UNIVERSIDADE TÉCNICA DE LISBOA

Instituto Superior de Agronomia

Metodologias integradas para a conservação de kiwi minimamente processado

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Dissertação apresentada neste Instituto para obtenção do grau de Doutor

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Resumo

O presente trabalho teve como principal objectivo o desenvolvimento de metodologias que permitam a melhoria e manutenção de qualidade de kiwi minimamente processado, pronto a consumir. Foram testados vários tratamentos em fases diferentes do diagrama de produção. O estado de maturação dos frutos mostrou-se determinante na sua adequação ao processamento e na selecção dos tratamentos a aplicar. A aplicação de pré-tratamentos térmicos moderados mostrou ser uma metodologia adequada conduzindo à preservação da firmeza do kiwi fatiado durante o armazenamento, mas apenas quando este se encontra num estado de maturação precoce. O tratamento optimizado foi de 45 ºC / 25 minutos. O efeito benéfico destes tratamentos pode ser incrementado através da imersão do fruto em soluções de cálcio ocorrendo apenas efeito sinérgico se os tratamentos forem em sequência e não em simultâneo. O principal mecanismo envolvido na preservação da dureza do fruto foi a activação da enzima pectina metilesterase e subsequente formação de pectatos de cálcio. Os estudos de descontaminação efectuados revelaram que a utilização da radiação UV-C como agente descontaminante foi eficaz mantendo os níveis microbiológicos sempre inferiores aos limites recomendados. Por outro lado induziu alterações na fisiologia do fruto permitindo uma melhor manutenção de firmeza do kiwi minimamente processado.

Palavras Chave: *Actinidea deliciosa*, processamento mínimo, pectina metilesterase, tratamentos térmicos moderados, cálcio, descontaminação

Combined methods for minimally processed kiwifruit preservation

Abstract

The main objective of the present work was the development of methodologies able to increase and/or maintain the quality of minimally processed kiwifruit during storage. Several treatments were tested at different steps of fresh-cut kiwifruit production process. Fruit maturity stage was a key factor determining both fruit ability for minimal processing and the appropriate treatment selection for appliance. Moderate heat treatments, applied to whole fruit, were efficient in preserving firmness of ready-to-eat kiwifruit during storage, but fruits must be at a firm ripe stage. Heat treatment that conduces to better results was 45 ºC/25 min. The beneficial effect of this treatment could be enhanced with calcium dips but, the synergistic effect between treatments is only observed if the treatments were done sequentially. Pectin methylesterase activation by heat treatment with calcium pectates formation was the main mechanism involved in fruit slices firmness maintenance. From tested methods, UV-C treatment was the most effective in reducing microbial loads of minimally processed kiwifruit, maintaining the levels always below the recommended limits. This treatment also induced fruit physiology changes allowing a better retention of fruit slices firmness during storage.

Keywords: *Actinidea deliciosa*, minimal processing, pectin methylesterase, moderate heat treatments, calcium, decontamination

Índice

Lista Abreviaturas

PPO	Polifenoloxidase
PAL	Fenilalaninaliase
PME	Pectinametilesterase
PG	Poligalacturonase
α - Gal	α - Galactosidase
β - Gal	β - Galactosidase
β - Xil	β - Xilosidase
HSP	Proteínas de choque térmico
RSM	Metodologia de superfície de resposta
1-MCP	1-Metilciclopropeno
IP	Pectina insolúvel
SP	Pectina solúvel
TP	Pectina total
SEM	Microscopia electrónica de varrimento
UV-C	Radiação ultra violeta de baixo comprimento de onda

Enquadramento geral e objectivos

É hoje geralmente reconhecido que a dieta alimentar desempenha papel fundamental na manutenção da saúde e em muitos casos na prevenção da doença. Segundo a Organização Mundial de Saúde (OMS) o baixo consumo de produtos hortofrutícolas é responsável por cerca de 19% dos cancros gastrointestinais, 31% da doença cardiovascular isquémica e por 11% dos enfartes do miocárdio, estando também associado a carências vitamínicas e minerais graves bem como distúrbios do tracto intestinal.

Os hortofrutícolas constituem um grupo de alimentos ricos em compostos bioactivos com propriedades sensoriais apelativas. Segundo a nova roda de alimentos o seu consumo deve constituir 43 % da alimentação diária. A OMS recomenda um consumo mínimo diário de hortofrutícolas de 400 g para se usufruir do efeito protector destes alimentos. Verifica-se que o seu consumo é reduzido por vezes por na grande maioria, necessitarem de operações de preparação, como o descasque e corte, o que nem sempre é compatível com o estilo de vida da sociedade moderna.

Torna-se assim importante o desenvolvimento a nível industrial de novos produtos hortofrutícolas com as características do produto fresco e que aliem a elevada qualidade à conveniência.

Frutos e vegetais minimamente processados são produtos frescos com elevada qualidade nutricional que se encontram prontos para o consumo e/ou utilização. Contudo, a sua taxa de degradação é superior à dos produtos inteiros que lhes deram origem, implicando que um período de conservação do produto inteiro que pode ser de vários meses seja reduzido apenas a alguns dias, depois de minimamente processado. São assim prioritárias a identificação e melhoria de metodologias de conservação que permitam manter a qualidade sensorial e nutricional dos produtos minimamente processados, por um período de tempo compatível com a actividade industrial e comercial. Por outro lado, o recurso a metodologias de conservação isentas ou com reduzida utilização de aditivos químicos é também uma exigência do consumidor actual, cada vez mais informado do impacto que esses produtos podem ter na saúde, sendo também mais restringidos na legislação actual.

Constituiu assim objectivo principal do presente trabalho o desenvolvimento de metodologias de conservação, em diferentes fases do diagrama de produção, que permitam manter elevados níveis de qualidade de kiwi minimamente processado, aumentando o seu período de vida útil.

Para concretizar este objectivo torna-se necessário:

- Identificar os processos de degradação envolvidos na perda de qualidade de kiwi minimamente processado.
- Estudar a importância da adequação da matéria-prima ao processamento mínimo.
- Desenvolver novas metodologias capazes de minimizar as perdas de qualidade sofridas por kiwi minimamente processado, durante o processo de armazenamento.
- Avaliar o impacto que as metodologias aplicadas apresentaram na qualidade global do produto.

O presente trabalho encontra-se estruturado em 5 capítulos.

No **Capítulo I,** capítulo introdutório**,** é efectuada uma breve descrição do que são produtos hortofrutícolas minimamente processados, quais os principais mecanismos de perda de qualidade que sofrem durante o armazenamento e de algumas possíveis metodologias de conservação visando minimizar e retardar esses mesmos mecanismos. É ainda efectuada uma breve descrição do fruto kiwi, matéria-prima seleccionada para este trabalho.

No **Capítulo II** é avaliada a influência da aplicação de tratamentos térmicos moderados a kiwi inteiro, na melhoria da qualidade do fruto minimamente processado. É estudada a importância do estado de maturação do fruto na eficácia do tratamento, bem como os mecanismos envolvidos nas alterações físicas, químicas e sensoriais sofridas pelo fruto durante o armazenamento.

No **Capítulo III** é estudada a eficácia da aplicação de cálcio na manutenção da firmeza de kiwi minimamente processado. É também avaliado o efeito sinérgico com

a aplicação de tratamentos térmicos moderados. São avaliadas as alterações físicas, químicas e estruturais sofridas pelo fruto durante o período de conservação.

No **Capítulo IV** é avaliada a importância do processo de descontaminação e o seu impacto na qualidade de kiwi minimamente processado. São estudados métodos de descontaminação alternativos ao método convencional que utiliza o cloro como agente descontaminante. É avaliada a eficácia dos vários processos utilizados na redução da carga microbiológica do fruto durante o período de armazenamento bem como os efeitos na sua fisiologia e características físicas e químicas.

No **Capítulo V** sumariam-se as principais conclusões deste estudo, identificando-se também algumas questões consideradas importantes e interessantes a serem desenvolvidas em trabalhos futuros.

Capítulo I

Introdução Geral

1 Produtos Hortofrutícolas minimamente processados

1.1 Definição

Os produtos hortofrutícolas minimamente processados surgiram na década de 70 do século passado nos Estados Unidos da América tendo, nas últimas décadas, o seu mercado assistido a um crescimento exponencial, devido a uma mudança no poder económico e na atitude do consumidor. Também na Europa, a partir do final da década de 80, se assistiu à procura de produtos minimamente processados, inicialmente em França e Inglaterra mas hoje em dia alargado a quase todos os países. No entanto existe ainda um desequilíbrio entre hortícolas e frutos, estando o mercado dos primeiros muito mais desenvolvido pela comercialização de saladas e preparados para sopa.

Produtos hortofrutícolas minimamente processados, também denominados de frescos cortados ou produtos da IV Gama, são todos aqueles que sofreram alterações físicas à sua forma original mantendo no entanto as características de frescura e qualidade do congénere inteiro. Podem ser constituídos por frutos e vegetais frescos sujeitos a operações de lavagem, descasque e corte que os tornam produtos de conveniência, encontrando-se prontos a consumir, sendo que o processo tecnológico a que foram sujeitos permite ainda manter uma elevada qualidade sensorial e nutricional (Wiley, 1994; Ahvenainen, 2000a). Depois de embalados são mantidos em refrigeração e conservados por um período de tempo suficiente para ser compatível com a distribuição e comercialização (Laurila e Ahvenainen, 2002; Ahvenainen, 2000b). Este período varia em função do produto e condições de conservação mas não deve ser inferior a 4 - 7 dias podendo atingir os 21 dias dependendo do mercado (Watada e Qui, 1999; Ahvenainen, 2000a).

Na Fig. 1 encontra-se representado um diagrama geral de produção de hortofrutícolas minimamente processados.

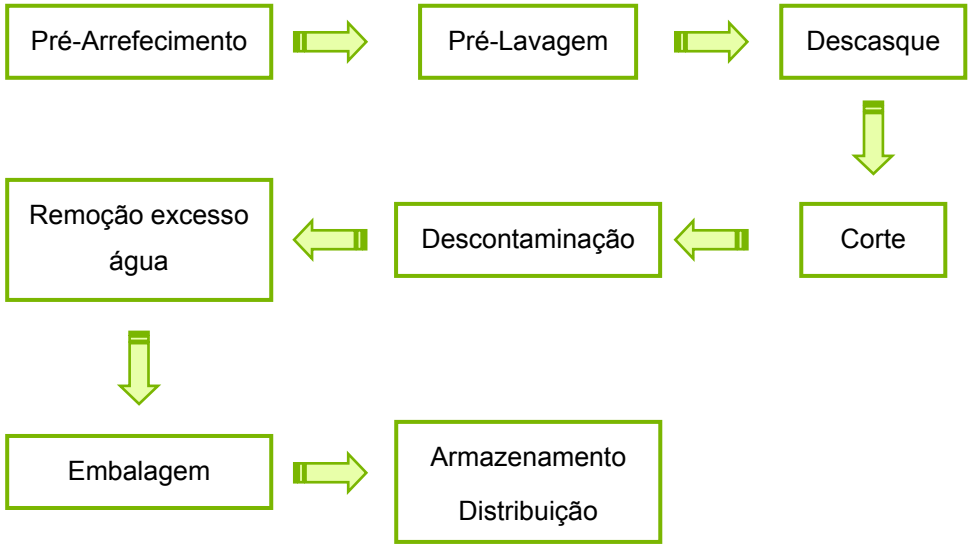

Fig.1 Diagrama tipo de produção de hortofrutícolas minimamente processados

1.2 Mecanismos de degradação

Os produtos hortofrutícolas frescos mantêm-se vivos, e portanto metabolicamente activos, no período pós-colheita. Quando sujeitos a processamento mínimo a sua taxa de deterioração é incrementada, por um lado como consequência da remoção do principal elemento protector, a casca, por outro devido aos danos mecânicos sofridos pelo produto (Watada e Qi, 1999). `

As operações de processamento mínimo a que o fruto ou vegetal é sujeito conduzem ao aumento da actividade fisiológica e de alterações bioquímicas, potenciando ainda a deterioração microbiológica dos mesmos (Laurila e Ahvenainen, 2002; Beaulieu e Gorny, 2001). As lesões provocadas nos tecidos durante o processamento, assim como o aumento da superfície específica devido ao corte, induzem aumento do metabolismo dos produtos, levando ao incremento da actividade respiratória, da perda de água e, no caso dos produtos climatéricos, da produção de etileno (Varoquaux e Willey, 1994). O aumento da taxa respiratória

pode variar de 20 a 700 % em função do produto, do tipo de corte e da temperatura de armazenamento (Laurila e Ahvenainen, 2002). Em kiwi, descascado e cortado em fatias, foi observada uma taxa respiratória duas vezes superior à do produto inteiro (Agar *et al*., 1999) e em rabanetes minimamente processados (Aguila *et al.*, 2006) bem como em cenoura o corte levou a um aumento de 62 % na taxa de produção de CO_2 (Cantwell, 1992).

O corte, para além de levar à ruptura de células à superfície, induz também lesões nos tecidos subadjacentes. Enzimas e substratos, normalmente localizados em compartimentos diferentes da célula são, através do corte, colocados em contacto, desencadeando assim uma série de reacções bioquímicas conducentes a alterações de cor, aroma e firmeza dos tecidos (Varoquaux e Willey, 1994; Dorantes-Alvarez e Chiralt, 2000).

As principais alterações de cor observadas nos produtos minimamente processados são catalisadas pela polifenoloxidase (PPO), enzima promotora da oxidação de compostos fenólicos com formação de compostos corados castanhos (Laurila *et al.*, 1998). Esta enzima é a principal responsável pelo escurecimento e consequente perda de qualidade de maçã (Rocha e Morais, 2001; Rocha e Morais, 2003) e pêra minimamente processadas (Dong *et al.*, 2000; Gorny *et al.*, 2002). Por outro lado, o incremento da actividade da fenilalanina amónia liase (PAL) como consequência do aumento da concentração de etileno, conduz à biossíntese de compostos fenólicos aumentando assim o conteúdo de substratos para a PPO (Garcia e Barrett, 2002; Saltveit, 2000). Alterações de cor decorrentes da oxidação de outros grupos de pigmentos como os carotenos e carotenóides, antocianinas e clorofilas, pelas actividades das lipoxigenase, clorofilase ou peroxidase são também relevantes na deterioração da qualidade visual e /ou nutricional do produto (Dorantes-Alvarez e Chiralt, 2000).

Durante o armazenamento, os produtos minimamente processados podem também sofrer alterações de aroma, principalmente devido à actividade da lipoxigenase. Esta enzima catalisa a peroxidação de ácidos gordos insaturados, que apesar de representarem uma pequena fracção da composição dos produtos hortofrutícolas, leva ao desenvolvimento de aromas indesejáveis resultantes da

formação de aldeídos e cetonas, contribuindo para a perda de qualidade sensorial do produto (Varoquaux e Willey, 1994; Laurila e Ahvenainen, 2002).

O corte dos tecidos vegetais conduz ainda à alteração de textura do produto traduzindo-se frequentemente numa perda de firmeza dos tecidos. Os três principais factores responsáveis pelas propriedades texturais dos tecidos vegetais são a turgescência das células, a rigidez da parede celular e a adesão célula a célula (Alzamora *et al.*, 2000). A parede celular é a principal estrutura que confere rigidez à célula. A libertação de enzimas pectinolíticas das células cortadas e a sua difusão para tecidos internos, vai conduzir a quebras sucessivas nos polissacáridos constituintes da parede com consequente fragilização e perda de estrutura da mesma (Varoquaux *et al.*, 1990; Varoquaux e Willey, 1994; Beaulieu e Gorny, 2001). Por outro lado, em produtos climatéricos, o incremento na produção de etileno devido ao corte e posterior acumulação no interior da embalagem acarreta também uma perda de firmeza dos tecidos (Varoquaux e Willey, 1994; Watada e Qi, 1999). A degradação rápida na textura devido ao amolecimento excessivo dos tecidos foi observada, entre outros, em papaia (Karakurt e Huber, 2003), morango (Hernández-Munoz *et al.*, 2006), meloa (Luna-Guzmán e Barrett, 2000; Saftner *et al.*, 2003) e kiwi (Varoquaux *et al.*, 1990; Agar *et al.*, 1999).

Existe a percepção generalizada de que os frutos e vegetais frescos cortados, apesar de apresentarem a grande vantagem da conveniência, são menos ricos do ponto de vista nutricional que os produtos inteiros, apresentando níveis inferiores de vitaminas e compostos bioactivos com propriedades antioxidantes, principalmente devido às metodologias de processamento e conservação. Se por vezes esta perda de qualidade é observada (Klein, 1987; McCarthy, 1994) outros trabalhos revelam que o valor nutricional e antioxidante podem ser mantidos ou mesmo incrementados como resultado das operações de processamento mínimo. Gil *et al.* (2006) observou que, após 6 dias a 5 ºC, manga, morango e melancia minimamente processados apenas apresentaram uma perda de vitamina C de 5% em relação aos frutos inteiros. Cubos de ananás e fatias de kiwi apresentaram perdas ligeiramente superiores (10-12%); em meloa "cantaloupe" o processamento mínimo levou a uma redução de 25% do teor inicial desta vitamina. Contudo, em todos os frutos, não ocorreram perdas significativas nos compostos fenólicos totais, mesmo quando expostos à luz. Os

teores de vitamina C e capacidade antioxidante de pêra minimamente processada permaneceram inalterados durante 9 dias de armazenamento a 4 ºC (Piga *et al.*, 2003). O mesmo se verificou em citrinos cortados em segmentos e armazenados durante 12-15 dias a 4 ºC apresentando mesmo, em alguns casos, um incremento na concentração de flavonóides totais e capacidade antioxidante (Del Caro *et al.*, 2004). Efeito semelhante foi observado em cubos de manga e melancia frescos cortados e armazenados a 5 ºC/ 9 dias e expostos à luz, acompanhados de um incremento da concentração em carotenóides totais (Gil *et al.*, 2006).

1.3 Metodologias de preservação da qualidade

A manutenção da qualidade de produtos frescos cortados baseia-se na tecnologia de barreiras, tirando benefício do efeito sinérgico entre vários factores individuais (Willey, 1994b; Ahvenainen, 2000b; Laurila e Ahvenainen, 2002; Allende *et al.*, 2006). A adequada selecção das barreiras, tanto em número como em intensidade e sequência de aplicação, apresenta-se como o futuro do processamento mínimo de frutos e vegetais (McMeekin e Ross, 2002).

Os principais factores que afectam a qualidade dos produtos minimamente processados são o grau de maturação e o estado fisiológico da matéria-prima, as metodologias de processamento, nomeadamente as técnicas de corte e descontaminação do produto, a embalagem e a temperatura, não só de processamento mas também de conservação e transporte (Watada e Qi, 1999; Ahvenainen, 2000b; Beaulieu e Gorny, 2001; Allende *et al.*, 2006).

A qualidade da matéria-prima é um factor determinante da qualidade do produto minimamente processado. A escolha de variedades mais adaptadas ao processamento mínimo, com melhores características de gosto e aroma e que sofram menores alterações de cor e firmeza durante o processamento e armazenamento apresentam-se como factores de grande relevância. É igualmente fundamental que aquela se encontre no estado fisiológico adequado, sem a presença de defeitos físicos que acarretam taxas mais elevadas das reacções de degradação (Gorny *et al.*, 2000). O estado de maturação em que a matéria-prima se encontra afecta não só a qualidade sensorial, nomeadamente a cor e textura percebidas pelo consumidor, mas também as

taxas respiratória e de produção de etileno (Soliva-Fortuny *et al.*, 2002; Soliva-Fortuny *et al.*, 2004; Oms-Oliu *et al.*, 2007).

Por outro lado operações de descasque e corte devem ser efectuadas recorrendo a técnicas que conduzam à lesão do menor número possível de células (Portela e Cantwell, 2001; Laurila e Ahvenainen, 2002). Assim, deverão ser utilizadas facas ou lâminas devidamente afiadas para que ocorra o corte e não o esmagamento das células. Metodologias como descasque por abrasão, muito utilizado para batata e cenoura devem portanto ser evitadas (Laurila e Ahvenainen, 2002).

A embalagem constitui mais uma barreira na preservação da qualidade do produto minimamente processado. A actividade fisiológica e algumas reacções de degradação enzimática, como a alteração de cor por via da PPO, podem ser minimizadas através da utilização de embalagem em atmosfera modificada. A alteração da composição da atmosfera no interior da embalagem pode ser conseguida de duas formas: passivamente, através do equilíbrio entre a produção de CO_2 e consumo de O_2 na actividade respiratória do produto e a embalagem de permeabilidade adequada, ou activamente colocando no interior da embalagem uma mistura gasosa específica. Em ambos os casos o objectivo é criar o equilíbrio gasoso ideal, no qual a actividade respiratória é conduzida ao mínimo possível, sem no entanto se atingirem níveis de O_2 e de CO_2 que afectem negativamente o produto (Ahvenainen, 2000b; Laurila e Ahvenainen, 2002; Sivertsvik *et al.*, 2002; Soliva-Fortuny e Martín-Belloso, 2003).

A manutenção da cadeia de frio desde o processamento até ao consumidor será talvez o parâmetro mais difícil de controlar tendo todavia uma importância fulcral. A baixas temperaturas toda a actividade fisiológica é reduzida, observando-se um decréscimo das taxas de respiração e produção de etileno. O desenvolvimento de reacções de degradação enzimática, bem como o desenvolvimento de microrganismos são minimizados a baixas temperaturas. O período de vida útil de durião (*Durio zibethinus* cv. D24) minimamente processado sofreu uma redução de 14 para 2 dias quando a temperatura de armazenamento aumentou de 4 ºC para 28 ºC (Voon *et al.*, 2006). De igual modo, kiwi fatiado e armazenado entre 0 - 2 ºC apresentou um período de vida útil superior ao armazenado a 5 ºC ou 10 ºC, sofrendo

neste caso um amolecimento acelerado, perda de massa e degradação de ácido ascórbico (Agar *et al.*, 1999).

Para além das apresentadas existem ainda outras metodologias de conservação que podem ser aplicadas durante o processamento, de forma a melhorar a qualidade e prolongar o período de vida útil do produto minimamente processado. A aplicação de tratamentos térmicos moderados e tratamentos de imersão em cálcio apresentam-se como metodologias com resultados benéficos na manutenção da qualidade de hortofrutícolas minimamente processados, sem efeitos negativos do ponto de vista do consumidor.

Por outro lado, a lavagem / descontaminação é um ponto crítico das operações de processamento mínimo pois, permite não só a remoção de microrganismos presentes na matéria-prima mas também de exsudados libertados durante o corte, minimizando o desenvolvimento microbiológico e reacções enzimáticas, garantido assim a maior segurança do produto. É assim relevante o estudo de técnicas que sejam eficientes no processo de descontaminação que não afectem a qualidade global do produto.

1.3.1 Tratamentos térmicos moderados

Num período em que o consumidor está cada vez mais preocupado e informado dos potenciais riscos que os tratamentos químicos podem representar para a saúde, o desenvolvimento de metodologias de conservação que tenham como princípio tratamentos físicos eficazes e sem resíduos, apresenta cada vez maior importância.

A aplicação de tratamentos térmicos pós-colheita, inicialmente tendo como objectivo a eliminação de insectos e microrganismos patogénicos, veio mais tarde a ser utilizada como método para controlar doenças e modificar o mecanismo de resposta dos frutos a situações de *stress* posteriores, permitindo a manutenção da qualidade durante o armazenamento (Lurie, 1998; Paull e Chen, 2000; Falik, 2004).

O processo de maturação de frutos climatéricos é acompanhado do aumento da actividade respiratória e de produção de etileno, amolecimento dos tecidos, aumento da relação açúcar : ácido, desenvolvimento e / ou alterações da cor. A exposição do fruto a temperaturas mais elevadas que a temperatura ambiente pode atenuar alguns destes processos mas também conduzir ao aumento de outros (Lurie, 1998).

Os efeitos da aplicação de tratamentos térmicos moderados ao fruto inteiro são condicionados por vários factores nomeadamente a espécie e variedade utilizada, estado de maturação mas também pelo historial de temperaturas a que o fruto esteve sujeito antes do tratamento térmico. Estes tratamentos conduzem a alterações do processo de maturação dos frutos devido a modificações na expressão de genes e síntese proteica (Lurie, 1998; Paull e Chen, 2000).

A taxa respiratória dos frutos, dependendo da idade fisiológica dos tecidos, é incrementada pela exposição a temperaturas elevadas mas, após o tratamento térmico esta taxa decresce para níveis semelhantes ou mesmo inferiores aos dos frutos controlo, não sujeitos a tratamento térmico (Falik, 2004; Paull e Chen, 2000). Por outro lado, a síntese de etileno apesar de inibida pela exposição a temperaturas elevadas é rapidamente recuperada quando o fruto é retirado do calor, sendo no entanto necessário nova síntese proteica (Lurie, 1998; Paull e Chen, 2000). Ameixas sujeitas a danos mecânicos após tratamento térmico a 45 ºC / 10 minutos apresentaram taxas de respiração e produção de etileno mais baixas que os frutos sem tratamento (Serrano *et al.*, 2004). A aplicação de tratamentos térmicos entre 60-70 ºC durante 10-30 segundos não apresentou qualquer efeito na taxa de respiração de pêra (*Opuntia ficus-indica* Miller (L.)) (Dimitris *et al.*, 2005) e, a influência destes tratamentos na taxa de produção de etileno de pêssegos, só foi relevante quando aplicados durante períodos longos e por aquecimento através de ar quente, resultando então num incremento da mesma (Budde *et al.*, 2006).

Os efeitos da aplicação de tratamentos térmicos moderados ao fruto inteiro prolongam-se para além das operações de processamento mínimo e durante o armazenamento do produto minimamente processado, sendo diferentes dos observados quando o tratamento é aplicado após o corte. A imersão de meloa em água a 50 ºC durante 1 hora conduziu a uma redução da taxa respiratória do fruto minimamente processado (Lamikanra *et al.*, 2005) enquanto que o tratamento térmico dos cubos deste fruto não apresentou qualquer efeito nas taxas respiratória e de produção de etileno (Luna-Guzmán *et al.*, 1999). Kim *et al.* (1993) referem uma redução na taxa respiratória de maçã minimamente processada como resultado da aplicação de um tratamento térmico a 45 ºC ao fruto inteiro, sendo no entanto a eficácia do tratamento dependente da variedade do fruto. Ketsa e colaboradores (1999) referem que manga mantida a 38 ºC apresenta um decréscimo gradual na taxa de produção de etileno sendo esta totalmente inibida ao final de três dias. Quando

transferidos para a temperatura ambiente os frutos recuperam a capacidade de produção de etileno mas, o pico climatérico acontece dois dias mais tarde que nos frutos não tratados termicamente. No entanto, o corte do fruto altera este comportamento. Quando após o tratamento térmico a manga é descascada e cortada em cubos a taxa de produção de etileno é muito reduzida nos frutos sujeitos a tratamento térmico contrariamente aos cubos dos frutos controlo que apresentaram um incremento desta ao longo do armazenamento.

Concomitantemente é muitas vezes observada a preservação, ou mesmo um incremento, da firmeza dos tecidos vegetais como consequência da aplicação de tratamentos térmicos moderados, podendo estar envolvidos vários mecanismos neste processo. A aplicação de tratamentos térmicos moderados conduz a alterações na actividade das enzimas responsáveis pela modificação da parede celular constituída maioritariamente por pectinas. Estes compostos são polissacáridos constituídos por uma cadeia principal linear de ácidos galacturónico, com alguns grupos carboxilo esterificados com grupos metilo. A linearidade da cadeia é interrompida aleatoriamente por uma molécula de ramnose, à qual se ligam várias cadeias laterais constituídas por açúcares neutros.

