann, A., Thewes, F. R., Anese, R. O., Both, V and Gasperin, A. R. (2014) Respiration rat and its effect on mass loss and chemical qualities of "Fuyu" persimmon fruit stored in controlled atmosphere. Ciênc. Rural, 44(4): 612-615

Chen, H.,Cheng, Z.,Wisniewski, M., Liu, Y., Liu, J. 2015. Ecofriendly hot water treatment reduces postharvest decay and elicits defense response in kiwifruit. Environ. Sci Pollut. R. 22, 15037-15045.

Chitarra, M. I. F., and Chitarra, A. B. (2005) Pós - colheita de frutas e hortaliças: Fisiologia e manuseio 2 edição, Lavras: UFLA 785pp, pag 73.

Conway, W.S., Sams, C.E., Wang, C.Y., Abbott, J.A., 1994. Additive effects of postharvest calcium and heat treatment on reducing decay and maintaining quality in apples. J. Am. Soc. Hort. Sci. 119, 49–53

Dincer, I.1994. Unsteady Heat-transfer analysis of spherical fruit to air flow, Energy 19 (1) 117-123

Dincer, I. 1995 Transiente Heat-transfer analysis in air cooling of individual spherical products, J. Food Eng 26 (4) 453-467.

Dissing, B. S.,Nielsen, M.E., Ersboll, B.K.. and Frosch, S. (2011) Multispectral Imaging for Determnation of Astaxanthin Concentration in Salmonids. PlosOne 6, e19032.

Escalona, V. H., Aguayo, E., Martínez-Hernández, G.B., Ártes, F (2010) UV-C doses to reduce pathogen and spoilage bacterial growth *in vitro* and in baby spinach. Postharvest Biol. Tec. 56, 223-231.

Fallik, E. Grinberg, S. Gambourg, M. Klein, J.D.And Lurie, S. (1995) Prestorage Heat Treatment Reduces Pathogenicity Of *Penicillium Expansum* In Apple Fruit. Plant Pathol 45**,** 92–97

Fallik, E., Tuvia-Alkalai, S., Feng, X., Lurie, S. 2001. Ripening Characterisation and Decay Development of Stored Apples after a Short Pre-Storage Hot Water Rinsing and Brushing. Innov. Food Sci. Emerg. Technol. 2,127-132

Fan, L., Song,J., Forney, C. F., Jordan, M. A. 2011. Fruit maturity affects the response of apples to heat stress, Postharvest Biol. Technol.62 , 35–42

Fellows, P. J. 2006. Tecnologia do Processamento de alimentos: Princípios e Prática, 2 edição, Porto Alegre:Artmed 602pp, pag 43

Ferreira, E. B., Cavalcanti, P. P., Nogueira, D. A. (2013). ExpDes.pt: Experime ntal Designs pacakge (Portuguese). R package version 1.1.2.

Fischer, M and Fischer, C. (2002) Pinova Apple Cultivar. The Compact fruitTre e 35(1), 19-20

Fonseca, S.C., Oliveira, F.A.R., Brecht, J.K., (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. J. Food Eng. 52, 99-119.

Fruk , G., Nišević, L., Sever, Z., Miličević , T., Jemrić, T ., (2012). Effects of different Postharvest Heat Treatments on Decreasing Decay, Reducing Chilling Injury and Maintaining Quality of Nectarine Fruit. Agric. Conspec. Sci 77 (1),27-30.

Gwanpuaa, S.G., Verlindenb, B.E., Hertoga, M.L.A.T.M., Van Impec, J., Nicolai,B.M., Geeraerda, A.H., 2013. Towards flexible management of postharvest variation in fruit firmness of three apple cultivars. Postharvest Biol. Tec. 85, 18-29.

Hansen, J. 1992. Heating curve models of quarantine treatments against insect pests. J. Econ. Entomol. 85, 1846-1854.

Hellebrand, H. J.,Herppich, W. B., Beuche, H., Dammer, K-H., Linke, M., Flath, K.. 2006. Investigations of plant infections by thermal vision and NIR imaging. Int. Agrophys.20, 1–10.

Huang,W., Li, J., Wang, Q., Chen,L 2015. Development of a multispectral imaging system for online detection of bruises on apples, J. Food Eng 146 , 62–71.

Irving, D. E**.,** Pallesen, J. C. and Cheah, L. H (1991) Respiration and ethylene production in kiwifruit following hot water dips. Postharvest Biol. Tec. 1, 137-142.

Kaewsuksaeng,S., Tatmala, N., Srilaong, V., Pongprasert, N. 2015 Postharvest heat treatment delays chlorophyll degradation and maintains quality in Thai lime (Citrus aurantifolia Swingle cv. Paan) fruit. Postharvest Biol. Tec. 100 ,1–7

Klein, J. D., Lurie, S. 1992 Prestorage heating of apple fruit for Enhanced Postharvest quality: Interaction of time and temperature. HortSci**.** 27**,** 326–328.

Korsgaard M, Toldan-Andersen T, Nordling J. 2009 The Pometum apple Key.

Avaiable from: Http://www.nordgen.org/nak/?chglang=ENG.

Kluge, R.A.,Nachtigal, J.C., Fachinelo, J.C., Bilhalva, A. B. (2002) Fisiologia e manejo pos-colheita de frutos de clima temperado 2 ed. Livraria editora Rural,Campinas. 214p.

Lay-Yee,M. Ball, S. Forbes,S.K. Woolf, A.B. 1997 .Hot-water treatment for insect disinfestation and reduction of chilling injury of 'Fuyu' persimmon. Postharvest Biol. Tec. 10, 81-87.

Li Yuan, Y Bi., Yonghong Ge, Y W., Yaoyao Liu, G L. 2013. Postharvest hot water dipping reduces decay by inducing diseaseresistance and maintaining firmness in muskmelon (*Cucumis melo* L.) fruit, Sci. Hort. 161,101–110.

Link, S. O., Drake, S. R., Thiede, M. E. 2004. Prediction of Apple Firmness from Mass Loss and Shrinkage. J. Food Quality 27, 13-26**.**

Liu,C.,Liu,W., Chen,W.,Yang,J., Zheng,L 2015. Feasibility in multispectral imaging for predicting the content of bioactive compounds in intact tomato fruit. Food Chem. 173, 482–488

Liu, C., Liu, W., Lu, X., Ma, F., Chen, W., Yang, J., Zheng, L. 2014 Application of Multispectral Imaging to Determine Quality Attributes and Ripeness Stage in Strawberry Fruit, Plos One 9(2) e87818.

Lokke, M. M., Seefeldt, H. F., Skov, T., Edelenbos, M. 2013 Color and Textural quality of packaged wild rocket measured by multispectral imaging, Postharvest Biol. Tec., 75, 86–95.

Lu, R., 2003. Detection of bruises on apples using near-infraredhyperspectral imaging. Trans. ASAE 46 (2), 523–530.

Luria, N., Sela, N., Yaari, M., Feygenberg, O., Kobiler, I., Lers, A. 2014. De-novo assembly of mango fruit peel transcriptome reveals mechanisms of mango response to hot water treatment. BMC Genomics, 15, 957.

Lurie, S. 1998. Postharvest Heat Treatments. Postharvest Biol. Tec. (14), 257-269

Lurie, S. and Klein, J. D. 1990. Heat treatment of ripening apples; Differential effects on physiology and biochemistry. Physiol. Plant. 78; 181-186.

Lurie, S. and Nussinovitch, A. 1996. Compression characteristics, firmness and texture perception of heat treated and unheated apples. International Jour. of Food Sci. and Techn., 31, 1-5

Lurie, S., and Pedreschi, R. 2014 Fundamental aspects of postharvest heat treatments. Horticulture Research 30,1-7

McGuire, R 1992. Reporting of Objective Color Measurements, HortSsc., 27(12), 1254-1255

Maguire, K. T., Banks, N. H., Lang, A., Gordon, I. L. 2000. Harvest Date, Cultivar, Orchard, and Tree Effects on Water Vapor Permeance in Apples. Journal. Amer. Soc. Hort. Sci. 125(1), 100–104.

Martinez Vega, M., Varming, C., Skov, T., Toldam-Andersem, T. B. 2014 Post-harvest ripening increase cultivar specific sensory and analytical aroma profile in apple juice: A study of four commercial cultivars in Denmark, Acta Agriculturae Scandinavica Section B: soil and Plant Science

Maxin, P. & Weber ,R W.S. 2011. Control of *Phacidiopycnis washingtonensis* storage rot of apples by hot-water treatments without the ethylene inhibitor 1-MCP**.** J. Plant Dis. Protect. 118 (6), 222–224

Maxin, P., Weber, R.W.S., Pedersen, H. L.,Williams, M. 2012a. Control of a wide range of storage rots in naturally infected apples by hot-water dipping and rinsing. Postharvest Biol. Tec. 70, 25–31.

Maxin,P., Weber,R. W. S., Pedersen, H. L., Williams, M. 2012b. Hot-Water Dipping of Apples to Control *Penicillium expansum*, *Neonectria galligena* and *Botrytis cinerea*: Effects of Temperature on Spore Germination and Fruit Rots. Eur. J. Hortic. Sci. 77 (1). S. 1–9,

Montero, C. R. S., Antes, R. B.,Santos, R.P., Santos, L. G., Cristiane Salete Andreazza, C. S., Bender,R. J (2010) Alteraçoes na cuticula de maçãs 'Fuji' e 'Gala' em funçao do tratamento térmico e da armazenagem refrigerada, Acta Sci. Agron.32 (3) 441-447

Neri, F., Mari, M., Brigati**,** S., Bertolini, P., 2009. Control of Neofabraea alba by plant volatile compounds and hot water. Postharvest Biol Tec 51, 425–430.