A pectina metilesterase (PME) é a enzima responsável pela desesterificação dos grupos metilo das cadeias de pectina, promovendo a libertação de metanol e produzindo polímeros carregados negativamente (ácidos pécticos) (Fig. 2) (Jackman e Stanley, 1995; Willats *et al.*, 2001; Brummell, 2006). Esta enzima tem o seu óptimo de actuação, a temperaturas elevadas, dependendo do produto podendo variar entre os 40 - 70 ºC, sendo apenas inactivada a temperaturas superiores a 70 ºC (Dijk e Tijskens, 2000). Durante a aplicação de tratamentos térmicos nesta gama de temperaturas é promovida a activação da PME com consequente formação de ácidos pécticos, ficando estes disponíveis para estabelecer ligações com catiões bivalentes, maioritariamente cálcio endógeno, e formando assim uma estrutura mais estável ("egg box") que vai conferir maior resistência e integridade à parede celular (Fig.3).

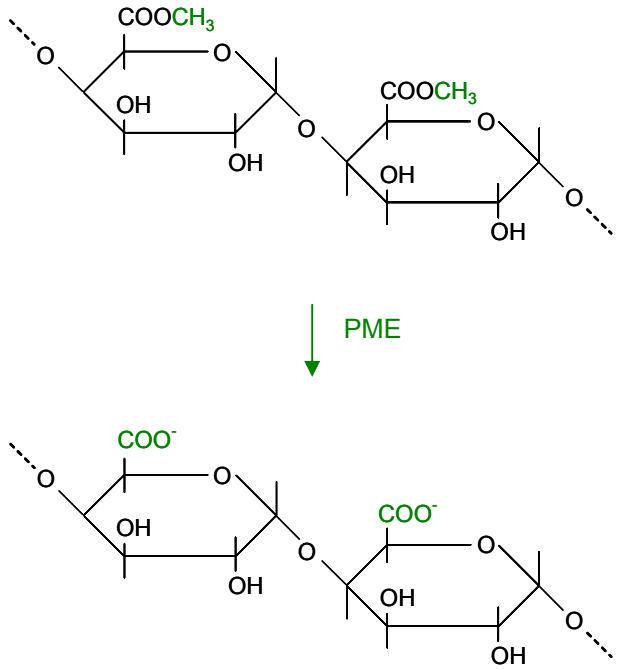

Fig. 2 Desesterificação da cadeia de pectina por acção da pectina metilesterase (PME).

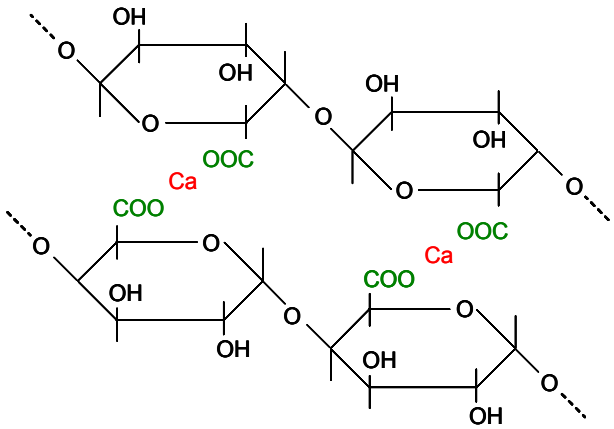

Fig. 3 Interacção entre cadeias de ácidos pécticos através de ligações iónicas com cálcio na formação de uma estrutura estável ("egg box").

A manutenção da firmeza e a redução da taxa de amolecimento após branqueamento, foi observada em vários vegetais quando os produtos foram previamente sujeitos a tratamentos térmicos moderados, sendo estabelecida a relação com a activação e estabilidade da PME registada nos tratamentos entre 45 ºC e 70 ºC durante 15-30 minutos (Ni *et al.*, 2005). Em beringela verificou-se que a aplicação de tratamentos térmicos de imersão influenciava a preservação da textura, devido à activação progressiva da PME entre 30 e 65 ºC, sendo o tratamento óptimo de 53 ºC durante cerca de 20 minutos (Zhang e Chen, 2006). Cubos de meloa minimamente processada apresentaram valores de firmeza mais elevados quando os frutos inteiros foram sujeitos a um tratamento térmico de 60 ºC durante 60 minutos, sendo o efeito mais pronunciado quando as operações de processamento mínimo foram conduzidas 24 horas após o tratamento térmico (Lamikanra e Watson, 2007). Efeito semelhante foi observado em fatias de maçã (Kim *et al.*, 1993) quando o fruto inteiro foi sujeito a tratamento térmico prévio sendo optimizada a temperatura de tratamento de 45 ºC. Em morangos sujeitos a tratamento à mesma temperatura durante 3 horas, a redução da taxa de amolecimento foi acompanhada pelo incremento da actividade da PME (Vicente *et al.*, 2005). Por outro lado, apesar das baixas temperaturas utilizadas, os elevados tempos de tratamento podem conduzir à inibição da síntese ou inactivação de outras enzimas pectinolíticas, designadamente a poligalacturonase (PG). A actividade das enzimas α- e β-galactosidase (α- e β-Gal) e β-xilosidase (β-Xil), é também alterada pela aplicação destes tratamentos, conduzindo a uma menor degradação das cadeias de pectina constituintes da parede celular. A perda dos açúcares neutros das cadeias laterais dos polímeros pécticos, permite uma maior interacção entre cadeias, resultando assim uma estrutura mais estável, contribuindo também para minimizar a perda de firmeza (Lurie, 1998; Paull e Chen, 2000).

Tratamentos de imersão de pêssego e nectarina em água a 46 ºC durante 25 minutos foram eficazes na manutenção de níveis elevados de qualidade, evitando o amolecimento dos frutos sendo o efeito atribuído à inactivação da PG (Malakou e Nanos, 2005). A avaliação da actividade de várias enzimas pectinolíticas em morangos, sujeitos a tratamento térmico através de ar a 45 ºC durante 3 horas, permitiu concluir que este conduziu por um lado à activação da PME enquanto reduziu a actividade da PG, β-Gal, β-Xil e 1,4-β-D-glucanase. O efeito combinado do tratamento térmico sobre as várias enzimas conduz à redução da solubilização das

pectinas e consequente redução da taxa de amolecimento do fruto (Vicente *et al.*, 2005a).

As alterações observadas tanto na taxa respiratória e de produção de etileno como na actividade das enzimas responsáveis pelo processo de perda de firmeza durante o processo de maturação podem estar relacionadas com alterações na expressão genética e síntese proteica (Lurie, 1998). A exposição dos produtos hortofrutícolas a temperaturas elevadas interfere com a síntese normal de proteínas podendo mesmo inibir a síntese de enzimas responsáveis por alguns processos de degradação, como já referido (Lurie, 1998; Paull e Chen, 2000; Saltveit, 2000; Wang, 2001). Por outro lado a exposição dos tecidos vegetais a temperaturas cerca de 10 ºC superiores à do seu desenvolvimento pode induzir à síntese de um grupo de proteínas, denominadas de proteínas de choque térmico ("HSP"), que desempenham função protectora dos tecidos em posteriores situações de *stress*.

Estudos conduzidos por Saltveit (2000) demonstram o efeito benéfico do tratamento térmico (45 ºC / 90 s) na diminuição do escurecimento de alface Iceberg, pela alteração do metabolismo dos compostos fenólicos inibindo a síntese de PAL, enzima responsável pela síntese de compostos fenólicos, e redireccionando-a para a síntese de HSP. A síntese de HSP foi também observada, entre outros, em manga (Zhu et al., 2003), abacate (Florissen et al., 1996) e tomate (Lurie e Klein, 1991; Sabehat *et al.*, 1996; Lurie e Sabehat, 1997) com consequente efeito protector para posteriores danos provocados pelo frio. A aplicação de um tratamento de 60 ºC durante 60 minutos a meloa inteira levou à produção de HSP, podendo estas estar envolvidas na manutenção de qualidade do produto minimamente processado (Lamikanra e Watson, 2007).

Pelo exposto, é possível verificar que a acção dos tratamentos térmicos moderados aplicados ao fruto inteiro se revela benéfica a vários níveis tanto na consevação do fruto inteiro como na manutenção de qualidade do produto minimamente processado. No entanto verifica-se que estes tratamentos induzem mecanismos de resposta complexos e ainda não totalmente esclarecidos.

1.3.2 Tratamentos de imersão

O principal objectivo da aplicação de tratamentos de imersão durante as operações de processamento mínimo de produtos hortofrutícolas é a remoção de substâncias, particularmente enzimas e substratos, libertados durante o corte dos tecidos vegetais que, como descrito anteriormente podem comprometer a qualidade e o período de vida útil do produto minimamente processado.

No entanto, estes tratamentos são também utilizados com o objectivo de veicular ao produto final compostos com acção específica. A aplicação de agentes inibidores de reacções de escurecimento enzimático com acção antioxidante, acidulante, complexante ou quelante, como o ácido ascórbico, ácido cítrico, EDTA ou 4-hexilresorcinol é efectuada mediante tratamentos de imersão em soluções simples ou combinadas de concentração adequada (Laurila *et al.*, 1998; Ahvenainen, 2000; Garcia e Barrett, 2002). Estes tratamentos mostraram-se eficazes na prevenção do escurecimento e manutenção de qualidade de vários produtos minimamente processados nomeadamente pêra (Dong *et al.*, 2000; Gorny *et al.*, 2002), pêssego (Gorny *et al.*, 1999) e maçã (Luo e Barbosa-Cánovas, 1997; Gil *et al.*, 1998; Buta *et al.*, 1999).

Tratamentos de imersão em soluções de cálcio, normalmente em concentração entre 0,5 e 3% são também largamente utilizados para a preservação de qualidade de produtos hortofrutícolas minimamente processados (Martín-Diana *et al.*, 2007). Este ião desempenha uma função importante na estabilização e estruturação da parede celular e lamela média pois apresenta capacidade para estabelecer ligação entre os grupos carboxilo das cadeias de ácido galacturónico que compõe as pectinas (Fig. 2) (Sams, 1999; Lara *et al.*, 2004; Rico *et al.*, 2007). Por outro lado, tem também um efeito inibitório do desenvolvimento de fungos e desordens fisiológicas (Serrano *et al.*, 2004). Entre outros, melancia (Mao *et al.*, 2006), morango (Aguayo *et al.*, 2006; Hernández-Munõz *et al.*, 2006), maçã (Soliva-Fortuny *et al.*, 2003) e manga (Souza *et al.*, 2006) minimamente processados, sujeitos a tratamentos de imersão em soluções de cálcio, apresentaram uma taxa de amolecimento inferior à dos frutos controlo, não sujeitos ao tratamento de imersão. Cubos de meloa sujeitos a tratamentos de imersão em soluções de cloreto de cálcio e lactato de cálcio mantiveram a firmeza, evitando o amolecimento excessivo do fruto minimamente processado durante o armazenamento (Luna-Guzmán *et al.*, 1999; Luna-Guzmán e Barrett, 2000). Saftner *et al.* (2003) referem ainda que o tratamento de imersão de

cubos de meloa em solução propionato de cálcio inibe a taxa respiratória e de produção de etileno bem como o desenvolvimento de microrganismos, prolongando o período de vida útil do fruto minimamente processado.

Apesar da grande maioria dos tratamentos de enriquecimento em cálcio ser efectuada por imersão em soluções de cloreto de cálcio vários estudos referem a ocorrência de sabores estranhos atribuídos a este sal (Luna-Guzmán e Barrett, 2000; Lawless *et al.*, 2003). Vários esforços têm assim sido efectuados no sentido de estudar alternativas ao uso de cloreto de cálcio. A utilização de propionato de cálcio mostrou-se eficaz na preservação de qualidade de meloa (Saftner *et al.*, 2003) e maçã (Buta *et al.*, 1999) minimamente processadas, assim como a utilização de glucanato de cálcio em morango (Hernández-Munõz *et al.*, 2006). Luna-Guzmán e Barrett (2000) referem que o tratamento com lactato de cálcio não só é igualmente efectivo na preservação da textura de meloa fresca cortada como evita o aparecimento de sabores estranhos normalmente associado ao cloreto de cálcio.

1.3.3 Tratamentos de descontaminação

Durante as operações de descasque e corte a superfície dos produtos é exposta ao ar e consequentemente à contaminação com bactérias, fungos e leveduras. Frutos e hortícolas não apresentam a mesma susceptibilidade à degradação microbiológica. Os produtos hortícolas, além de frequentemente apresentarem cargas microbianas superiores, caracterizam-se por apresentar valores relativamente elevados de pH (4,5 – 6,0) associados a humidade elevada e grande número de superfícies cortadas, com consequente libertação de nutrientes. Estas características permitem o desenvolvimento de uma grande diversidade de microrganismos, sendo o controlo da temperatura de armazenamento, que deverá ser inferior a 8 – 10 ºC, fundamental para evitar este desenvolvimento (NACMCF, 1999; Martínez *et al.*, 2000; Laurila e Ahvenainen, 2002; Ragaert *et al.*, 2007).

Em oposição aos produtos hortícolas, os frutos apresentam características que contribuem para um menor desenvolvimento microbiano. Apesar de algumas excepções como a banana, melão melancia e meloa, estes produtos apresentam geralmente baixos valores de pH (< 4,6) e a própria natureza dos ácidos orgânicos presentes não permite o desenvolvimento de bactérias para além das bactérias lácticas (Brackett, 1994; Martínez *et al.*, 2000). Por outro lado, os microrganismos de

deterioração presentes nestes produtos são psicrotróficos, com vantagem competitiva sobre a maioria dos patogénicos (Laurila e Ahvenainen, 2002).

Como por definição os produtos hortofrutícolas minimamente processados estão prontos a ser consumidos, é fundamental que seja garantido ao consumidor a segurança alimentar. Esta preocupação é aumentada quando se trata de produtos que não necessitam de operações de processamento adicionais (ex. tratamento térmico) que possam reduzir / eliminar os eventuais perigos.

As principais fontes de contaminação por microrganismos patogénicos dos produtos minimamente processados são, em primeiro lugar, as próprias matérias-primas que ao contactarem na grande maioria directamente com o solo podem possuir uma carga microbiana elevada, normalmente composta por *Clostridium botulinum*, *Bacillus cereus* e *Listeria monocytogenes*. Pode ainda ocorrer contaminação durante as operações de processamento através dos operadores responsáveis pelo manuseamento do produto ou por descontaminação deficiente dos equipamentos e ambiente fabril, estando neste caso associado ao desenvolvimento de *Salmonella*, *Shigella*, *Escherichia coli* e *Campylobacter*. Adicionalmente existe uma vasta flora de microrganismos que, apesar de não ser patogénica e não apresentar qualquer problema para a saúde do consumidor, conduz à degradação do produto reduzindo assim o seu período de vida útil (Brackett, 1994; Beauchat, 1998; NACMCF, 1999; Martínez *et al.*, 2000; Laurila e Ahvenainen, 2002).

É assim essencial a observação de processos de boas práticas agrícolas e de fabrico bem como de controlo de pontos críticos (HACCP) para garantir a qualidade microbiológica do produto final.

A lavagem / descontaminação dos produtos minimamente processados apresenta-se assim como um ponto crítico na produção de hortofrutícolas IV gama, visto ser a única fase do processo capaz de reduzir o número de microrganismos responsáveis pela perda de qualidade (Sapers, 2003) e a embalagem em atmosfera modificada não apresentar resultados consistentes na redução do desenvolvimento microbiano (Ragaert *et al.*, 2007).

Existem várias metodologias, químicas e físicas, passíveis de serem utilizadas na lavagem e higienização de produtos hortofrutícolas minimamente processados dependendo a sua escolha, entre outros factores, das características do produto e do tipo de microflora presente (Parish *et al.*, 2003). O método mais divulgado e

utilizado desde há várias décadas na indústria, para a descontaminação de frutos e hortícolas bem como equipamentos e superfícies, é a descontaminação através de cloro, na sua forma elementar ou sob a forma de hipoclorito, em soluções com concentração de 50 – 200 ppm durante um período de 1 – 2 minutos (Beauchat, 1998; Parish *et al.*, 2003). Contudo, vários factores podem reduzir a eficácia do cloro pois a actividade letal depende da quantidade de ácido hipocloroso, forma com maior poder bactericida, e consequentemente do pH e temperatura da solução. A dissociação deste ácido é favorecida a baixa temperatura e pH pelo que a solução deverá ter um pH entre 6 e 7,5 e encontrar-se a temperatura inferior a 10 ºC. Todavia tratamentos de descontaminação com cloro mostraram não ser eficazes na redução da população inicial de microrganismos em mais de 1 ciclo logarítmico em alface e cenoura, entre outros (Nguyen-the e Carlin, 1994; Delaquis *et al.*, 2004; Ruiz-Cruz, 2007).

Por outro lado, é hoje conhecido que o cloro pode não oxidar completamente a matéria orgânica e produzir subprodutos como o clorofórmio e outros trihalometanos ou ainda cloroaminas, compostos com acção tóxica e potencialmente carcinogénica (Suslow, 2003; Rico *et al.*, 2007). Torna-se assim necessário o desenvolvimento de metodologias que apresentem maior eficácia que os tratamentos com cloro e em simultâneo não representem perigo para o consumidor e ambiente.

A aplicação de tratamentos de branqueamento usualmente por imersão em água ou com vapor, não envolvendo a utilização de qualquer agente químico, pode constituir uma metodologia alternativa na descontaminação de produtos minimamente processados. Estes tratamentos, consistindo na exposição a temperaturas entre 85 -100 ºC durante poucos segundos, podem conduzir ao decréscimo significativo da flora inicial dos produtos (Rico *et al.*, 2007). Tratamentos de branqueamento em saladas compostas por vegetais de folha conduziram a reduções de 3 ciclos logarítmicos na contagem inicial de microrganismos mesofílicos sendo todavia menos efectivos na redução de *Enterobacteriacea* (Gartner *et al.*, 1997). Também em frutos a aplicação de tratamentos de branqueamento se têm revelado efectivos como metodologia de descontaminação. Morango sujeito a branqueamento a 100 ºC durante 30 s manteve a estabilidade microbiológica do produto minimamente processado durante o armazenamento a 5 ºC por 30 dias (Moreno *et al.*, 2000).

Apesar de a estes tratamentos poderem estar associadas alterações negativas da qualidade, nomeadamente perda de valor nutricional por degradação térmica ou lexiviação / difusão de nutrientes (Negi e Roy, 2000), bem como alterações de cor e perda de firmeza, se forem aplicados antes das operações de processamento mínimo podem conduzir à melhoria da qualidade do produto final (Rico *et al.*, 2007). A inactivação da PPO, com consequente preservação da cor foi ainda observada em morango, no estudo referido anteriormente (Moreno *et al.*, 2000).

O ozono é um forte agente com acção anti-microbiana, actuando sobre vários tipos de microrganismos e em concentrações muito reduzidas, devido ao seu elevado potencial de oxidação-redução, o que lhe confere capacidade para oxidar estruturas fundamentais da célula como os lípidos da membrana celular, enzimas e proteínas provocando a morte da célula bacteriana (Beuchat 2000; Guzel-Seydim *et al.*, 2004; Mahapatra *et al.*, 2005). É uma molécula muito instável que em poucos minutos se quebra formando apenas oxigénio, não tendo sido identificados quaisquer subprodutos potencialmente perigosos. Ao contrário do cloro, a sua eficácia é marginalmente afectada pelo pH da água contudo, na presença de matéria orgânica decompõe-se muito rapidamente formando cetonas, aldeídos e ácidos carboxílicos (Guzel-Seydim *et al.*, 2004), o que pode ser minimizado utilizando água a baixa temperatura (Suslow, 2003).

Devido ao seu elevado poder oxidante, tratamentos de descontaminação através de água ozonada podem, para além de acção descontaminante, influenciar a fisiologia dos produtos hortofrutícolas. O ozono oxida a molécula de etileno retardando os fenómenos envolvidos no processo de maturação (Beauchat, 1998; Guzel-Seydim *et al.*, 2004; Rico *et al.*, 2007). Zhang e colaboradores (2005) verificaram que tratamentos de imersão em água ozonada conduziram à diminuição da taxa respiratória e actividade da PPO de aipo minimamente processado. Contudo, o seu elevado poder oxidante pode também conduzir a danos fisiológicos (Beauchat, 2000), tal como foi observado em cenouras (Forney *et al.*, 2007), e perda de valor nutricional com decréscimo do teor de vitamima C e compostos fenólicos (Allende *et al.*, 2007). A aplicação de tratamentos de imersão em água ozonada pode ainda ser efectiva na degradação de alguns pesticidas (Wu *et al.*, 2008a; Wu *et al.*, 2008b), contribuindo assim para o aumento da segurança alimentar associada ao consumo do produto minimamente processado.

A utilização de radiação artificial ultravioleta não ionizante de comprimento de onda entre 200 – 280 nm (UV-C) apresenta-se como um método físico de tratamento com potencialidade na descontaminação de hortofrutícolas minimamente processados. Para além do reduzido custo associado à sua utilização não deixa qualquer resíduo no produto final, indo assim de encontro às expectativas do consumidor. O mecanismo pelo qual é atingida a morte dos vários microrganismos relaciona-se com a absorção da radiação e consequente dimerização das cadeias de DNA, e inibição de transcrição e tradução celular (Morgan, 1989; Sastry *et al.*, 2001; Ohlsson, 2002).

Estudos efectuados, *in vitro*, demonstraram a eficácia da aplicação de tratamentos com radiação UV-C na inibição do desenvolvimento de vários grupos de microrganismos na superfície de materiais sólidos (Gardner e Shama, 2000). Adicionalmente vários autores referem que a aplicação de radiação ultravioleta de baixo comprimento de onda reduz significativamente o desenvolvimento dos diferentes microrganismos presentes nos produtos alimentares. Tratamentos de UV-C revelaram ser efectivos na redução do desenvolvimento microbiano em frutos e derivados (López-Rubira *et al.*, 2005; Lamikanra *et al.*, 2005b; Fonseca e Rushing, 2006; Tran e Farid, 2004) e vegetais (Allende e Artes, 2003; Gómez-López *et al.*, 2005; Allende *et al.*, 2006b).

A radiação ultra violeta conduz também a uma situação de *stress* biológico, activando assim, nos tecidos vegetais mecanismos de defesa envolvendo modificações da parede celular, produção de poliaminas com acção anti-senescente, fitoalexinas e várias enzimas de defesa (Maharaj *et al.*, 1999; Vicente *et al.*, 2005b; Lamikanra *et al*., 2002; Lamikanra *et al.*, 2005b). Meloa minimamente processada sob radiação UV-C apresentou, durante o armazenamento a 10 ºC, uma redução da taxa respiratória e actividade das enzimas esterase e lipase e uma melhor manutenção da firmeza dos tecidos (Lamikanra *et al.*, 2005b) tendo também induzido a síntese de fitoalexinas (Lamikanra *et al*., 2002), melhorando a sua qualidade durante o período de vida útil em relação aos frutos não tratados. Este tratamento foi ainda eficaz na manutenção de qualidade de pimento, evitando o amolecimento dos frutos, retardando o processo de senescência e diminuindo a incidência de danos provocados pelo frio (Vicente *et al.*, 2005b), bem como na redução da degradação da clorofila e da perda de estrutura dos tecidos mantendo ainda a capacidade antioxidante de brócolos (Costa *et al.*, 2006).

Indo de encontro às expectativas quer do industrial quer do consumidor, os tratamentos referidos anteriormente apresentam-se como uma alternativa promissora à utilização do cloro.

2 Kiwi

O kiwi é um fruto pertencente ao género *Actinidia* oriundo da Ásia. As primeiras plantas, mais tarde identificadas como pertencentes a este género, foram encontradas na China, nordeste da Índia e Japão. O género *Actinidia* apresenta várias espécies com frutos edíveis como *A. chinensis*, *A. arguta*, *A. kolomikta*, *A. polygama* e *A. eriantha*. A espécie *A. deliciosa* foi a única explorada comercialmente em grande escala, inicialmente na Nova Zelândia. A sua produção neste país remonta à década de 30 do século passado mas foi nos anos 70 que se iniciou a sua exportação para a Europa e Estados Unidos da América, desencadeando também a sua produção em França, Itália, Espanha e Grécia (Fergunson, 1990; Given, 1993; Crisosto e Kader, 1999). Da espécie *Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Fergunson var *deliciosa* conhecem-se várias variedades sendo a preferida pelo consumidor, e portanto mais divulgada, a variedade 'Hayward' de frutos com melhores características de gosto e aroma (*flavour*) e uma maior capacidade de conservação (Cheah e Irving, 1997).

Actualmente, e a nível mundial, os principais países produtores de kiwi são Itália, China, Nova Zelândia e Chile. Ainda a nível europeu a França e a Grécia apesar de apresentarem níveis de produção muito inferiores assumem também alguma importância (Fig 4) (IKO, 2004).

Em Portugal a cultura do kiwi é recente surgindo as primeiras plantações no início da década de 80. A produção nacional representa cerca de 1% da produção total de frutos frescos e, responde apenas a 50% do consumo nacional do fruto, que se encontra nas 20 000 toneladas/ano. As zonas do país líderes na produção de kiwi são a região de Entre Douro e Minho seguindo-se a Beira Litoral (MADRP, 2007).

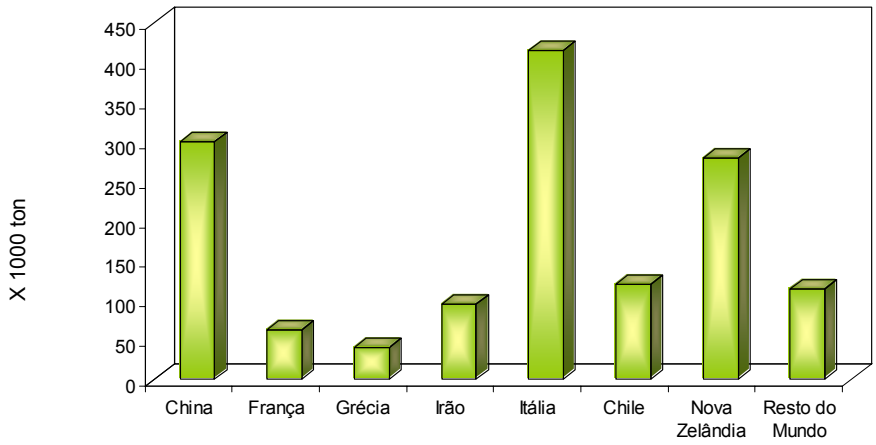

Fig. 4 Produção mundial de kiwi em 2004 (Fonte IKO)

A cultura encontra-se bem adaptada às condições edafo-climáticas das zonas onde está inserida e a fileira do kiwi apresenta actualmente um nível de organização interessante, tendo sido realizados investimentos significativos em novas plantações e em estruturas de comercialização, apresentando-se assim como uma cultura com grande potencial de produção no país (MADRP, 2007).