Nimitkeatkai, H. and Kulthip, J. 2016. Effect of sequential UV-C irradiation on microbial reduction and quality of fresh-cut dragon fruit. Int. Food Res. J. 23(4),1818-1822

Palmer, JohnW ; Harker, F Roger; Tustinc, D Stuart and Jason Johnstonb 2010. Fruit dry matter concentration: a new quality metric for apples. J. Sci. Food Agr. 90, 2586–2594

Parkin, K.L. 2010. Enzimas In: Damodaran, S., Parkin,K.L.,Fennema,O.R. Química de Alimentos de Fennema.Artmed, Porto Alegre,pp 336-337.

Pavoncello, D., Lurie, S., Droby, S., Porat, R. 2001. A hot water treatment induces resistance to Penicillium digitatum and promotes the accumulation of heat shock and pathogenesis-related proteins in grapefruit flavedo. Physiol. Plant.111, 17–22.

Planton, G. 1994. Maturité de Cueillette de Pommes: l'amidomiètre AM 93. Infos-CTIFL 98, 24-26.

Porat, R., Daus, A.,Weiss, B., Cohen, L., Fallik, E., Droby, S. 2000. Reduction of postharvest decay in organic citrus fruit by a short hot water brushing treatment Postharvest Biol Tec 18, 151–157

R Core Team 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-proje ct.org/.

Ryalls, J.A., Neuenschwander, U.H., Willits, M.G., Molina , A., Steiner, H.Y., Hunt, M.D. 1996. Systemic acquired resistance. Plant cell, 8,1809-1819.

Schirra, M, D'hallewin, G., Ben-Yehoshua, S. Fallik, E. 2000 Host–pathogen interactions modulated by heat treatment. Postharvest Biol Tec 21,71–85

Schönherr, J., Eckl ,K., Gruler, H. 1979. Water Permeability of Plant Cuticles: The Effect of Temperature on Diffusion of Water, Planta 147, 21 -26.

Smith, K.J., Lay-Yee, M. 2000. Response of 'Royal Gala' apples to hot water treatment for insect control, Postharvest Biol Tec 19 : 111–122

Spadoni, A., Guidarelli,M., Phillips, J., Mari, M., Wisniewski, M. 2015 Transcriptional profiling of apple fruit in response to heat treatment: Involvement of a defense response during *Penicillium expansum* Infection. Postharvest Biol Tec 101, 37–48.

Spadoni,A. Neri,F. Bertolini,P. Mari,M. 2013. Control of Monilinia rots on fruit naturally infected by hot water treatment in commercial trials. Postharvest Biol Tec. 86, 280–284.

Sui, Y., Droby, S., Zhang, D.,Wang,W., & Liu, Y. 2014. Reduction of Fusarium rot and maintenance of fruit quality in melon using ecofriendly hot water treatment. Environ. Sci. Pollut. R., 21, 13956-13963

Tahir, I.I., Eva Johansson., Olsson, M. E. 2009. Improvement of Apple Quality and Storability by a Combination of Heat Treatment and Controlled Atmosphere Storage, Hortscience 44(6):1648–1654.

Tang, J.,Ikediala, J. N., Wang, S., Hansen, J. D., Cavalieri, R.P. 2000. High-temperature-short-time thermal quarantine methods Postharvest Biol. Tec. 21, 129–145

Varith, J., Hyde, G. M.,Baritelle, A. L., Fellman, J. K., Sattabongkot, T. 2003. Non-contact bruise detection in apples by thermal imaging. Innov. Food Sci. Emerg. Technol. 4 ,211-218

Veraverbeke,E. A., Verboven, P., Lammertyn, J., Cronje ,P, De Baerdemaeker, J., Nicolaı, B. M. 2006. Thermographic surface quality evaluation of apple J. Food Eng. 77, 162–168

Vincente, A.R., Costa, M.L., Martìnez, G.A., Chaves, A.R., Civello, P.M. 2005. Effect of heat treatments on cell wall degradation and softening in strawberry fruit. Postharvest Biol Tec. 38, 213–222.

Wang, S. Tang, J. Cavalieri, R. P. 2001. Modeling fruit internal heating rates for hot air and hot water treatments. Postharvest Biol Tec 22, 257–270

Wang, S., Birla, S. L., Tang, J., Hansen, J. D. 2006. Postharvest treatment to control codling moth in fresh apples using water assisted radio frequency heating. Postharvest Biol Tec 40:89–96.

Wei, J., Ma, F., Shi, S., Qi, X., Zhu, X., Yuan, J. 2010. Changes and postharvest regulation of activity and gene expression of enzymes related to cell wall degradation in ripening apple fruit Postharvest Biol Tec 56: 147-154

Zhang, B.,Li, J., Zheng,L., Huang, W., Fan, S., Zhao, C., Meng, Q 2015 Development of a Hyperspectral Imaging System for the early detection of Apple Rottenness caused by *Penicillium.* J. Food Process Eng. 38**,** 499–509