Botanicamente o fruto, desenvolvendo-se a partir de um ovário multicarpelar, classifica-se como uma baga, de cor verde acastanhada clara e totalmente coberto por pequenos filamentos (tricomas). A polpa (pericarpo) que envolve a zona central do fruto (columela), é de cor verde-claro apresentando inclusas centenas de pequenas sementes pretas (Fig.5) (Fergunson, 1990; Cheah e Irving, 1997; Crisosto e Kader, 1999).

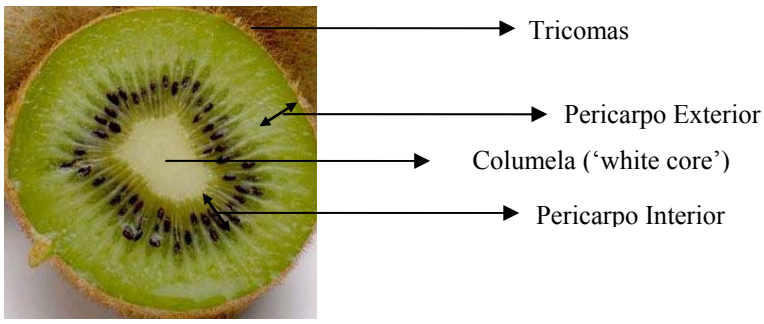

Fig. 5 Kiwi. Representação esquemática da morfologia do fruto.

Sendo um fruto climatérico, apresenta todavia uma baixa produção de etileno (C_2H_4) durante a maturação. No entanto, durante a maturação pós-colheita a taxa de produção de C_2H_4 aumenta de aproximadamente 0,1 – 0,5 $\mu Lkg^{-1}h^{-1}$ para 50 – 100 $\mu Lkg^{-1}h^{-1}$. Durante o armazenamento exibe uma elevada sensibilidade ao mesmo e níveis de 5 – 10 ppb de C_2H_4 induzem o amolecimento do fruto.

Pela sua composição o kiwi apresenta-se como um fruto nutricionalmente rico podendo no entanto esta ser diferente em função da variedade, origem geográfica e condições de produção. A composição média da variedade 'Hayward', uma das mais comercializadas, encontra-se sistematizada na Tabela 1.

Tabela 1 Composição bioquímica de kiwi, variedade 'Hayward' (Adaptado de Beever e Hopkirk, 1990)

Composto	Concentração	Composto	Concentração
Água (%)	80 – 88	Cinza (%)	0,45 – 0,74
Proteína (%)	0,11 – 1,2	Composição Mineral (mg / 100g)	
Lípidos (%)	0,07 – 0,9	Cálcio	16 – 51
Fibra (%)	1,1 – 3,3	Magnésio	10 – 32
Glúcidos (%)	17,5	Azoto	93 – 163
		Fósforo	22 – 67
Vitaminas		Potássio	185 – 576
C (mg / 100g)	80 – 120	Ferro	0,2 – 1,2
A (I.U. / 100g)	175	Sódio	2,8 – 4,7
B6 (mg / 100g)	0,5	Cloro	39 – 65
Tiamina (mg / 100g)	0,014 – 0,02	Manganês	0,07 – 2,3
Riboflavina (mg / 100g)	0,01 – 0,05	Zinco	0,08 – 0,32
Niacina (mg / 100 g)	0 – 0,5	Cobre	0,06 – 0,16

O kiwi é um fruto muito rico do ponto de vista vitamínico fornecendo cerca de 150% da dose diária recomendada (DDR) de vitamina C. É ainda rico em minerais nomeadamente potássio e fornece entre 4-8% da DDR de cálcio, ferro, magnésio e cobre e apresenta baixos níveis de sódio.

As relevantes características de composição deste fruto, conforme descrito, aliadas a limitações de manuseamento sentidas por alguns segmentos dos

consumidores, tornam-no interessante do ponto de vista industrial para ser sujeito a processamento mínimo, justificando assim o tema do presente estudo.

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Capítulo II

Efeitos da aplicação de tratamentos térmicos moderados na conservação de kiwi minimamente processado. Mecanismos de actuação.

No presente capítulo é avaliada a influência da aplicação de tratamentos térmicos moderados a kiwi inteiro na melhoria da qualidade do fruto miniamemnte processado. O delineamento experimental permitiu selecionar o binómio tempo / temperatura adequado. É estudada a importância do estado de maturação do fruto na eficácia do tratamento, bem como os mecanismos envolvidos nas alterações físicas e químicas sofridas pelo fruto durante o armazenamento.

Effects of maturity stage and mild heat treatments on quality of minimally processed kiwifruit

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Abstract

A central composite rotatable experimental design was used on kiwifruits of two distinct maturity stages (firm ripe and soft ripe), subjected to mild heat pre-treatments by immersion during 10–90 min in water at temperatures of 25-50 ºC. Minimal processing of the fruits was performed 24 h after the heat treatment and soluble solid content, colour and texture properties were analysed in the samples during the whole storage period (0-10 days). For both maturity stages, the effect of heat treatment on colour was negligible. For fruits of the early maturity stage (firm ripe), total content of soluble solids increased with the mild heat pre-treatments. Pre-treatments avoided texture breakdown in firm ripe kiwi slices. Firmness, the most sensitive parameter, is increased or preserved using treatment periods of up to 40 minutes. Favourable responses were evidenced immediately after them and during the whole storage period. Therefore, mild heat pre treatments, when applied to firm ripe kiwi at temperatures below 45 ºC during less than 25 minutes improve the quality, mainly the firmness, colour being only marginally affected.

Keywords

Kiwifruit (*Actinidea deliciosa*), mild heat pre treatments, minimal processing, RSM, firmness.

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1 Introduction

Minimally processed or fresh cut products are a rapidly growing segment of the retail and food service horticultural industry (Anon., 1998).

Minimal processing of fruits and vegetables, yielding convenient produce with fresh-like quality and high nutritional value, is one of the major goals for processors, in response to increased demand.

The physiology of minimally processed products is essentially that of the wounded tissues leading to excessive tissue softening and superficial browning, and represents the greatest hurdle to product development and marketing. The intensity of the wounded response is affected by several factors, depending obviously from the species, variety and initial maturity state. Studies show that the more advanced the stage of ripeness, the more susceptible the fruit is to wounds, hence to minimal processing (Brecht, 1995; Gorny, Cifuentes, Hess-Pierce & Kader, 2000; Gorny, Gil & Kader, 1998; King & Bolin, 1989; Soliva-Fortuny & Martín-Belloso, 2003; Watada & Qi, 1999).

For better preservation of fresh peeled, diced or sliced commodities, research and development work has mostly been centred on the use of preservatives such as antibrowning (Sapers, 1993) and/or firming agents (Soliva-Fortuny & Martín-Belloso, 2003).

The use of mild treatments, without any chemical additives, and employing mostly physical preservation techniques seems ever more desirable.

From the nutritional point of view, kiwi is an interesting fruit due to its very low content in saturated fat, cholesterol and sodium, and because it is a good source of dietary fibre, potassium and copper and has a high vitamin content, namely of vitamin C (≈ 155% daily value) and vitamin K (≈ 50% daily value).

In kiwifruit, minimal processing operations are known to lead to excessive tissue softening (O'Connor-Shaw, Roberts, Ford & Nottingham, 1994; Varoquaux, Lecendre, Varoquaux & Souty, 1990), to increased CO_2 and ethylene production, to larger mass loss (Agar, Massantini, Hess-Pierce & Kader, 1999) and to decrease flavour intensity (O'Connor-Shaw *et al.*, 1994).

Heat treatments have already been used to control post harvest decay and to improve the storage quality in intact fruits, due to changes it induces in physiological and physicochemical characteristics, and post-processing quality. Mild heat pre-treatments have been shown to induce a firming effect on several processed vegetables (Aguilar, Anzaldúa-Morales, Talamás & Gastelúm, 1997; Anderson, Gekas, Lind, Oliveira & Öste, 1994; Bourne, 1987; Greve, Shackel, McArdle, Gohlke & Labavitch, 1994) and to minimally processed fruits evidencing improved quality. Kim, Smith & Lee (1993) reported that apples, heat treated at 45 ºC, yielded slices evidencing less browning and firmer texture, when compared to non-treated apples, probably due, respectively, to polyphenol oxidase inhibition and to pectin methylesterase activation, these effects being strongly dependent upon cultivar. Similar results were obtained by Barrancos *et al.* (2003), concluding that for Golden Delicious apple quarters, heat pre-treatments at 34 - 42 ºC for up to 70 minutes, avoided browning of the cut surface and evidenced a firming effect.

The use of a heat treatment at 35 ºC during 20 minutes, in association with dipping into a 3.5 % (w/w) $CaCl_2$ solution and a modified atmosphere containing 5 % O_2 and 5 % CO_2, allowed firmness preservation of mango cubes (Trindade *et al.*, 2003). The combined use of heat treatments and calcium dips had also been studied too by Luna-Guzmán, Cantwell & Barret (1999). Authors reported that the firming effect provided by dipping fresh-cut cantaloupe melons in 2.5 % calcium chloride solution was improved when combined with higher dip temperatures, probably due to temperature-induced diffusion rather than to pectin methylesterase activation. Results obtained by Abreu, Beirão-da-Costa, Gonçalves, Beirão-da-Costa & Moldão-Martins (2003) showed that pears (*cv* Rocha), subjected to heat treatments at 35 – 45 ºC for 40 - 150 minutes, did not show cut surface browning and that their firmness was preserved when stored 7 days at 2 ºC.

The mechanism(s) by which these mild heat pre treatments act are not undisputable established. Several pathways are identified, in fruits, as responses to heat treatments. Fallik (2004) reports that the use of this kind of treatment allows a better preservation of fruits, inhibiting ripening processes or inducing resistance to chilling injuries. On the other hand, pathogen spread inhibition results from induction of defence mechanisms in the outer layers of the epicarp.

Another effect of heat treatments on fruits is on protein synthesis. Saltveit (2000) reported that the application of a heat treatment inhibits normal protein synthesis,

while it induces heat shock proteins production. When applied to lettuce it can prevent browning by inhibition the synthesis of phenylalanine ammonia lyase (PAL). Synthesis of proteins involved in the complex of fruit disease resistance mechanisms can also occur (Fallik, 2004).

Heat treatments also have an important effect on rate of respiration and of ethylene production. In some products those rates were significantly lower in treated fruits. Plums subjected to a mechanical damage after being heat treated (45 ºC / 10min) evidenced lower respiration and ethylene production rates than fruits not subjected to heat treatment (Serrano, Martínez-Romero, Castillo, Guillén & Valero, 2004). However, other authors report that mangoes subjected to a heat treatment showed higher respiration and ethylene production rates than untreated fruits (Ketsa, Chitragool, Klein & Lurie, 1999).

The well known effect of heat treatments on horticultural products is the improvement and/or maintenance of textural characteristics (Garcia & Barrett, 2002) during a longer period. The treatment activates the enzyme pectin methylesterase (PME) promoting de-esterification of pectin molecule, thus increasing the number of calcium binding sites, and resulting in strengthening of the cell wall.

The objective of the present work is to evaluate the effect of mild heat pre treatments on minimally processed kiwifruit quality, when applied at two different stages of maturity, both immediately after the treatment and during the whole storage period.

2 Material and methods

2.1 Raw Material

Kiwifruit (*Actinidia deliciosa* (A Chev) Liang et Ferguson var *deliciosa* cv Hayward) at two different maturity stages were used. Batch I kiwis were fully ripe (soft ripe) while batch II kiwis presented a partially ripe (firm ripe) maturity stage. The difference in maturity stage is based on TSS, pH and firmness of fruit flesh. The physio-chemical characteristics of the two batches of kiwifruit prior to heat treatment are shown in Table 1. As can be seen fruits of Batch I presented a higher TSS (*ca* 45 %) and a lower firmness (*ca* 50%) values. Fully ripe kiwis were darker (L value

44.13 *vs* 54.41) than partially ripe fruits, but these show a higher chroma value (36.77 *vs* 15.69), revealing a higher content of green pigments.

Table 1 Physio-chemical characterisation of kiwifruit.

	Ca^{2+} (mg/100g fw)	TSS	pH	Firmness (N/mm^2)	Colour				
					L^*	a^*	b^*	Chroma	Hue angle
Batch I	25	14.4	3.2	0.08	44.13	-5,67	14.63	15.69	111.18
Batch II	59	10.0	3.4	0.19	54.41	-17.47	32.35	36.77	118.36

2.2 Mild heat treatment and minimal processing

Whole kiwis were subjected to different time/temperature treatments (according to the experimental design) by immersion in temperature controlled water baths. In order to avoid loss of internal solutes, as result of the thin skin of kiwifruit, fruits were packed under vacuum in polypropylene films. After heat treatment packages were opened and fruits cooled and kept in cold storage (4 ºC) for 24 h. Later fruits were hand peeled and cut in slices (≈1.5 cm) with sharpened knives, packed in low-density polyethylene and vinylidene chloride bags [3000-4000 and 11000-15000 ($ml/m^2/24h/atm$) O_2 and CO_2 permeability) and stored at 4 ºC according to the experimental design.

2.3 Experimental design

A central composite rotatable design (CCRD) was used. Central composite experiments consist of three sets of experimental points (Meilgaard, Civille & Carr, 1991): (1) a traditional factorial design with 2^k points, k being the number of x_i variables (factors) with coded levels +1 and –1 for each; (2) a star of 2^k points, coded as $+\alpha$ and $-\alpha$ on the axis of the system at a distance of $2^{k/4}$ from the origin, that accounts for non-linearity; (3) central points, which are replicated to provide an estimate of the lack of fit of the linear statistical model obtained as well as the pure error of the experiments (Montgomery, 1996). The main advantage of this methodology is to diminish the number of experimental trials needed to evaluate

multiple parameters and their interactions (Lee, Ye, Landen & Eitenmiller, 2000; Porreta, Birzi & Vicini, 1995).

No previous reports were found on the use of mild heat pre treatments for kiwifruit. Therefore, no data is available regarding the most adequate temperature and time period conditions or on the impact of treatments on quality of minimally processed kiwi. The ranges these authors chose to test were temperatures of 25 – 50 ºC, time periods of 10 - 90 min, and storage periods of 0 - 10 day. Table 2 shows the coded and uncoded experimental design.

Table 2 Experimental design.

Coded independent variables			Decoded independent Variables		
Treatment Temperature	Treatment Time	Storage Time	Treatment Temperature (ºC)	Treatment Time (min)	Storage Time (day)
-1	-1	-1	30	26	2
-1	-1	1	30	26	8
-1	1	-1	30	74	2
-1	1	1	30	74	8
1	-1	-1	45	26	2
1	-1	1	45	26	8
1	1	-1	45	74	2
1	1	1	45	74	8
-1.68179	0	0	25	50	5
1.68179	0	0	50	50	5
0	-1.68179	0	37.5	10	5
0	1.68179	0	37.5	90	5
0	0	-1.68179	37.5	50	0
0	0	1.68179	37.5	50	10
0	0	0	37.5	50	5
0	0	0	37.5	50	5
0	0	0	37.5	50	5
0	0	0	37.5	50	5
0	0	0	37.5	50	5
0	0	0	37.5	50	5

Coded and decoded matrix of independent variables.

2.4 Quality measurements

Samples were analysed for colour, firmness and total soluble solids content (% TSSC) in raw material, before heat treatment and after mild heat pre treatment, according to the experimental design. pH was also evaluated in the raw material in order to establish sugar / acid ratio.

The colour of minimally processed kiwi was evaluated at the green section of slices with a colorimeter (CR 300 Minolta, Osaka, Japan) by measuring *L*a*b** (CIE) parameters. From those, chroma and hue angle were determined. A white tile (*L**= 97.46; *a** = - 0.02; *b** = 1.72) was used as reference. Thirty measures were performed for fifteen slices under each condition, set by the experimental design.

Firmness was evaluated by performing a puncture test on kiwi slices' flesh using a TA-XT2 texture analyser from *Stable Micro Systems* with a 25 kg load cell. Firmness measurements were taken as the first peak force value obtained during the test to penetrate the fruit 6 mm, at 1 mm/s. Mean values were calculated from results of 30 fruit slices for each condition set by the experimental design.

A combined sample of juices extracted from 10 slices from each treatment was used to evaluate TSSC and pH. TSSC were assessed with a table digital refractometer. pH was measured using a pH-meter.

2.5 Statistics

Data were fitted to second-order polynomial equations (1) for each dependent Y variable, through a stepwise multiple regression analysis using "Statistica v. 6.0" software.

$$Y = b_0 + b_1T + b_{11}T^2 + b_2t + b_{22}t^2 + b_3St + b_3St^2 + b_{12}T\,t + b_{13}T\,St + b_{23}t\,St \quad (1)$$

where b_n are constant regression coefficients and T (treatment temperature), t (treatment period) and St (storage period) independent variables.

Analysis of variance was performed to determine the lack of fit and the significance of the effects of each of the three independent factors.

3 Results and discussion

3.1 Effects of mild heat treatment on quality parameters

Table 3 and Table 4 summarize the results of the analysis of variance of minimally processed soft and firm ripe kiwi. Experimental data from dependent variables analysed were fitted to Eq. 1 (Table 5). Only parameters adjusted by the model are shown.

3.1.1 Effect on total soluble solid content

When fully ripe kiwis were subjected to mild heat treatment no effect of treatment temperature was revealed on total soluble solid content (Figure1). This parameter is mainly dependent on storage time ($P < 0.005$), its effect being negative, linear and quadratic, and marginally dependent on treatment time ($P < 0.1$). However, high treatment temperatures (> 45 ºC) for short periods (< 30 min) seem to yield better retention of soluble sugar content (14 %). Fruit subjected to longer treatments (> 40 min) evidenced an accelerated consumption of sugars during the first 2 days, while short treatment period appear be effective in retention of initial TSSC up until the sixth day of storage.

Table 3 ANOVA of total soluble solids, lightness and firmness as function of treatment temperature, treatment time and storage time of minimally processed soft ripe kiwis.

		Factor											
		T (L)	T (Q)	t (L)	t (Q)	St (L)	St (Q)	T×t	T×St	t×St	Lack of fit	Pure Error	Total SS
TSS	SS	0,07426	0,17757	0,80715	0,01972	3,82958	5,89044	0,04961	0,16917	0,12583	1,94137	0,89579	14,19974
	df	1	1	1	1	1	1	1	1	1	5	5	19
	MS	0,074263	0,177569	0,807152	0,01972	3,829576	5,890444	0,049613	0,169168	0,125835	0,388274	0,179157	
	P	0,548052	0,365173	0,087222	0,753519	0,005718	0,002259	0,621225	0,375818	0,4402	0,207983		
Firmness	SS	0,000003	0,000005	0,000014	0	0,000954	0,000634	0	0,000001	0,000079	0,000519	0,000056	0,002265
	df	1	1	1	1	1	1	1	1	1	5	5	19
	MS	0,000003	0,000005	0,000014	0	0,000954	0,000634	0	0,000001	0,000079	0,000104	0,000011	
	P	0,613985	0,550203	0,310554	0,941384	0,000254	0,000667	0,878985	0,803553	0,045504	0,014665		

Table 4 ANOVA of total soluble solids, lightness and firmness as function of treatment temperature, treatment time and storage time of minimally processed firm ripe kiwis.

		Factor											
		T (L)	T (Q)	t (L)	t (Q)	St (L)	St (Q)	T×t	T×St	t×St	Lack of fit	Pure Error	Total SS
TSS	SS	0,07877	0,06851	20,38266	4,83113	1,9608	-	-	0,23347	0,13347	8,94754	3,13356	39,90082
	df	1	1	1	1	1	-	-	1	1	7	5	19
	MS	0,07877	0,06851	20,38266	4,83113	1,9608	-	-	0,23347	0,13347	1,27822	0,62671	
	P	0,737407	0,754335	0,002314	0,039066	0,137157	-	-	0,5683	0,663829	0,224983		
Lightness (L*)	SS	2,5548	20,2619	22,2439	1,2916	24,7456	12,309	2,6555	4,4699	2,8662	16,8696	8,9795	122,6342
	df	1	1	1	1	1	1	1	1	1	5	5	19
	MS	2,55478	20,2619	22,2439	1,29161	24,74559	12,30904	2,65553	4,46988	2,86622	3,37393	1,7959	
	P	0,286491	0,020132	0,016931	0,435114	0,013826	0,047211	0,278256	0,175478	0,262177	0,252805		
Firmness	SS	0,001619	0,00066	0,022269	0,002889	0,003436	0,006463	0,003025	-	-	0,010447	0,004853	0,055668
	df	1	1	1	1	1	1	1	-	-	7	5	19
	MS	0,001619	0,00066	0,022269	0,002889	0,003436	0,006463	0,003025	-	-	0,001492	0,000971	
	P	0,253034	0,447066	0,004928	0,145081	0,118656	0,049406	0,137782	-	-	0,328593		

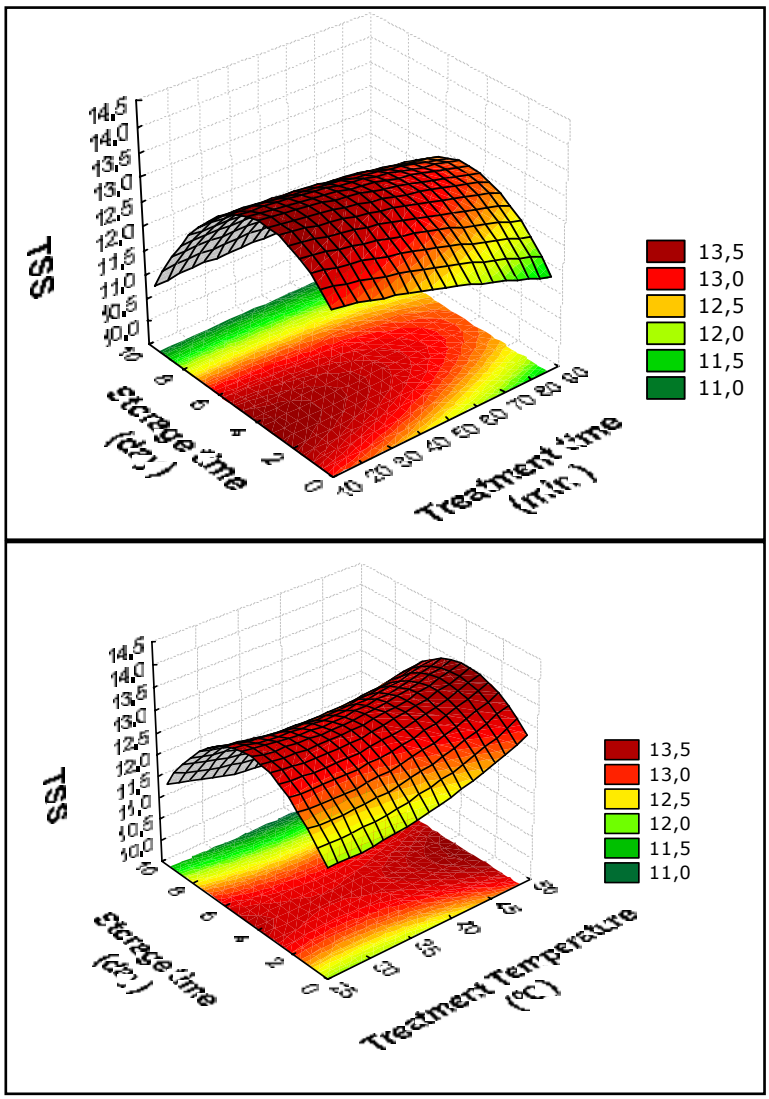

Fig. 1 Response surfaces of total soluble solid content of minimally processed soft ripe kiwi.

Mild heat treatment applied to partially ripe fruits yielded fruits with higher TSS, also sensorially characterised by a trained panel as sweeter fruit (data not shown), the effect being mainly dependent on treatment time ($P < 0.05$). Treatments for periods shorter than 25 minutes allow maintaining the initial values of soluble solid content during the whole studied storage period, while longer treatment periods increased up to 13 % soluble solids contents of fruits (Figure 2).

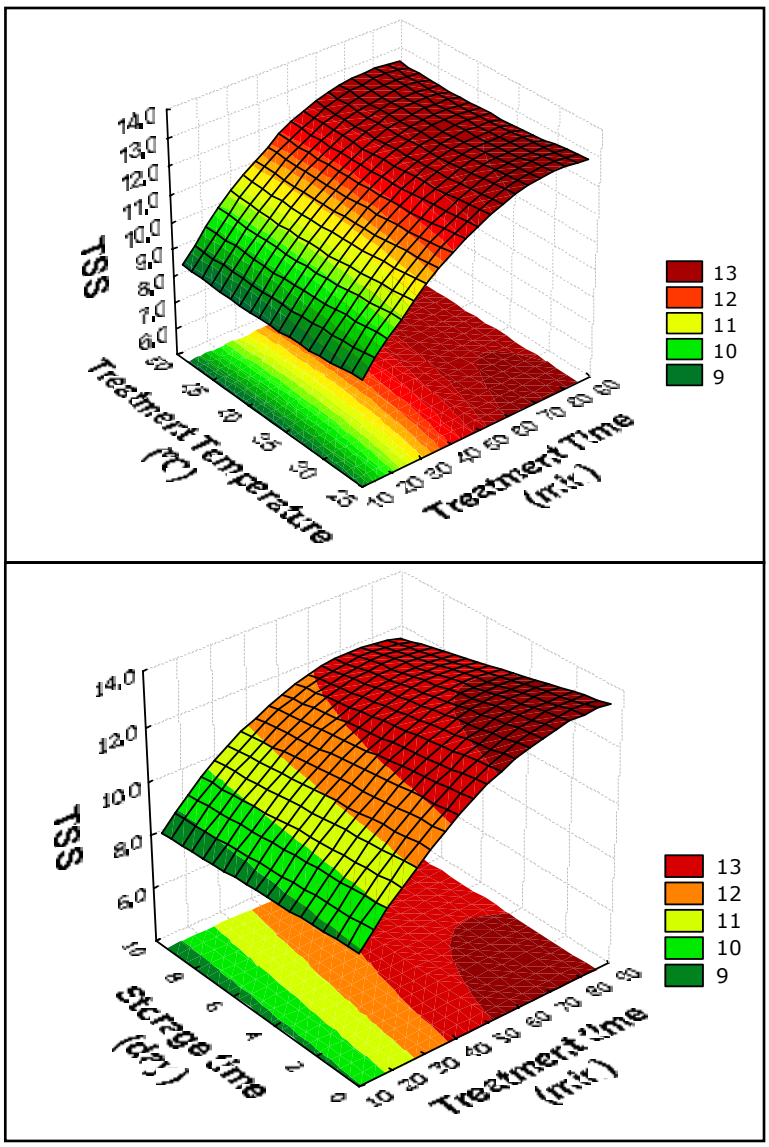

Fig. 2 Response surfaces of total soluble solid content of minimally processed firm ripe kiwi.

The different effects, depending on kiwifruit maturity stage, may be explained both by metabolic processes, such as respiration as a response to heat treatment, and by changes on pectic polymer structures. When fruits are subjected to heat treatment and minimal processing stress conditions are created, an increase of fruit respiration rates ensues, and consequently sugar consumption also increases. Fully ripe fruits subjected to treatment in the range of 30 - 44 ºC evidenced a more pronounced O_2 depletion inside their packages than the others, suggesting an enhanced respiratory activity (data not shown). On the other hand a TSS increase in the fruit is probably

due to the solubilization of neutral sugars, from pectic polymer residues. In kiwifruit the most representative pectin fraction is the water soluble one, containing 60 % of neutral sugars and 40 % of uronic acid (Varoquaux *et al.*, 1990). Gallego & Zarra (1998) identified at harvest, in ripe kiwi cell walls, mainly galactose, glucose and xylose, some arabinose and rhamnose, and traces of manose and fucose.

At a partially mature stage, when fruits are firm, the pectic polysaccharides remain unbroken, with many neutral sugar residues. The application of a mild heat treatment probably activates enzymes such as α-galactosidase, glucosidase and arabinase that will promote the release of these neutral sugars, thus fruits become sweeter. When kiwi is fully ripe most of these sugars are already solubilized, as result of the maturation process, and so heat treatment has only a marginal effect.

It may therefore be stated that the observed evolution of soluble solid content of kiwifruit at both maturity stages seems to be the result of the balance of two different processes.

3.1.2 Effect on colour

When a full maturity stage is tested the more severe heat treatment (50 ºC / 50') lead to great undesirable changes in fruit appearance. Is observed that the application of this treatment promote, in addition to development of brown pigments, the conversion of chlorophylls to pheophytins inducing the change of the vivid green colour to a dull olive brown. Although the colour parameters, either express as L^*, a, b or chromaticity (c) and hue angle (h), were not adjusted by the model, the other temperature / time combinations did not affect the colour of kiwi slices. Neither browning nor colour loss of samples was observed during the storage period.

The applied mild heat treatment affected partially ripe kiwi colour in a different way. In this batch of experiments the three independent variables significantly influence ($P < 0.05$) the lightness of fruit slices. As can be seen in Fig. 3 when treatment time is shorter than 25 minutes, lightness of the treated samples is not different of that of the raw material and is preserved during all the storage period. Treatments at 25 - 40 min only preserve this value for two days. Treatments longer than 40 minutes at temperatures lower than 45 ºC induced a more pronounced decrease of shine from the fourth day of storage onwards. For higher treatment temperatures the deterioration in lightness value occurs immediately after treatment.

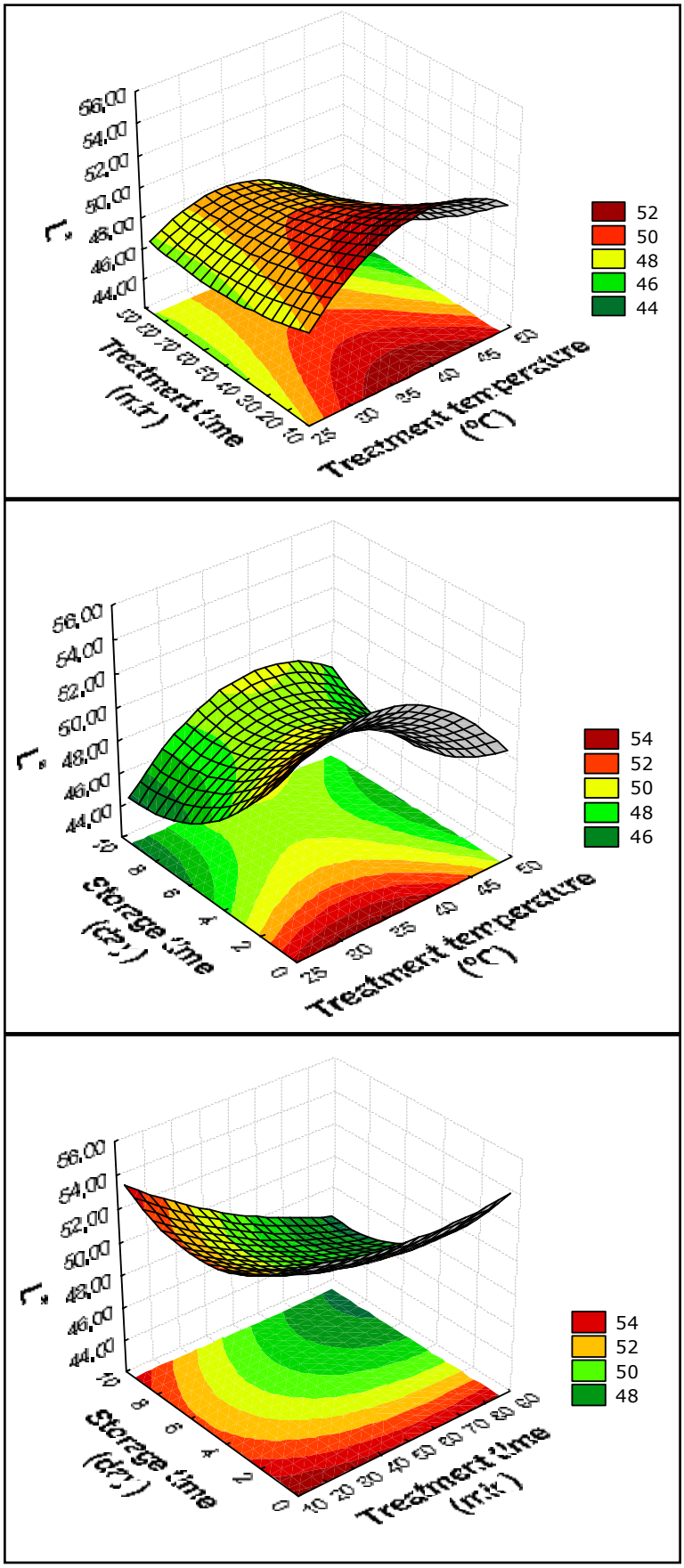

Fig. 3 Response surfaces of lightness (L*) of minimally processed firm ripe kiwi.

Usually a decrease in the L^* value meant a surface darkening. Agar *et al.*, (1999) report that in the case of kiwifruit slices such decrease is due to induction of translucent water-soaked tissue in spite of traditional enzymatic browning. These authors also referred previous works reporting that low tannin and polyphenoloxidase and high ascorbic acid contents, did not allow surface browning.

3.1.3 Effect on firmness

Texture is the limiting factor of minimally processed kiwi quality, firmness being the parameter that better characterises the evolution of textural quality.

The response surfaces representing heat treatment effect on firmness of soft ripe kiwifruit slices are shown in Fig. 4 and are described by equation 3 (Table 5). Only storage period had a significant effect ($P < 0.001$) on firmness reduction. The linear interaction between treatment time and storage time also had a significant effect ($P < 0.05$) but this last factor alone did not appear to be significant ($P > 0.05$). Treatment times longer than 55 min, independently of treatment temperature, prevented a sharp decrease in firmness only for the first 2 days while shorter treatments did not avoid a fast softening rate. However, the model showed a significant lack of fit ($P < 0.05$) suggesting that higher order interactions and/or other variables not considered in the experimental design may contribute to a better explanation of the data.

Table 5 Adjusted equations from RSM

Maturation Stage	Dependent Variable	Equation	R^2	Eq. number
Soft Ripe	TSS	$13.42 + 0.086T + 0.002T^2 + 0.004t + 0.698\ St - 0.072St^2 - 0.006T*St + 0.002t*St$	0.80	(2)
	Firmness	$0.05755 - 0.00068T + 0.00001T^2 + 0.00021t - 0.00756St + 0.00075St^2 - 0.00001T*St - 0.00004t*St$	0.75	(3)
Firm Ripe	TSS	$8.09 - 0.065T + 0.001T^2 + 0.162t - 0.001t^2 + 0.248St - 0.008T*St - 0.002t*St$	0.70	(4)
	Lightness (L^*)	$28.36 + 1.54T - 0.02T^2 + 0.06t - 2.32St + 0.1St2 + 0.03\ T*St - 0.01t*St$	0.79	(5)
	Firmness	$-0.08057 + 0.01601T - 0.00012T^2 - 0.00013t + 0.00002\ t^2 - 0.02916St + 0.00238St^2 - 0.00011T*t$	0.72	(6)

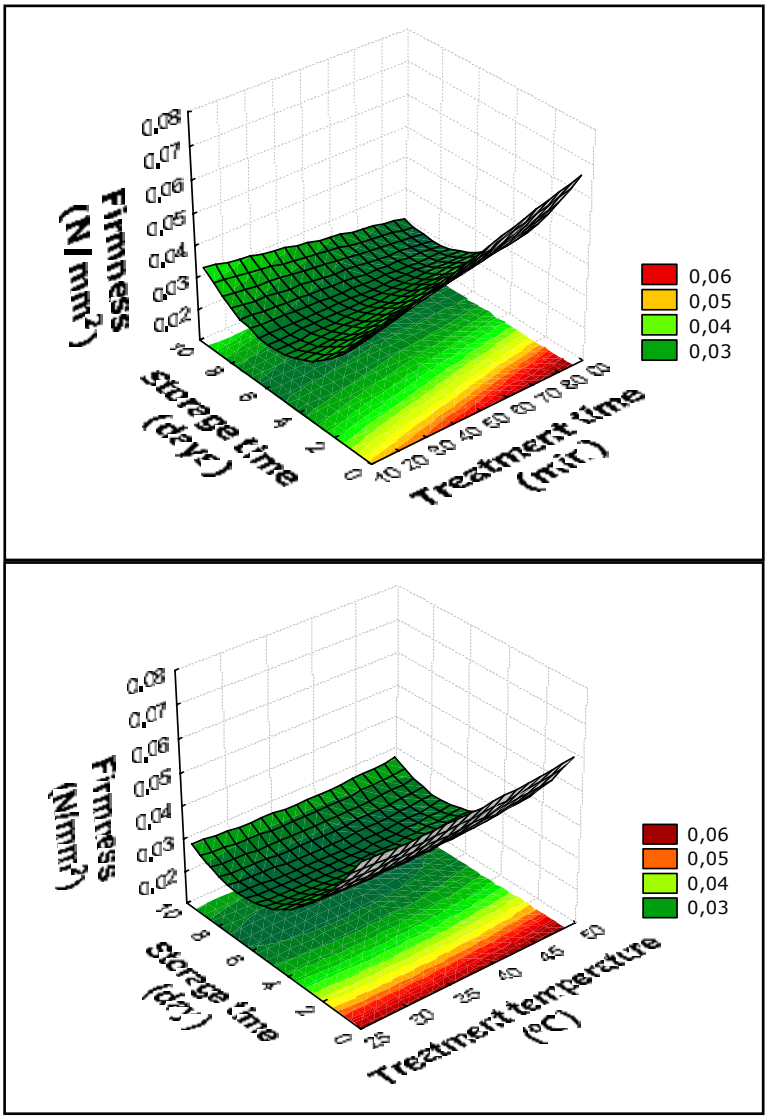

Fig. 4 Response surfaces of firmness evaluation of minimally processed soft ripe kiwi.

The application of heat pre treatments to firm ripe fruits showed that the main factors affecting firmness of minimally processed kiwi slices were treatment period ($P < 0.005$) and storage period ($P < 0.05$). The results illustrated that the period during which fruits were subjected to treatment was more important than treatment temperature, within the tested range. Fig. 5 represents the response surfaces of the influence of the three independent variables on firmness. From the analysis of graphs it can be stated that treatments performed during up to 40 min promote an increase of about 28 % of firmness, for the two first days of storage, preserving a value higher or similar to the one evidenced by the raw material during the remaining storage period.

Treatments longer than 60 min lead to a decrease of *ca.* 36 %, for storage periods longer than 4 days. Despite the significant lack of fit ($P = 0.32$) shown by the model,

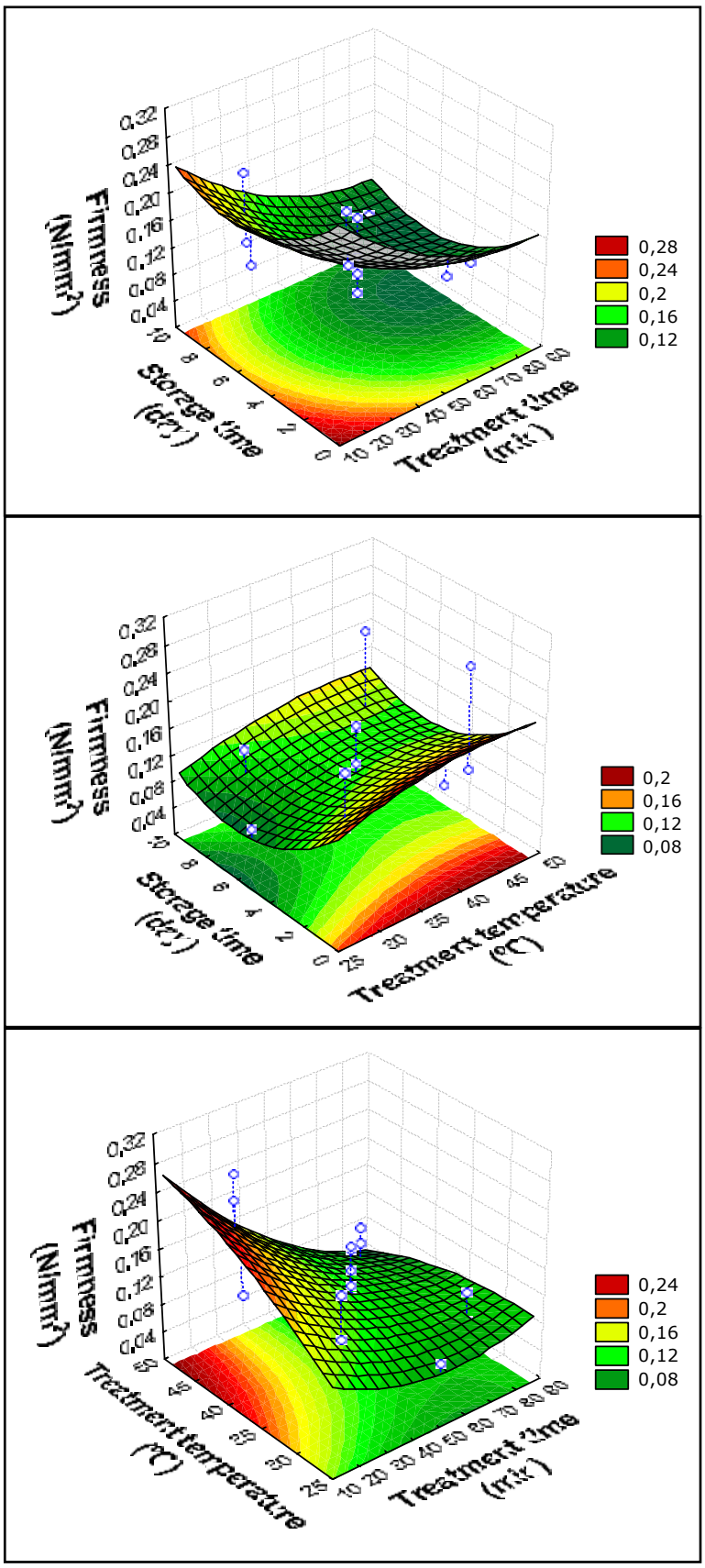

Fig. 5 Response surfaces of firmness evaluation of minimally processed firm ripe kiwi.

the regression coefficient is not high (0.72), suggesting again that higher order effects/interactions or other variables may be involved in these phenomena.

In spite of the lack of literature on the effect of mild heat pre-treatments on texture of kiwi slices, some previous works report the effect of the temperature on quality parameters. Even at 2 ºC, a fast loss of firmness was noticeable few hours after packaging (Varoquaux *et al.*, 1990). Agar *et al.*, (1999) reported that softening of kiwi slices increased as storage temperature and time increased. At 0 ºC kiwi slices firmness reduced about 62 % during 12 days of storage, while slices kept at 5 ºC exhibit a rate of softening about 75 % after 9 days. Varoquaux *et al.*, (1990) stated that texture loss in kiwi slices seems to be a consequence of the enzymatic breakdown of pectic compounds, due to demethoxylation and depolymerization by pectinolytic enzymes such as PME and polygalacturonase. As other authors already reported (Abreu *et al.*, 2003; Kim *et al.*, 1993), promoting PME activation, heat treatment contributes to increased cross-linkage between calcium (endogenous and/or supplied Ca^{2+}) and pectin molecules, forming calcium pectates and increasing rigidity of middle lamella of cell walls.

The mild heat pre-treatments affect the firmness of fruits in a different way according to the maturity stage, probably also due to the different Ca^{2+} contents. Totally ripe fruits showed calcium contents of about 25 mg/100 g (fresh weight basis), while partially mature fruits presented higher values for this cation (59 mg/100 g (fresh weight basis)). In this case the formation of calcium pectates is higher.

4 Conclusions

Maturity stage proved to be an important parameter deserving consideration when producing minimally processed kiwi. On the other hand, mild heat treatments applied to the intact fruit can constitute a useful hurdle against quality loss, but only if the fruit is at an early maturity stage.

Mild heat pre-treatments below 45 ºC, for periods shorter than 25 min, applied to fruits at a firm ripe stage, improved quality attributes of minimally processed kiwi

slices, increasing / preserving tissues' firmness and total soluble solids content, their colour being only marginally affected, and allowing a shelf-life period of 10 days.

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Influence of moderate heat pre- treatments on physical and chemical characteristics of kiwifruit slices

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Abstract

The effect of mild heat treatments, applied to whole kiwifruit, on physical characteristics and chemical composition of minimally processed fruit was studied. Fruits were subjected to heat treatments at 45 ºC for 25 and 75 min, cooled for 24 h, minimally processed and stored at 4 ºC for 12 days. Heat-treated fruits showed increased respiration rates in the first 2 days of storage. Samples colour was marginally affected either by heat treatments or minimal processing. The application of heat treatments leads to an increment of slices firmness due to cross linking between demethylated galacturonic acid chains and endogenous calcium. An increment in sucrose, L-malic, citric, quinic and ascorbic acids was observed as a consequence of applied heat treatments. Heat treatment at 45 ºC for 25 min applied to whole fruits allows quality retention of minimally processed fruit during 9 days at 4 ºC.

Keywords

Kiwifruit, Minimal processing, Composition, Moderate heat treatments.

1 Introduction

The most important motivation for purchasing minimally processed products relates to convenience [1] but the health benefits associated to the consumption of fruits and vegetables have led to a growing attention to this kind of products [2, 3].

Convenience and quality are the two aims difficult to harmonize because minimal processing operations lead to physiological and biochemical changes typical of the senescence process such as increased respiration, ethylene production and loss of membrane integrity [3 - 5].

Minimal processed kiwifruit undergoes several deteriorative reactions leading to loss of firmness, increased respiration and ethylene production rates, mass and flavour loss [6 - 8]. Some efforts had been made in order to get a better preservation of the sliced fruit, such as application of 1- methylcyclopropene (1-MCP) prior to cutting, packaging in non-conventional modified atmosphere with argon and nitrous oxide [9], application of volatile compounds such as methyl jasmonate, ethanol and other alcohols [10], treatments with hydrogen peroxide, calcium lactate and controlled atmosphere packaging [11].

The effects of mild heat treatments on the postharvest physiology of fruits and vegetables were already discussed [12 - 16]. This kind of treatments was effective in quality maintenance of fresh cut cantaloupe melon [4, 17, 18], apples [19] and mango [20].

Moderate heat treatments applied to whole kiwifruit were found to be an interesting methodology able to better preserve minimally processed fruit slices [12] but the underlying mechanisms remain somewhat unknown. The objective of the present work was to understand the physical and chemical changes involved in minimally processed kiwifruit previous subjected to moderate heat treatments.

2 Materials and methods

2.1 Material

Kiwifruits (*Actinidia deliciosa* (A Chev) Liang et Ferguson var *deliciosa* cv Hayward) were purchased at a local market. Fruits selected by uniform size and the absence of visual wounds were stored under refrigeration (4 ºC) prior to pre-treatments and / or minimal processing. Physical and chemical fruit characteristics are summarized in Table 1.

As it was apparent from previous works [12] that maturity stage has relevance in the treatments' efficiency, so total soluble solids and pH were measured for the raw material.

Table1 Physical and chemical characterization of raw material.

Firmness (N/mm^2)	0.052 ± 0.0025
Colour (outer pericarp / white core)	
L*	45.30 ± 1.254 / 67.93 ± 1.047
c	20.14 ± 1.001 / 23.27 ± 0.718
h	116.77 ± 0.431 / 108.38 ± 0.312
Total soluble solids	12.8
pH	3.5
Calcium (mg/100g fw)	33.97 ± 1.212
Sugars (g/100g dw)	
Sucrose	4.28 ± 0.133
Fructose	23.91 ± 2.846
Glucose	17.45 ± 1.812
Acids (g/100g dw)	
Malic	0.14 ± 0.003
Citric	2.42 ± 0.016
Quinic	3.09 ± 0.063
L-Ascorbic	0.17 ± 0.003

(Mean values ± standard error. Firmness $n = 30$; colour $n = 20$; other parameters $n = 3$)

Despite the low firmness value, total soluble solids and pH denote that fruits were at a ripe stage, adequate for consumption (TSS ≥ 12.5%). The lowest firmness value may be indicative of early harvested fruits followed by storage, because as [21] state, late harvested kiwifruit retain their flesh firmness during storage better than early harvested fruit.

2.2 Methods

2.2.1 Heat treatments

Whole kiwifruits were subjected to immersion heat treatments at 45 ºC for 25 min and for 75 min, [12], rapidly cooled in a cold chamber (4 ºC) and kept under cold storage (4 ºC) for 24 h. Non-treated fruits were used as control.

2.2.2 Minimal processing

Heat-treated and control fruits were hand peeled, washed in a cold sodium hypochlorite solution (125 mg/L free of Cl^-, at pH 6), gently dried with blotting paper and cut into slices (≈1.5 cm thickness) with sharpened knives. Slices from different fruits were randomly distributed and each 250 g packed in low-density polyethylene and vinylidene chloride bags [3,000 – 4,000 and 11,000 – 15,000 ($mL/m^2/24h/atm$) permeability to O_2 and CO_2, respectively], and stored at 4 ºC. At each date of analysis three packages per sample were evaluated.

2.2.3 Analytical procedures

During cold storage, samples were analysed for package atmosphere composition, colour, firmness, free and linked calcium, sugars, acids and pectic polymers.

Sensory analysis was performed by a ten elements trained panel, concomitantly with those objective determinations.

2.2.3.1 Atmosphere composition

Headspace gas samples were taken with a hypodermic needle through an adhesive septum previously fixed on the bags and analysed using a checkmate 9,900 O_2/CO_2 gas analyzer (PBI-Dansensor, Denmark).

2.2.3.2 Colour

The colour of kiwifruit slices was evaluated both on outer pericarp and on inner white core. Measurements, made with a Minolta CR300 colorimeter, were taken by L*, a*, b* (CIE) parameters. Hue angle (h^o) and chroma (c) were calculated [22]. Results were the mean of 20 measures on each section of fruit slices.

2.2.3.3 Firmness

Firmness was evaluated by performing a puncture test on flesh of kiwifruit slices using a TA-XT2 texture analyser from *Stable Micro Systems*, equipped with a 25 kg load cell. Firmness measurements were taken as the medium force attained in a puncture at 4 mm distance, at 1 mm/s with a 4 mm diameter stainless steel probe. Results were the mean of 30 measures for every sample. As firmness is the critical parameter in minimally processed kiwifruit's shelf life, this parameters' analysis was extended for 12 days of storage.

2.2.3.4 Calcium

Calcium analysis was performed on the fruit's outer pericarp, without seeds, using the method described by [17], slightly modified. About 2 g of kiwifruit cut in small cubes and 20 mL of cold deionized water were homogenized. About 5 mL of water used to wash apparatus was added. The mixture was stirred at low speed for 1 h. About 5 mL of rinse water used was added to the homogenate before centrifugation at 20,000 *g* for 1 h, at 4 ºC using a Hermle Z 383K (Germany). Samples were filtered through the Whatman 541 ashless filter paper. About 30 mL of filtrate was added of 30 mL of 3 mol/L HCl to determine free calcium. The insoluble material was incinerated with the filter paper at 550 ºC for 24 h and re-suspended in 10 mL of 3 mol/L HCl and 40 mL of deionized water. Strontium chloride was added to control ionization interferences. Calcium levels were determined at 422.7 nm using an atomic absorption spectrophotometer equipped with an air-acetylene flame.

2.2.3.5 Sugars and organic acids

Sugars and organic acids were extracted and analysed by HPLC according to the method described by [23]. About 10 g of kiwi were homogenized in 25 mL distilled water and the sample clarified by centrifugation at 20,000 *g* for 20 min. The pellet was washed with 25 mL distilled water, centrifuged again, and the supernatants collected and pooled. Samples were filtered through 0.45 μm filters prior to HPLC analysis.

Sugar content was evaluated by an HPLC system consisting of a 125 Beckman pump, a differential refractometer model 401, a R460 Waters detector and a Waters 745 integrator. A Sugar Pak 1 column from Waters was used. The mobile phase was 50 mg/L calcium EDTA solution and the flow rate 0.5 mL/min at 90 ºC. The sample volume injected was 20 μL.

For the determination of organic acids a Beckman system Gold chromatograph was used. The HPLC system consisted of a 126 Beckman pump, a diode array detector model 168, operated by Gold 8.10 software. An Aminex HPX-87H column from Biorad was used. The mobile phase consisted of 5 mmol/L sulphuric acid, pH 2.2, and the flow rate was 0.5 mL/min. The sample volume injected was 20 μL and detection was at 214 nm.

Ascorbic acid was extracted according to a method described in [24]. About 50 g of kiwifruit were homogenized with 50 mL of metaphosphoric acid, during 15 min. Then, another 50 mL of metaphosphoric acid were added. The slurry was filtered using the Whatman 42 filter paper. The filtrate was filtered again through 0.45 μm filters prior to HPLC analysis. Ascorbic acid content was determined as referred for organic acids.

2.2.3.6 Pectic polymers

Pectin extraction / determination was performed according to [25]. Pectins were extracted from the alcohol insoluble residue (AIR) obtained homogenizing 20 g of fruit with 100 mL of boiling absolute ethanol. The homogenate was vacuum filtered, the residue washed with 80% ethanol until discoloration and the residue was dried at 35 °C ± 2 ºC.

100 mg of AIR were homogenized with 50 mL of distilled water and stirred for 30 min. The mixture was centrifuged at 15,000 *g* for 15 min, the pellet resuspended in 50 mL of distilled water, stirred and centrifuged again. These procedures were repeated twice. The three supernatants were pooled and represent the water soluble fraction (WSF). The pellet was resuspended in 50 mL of 0.05 mol/L HCL and incubated for 30 min at 98 ºC under reflux. All extractions were performed in triplicate.

Uronic acid concentrations were estimated by the *m*-hydroxydiphenyl method using galacturonic acid as standard [25].

2.2.3.7 Sensory Analysis

Sensory evaluation of minimally processed kiwi samples was conducted in the sensory laboratory of the Department of Food Science and Technology, ISA/UTL. The sensory evaluation was performed by a total of ten trained judges (food engineering students and staff). All of them were non-smokers and their age ranged from 25 to 63 years old. The room, at 20 ºC, was equipped with seven isolated sensory booths. The tasting sessions occurred in the period from 10 AM to12.30 PM. Panellists performed a descriptive test and were asked to analyse the samples' colour, typical aroma, untypical aroma, typical taste and firmness, in a scale of five points. Those descriptors were selected in previous trials.

2.3 Statistics

Analysis of variance was applied to the results, as well as a mean comparison test (Fisher LSD), to analyse differences between treatments along storage time. Principal component analysis was also performed. "Statistica" v. 6.1 software from Statsoft, Inc.was used.

3 Results and discussion

Figure 1 shows the evolution of CO_2 concentration in the headspace of minimally processed kiwifruit packages along storage period. The analysis of atmosphere composition inside the packages during 9 days of storage revealed that minimal processing operations led to an enhancement of respiration rate with an accumulation of CO_2 inside packaging of all samples, producing a modified atmosphere.

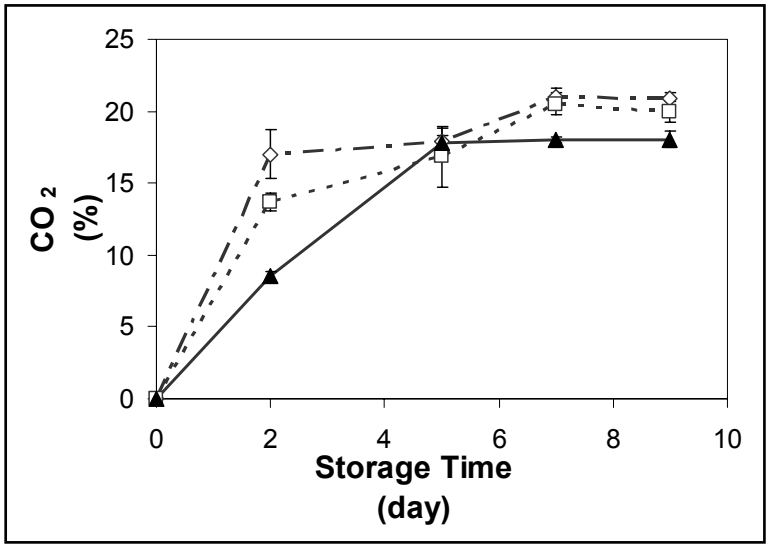

Fig. 1 CO2 headspace composition of minimally processed kiwifruit packaging during storage. Open diamond 45 ºC / 25 min; open square 45 ºC / 75 min; filled triangle Control. Each data point represents mean ± SE.

Agar *et al.* [8] reported that kiwifruit slices had doubled their CO_2 production rates of whole fruit few hours after peeling and slicing operations at 20 ºC and within 1 to 3 days at 2 ºC. Samples previously subjected to heat treatments exhibit a higher accumulation in the first two days of storage, 17 and 14 % for treatments during 25 and 75 min, respectively. From the fifth day to the end of storage period the concentration of CO_2 inside the packaging was similar for treated and control fruits (*c.a.* 19%). Paull and Chen [16] refer that the respiration rate of ripening fruit is initially increased by exposure to higher temperatures but, after treatment the respiration rate declines to near or below the level of non-heated control. In

minimally processed kiwifruit a similar pattern was found between heat-treated and control samples.

The accumulation of CO_2 was followed by depletion in O_2 levels, reaching about 4.5% of O_2 inside packages of all samples at the end of storage period.

Changes in respiration rate as result of whole fruit heat treatments and/or minimal processing operations were observed in sweet cherry [26], cantaloupe melon [4], peaches [27], plums [28] apples [29, 30] and pears [31] among others, depending beside other factors on cultivar, maturity stage, age of tissue and storage temperature [16]. In our work, we notice that the effect of the minimal processing operations on respiratory activity of kiwifruit could not be minimized by the application of moderate heat pre-treatments.

The tolerance of minimally processed products to low O_2 and high CO_2 levels depends upon the commodity. Low levels of O_2 combined with high concentration of CO_2 may induce fermentative reactions with the production of ethanol and acetaldehyde imparting off-flavours.

The O_2 level can be allowed to drop to near or at the respiratory quotient breakpoint, the O_2 concentration which corresponds to a sudden raise of the respiratory coefficient [32], without injury [33].

Some works [8] refer that O_2 levels below 0.5 % can enhance the production of acetaldehyde and ethanol, while modified atmospheres with 2 - 4 kPa O_2 and/or 5 - 10 kPa CO_2, in combination with calcium treatments, were effective in maintaining the quality of fresh-cut kiwifruit slices for 9-12 days. In minimally processed pineapple wedges no off-odour or off-flavours were detected after a 2-week storage period, at O_2 and CO_2 levels lower than 2 % and higher than 15 %, respectively [34].

In the present work, the O_2 concentrations inside packaging never drops below the referred levels.

Samples' pericarp colour evolution during a 9-day storage period is summarized in Table 2. The major changes occurred in the first 2 days of storage. Samples' L* and *c* underwent a decline indicating some yellowing resulting from chlorophyll degradation. These changes were mainly due to minimal processing, as followed by control as well, and were marginally affected by heat treatments. Cut surface browning was not noticed. Hue angle, the parameter that better describes the human eye perception of colour, underwent marginal differences in all samples along the whole storage period; hence, these changes were not visually perceptible.

These results are in accordance with previous results [12] in which the adjusted model showed a similar pattern of evolution of L* value for firm ripe kiwifruit.

Loss of kiwifruit's pericarp green colour, yielding an increase in a* values, and induced by cutting had already been observed, probably due to chlorophyll degradation and consequently to pheophytin formation [35]. Blanching treatments at 99.8 ºC for periods longer than 5 min also leads to an increase in a* values of outer pericarp tissue of kiwifruit halves [36]. Rocculi *et al.* [9] observed similar colour changes in minimally processed kiwifruit, *c* being the most affected parameter. The authors conclude that a storage atmosphere of 90 % N_2O, 5 % O_2 and 5 % of CO_2 allowed a better preservation of L* and h^o, probably due to an indirect inhibitory effect of clorophyllase by N_2O. Nevertheless, other authors referred that there did not appear to be any consistent relationship between the changes occurred in the total chlorophyll content (degraded between 70-80%) and colour of kiwifruit slices subjected to blanching and vacuum solutes impregnation [37]. Agar *et al.* [8] report a slight brown discolouration in slices exposed to 10 kPa or higher of CO_2 but this effect was observed just in 1 year experiment, not being confirmed in the second one. On the other hand, the same authors state that kiwifruit slices' surface darkening, expressed by a decreased L* value, was due to the induction of a translucent water soaked tissue and not to enzymatic browning.

Colour was also measured in the white core of slices (Table 3.). In parallel with the pericarp, heat treatments only marginally affect slices colour. The observed differences were mainly due to minimal processing as both treated and control samples underwent the same colour evolution. Chroma was the only parameter slightly affected by heat treatments. Despite the fact that at the beginning of the storage period treated samples showed lower *c* values than control, from the second day onwards the three samples did not show significant differences ($P > 0.01$).

Table 2 Colour of minimally processed kiwifruits' outer pericarp during storage.

Storage Time	45 ºC / 25 min			45 ºC / 75 min			Control		
(day)	L	c	h	L	c	h	L	c	h
0	43,18 ^{ab}	19,00 ^{ab}	117,91 ^{a}	40,38 ^{bc}	15,94 ^{bc}	113,09 ^{abcd}	45,30 ^{a}	20,14 ^{a}	116,77 ^{ab}
2	37,53 ^{cd}	12,00 ^{cde}	110,70 ^{cde}	36,00 ^{d}	13,42 ^{cdef}	115,00 ^{abc}	40,19 ^{bc}	14,17 ^{cd}	110,70 ^{cde}
5	37,69 ^{cd}	10,86 ^{def}	112,11 ^{bcde}	37,56 ^{cd}	10,61 ^{f}	107,96 ^{e}	38,71 ^{cd}	12,83 ^{cdef}	109,01 ^{de}
7	38,38 ^{cd}	13,91 ^{ef}	112,88 ^{bcd}	37,04 ^{d}	11,60 ^{def}	112,01 ^{bcde}	40,29 ^{bc}	12,32 ^{def}	109,60 ^{de}
9	38,38 ^{cd}	10,42 ^{f}	107,48 ^{e}	37,31 ^{cd}	11,47 ^{def}	110,66 ^{cde}	37,81 ^{cd}	12,94 ^{cdef}	110,94 ^{cde}

For each colour parameter values followed by the same lowercase letter are not significantly different at $P < 0.01$, Fisher LSD Test.

Table 3 Colour of minimally processed kiwifruits' white core during storage.

Storage Time	45 ºC / 25 min			45 ºC / 75 min			Control		
(day)	L	c	h	L	c	h	L	c	h
0	65.10 ^{a}	20.65 ^{b}	109.24 ^{a}	65.21 ^{a}	19.88 ^{bc}	108.09 ^{a}	67.93 ^{a}	23.27 ^{a}	108.38 ^{a}
2	60.96 ^{b}	19.82 ^{bc}	105.58 ^{bc}	58.14 ^{bcde}	18.67 ^{bcd}	105.04 ^{bc}	58.62 ^{bcde}	19.28 ^{bcd}	106.25 ^{b}
5	60.32 ^{bc}	17.98 ^{cde}	103.32 ^{de}	59.01 ^{bcde}	19.26 ^{bcd}	102.73 ^{ef}	59.87 ^{bcd}	19.66 ^{bcd}	105.06 ^{bc}
7	57.20 ^{cde}	18.52 ^{bcde}	102.81 ^{ef}	56.68 ^{de}	17.55 ^{de}	102.29 ^{ef}	60.06 ^{bc}	18.43 ^{bcde}	104.47 ^{cd}
9	59.66 ^{bcde}	18.16 ^{cde}	101.78 ^{f}	58.62 ^{bcde}	18.20 ^{cde}	101.72 ^{f}	56.52 ^{e}	16.48 ^{e}	102.91 ^{ef}

For each colour parameter values followed by the same lowercase letter are not significantly different at $P < 0.01$, Fisher LSD Test.

Limited information is available about colour changes of kiwifruits' white core. Rocculi *et al.* [9] measured colour changes of kiwifruit white core by image analysis and conclude that just a modified atmosphere with N_2O could minimize the occurrence of brown area, which represented more than 90% in control after 4 days. In our work, colour was evaluated both by the tristimulus reflectance method and by sensory analysis but such noticeable changes were not detected.

The influence of the application of mild heat treatments in kiwifruit firmness is shown in Fig. 2.

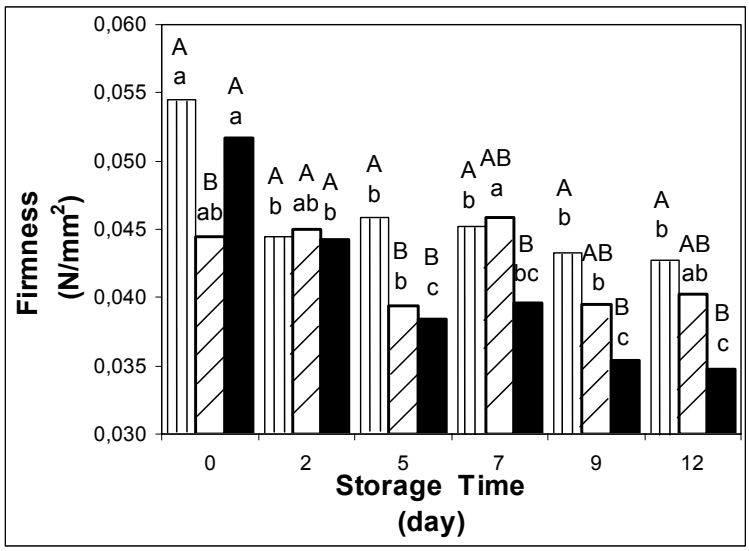

Fig. 2 Effect of heat treatment and storage time on firmness of minimally processed kiwi during storage. Above each bar, different uppercase indicate significant differences at each time for different samples, while different lowercase indicate significant differences of each sample at different times (Fisher LSD). Bar with vertical lines 45 ºC / 25 min; bar with slanting lines 45 ºC / 75 min; filled bar Control

It is apparent that all samples evidenced a gradual softening along the 12-day storage period. Nevertheless, in control samples, the firmness decrease was more pronounced than in treated samples. Immediately after minimal processing, samples treated at 45 ºC during 75 min showed a lower firmness (≈ 15 %) than the other two, but this value was almost constant until the end of the storage period. At the second day of storage no significant differences were found between the three samples. After 5 days, control samples continued evidencing a faster softening pattern while samples subjected to treatment at 45 ºC / 25 min preserve the firmness of the second day of storage. After 12 days of storage, control samples showed a reduction of

firmness of about 49 % while samples treated at 45 ºC / 25 min exhibit a softening of 22 %, in comparison with the raw material.

Firmness is the most limiting parameter in quality maintenance of kiwifruit slices. Agar *et al.* [8] conclude that the hurdle effects of storage temperature, ethylene scrubbing, calcium treatments and modified atmosphere, could minimize kiwifruit softening. However, even the most effective treatments used lead to a decline in firmness values between 25% and 50 % of initial raw material. Rocculi *et al.* [9] also describe positive effects on firmness preservation of kiwifruit slices by packaging atmospheres with argon or nitrous oxide. Slices underwent a firmness decrease about 10% after 8 days of storage and a 36% decrease after 12 days. As pre-treatment, the application of 1-MCP to whole kiwifruit results in a firmness decrease of just 12.5% in fruit halves, after 10 days of storage [35].

The firming effect of moderate heat pre-treatments in kiwifruit slices had already been observed in previous work [12], and it was supposed that the preservation of firmness was due to the activation of pectin methylesterase (PME) (EC 3.1.1.11) and subsequent formation of calcium pectates. In our results the analysis of pectic polymers (Fig. 3) and free and linked calcium (Fig. 4) support this hypothesis. In control samples, the period of faster softening was accompanied by a marked increase in soluble pectin content. From the fifth day and until the end of storage, the level of those polysaccharides remained constant but tissue softening proceeds. In heat-treated samples, the levels of soluble pectin remain constant during the same period. Samples heated during 75 min showed at the beginning a higher level of soluble pectin, explaining the lower firmness, but this value was maintained until the end of storage. The prolonged heat treatment promotes the release of strongly linked polysaccharides; therefore, this sample also exhibits higher content of total pectin, at the beginning of storage (Fig.3).

In samples treated during 25 min, soluble pectin just began to increase after 5 days storage. Despite the differences observed in firmness, at the end of storage, all samples showed similar levels of soluble pectin.

Varoquaux *et al.* [6] already suggested that the texture breakdown of kiwi slices is probably due to enzyme catalysed hydrolysis of cell wall components.

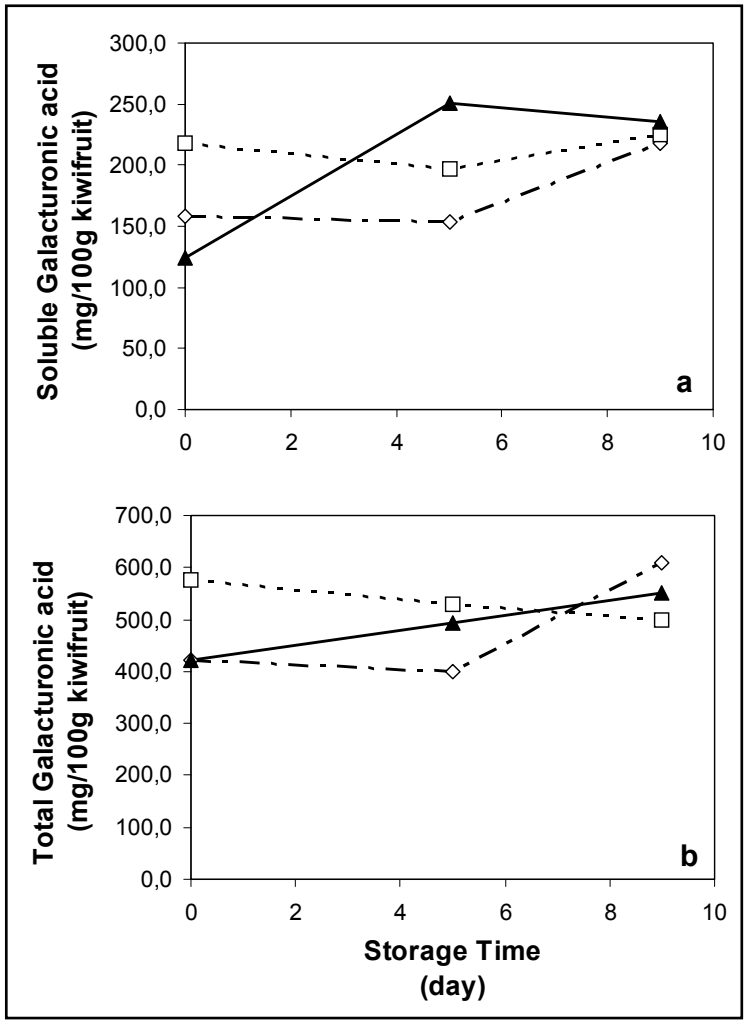

Fig. 3 Effect of heat treatment and storage time on (a) soluble galacturonic acid and (b) total galacturonic acid of minimally processed kiwifruit during storage. Open diamond 45 ºC / 25 min; open square 45 ºC / 75 min; filled triangle Control.

One day after heat treatments, both treated samples showed similar levels of free calcium, about 7-9 % lower than control samples, suggesting that in heat treatments of samples calcium pectates formation occurred.

At the end of 9 days packaging all samples showed similar levels of free calcium. During the storage period the progressive PME activity induced by slicing allows, in control samples, the formation of calcium pectates as well. Nevertheless, this slower process was superseded by a faster and stronger softening of samples as observed.

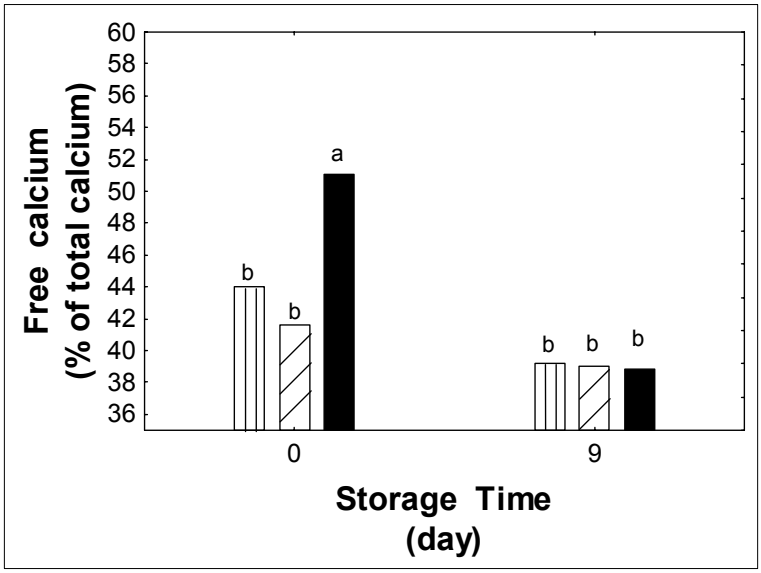

Fig. 4 Free calcium (% of the total calcium in samples) of minimally processed kiwifruit. Bars with the same lower case are not significant different at 95%. Bar with vertical lines 45 ºC / 25 min; bar with slanting lines 45 ºC / 75 min; filled bar Control.

Despite the fact that PME activity was not measured during and after the heat treatments, the results seem to prove that those heat treatments promote PME activation allowing cross linking between endogenous calcium and demethylated galacturonic acid chains from pectin polymers in the cell wall and middle lamella.

Zhang and Chen [38] showed that soaking treatments influence the texture of green eggplants and optimum results were obtained at 52.6 ºC for 18.9 min in a NaCl solution at 0.224 mol/dm^3. In eggplants a progressive activation of PME was clear between 30 ºC and 65 ºC, but the enzyme was stable at 50 ºC for 20 min. When incubated at 60 ºC, the residual activity decreased significantly during the first 10 min of incubation, but the residual activity decreased slightly as incubation time progressed, perhaps due to different isoforms of PME present, with different thermal resistances.

Vicente *et al.* [39] refer that air heat treatments at 45 ºC for 3 h delay strawberries' softening by enhancing PME activity, diminishing endo-1,4-β-D-glucanase, β-xylosidase, β-galactosidase and polygalacturonase activities, delaying hemicellulose degradation and pectin solubilization.

Luna-Guzmán *et al.* [17] describe as well, for cantaloupe melon, the firming effect of heat treatments at 20, 40 and 60 ºC in association with dips in 2.5 % calcium chloride solutions and, therefore it may be possible that the observed effect in kiwi slices quality maintenance could be enhanced by the application of calcium.

Fructose and glucose were the main sugars in analysed kiwifruit (Fig. 5). Fructose (≈ 53 %) was present in slightly larger amounts than glucose (≈ 38 %). Sucrose is also present but at lower levels (≈ 9%).

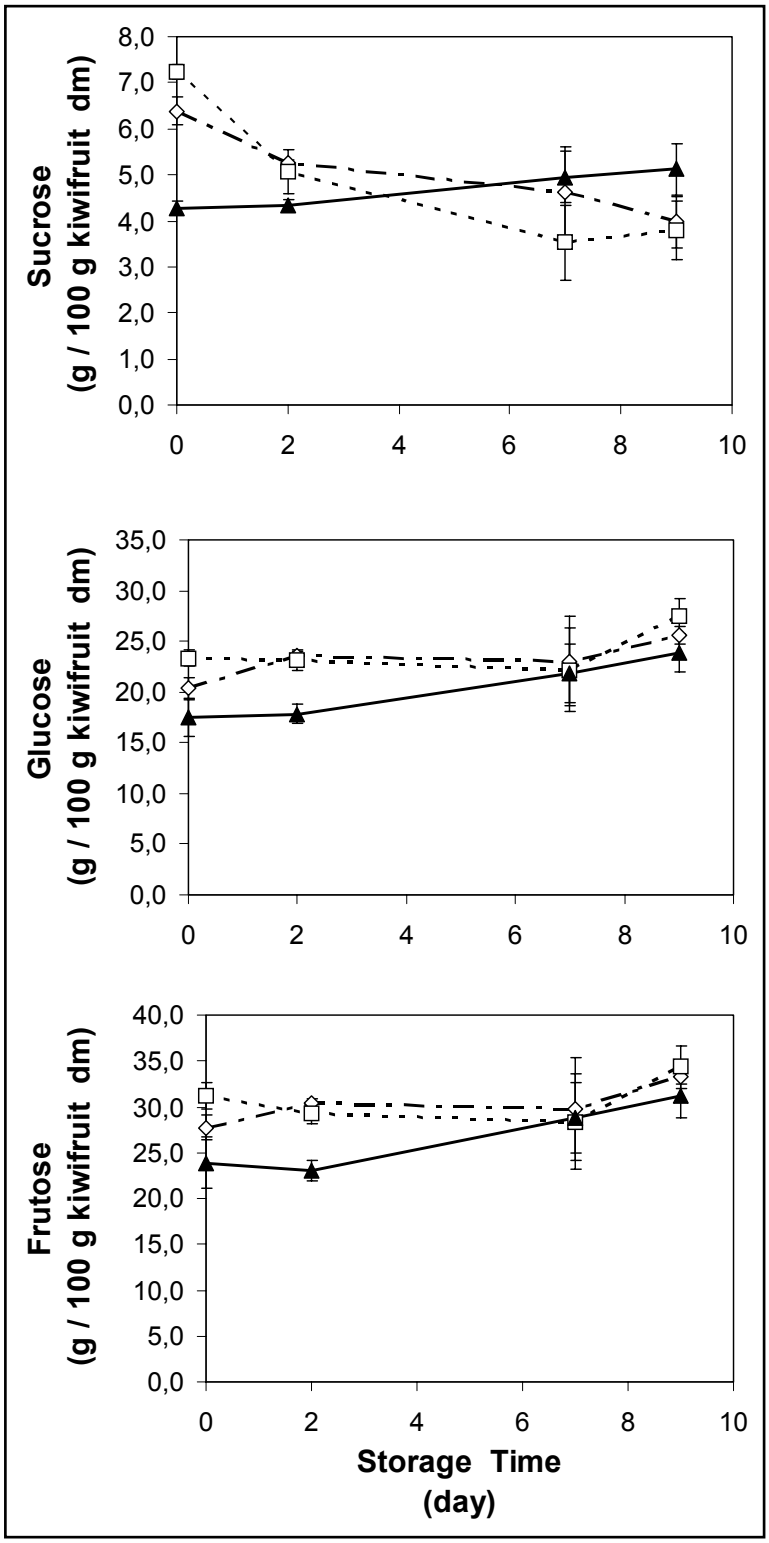

Fig. 5 Effect of heat treatment and storage time on sugars content of minimally processed kiwi during storage. Open diamond 45 ºC / 25 min; open square 45 ºC / 75 min; filled triangle Control. Each data point represents mean ± SE. Bars not revealed are hidden with symbols.

Other authors [8, 10, 40] refer a similar relation between these different sugars found in kiwifruit. Both heat-treated samples showed higher ($P < 0.05$) levels of sucrose than control samples that retain the initial content of sucrose during all storage period, indicating that this enhancement was due to heat treatment rather than to minimal processing.

Despite the results demonstration of a clear connection between heat treatment and sucrose content increase, the mechanisms involved are somewhat unknown. The hydrolysis of larger polysaccharides by the heat treatments is a possibility which should be considered. On the other hand, the activation of enzymes responsible for sucrose synthesis, like sucrose phosphate synthase (SPS) and sucrose synthase, as response to stress imposed by heat treatment is also possible. The activity of these enzymes barely changes before and during the period of rapid sugar increase but it is known that a competition between sucrose synthesis and degradation exists during postharvest ripening [41]. Langenkämper *et al.* [42] report that SPS mRNA increases in kiwifruit in response to ethylene. It is possible that heat treatment led to a higher production of ethylene, contributing as well to sucrose synthesis.

In the first 2 days both samples exhibit a decline in sucrose concentration, to levels similar to control. As fructose and glucose concentration remained identical in the same period, the assumption of disaccharide hydrolysis is improbable. As already observed, heat treatments lead to a rise in respiration rate, confirmed by the greater accumulation of CO_2 inside packages. The decline in sucrose level may suggest that this sugar was consumed as respiration substrate.

In control samples glucose and fructose content increased ($P < 0.05$) from day 7, revealing the occurrence of hydrolysis, but sucrose content remained at the same, probably due to continued ripening.

The acids analysed in samples were quinic, citric, malic and ascorbic (Fig. 6). Quinic acid is the one present in major amount (≈ 53 %) followed by citric acid (≈ 42 %). L-malic acid is present in about 2 % of the total acid amount and L-ascorbic acid, the most active form of vitamin C [43], represents about 3 % of total acids.

Some authors have referred different levels of these acids in kiwifruit [8, 10, 44]. The nutrient content of fruits is subject to variation within a species but there are also differences due to environmental and cultural practices [45, 46]. Storage temperature of whole fruit can change the balance of quinic, citric and L-malic acids in the fruit [44].

Heat-treated samples showed higher levels of all analysed organic acids, at the beginning of the storage period.

As consequence of minimal processing, L-malic acid content decreases during the first two storage days, which may be due to the more pronounced respiratory activity for heat-treated fruits. Afterwards, the three samples showed a progressive decrease but with no significant ($P > 0.05$) differences between each other.

Citric acid contents were higher ($P < 0.05$) than control in samples treated, respectively during 25 min and 75 min, for 2 and 5 days. Thereafter, all samples maintained equal and unchanged values until the end of storage. A similar pattern was found for quinic acid. As quinic acid is the one responsible for the "Hayward" flavour [44], the maintenance / increment of its content may be desirable for the maintenance of flavour profile during storage.

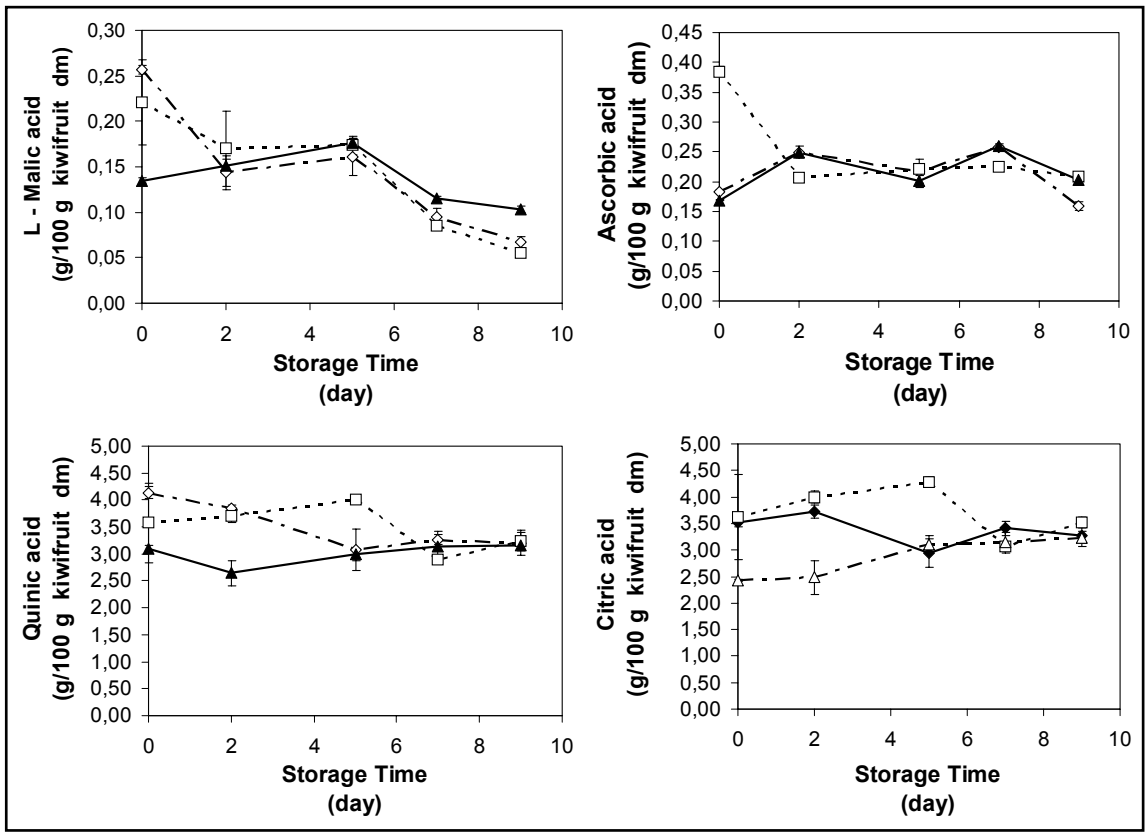

Fig.6 Effect of heat treatment and storage time on acids content of minimally processed kiwi during storage Open diamond 45 ºC / 25 min; open square 45 ºC / 75 min; filled triangle Control. Each data point represents mean ± SE. Bars not revealed are hidden with symbols.

For both of these acids, control samples evidenced no significant differences ($P > 0.05$) during storage. At the end of the storage period, acids levels are similar for all samples.

The stress imposed by minimal processing led to ascorbic acid synthesis, which is confirmed by the significant ($P < 0.05$) increase in its content in samples at the second day of storage. The antioxidant role played by L-ascorbic acid is what makes this small molecule a crucial component of the plant response to different stress agents [47]. The marked increased observed in samples treated at 45 ºC / 75 min was due to the higher stress caused by a longer heat treatment. All recent publications seem to indicate that multiple L-ascorbic acid biosynthetic pathways are functioning in plants. However, definitive proof of their occurrence must be obtained either by using quantitative radiolabelling studies or by reverse genetics [48]. Nevertheless, sample heat treated for 75 min showed higher soluble pectin content, as can be seen in Fig.3. It is known that D-galacturonic acid is metabolized to L-ascorbic acid-6-14C by an inversion pathway in detached ripening strawberry fruit. In this pathway, pectin-derived D-galacturonic acid is reduced to L-galactonic acid, which in turn is spontaneously converted to L-galactono-1,4 lactone. This compound is the substrate of the L-galactono-1,4-lactone dehydrogenase enzyme, producing L-ascorbic acid [48].

However, the benefit of the treatment from nutritional point of view was lost, since from the second day the vitamin content drops to level similar to other samples. Despite the higher levels of CO_2 inside packages, from that moment and until the end of storage, ascorbic acid content was maintained in all samples. The stability of ascorbic acid in kiwifruit slices can be partially justified by the pH of the fruit [45].

Our results are in agreement with others for citrus segments [49], cactus pear [50] and Golden delicious apples [51] in which ascorbic acid content was marginally affected by minimal processing, but not with those of [8] that report a total ascorbic acid progressive loss for kiwifruit slices during 12 days of storage, either packed in air or under various modified atmosphere packaging. Fergunson and MacRae [52] reported that the content in total ascorbic acid in *Actinidea* fruits, including *Actinidea deliciosa*, showed little or no decline during postharvest ripening.

The effect of heat treatments in ascorbic content is also ambiguous. The application of an air heat treatment of 50 ºC for 2 h slowed the decrease in ascorbic

acid content of broccoli florets [53], but the applications of air heat treatments at 34 and 38 ºC led to higher losses in ascorbic acid than for non-treated tomato fruits [54].

Principal components analysis allowed a better understanding of interactions between objective and sensorial measurements. The two first principal components (PC's) explained 73.32% of total variance. As can be seen in Fig.7, there is a strong correlation between sensorial parameters of firmness, typical aroma and taste and objective measurements like firmness, sucrose and L-malic contents, all positively associated with the PC1. It can be stated that these objective measurements are related to freshness attributes.

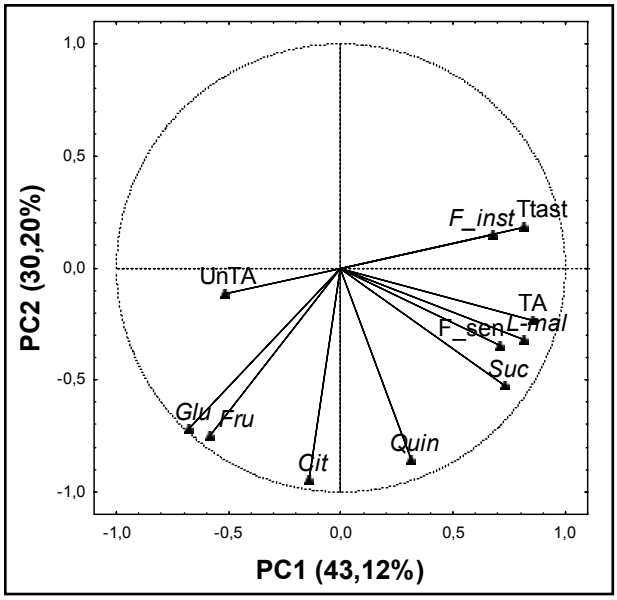

Fig.7 Principal components comparison of variables: aroma - typical (TA) and untypical (UnTA), typical taste (Ttast), firmness – sensorial (*F_sen*) and instrumental (*F_inst*), and chemical composition – glucose (*Glu*), fructose (*Fru*), sucrose (*Suc*), citric acid (*Cit*), L-malic acid (*L-mal*) and quinic acid (*Quin*).

After the observed correlations a new analysis was performed using only the objective parameters data. The two first PC explained 84.02% of the total variance of the data. Nevertheless, firmness, a critical quality parameter in minimally processed kiwifruit, has a high correlation with PC3 (9.37%), and therefore can be useful in samples' differentiation. Fig. 8 and Fig. 9 show the projection of analysed variables and samples in the space of PC1, PC2 and PC3.

From Fig. 8 it can be observed that samples from day seven onward are correlated with higher concentration of simple sugars, denoting some polysaccharide hydrolysis. On the other hand, heat treated samples until the fifth day of storage, are

correlated with higher content of quinic and citric acid maintaining the flavour of fresh fruit.

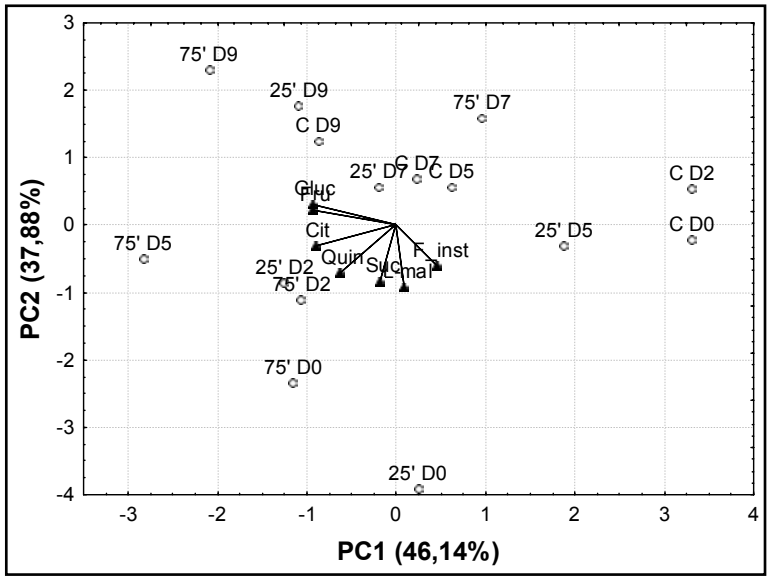

Fig.8 Principal components comparison (PC1 *vs* PC2) of firmness instrumental (*F_inst*) and chemical composition – glucose (*Glu*), fructose (*Fru*), sucrose (*Suc*), citric acid (*Cit*), L-malic acid (*L-mal*) and quinic acid (*Quin*). Samples: Heat treated 25 and 75 minutes (*25', 75'*respectively), control (*C*). 0, 2, 5, 9 days of storage - *D0, D2, D5, D9*, respectively.

The projection of PC also permits the confirmation that both heat treatments applied led to a better maintenance of firmness. In Fig. 9 the correlation of the heat treated samples during almost all storage period with firmness is apparent.

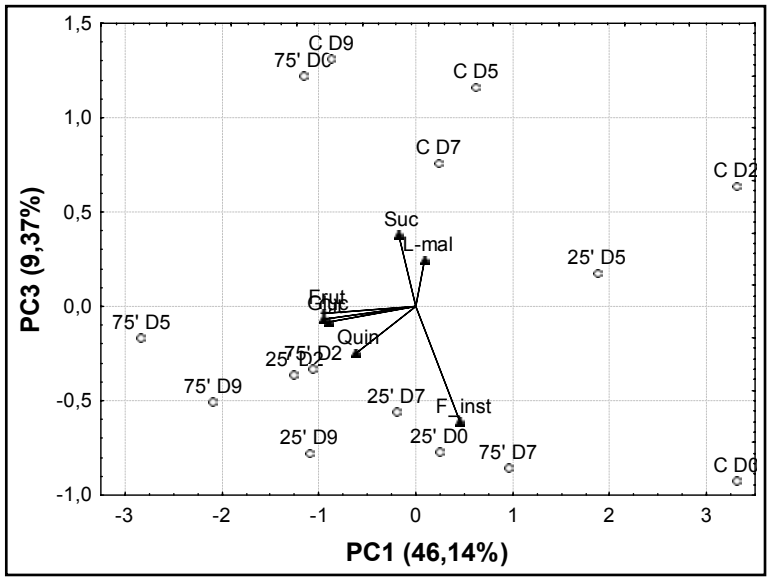

Fig.9 Principal components comparison (PC1 *vs* PC3) of firmness instrumental (*F_inst*) and chemical composition – glucose (*Glu*), fructose (*Fru*), sucrose (*Suc*), citric acid (*Cit*), L-malic acid (*L-mal*) and quinic acid (*Quin*). Samples: Heat treated 25 and 75 minutes (*25', 75'*respectively), control (*C*). 0, 2, 5, 9 days of storage - *D0, D2, D5, D9*, respectively.

4 Conclusions

Since softening is the crucial problem in sliced kiwifruit quality, the application of moderate heat pre treatments to whole kiwis allowed firmness preservation of the minimally processed fruit. Despite the fact that both heat treatments maintained the saleable quality of minimally processed kiwifruit for 9 days at 4 ºC, treatment at 45 ºC for 25 min maintained a better quality of the commodity. The mechanism involved in the process was the formation of calcium pectates from demethylated galacturonic acid chains and endogenous calcium, enhanced by heat treatment. Significant ascorbic acid loss was not observed in sliced fruit. On the other side it is noteworthy that an increment in this vitamin content was observed after heat treatment; it is nevertheless necessary to use supplementary techniques in order to preserve the concentration of biologically active compounds that tends to diminish.

Heat treatments at 45 ºC seem to be a "clean technology" useful in quality preservation of minimally processed kiwifruit.

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Capítulo III

Aplicação de cálcio na conservação de kiwi minimamente processado. Sinergias com tratamentos térmicos.

No presente capítulo é avaliada a eficácia da aplicação de cálcio na manutenção da firmeza de kiwi minimamente processado. Após se verificar a importância que a aplicação de tratamentos térmicos moderados apresenta na preservação de firmeza de kiwi fatiado foi testado o efeito sinérgico entre estes dois tratamentos. A aplicação de cálcio foi efectuada no fruto inteiro em simultâneo com o tratamento térmico, ou no fruto fatiado durante o processamento mínimo. Foram avaliadas as alterações físicas, químicas, sensoriais e estruturais sofridas pelo fruto.

The effect of calcium dips combined with mild heating of whole kiwifruit for fruit slices quality maintenance

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Abstract

The effect of moderate heat treatment combined with calcium dips on the quality of minimally processed kiwifruit was studied. Whole fruits were treated for 25 min at 45 ºC by dipping in deionised water or $CaCl_2$ solutions (1%, 2% and 3%(w/v)) and cooled to 4 ºC. Twenty-four hours later fruits were peeled, sanitized, cut into slices and packed. The firmness of kiwifruit slices' was subsequently evaluated during 8 days of storage. Calcium content, pectinmethylesterase activity and heat shock proteins accumulation were also investigated. Heat treatment conduced in water induced a firming effect and avoid softening of fruit slices while calcium dips had a marginal effect on this parameter. A calcium loss was observed due to dip treatment, but this effect was minimized when treatment was conduced in 3% $CaCl_2$ solution. The firming effect provided is due to the activation of pectinmethylesterase and the presence of calcium in treatment solution reduces or inhibits enzyme activation. Under the tested conditions, no heat shock proteins *de novo* synthesis was detected.

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Keywords:
Kiwifruit, mild heat treatments, calcium, pectinmethylesterase, minimal processing.

1 Introduction

Pre and postharvest calcium application has been demonstrated to produce beneficial effects on whole fruit quality, decreasing the incidence of physiological disorders (Gerasopoulos & Drogoudi, 2005; Manganaris, Vasilakakis, Diamantidis, & Mignani, 2007; Serrano, Martínez-Romero, Castillo, Guillén, & Valero, 2004), mould growth (Naradisorn, Klieber, Sedgley, Scott, & Able, 2006) and delaying softening (Antunes, Panagopoulos, Rodrigues, Neves, & Curado, 2005; García, Herrera, & Morilla, 1996; Naradisorn *et al.*, 2006).

Changes in texture occur due to changes in the chemistry of the primary cell wall components cellulose, pectins, and hemicelluloses which occur during growth and development (Sams, 1999). Calcium is directly involved in strengthening plant cell walls through its ability to cross link with carboxyl groups of polyuronide chains of pectins found in the middle lamella (Lara, García, & Vendrell, 2004; Sams, 1999).

Calcium ions help in the stabilization of cell membranes (Picchioni, Watada, Conway, Whitaker, & Sams, 1995) and cell turgor pressure can also be affected (Mignani *et al*., 1995), decreasing fruit softening.

Calcium salts have also been used to preserve the quality of minimally processed commodities. Calcium chloride dips allowed firmness retention of fresh-cut watermelon for 7 days of storage (Mao, Jeong, Que, & Huber, 2006) and improved firmness of fresh-cut strawberries (Aguayo, Jansasithorn, & Kader, 2006). If combined with 1-methylcyclopropene under controlled atmosphere it also slowed down the loss of appearance quality, changes in titrable acidity and microbial growth of strawberry wedges (Aguayo *et al.*, 2006). Fresh-cut 'Kensington' mango evidenced a smaller softening rate when treated with 3% CaCl2 however combining calcium application with low oxygen atmosphere was found to be the most effective treatment for extending the shelf-life of mango slices (Souza, O'Hare, Durigan, & Souza, 2006). Quality of fresh-cut cantaloupe (Luna-Guzmán & Barrett, 2000; Luna-

Guzmán, Cantwell, & Barrett, 1999) and honeydew (Saftner, Bai, Abbott, & Lee, 2003) melons was improved with calcium chloride, calcium lactate, calcium propionate and calcium amino acid chelate dips. However, post-cutting dips of ascorbic acid and calcium lactate slightly extended the shelf-life of peach and nectarine slices but the overall advantages of such treatments were marginal (Gorny, Hess-Pierce, & Kader, 1999).

Moderate heat treatments, applied to whole fruit, have been demonstrated to alter fruit physiology and biochemistry being helpful in quality maintenance of fresh-cut kiwifruit (Beirão-da-Costa, Steiner, Correia, Empis, & Moldão-Martins, 2006; Beirão-da-Costa *et al.*, 2006), cantaloupe melon (Lamikanra, Bett-Garber, Ingram, & Watson, 2005), apples (Barrancos *et al*., 2003), pears (Abreu, Beirão-da-Costa, Gonçalves, Beirão-da-Costa, & Moldão-Martins, 2003) and peaches (Steiner *et al*., 2006). This kind of treatments can lead to changes in respiratory and ethylene production rates, enzymatic activity, and in cuticle structure, together or not with protein synthesis, such as heat shock proteins (HSP) (Paull & Chen, 2000; Pavoncello, Lurie, Droby, & Porat, 2001).

Post-cut calcium treatments, when conducted at higher temperatures, seem to be more effective. Calcium chloride combined with heat treatment was effective in preserving the quality of fresh-cut mango cubes (Trindade *et al.*, 2003). A wash treatment solution of 1.5% calcium lactate at 50 ºC for 1 min was the best treatment in inhibiting browning and preserving firmness of minimally processed lettuce, by reducing the loss of turgor pressure and enhancing pectinmethylesterase (PME) activity (Martin-Diana *et al.*, 2005, 2006).The firming effect provided by dipping fresh-cut cantaloupe melons in 2.5% calcium chloride was improved when combined with higher dip temperatures (1 min at 60 ºC) but dip temperature did not affect fruit metabolism (Luna-Guzmán *et al.*, 1999). However, other authors refer that at lower temperatures (4 ºC during 3 min), calcium treatment appears to be beneficial for the storage of cut cantaloupe melon, reducing respiration rate and moisture loss, compared to treatment at room temperature (25 ºC during 3 min) (Lamikanra & Watson, 2004).

The combined effect of moderate heat treatments and calcium dips on the whole fruit is scarcely described. To the best of our knowledge, only one published work considered the biochemical effects of whole cantaloupe melon heat treatment, which

was performed during 60 min at 60 ºC in a 1% calcium lactate solution on fresh-cut fruit during storage (Lamikanra & Watson, 2007).

The objective of our research was to investigate the effects of moderate heat pre-treatments conducted in calcium solutions, on kiwifruit slices firmness and fruit response mechanisms.

2 Materials and methods

2.1 Fruit preparation

Unripe kiwifruit (*Actinidia deliciosa* (A Chev) Liang et Ferguson var deliciosa cv Hayward), without any wound signals, were purchased at a local market, selected for uniform size, and stored at 4 ºC prior pre-treatments and processing. Whole kiwifruits were subjected to a previously optimized heat treatment at 45 ºC during 25 min (Beirão-da-Costa *et al*., 2006) in 1%, 2% and 3% (w/v) calcium chloride solution. Non-heat treated and heat treated in deionised water fruits were used as control samples. Treatment time was recorded since the core of the fruit achieves 45 ºC.

After overnight storage, fruits were hand peeled, sanitized in chlorine water with 125 mg/L free Cl^-, supplied as sodium hypochlorite at pH 6, gently dried with blotting paper, cut in ≈1.5 cm slices, packed and stored at 4 ºC.

2.2 Firmness analysis

Firmness was evaluated by a puncture test on kiwi slices flesh using a TA-XT Plus texture analyser from Stable Micro Systems with a 5 kg load cell. Firmness measurements were taken as the peak load values obtained from a test in which a 4 mm diameter stainless steel probe penetrates the fruit by 4 mm at a crosshead speed of 1 mm s^{-1}. Mean values were calculated from results of 20 measurements in different slices for each sample.

2.3 Pectinmethylesterase (PME) extraction

Twelve grams of kiwifruit outer pericarp (without seeds) was homogenised with 40 mL of cold 1.5 M NaCl in a T25 basic, IKA LABORTECHNIK homogeniser. The homogenate was incubated with agitation for 30 min at 4 ºC and centrifuged at 15000*g* for 10 min, at 4 ºC. The resulting supernatant was used for PME activity assay.

2.4 Pectinmethylesterase assay

PME activity was assayed titrimetrically using a pH electrode measuring the H^+ produced by the carboxyl groups released by the hydrolysis of methyl esters of pectin, using the method described by (Kimball, 1991) modified. One hundred millilitres of substrate solution (0.25% (w/v) citric pectin in 0.2 M NaCl) was mixed with 5 mL of PME extract and the pH adjusted to 7.5 with 1 M and 0.05 M NaOH. After the pH reached 7.5, 0.2 mL of 0.02 M NaOH was added and the time required to reach pH 7.5 again was recorded. PME activity was measured at 25 ºC, as a reference temperature and at 45 ºC, the temperature used in heat treatment. One unit of PME activity was defined as the amount of enzyme that can cause the released of 1 μmol of COO^- per gram of fresh tissue and per minute (μmol g^{-1} min^{-1}).

Heat stability of PME from extracts of kiwifruits treated at the several conditions was evaluated by monitoring enzyme activity at 45 ºC for around 30 min. All values are the mean of three replicates.

2.5 Calcium analysis

Two grams of fruit outer pericarp (without seeds) selected from different slices, were ashed at 550 ºC. Ashes were suspended in 10 mL of HCl 3 N for 24 h, deionised water was added to a total volume of 50 mL and the mixture filtered through ashless filter paper. Strontium chloride was added to final solutions to control ionization interferences. Ca^{2+} concentration was determined at 422.7 nm using an atomic absorption spectrophotometer (Pye-Unicam SP9).

2.6 Heat shock proteins (HSP) analysis

Thirty grams of fruit outer pericarp (without seeds) selected from different fruits were homogenised with 100 mL of acetone, in a T25 basic IKA LABORTECHNIK

homogeniser, vacuum filtered and the residue was dried at 25 ºC. Crude protein extract was obtained using 500 mg of this residue macerated with 5 mL of 100 mM

Tris–HCl buffer (pH 7.5), containing 3 mM dithiothreithol (DTT) (Sigma) and 1 mM ethylenediamine-tetracetic acid (EDTA) (Pharmacia). The homogenate was centrifuged at 10000g for 20 min. Partial purification of protein fraction was performed using an Amicon centrifugal filter (microcon YM 10, Millipore) and centrifugation at 10000g for 30 min. Sample denaturation was performed using 0.05 M Tris–HCl buffer containing 2.5% SDS, 5% 2-mercaptoethanol and 12% glycerol and boiled for 15 min before SDS–PAGE.

SDS–PAGE was performed in a 10% polyacrylamide (Pharmacia) and 2.6% BIS gel slabs with 1% SDS (Pharmacia) in a vertical system (Midget Electrophoresis Unit LKB 2050) with refrigeration at constant 60 mA and maximum 250 V in 100 mM Tris–glycine buffer (pH 8.3) (Martins, Mourato, & Mendonça, 2002). Molecular weight standards from 14.4 to 94 kDa (Pharmacia) were also run. Staining was done using a silver staining kit (Pharmacia).

2.7 Statistics

To evaluate significant differences among samples with time an analysis of variance (factorial ANOVA) was performed together with Fisher0s LSD mean comparison test, using the ‘‘Statistica” v. 6.1 software from Statsoft, Inc.

3 Results and discussion

3.1 Firmness evaluation and calcium analysis

The effects of applied treatments on firmness are shown in Fig. 1. Heat treatment had a firming effect on slices during 8 days of storage while the presence of calcium ions in the dip treatment solution evidenced only a marginal effect. After minimal processing, no significant differences ($P \geq 0.166$) were observed among calcium heat

treated fruits (HT1%, HT2% and HT3%) and unheated control (Control). In contrast, heat treatment in water (HT) significantly (P = 0.004) increased slice firmness, 24% higher than unheated samples. Increasing calcium concentration in solution minimizes the firming effect of heat treatment, in the beginning of storage.

After 3 days control samples underwent faster softening, attaining a 64% of the initial firmness value, and being significantly different from the heat-treated samples.

Similar results were described by Lamikanra and Watson (2007) for cantaloupe melon. These authors concluded that the presence of calcium in the treatment water did not profoundly alter fresh-cut cantaloupe melon texture relative to treated fruit at 60 ºC for 60 min without added calcium, during storage.

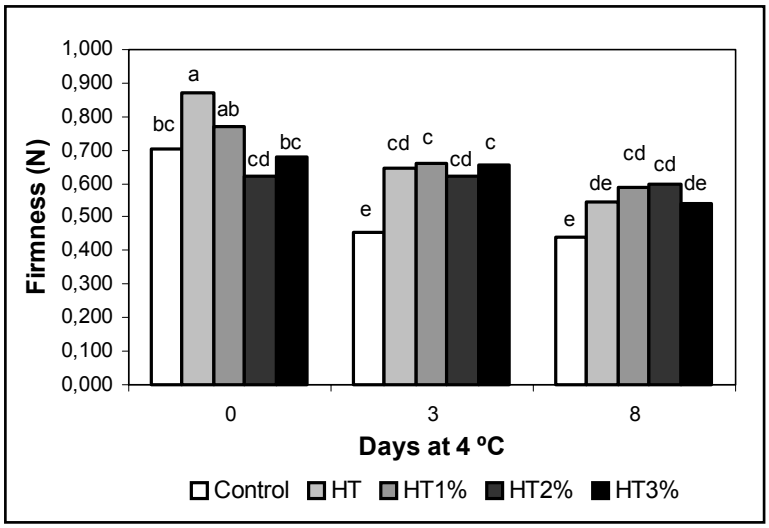

Fig.1. Firmness evolution of kiwifruit slices during storage. Whole fruits were unheated (Control) or heat treated in water (HT), 1% CaCl2 (HT1%), 2% CaCl2 (HT2%) and 3% CaCl2 (HT3%). Above each bar different letters indicate significant differences between samples ($P < 0.05$).

García *et al*. (1996) reported that dip treatments in 4% $CaCl_2$ at 25 ºC lead to strawberries with lower firmness than control, for 3 days of shelf-life. Nevertheless, fruits treated in 1% $CaCl_2$ at 45 ºC showed significantly higher firmness than fruits treated at the same temperature without calcium, with differences noted after 2 days of storage.

Several published works refer that $CaCl_2$ dip could impart a bitter flavour to the fruit. Performed sensory analysis (data not shown) did not reveal the presence of any strange flavour in fruit slices.

Calcium analysis (Table 1) showed that applied treatments did not allow the diffusion of calcium into the fruit and instead lead to some loss of calcium ions. Control samples had significantly ($P < 0.01$) higher amounts of calcium than heat treated samples, with the exception of fruits previously treated with 3% of $CaCl_2$.

Manganaris *et al*. (2007) reported that calcium application in peaches by immersion of the whole fruit in calcium chloride, calcium lactate and calcium propionate solutions at two different calcium concentrations, for 5 min, lead to a flesh calcium increase by 50–74% compared to control fruits. $CaCl_2$ (1%) dips of whole strawberries lead to an increase in calcium content but heating at 45 ºC enhanced the penetration of ions into the fruit (García *et al*., 1996).

Table1. Calcium content of kiwifruit slices outer pericarp

	Calcium (mg / 100g)
Control	18.43 ± 0.60 [a]
HT	14.48 ± 0.71 [b]
HT 1%	15.46 ± 0.11 [b]
HT 2%	14.13 ± 0.44 [b]
HT 3%	16.31 ± 0.79 [ab]

Results are the mean ± SE of 6 measurements.
Values followed by the same lower case are not significantly different at 99 %.

In our experiments, the initial temperature gradient between the fruit (4 ºC) and the water/calcium solutions (45 ºC), despite the higher calcium concentrations in the bath, could render the ion exit easier. This process was minimized at 3% of $CaCl_2$ where probably the osmotic pressure of the solution balanced the effect of temperature gradient.

Lurie, Fallik, and Klein (1996) reported that heat treatment fills in cracks present in apple's epicuticular wax decreasing the ability of applied calcium to be transported into fruit tissue. In kiwifruit the thin brown skin covered with small hairs (trichomes) includes a periderm and hypodermal cells, comprising a thick layer of dead, radially compressed cells with suberized cell walls over the hypodermis. Parenchyma cells beneath the hypodermis gradually merge into the fleshy tissue that forms most of the outer pericarp (Crisosto & Kader, 1999; Hallett & Sutherland,

2005). It was this complex and closed structure that probably did not allowed the calcium flow into the fruit.

3.2 Pectinmethylesterase activity

Previous works have justified the firming effect resulting from heat treatments at low temperatures by activation of PME. PME activity of samples obtained in our study is shown in Table 2. It was found that increasing temperature in PME activity assay from 25 ºC to 45 ºC leads to a significant 2–3 fold increase in measured activity. At treatment temperature (45 ºC), the sample treated in water evidenced higher PME activity ($P < 0.03$) than samples treated in $CaCl_2$ solutions at all tested concentrations. Despite the lowest significance level ($P = 0.061$) it can be stated that PME activity is also higher in HT than in control fruits.

Table 2. PME activity of kiwifruit slices outer pericarp

	μmol COO^- g^{-1} min^{-1}	
	25 ºC	45 ºC
Control	2.88 ± 0.07 [ab]	7.35 ± 0.24 [ab]
HT	3.19 ± 0.32 [b]	9.00 ± 0.98 [b]
HT 1%	2.12 ± 0.17 [a]	5.86 ± 0.36 [a]
HT 2%	2.89 ± 0.10 [ab]	6.86 ± 0.41 [a]
HT 3%	3.60 ± 0.48 [b]	6.87 ± 0.47 [a]

Results are the mean ± SE of 3 measurements. In each column values followed by the same lower case are not significantly different at 95 %.

These results may explain why HT samples showed higher firmness values than the other samples. Moreover, in previous works (Beirão-da-Costa *et al*., 2006) whole kiwifruits subjected to heat treatment at 45 ºC during 25 and 75 min showed higher levels of linked calcium than non-heated fruits. Both results evidence that the formation of calcium pectates within the cell wall as consequence of heat treatment was the mechanism involved in firmness increment.

Similar results were obtained for strawberries where fruits heat-treated at 45 ºC for 3 h showed higher PME activity than the control, slowing down pectin solubilization by increasing the amount of putative sites for calcium bridge formation

within the cell wall, and higher firmness (Vicente, Costa, Martínez, Chaves, & Civello, 2005).

In previous works (Beirão-da-Costa, Steiner, Correia, Empis *et al.*, 2006, Beirão-da-Costa et al., 2006) it was established that, at 45 ºC, 25 min was the optimum time for quality preservation of kiwifruit slices, and so PME stability at 45 ºC as a function of time was also studied (Fig. 2). Analysis of variance shows the effects of the applied treatments (water, CaCl2 and control) and treatment time, in PME activity of kiwifruits' outer pericarp. A significant effect on enzyme activity was observed by the kind of applied treatment (P = 0.000), the duration of treatment (P = 0.003) and the interaction of the two effects (P = 0.000).

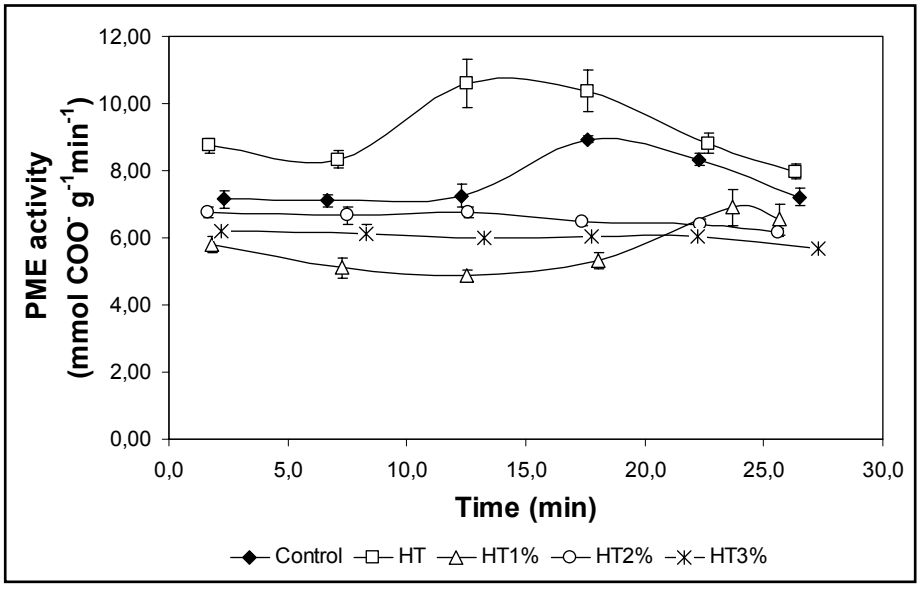

Fig.2. Effect of treatment time on PME activity of kiwifruit. Whole fruits were unheated (control) or heat treated in water (HT), 1% $CaCl_2$ (HT1%), 2% $CaCl_2$ (HT2%) and 3% $CaCl_2$ (HT3%). Vertical bars represent the *standard error* of the mean. Bars not revealed are hidden with symbols.

In all samples, there was a measurable PME activity during the whole period analysed. As can be seen in Fig. 2, PME activity on enzyme extract from control fruits was quite stable during the initial period of 10–15 min of the heating process; however after that, a significant (P = 0.003) increase in enzyme activity was recorded and maintained for 10 min more. In the authors opinion, despite the fact that in the assay the optimum enzyme activity pH was used, these results might indicate what is occurring during whole fruit heat treatment, and can explain why 25 min was the most suitable treatment time previously established.

When fruits were treated at 45 ºC in water, enzyme extract exhibits a significantly ($P \leq 0.001$) higher PME activity up to 20 min of heating time but a rapid increase in enzyme activity was observed after 5 min. After 20 min at 45 ºC PME activity underwent a decline to values similar to the ones at the beginning of the heating process. These results show that despite the long treatment time, moderate heat treatment applied to whole kiwifruit did not inactivate PME and on the other hand, that an irreversible activation seems to occur.

When samples were previously treated in $CaCl_2$ a decrease of PME activity was observed. These samples showed a lower but quite stable PME activity during the analysed heating period with exception of extracts from fruits that had been heat treated in 1% CaCl2 which showed a small increase in enzyme activity in the last minutes of measurement, although still significantly ($P = 0.008$) lower than control extracts.

Although the role of calcium and temperature as regulators of enzyme activity has been described, the results are still somewhat contradictory. Ni, Lin, and Barrett (2005) evaluated PME activity in leafy, fruit, root and flower vegetables and conclude that, with exception of red bell peppers, all present measurable enzyme activity is enhanced, for 30 min, at temperatures between 50 ºC and 70 ºC. Anthon, Blot, and Barret (2005) reported that diced tomatoes heated in water at 70 ºC showed a rapid methanol production, resulting from PME activity, in the first 10 min but both the initial rate and final extent of methanol production were increased if the tomatoes were heated in 0.5% $CaCl_2$ instead of water. Some authors report that temperature is the most important factor in raising PME activity on lettuce and calcium presence has no significant effect (Martin-Diana *et al*., 2005, 2006). There was no significant difference in PME activity between carrots treated with and without calcium at 25 ºC but carrots dipped in calcium lactate at 50 ºC showed higher PME activity (Rico *et al*., 2007). Whole peaches dipped in calcium salts also showed higher PME activity compared to control fruits but the differences were only detected after 4 weeks of storage (Manganaris *et al*., 2007).

Our results showed that despite the fact that no diffusion of calcium into whole kiwifruit was observed, heat treatment conducted in those solutions interfered with enzyme activity. It is hypothesized that $CaCl_2$ solutions promote the diffusion of several ions from the fruit to the external solution, changing the ionic balance in fruit cells and leading to internal conditions less favourable to enzyme activity.

3.3 Heat shock proteins analysis

One of the effects of heat treatments is the synthesis of small HSP with low molecular mass (16–20 kDa) able to protect the fruit from later injuries and stress. HSP are involved in heat stress, and might be important in the increase of plasma membrane resistance or in preventing membrane damage, due to their function as a protein protector under severe abiotic stress (Hall, 2002).

To the best of our knowledge, there are no published works reporting heat shock proteins accumulation in kiwifruit as consequence of thermal treatments. Fig. 3 shows the protein analysis of kiwifruit samples by SDS–PAGE. Electrophoresis gels analysis showed that kiwifruit exhibits several protein bands from 68 kDa to 10.5 kDa molecular weight but, despite the different intensity of some bands, no differences were detected in protein profile of samples. It seems therefore that heat treatment at 45 ºC for 25 min, either conducted in water or $CaCl_2$ solutions, did not promote HSP synthesis, within this molecular weight range, as it no novel bands were detected in heat-treated samples, compared to control. Another hypothesis is that the synthesis of HSP is in fact occurring but band overlap does not allow the identification of the different proteins with the same molecular weight, but only a two-dimensional electrophoresis could clarify this.

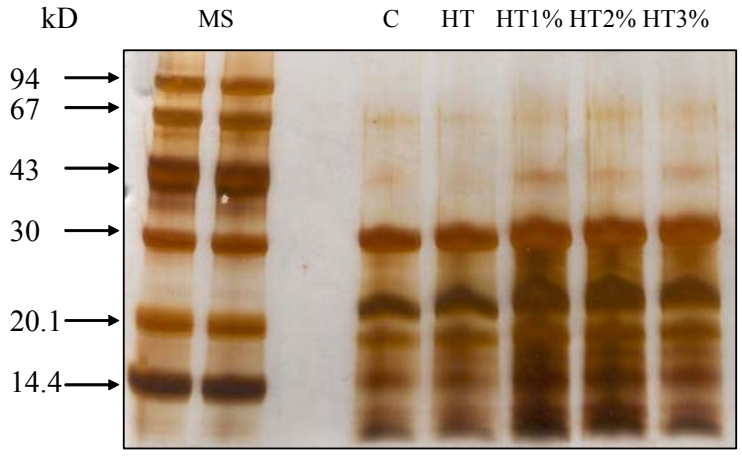

Fig. 3. Protein profile by SDS–PAGE of kiwifruit outer pericarp. Whole fruits were unheated (control) or heat treated in water (HT), 1% $CaCl_2$ (HT1%), 2% $CaCl_2$ (HT2%) and 3% $CaCl_2$ (HT3%). Exemplificative gel of the global protein profile.

Our results did not agree with others that report the expression of HSP in many horticultural products. For avocado fruit the optimal range for induction of HSP synthesis in mesocarp was found to be between 34 and 38 ºC and those proteins are maximally expressed after a heat shock at 38 ºC for 4 h (Florissen *et al*., 1996). Exposure of papaya to 38 ºC for 2 h leads to protein synthesis changes (Paull & Chen, 1990) occurring the same with apples held 4 days at 38 ºC. The new bands in electrophoresis gels correspond to proteins with low (14–22 kDa) and high (68 and 92 kDa) molecular weight (Lurie & Klein, 1990). A hot water brushing treatment (62 ºC for 20 s) induced the accumulation of several heat shock proteins probably involved in a complex fruit disease resistance mechanism (Pavoncello *et al*., 2001). Zhu, Ji, Lu, and Zhang (2003) reported that heat-treated mango at 55 ºC during 5 min accumulated three new polypeptides with molecular weights of 13.7, 15.7 and 15.7 kDa involved in chilling tolerance of mangoes. Lamikanra and Watson (2007) reported the development of two proteins bands, in isoelectric focussing, at pI 5.1 and 6.5 in fresh-cut cantaloupe pre-cut heat treated at 60 ºC for 60 min.

Conclusions

Hot water treatment at 45 ºC/25 min of whole kiwifruit is an effective methodology in quality maintenance of fruit slices, increasing fruit firmness and avoiding an excessive softening. The presence of calcium in dip solutions during heat treatment had a marginal effect in fruit firmness. Dip treatment leads to partial calcium loss from the fruit, this effect being minimized when treatment was conducted in 3% $CaCl_2$ solution. PME is activated by heat treatment but in the presence of calcium this effect is reduced or even inhibited. Therefore, it is tentatively concluded that firmness preservation is due to PME activation, though the degree of methylesterification of pectin for the different samples might permit a definite conclusion. No HSP in the range of 94–14.4 kDa were detected as a consequence of the applied treatments which leads us to think that these proteins were not involved in quality maintenance of kiwifruit slices. Although calcium presence did not induce benefits on the quality preservation of fruit slices, it also did not cause negative effects when present in the heat treatment solution.

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Structure and firmness of fresh-cut kiwifruit as affected by heat treatments and pos-cut calcium dips

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Structure and firmness of fresh-cut kiwifruit as affected by heat treatments and pos-cut calcium dips

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Abstract

The effect of pre heat treatment (45 ºC / 25 min) and post cut calcium dips in 1 g/L and 2 g/L $CaCl_2$ on metabolic activity and texture preservation of kiwifruit slices was studied. During a 9-day period, packages' atmosphere composition, slices' firmness, pectin content and sensory scores were determined. Histological observations of samples were also performed. Neither heat treatment nor calcium dips alone were effective in diminishing fruit respiration rate but the application of both treatments revealed a synergistic effect in metabolic activity reduction. Calcium dipped fruit slices showed better firmness preservation. Post-cut calcium dips, alone or combined with heat treatments, yielded fruits presenting higher insoluble pectin / total pectin ratio, indicating formation of calcium pectates. Heat treated fruits, even in the absence of calcium, also revealed insoluble pectin / total pectin ratio similar to calcium treated fruits, supporting, despite the lower firmness value, a more structured tissue, as observed in SEM photos. A good correlation was observed between sensory and physical-chemical parameters. The effectiveness of calcium treatment was equally observed for both tested concentrations during a 9-day shelf life period.

Keywords: Heat treatment, calcium dip, fresh-cut kiwifruit, structure and softening, SEM.

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1 Introduction

Minimal processing operations including peeling and cutting bring about incremented metabolic activity and critically limit fresh-cut fruit shelf-life. Cell disruption due to the cutting process allows exudation of pectinolytic enzymes from damaged tissues to diffuse to inner tissues, resulting in important texture losses (Varoquaux, Lecendre, Varoquaux & Souty, 1990; Soliva-Fortuny & Martín-Belloso, 2003).

Mechanical properties of fruit tissue depend, at the molecular level, of the interactions between constituting polymers but also from the architecture of tissue cells and their interactions, and from the arrangement of cells into tissue. Therefore different approaches derived from this structural hierarchy, should be used in the study of texture of minimally processed fruits (Jackman & Staley, 1995; Alzamora, Castro, Nieto & Salvatori, 2000).

Cell turgor pressure, viscoelasticity of the cell wall and middle lamella and plasmalemma hydraulic permeability are determinant for mechanical characteristics, so the changes of these parameters as result of processing will determine textural properties of fruit tissue (Alzamora *et al.*, 2000; Rojas, Gerschenson & Marangoni, 2001).

Pectin is a group of polysaccharides that are rich in anhydrogalacturonic acid, covalently linked to form a network throughout the primary cell wall matrix and middle lamellae, being one of the major constituents of these structures, and can account for one third of all primary cell wall macromolecules (Willats, McCartney, Mackie & Knox, 2001). In general, fruit softening is associated with disassembly of the primary cell wall, with solubilization and depolymerization of pectins (Alzamora *et al.*, 2000; Brummell, 2006). On the other hand, the demethylesterification of these chains by pectin methylesterase (PME) also affects the rigidity of the pectin network, as the structure becomes available to cleavage by *endo*-polygalacturonase (Brummell, 2006). If calcium is available the free carboxylic acid groups in the unesterified regions of the polygalacturonic acid chains can bind to this cation and cross-link, forming calcium pectates which strengthen the cell wall middle lamella

and create greater cell to cell adhesion yielding a firmer structure (Anthon & Barrett, 2006).

Moderate heat treatments promote the activation of PME and the resulting lesser degree of methylesterification of the pectins allows for more cross-linking between pectin polymers (Ni, Lin & Barrett, 2005; Martín-Diana, Rico, Barry-Ryan, Frías, Mulcahy & Henehan, 2005; Anthon & Barrett, 2006).

The effectiveness of those treatments can be improved by calcium addition. The combined effect of heat treatment and calcium dips on firmness preservation had been observed, among others, in fresh-cut cantaloupe (Luna-Guzmán, Cantwell & Barrett, 1999), ready-to-eat carrots (Rico, Martín-Diana, Frías, Barat, Henehan & Barry-Ryan, 2007) and diced tomatoes (Anthon, Blot & Barrett, 2005).

The objective of the present work was to evaluate the effects of moderate heat treatments and post-cut calcium dips in kiwifruit slices CO_2 production and firmness as well as to correlate mechanical properties changes with related chemical composition and histological observation.

2 Materials and methods

2.1 Raw Material

Kiwifruit (*Actinidia deliciosa* (A Chev) Liang et Ferguson var *deliciosa* cv Hayward) produced in the centre of Portugal (Bairrada region), were purchased at a local market, transported the laboratory and selected for uniform size and absence of wound signals. Fruits, at a ripe stage with total soluble solids of 10 % and a pH of 3.5, were stored at 4 ºC prior pre treatments and processing.

2.2 Fruit preparation

Whole fruits were subjected to heat pre treatment at 45 ºC for 25 minutes as described in previous works (Beirão da Costa, Steiner, Correia, Empis & Moldão-Martins, 2006; Beirão da Costa, Steiner, Correia, Leitão, Empis & Moldão-Martins, 2008b). After over-night storage, fruits were hand peeled, sanitized in chlorinated

water with 125 mg/L free Cl^-, supplied as sodium hypochorite at pH 6, and cut in about 1.5 cm slices.

Slices from heat treated and non heat treated fruits were dipped in 1 g/L and 2 g/L $CaCl_2$ solutions for 5 minutes, gently dried with blotting paper, packed in low-density polyethylene and vinylidene chloride bags with 3000-4000 and 11000-15000 ($mL/m^2/24h/atm$) of permeability to O_2 and CO_2, respectively and stored at 4 ºC. Heat and non heat treated fruits without calcium dips were used as controls. At each date of analysis three different bags per sample were evaluated.

2.3 Analytical measurements

2.3.1 Packages gas composition

Headspace gas samples were taken with a hypodermic needle through an adhesive septum previously fixed on the bags and analysed using a checkmate 9900 O_2/CO_2 gas analyzer (PBI-Dansensor, Denmark).

2.3.2 Calcium analysis

2 g of fruit outer pericarp (without seeds) selected from different slices, were ashed at 550 ºC. Ashes were suspended in 10 mL of HCl 3 mol/L for 24 h, deionised water was added to a total volume of 50 mL and the mixture filtered through ashless filter paper. Strontium chloride was added to final solutions to control ionization interferences. Ca^{2+} concentration was determined at 422.7 nm using an atomic absorption spectrophotometer (Pye-Unicam SP9).

2.3.3 Pectin analysis

Pectins were extracted from the alcohol insoluble material (AIM) obtained homogenising 20 g of fruit with 100 mL of boiling absolute ethanol. The homogenate was vacuum filtered, the residue washed with 80 mL/100 mL ethanol until discoloration and dried at 35°C ± 2 ºC. Polyuronides were isolated from AIM. 70 mg of AIM were homogenized in 150 mL of water and stirred for 2 hours at room temperature. The homogenate was centrifuged and the supernatant was labelled as water soluble pectin (SP). The pellet was resuspended in 150 mL of HCl (0.05 mol/L), incubated for 1h at 98 ºC under reflux and centrifuged. The supernatant was labelled as insoluble pectin (IP). All extractions were performed in triplicate.

Uronic acid concentration of both fractions was estimated by *m*-hydroxydiphenyl method (NF V05 – 128, 1984) and expressed as galacturonic acid.

2.3.4 Firmness

Firmness was evaluated by a puncture test on kiwi slices flesh using a TA-XT Plus texture analyser (*Stable Micro Systems*) with a 5 kg load cell. Firmness measurements were taken as the medium force value obtained during the test to a stainless steel probe with 4 mm diameter penetrate the fruit 4 mm, at 1 mm/s. Mean values were calculated from results of at least 20 measurements in different slices for each sample.

2.3.5 Microstructure

Fruit tissue was observed by scanning electron microscopy (SEM) and samples were prepared using the method described by Figueiredo and Pais (1994). Small cubes (≈ 2 mm) were excised with a sharp blade from the outer pericarp of minimally processed kiwifruit. Tissues were fixed with 1.5 mL/100 mL glutaraldehyde in 0.05 mol/L sodium cacodylate buffer, pH 7 for 45 min at room temperature. After 1-2 min under vacuum (26 mm Hg, 3.46 kPa) the fixative was substituted by 3 mL/100 mL glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7 for 2 h at room temperature. The material was rinsed thoroughly in the same buffer and postfixed with a 2 mol/L osmium tetroxide aqueous solution for 2 h at room temperature. After dehydration in a graded acetone series, the material was dried by the critical point drying method. Dried samples were coated with gold and observed with a scanning electron microscope.

2.3.6 Sensory analysis

Sensory evaluation of minimally processed kiwi samples was conducted in the sensory laboratory of the Department of Food Science and Technology, ISA/UTL. The sensory evaluation was performed by a total of ten trained judges (food engineering students and staff). All of them were non-smokers and their age ranged from 25 to 63 years old. The room, at 20 ºC, was equipped with seven isolated sensory booths. The tasting sessions occurred in the period from 10 am to12.30 pm. Panellists performed a descriptive test and were asked to analyse the samples

appearance (translucency), presence of strange flavour, sweetness and firmness, in a scale of five points. Those descriptors were selected in previous trials.

2.4 Statistics

Analysis of variance was applied to the results, as well as a mean comparison test (Fisher LSD), to analyse differences between treatments along storage time. Principal component analysis and cluster analysis was also applied. "Statistica" v. 6.1 software from Statsoft, Inc.was used.

3 Results and discussion

Fig. 1 shows the evolution of CO_2 concentration inside packages during 9 days storage. The application of only one kind of treatment was not effective in decreasing fruit metabolism, as either heat treated fruits (Ht) and calcium dipped fruits (NHt1, NHt2) showed CO_2 production similar to control (NHt).

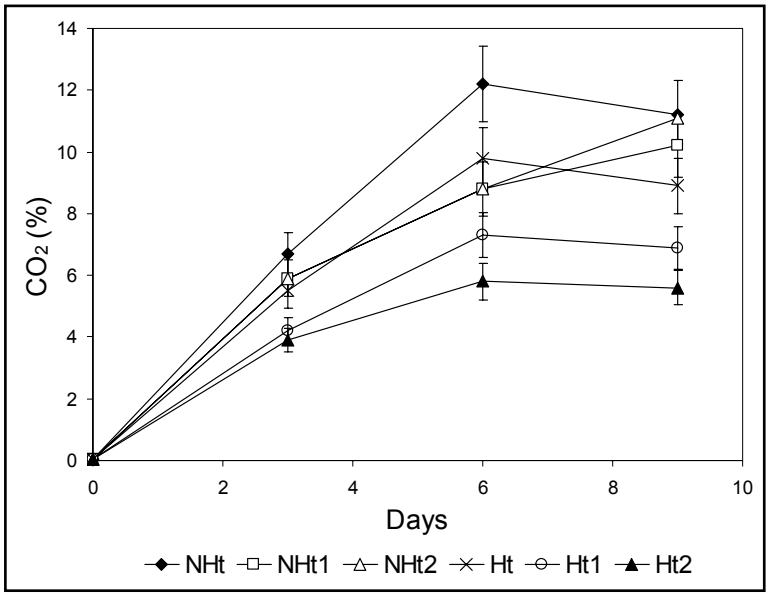

Fig.1. CO_2 headspace composition of minimally processed kiwifruit packages during storage. NHt – non heat treated; NHt1 – non heat treated with 1g/L $CaCl_2$; NHt2 – non heat treated with 2 g/L $CaCl_2$; Ht – heat treated; Ht1 –heat treated with 1g/L $CaCl_2$; Ht2 – heat treated with 2 g/L $CaCl_2$. Each data point represents mean ± *standard error*

On the other hand, results suggest a synergistic effect among moderate heat pre treatment of whole kiwifruit and post cut calcium dips, in the decrease of fruit slices CO_2 production observed from the third day throughout storage. A slight advantage is noted when calcium concentration is incremented from 1 to 2 g/L.

The enhancement of kiwifruit slices' respiration rate as consequence of heat treatments had already been observed (Beirão-da-Costa *et al.*, 2008b). The effect of post-cut calcium dips on fresh cut commodities is usually a decrease in respiration rate, as observed on cantaloupe melon (Lamikanra & Watson, 2004; Luna-Guzmán *et al.*, 1999), honeydew (Saftner, Bai, Abbott & Lee, 2003) and lettuce (Martín-Diana *et al.*, 2005). However, strawberries wedges (Aguayo, Jansasithorn & Kader, 2006) and watermelon cylinders (Mao, Jeong, Que & Huber, 2006) subjected to post-cut $CaCl_2$ dip exhibited respiration rates similar to those of control samples, but a synergistic effect was also noted if calcium treatment is combined with 1-methylcyclopropene.

Firmness was evaluated every three days during a nine days long storage period (Fig. 2).

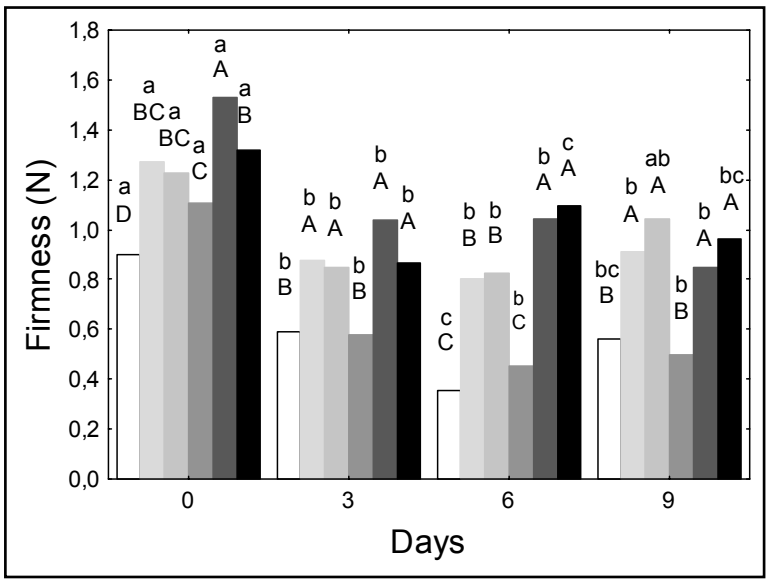

Fig.2. Firmness evolution of minimally processed kiwifruit during storage.
(□) NHt – non heat treated; (▭) NHt1 – non heat treated with 1g/L $CaCl_2$; (▭) NHt2 – non heat treated with 2 g/L $CaCl_2$; (▭) Ht – heat treated; (▭) Ht1 – heat treated with 1 g/L $CaCl_2$; (■) Ht2 – heat treated with 2 g/L $CaCl_2$. Above each bar: different lowercase indicate significant differences for each samples along storage time; different uppercase indicate significant differences between samples at the same storage time (Fisher LSD test, $P < 0.01$).

After minimal processing, non-heated samples showed significantly ($P < 0.01$) lower firmness than the other samples. Fruits previously subjected to heat treatment, even without post-cut calcium dip, exhibited an increased force value similar to those of non-heated but calcium treated fruits. Besides, calcium played a positive role in firmness enhancement as $CaCl_2$ dipped samples showed, after treatment, higher firmness values.

During storage all samples undergo a softening process being the major differences noted after 3 days of storage. Heat treated samples exhibited a higher loss of firmness, showing not significant differences to non-heated samples (NHt), from that time forwards. On the other hand, calcium had a beneficial effect in firmness retention since samples treated with calcium still remained firmer at the end of 9-day storage, being these samples not significantly different from the control sample (NHt) immediately after minimal processing.

At the 6^{th} storage day fruits subjected to heat treatment and calcium dips were firmer than non heated fruits. No significant differences ($P > 0.01$) were observed between previously heated and non heated samples at the end of storage. On the other hand no overall significant differences were noted in firmness between fruits treated with 1 and 2 g/L $CaCl_2$. Similar results were already reported by Agar, Massantini, Hess-Pierce and Kader (1999) for sliced kiwifruit.

Published works (Varoquaux *et al.*, 1990) reported a biphasic softening pattern for kiwifruit after slicing, achieving a reduction of the initial firmness of about 50 % in the two first days of storage, at 2 ºC. The same trend was observed in minimally processed kiwifruit previously subjected to moderate heat treatment (Beirão-da-Costa *et al.*, 2008b) being the effectiveness of the applied pre treatment highly dependent of fruit maturity stage and calcium content (Beirão-da-Costa *et al.*, 2006). In the present study fruits showed low calcium content (12 mg/100 g) (Fig. 2), almost four times lower than in previous studies, which may explain the higher firmness drop in heat treated samples during the first three days of storage and the inefficiency of the methodology for fruit firmness preservation. As had been seen is previous works (Beirão-da-Costa *et al.*, 2008a) the applied heat treatment promote the activation of PME, allowing calcium pectates formation. If just a small amount of calcium is available for cross-linkage with depolymerised pectin chains, a weaker

structure will result, which is more accessible for attack by other pectinolytic enzymes, *e.g.* polygalacturonase.

Non heated and heated fruits showed similar levels of calcium (Fig.3), both significantly ($P < 0.05$) lower than those of samples treated with calcium chloride after peeling and slicing. Dip treatment in 1 g/L $CaCl_2$ increased kiwifruit calcium content three fold. Increasing $CaCl_2$ concentration in the dipping solution also yielded a significant ($P< 0.05$) intake of calcium by samples.

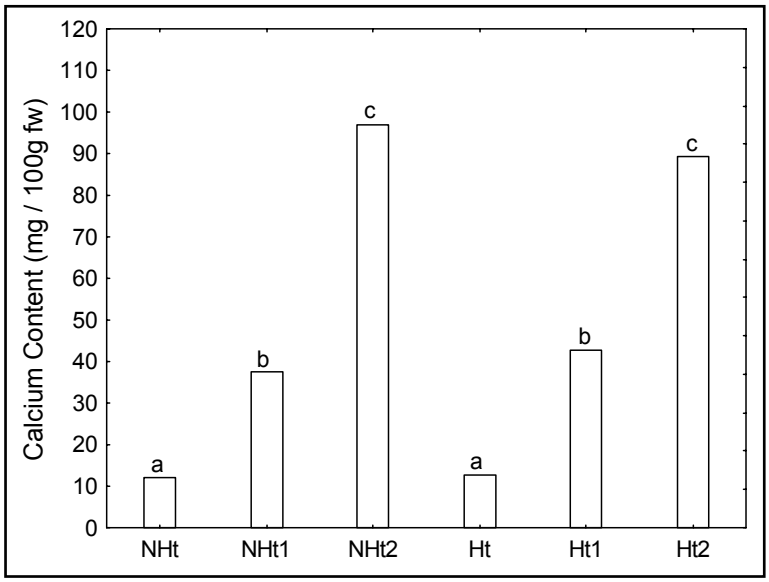

Fig.3. Calcium content of minimally processed kiwifruit. NHt – non heat treated; NHt1 – non heat treated with 1g/L $CaCl_2$; NHt2 – non heat treated with 2 g/L $CaCl_2$; Ht – heat treated; Ht1 – heat treated with 1 g/L $CaCl_2$; Ht2 – heat treated with 2 g/L $CaCl_2$. Above each bar: samples with same lowercase are not significant different (Fisher LSD test, $P < 0.01$).

Several authors referred the positive effect of higher temperature calcium dips in effectiveness of the dip treatments in quality preservation of minimally processed cantaloupe melon (Luna-Guzman *et al.*, 1999), lettuce (Martín-Diana *et al.*, 2005; Martín-Diana, Rico, Frías, Henehan, Mulcahy, Barat & Barry-Ryan, 2006) and carrots (Rico *et al.*, 2007), mainly due to the promotion of diffusion process. The heat treatment applied to whole kiwifruits had no significant effect ($P > 0.05$) in calcium intake by fruit slices.

When calcium was supplied, cross linked was promoted, as may be inferred by the lower soluble pectin / total pectin ratio (Fig. 4I). The three samples subjected to the moderate heat pre treatment presented ratio values of between 60 % - 68 %, against 74 % - 78 % of non heated samples.

The most representative fraction of kiwifruit total pectin is the water soluble fraction, in agreement with other works (Varoquaux *et al.*, 1990; Beirão-da-Costa *et al.*, 2007). Exception to non heated and non calcium dipped fruits, after minimal processing all samples exhibited similar values of total pectin. The difference found relative to non heated fruits was not probably due to the heat treatment, as no differences were found relative to non heated fruits with calcium dips, but to the biological variability of raw material. Vicente, Costa, Martínez, Chaves and Civello (2005) report that no change

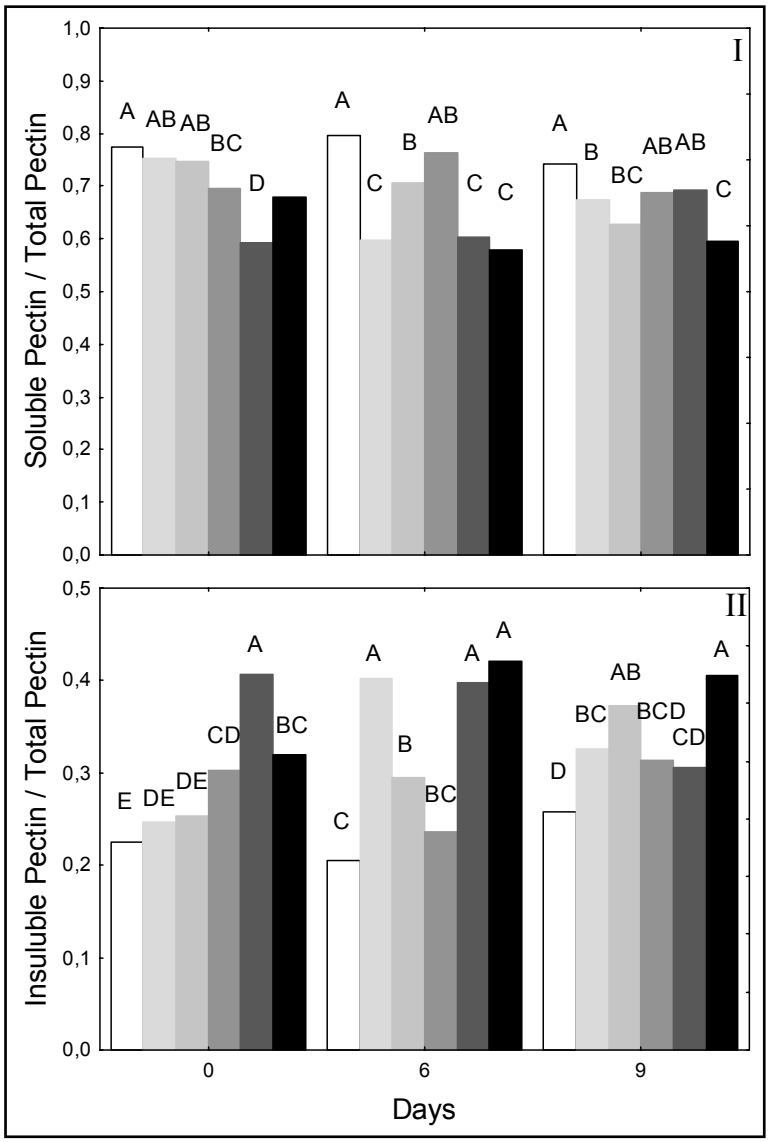

Fig.4. Soluble pectin / total pectin ratio (I) and insoluble pectin / total pectin ratio (II) of minimally processed kiwifruit during storage. ( ) NHt – non heat treated; ( ) NHt1 – non heat treated with 1g/L $CaCl_2$; ( ) NHt2 – non heat treated with 2 g/L $CaCl_2$; ( ) Ht – heat treated; ( ) Ht1 – heat treated with 1 g/L $CaCl_2$; ( ) Ht2 – heat treated with 2 g/L $CaCl_2$. Above each bar: for each day, samples with same uppercase are not significant different (Fisher LSD test, $P< 0.05$)

was found in total pectin content between heat treated and non heat treated strawberries. In previous works (Beirão-da-Costa *et al.*, 2008b) an increase in kiwifruit total pectin content was noted only as result of a prolonged heat treatment (45 ºC / 75 min) no differences being detected between control and heat treated fruits at 45 ºC for 25 minutes.

Fruits subjected to both heat treatment at 45 ºC for 25 min and post cut dip in 1 g/L $CaCl_2$ showed higher IP/TP ratio (Fig. 4II) explaining the higher firmness value (Fig. 2). All non heated fruits samples showed no differences in that ratio. Heat treated fruits without calcium dips, despite the higher value of SP than non heated fruits, had a lower SP/TP ratio. After minimal processing, this sample showed a firmness value which was not significantly different from non heated but calcium treated samples pointing out that the first formed calcium pectates were mainly due to cross linkage with endogenous calcium.

After six days of storage, calcium dipped samples, showed a decrease in SP/TP ratio concomitantly with a significantly increase in IP/TP due to calcium pectates formation (Fig. 4). On the other hand, heat treated fruits without calcium exhibited a significant increase in SP/TP ratio, resulting from pectin solubilization and explaining the observed tissue softening.

At the end of the 9-day storage slices dipped in 2 g/L $CaCl_2$ showed a higher IP/TP ratio (Fig. 4II) in opposition to non heated fruits that showed the lower value and the higher for SP/TP ratio. Despite the lower firmness (Fig. 2), heat treated sample only evidenced an IP/TP ratio similar to 1 g/L $CaCl_2$ dipped fruits.

Vicente *et al.* (2005) refer that air heat treatments at 45 ºC for 3 hours reduced the decrease in HCl soluble pectin and the increase in the water soluble fraction and, after heat treatment, higher levels of ionically bound pectins were found in treated fruits, correlated to the increase in PME.

In order to better understand and correlate textural changes and pectins fractions, the microstructure of samples was also analysed along the 9 day storage period. As stated by Jackman & Staley (1995), visual examination of plant materials as they are being subjected to an applied force or deformation provides critical information necessary for the interpretation of data associated with fracture or failure.

Fig. 5, microphotographs of samples after minimal processing, revealed a tissue composed by two different sizes oriented cells, the large with a spherical elongated

shape and the small mainly with angular shape, with thin walls and small intercellular spaces. Large vacuoles can be observed inside cells (white star). A similar structure for kiwifruit outer pericarp tissue was reported by Muntada, Gerschenson, Alzamora and Castro (1998).

Non heated fruits (Fig. 5A) showed a loss of structural features with many disrupted cells, supporting the lowest firmness value of this sample.

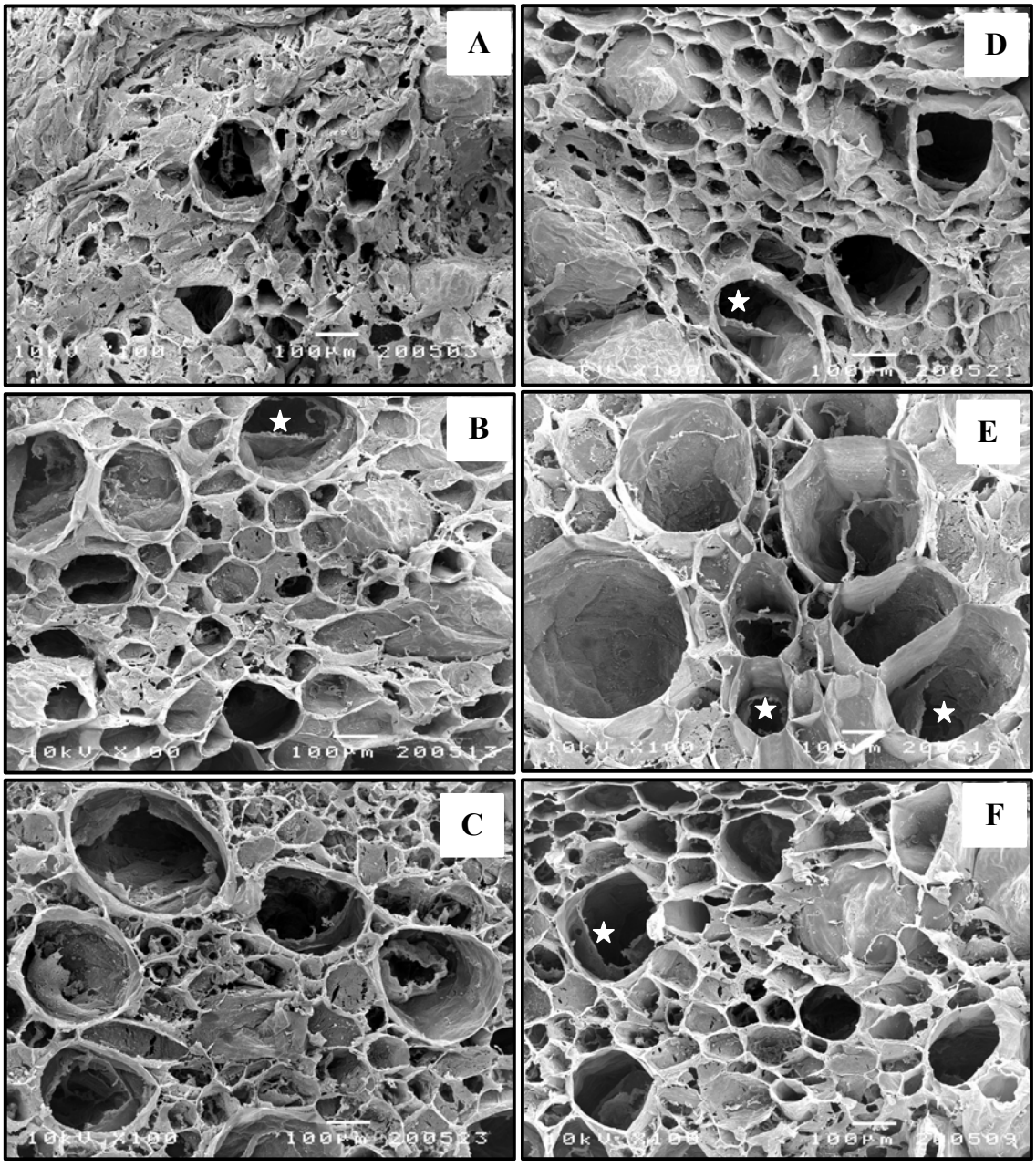

Fig.5. Microstructure of kiwifruit slices after minimal processing: A – non heat treated; B – non heat treated with 1g/L $CaCl_2$; C – non heat treated with 2 g/L $CaCl_2$; D – heat treated; E –heat treated with 1g/L $CaCl_2$; F – heat treated with 2 g/L $CaCl_2$. Bar represent 100 μm.

When fruits were previously subjected to the moderate heat treatment even without calcium addition (Fig. 5B) a more structured tissue was observed, with turgescent intact cells. When calcium was added the integrity of fruit outer pericarp was enhanced, the effect being more pronounced in heat treated fruits (Fig.5E and Fig. 5F). In all samples some cell membrane (plasmalemma) rupture can be observed. As this structure has little mechanical resistance, it is the pressure exerted by intracellular liquid on the cell wall which accounts for the turgor pressure-induced elasticity of cells and tissue. Nevertheless losses in the integrity of plasmalemma would lead to some decrease in cellular turgor (Rojas *et al.*, 2001).

After six days of storage firmness of all samples drop, nearly 60 % in non heated and heated samples and 30 % in calcium treated samples. Fig. 6 shows the microstructure of samples at that time.

As can be seen, a loss of turgidity occurred in all samples supporting the observed softening. Large parenchyma cells evidence many wrinkles (black arrow in Fig. 6A and D) resulting from water loss, enhancing intercellular spaces concomitantly with smaller cells collapse and loss of integrity (open arrow in Fig. 6A). In calcium treated samples, despite the loss of turgor pressure, the structure is better preserved due to integrity of the cell wall and middle lamella.

Between the 6^{th} and the 9^{th} day of storage little differences were noted in tissues structure. Calcium treated samples (Fig.7) still showed higher turgidity, with a little advantaged of samples treated with 2 g/L $CaCl_2$.

Although no significant differences ($P > 0.01$) were detected in firmness (Fig. 2) between heat treated (Ht) and non heat treated (NHt) fruits, in Fig. 7D can be observed that the first ones still maintained a less damaged structure, with minor loss of turgor, in accordance with the highest IP/TP ratio.

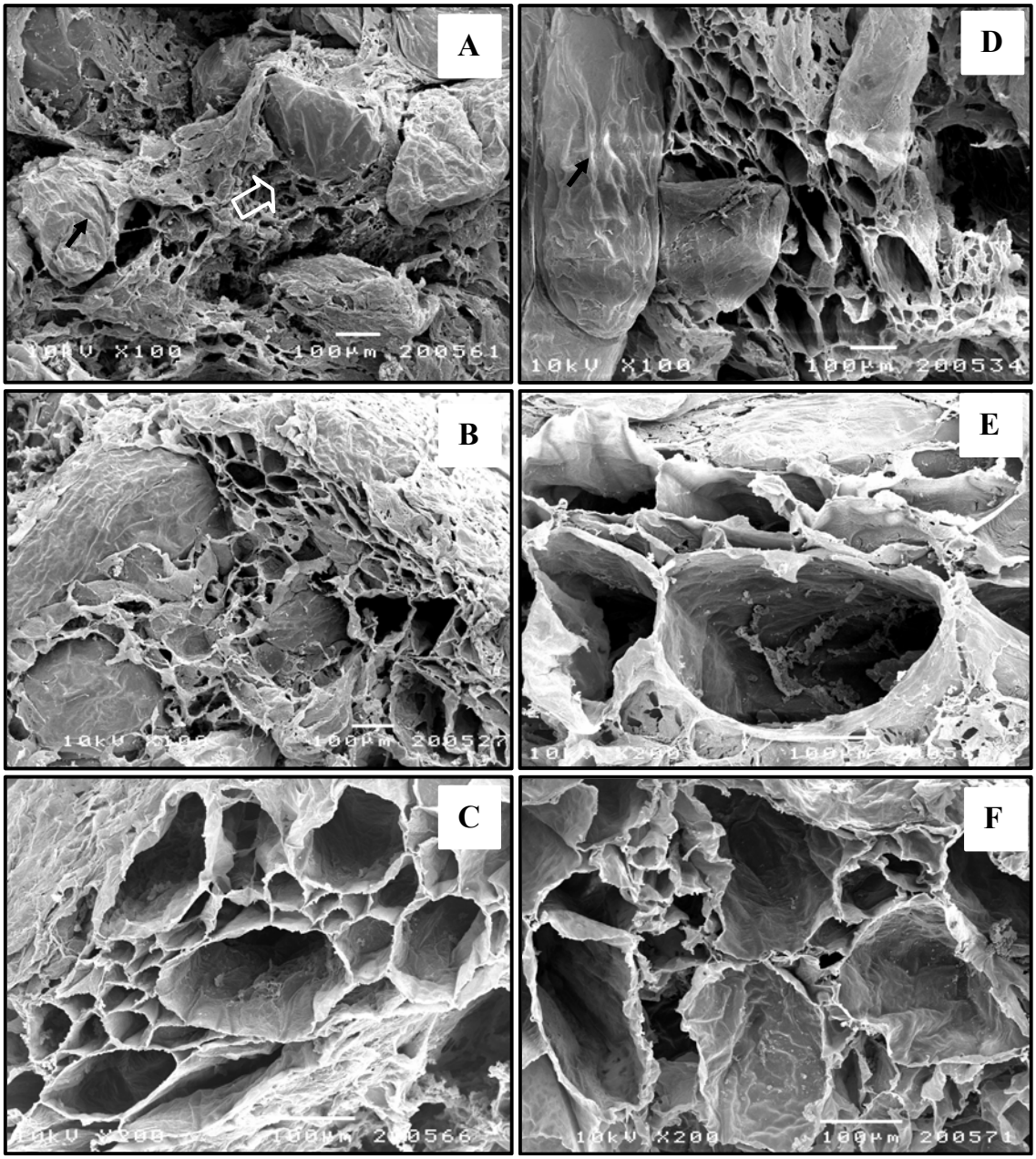

Fig.6. Microstructure of kiwifruit slices after 6-day storage: A – non heat treated; B – non heat treated with 1 g/L$CaCl_2$; C – non heat treated with 2 g/L $CaCl_2$; D – heat treated; E –heat treated with 1 g/L $CaCl_2$; F – heat treated with 2 g/L $CaCl_2$. Bar represent 100 μm.

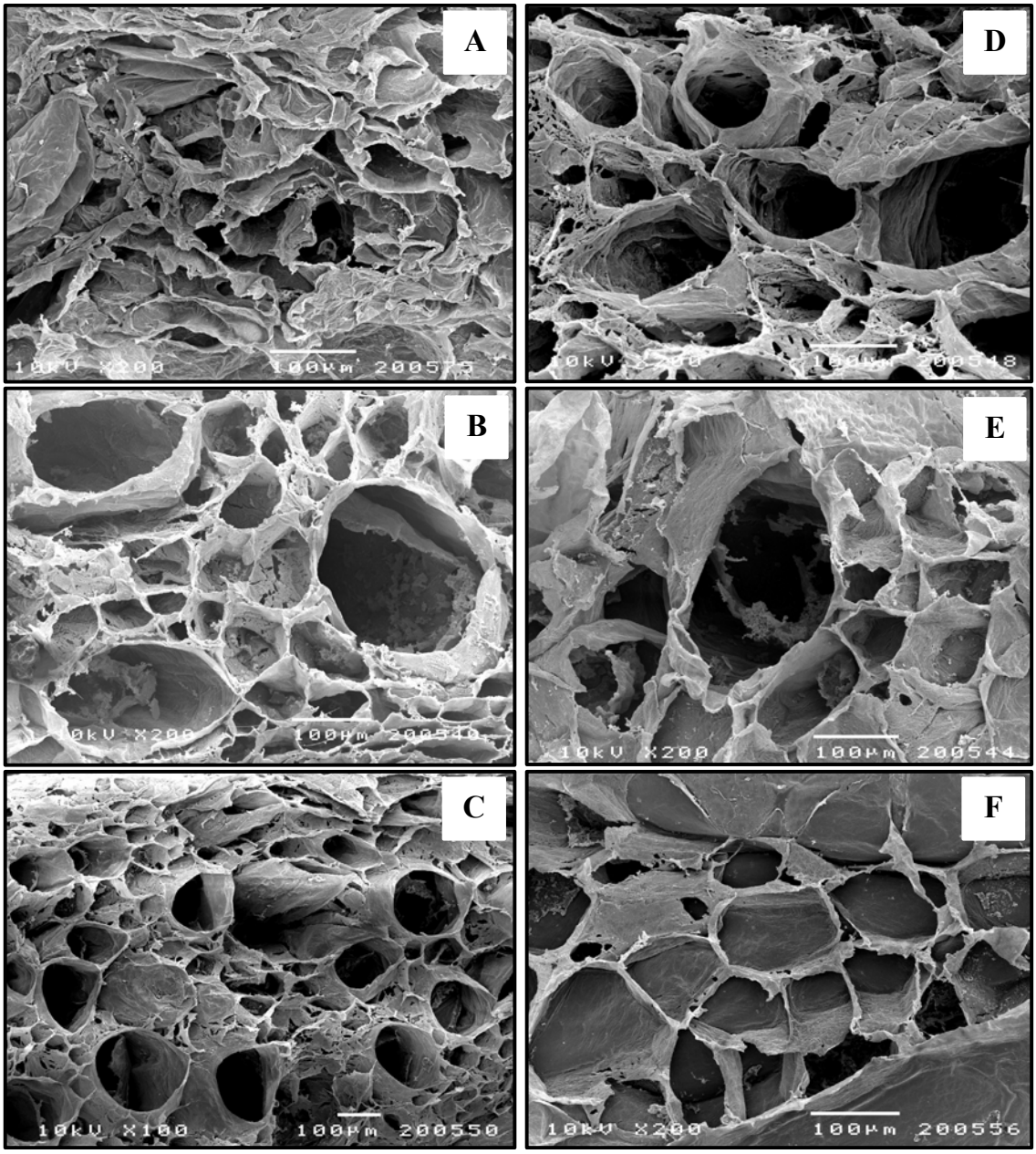

Fig.7. Microstructure of kiwifruit slices after 9-day storage: A – non heat treated; B – non heat treated with 1 g/L $CaCl_2$; C – non heat treated with 2 g/L $CaCl_2$; D – heat treated; E –heat treated with 1 g/L $CaCl_2$; F – heat treated with 2 g/L $CaCl_2$. Bar represent 100 µm.

Sensory analysis results were correlated with the instrumental measurement of firmness and insoluble and soluble pectins ratios, performing principal components analysis. The two first principal components (PC) explained 71.5% of the total variance of results. As can be seen (Fig. 8) the instrumental measure of firmness (F_I) is well correlated with panel sensorial perception (F_S), and both are also correlated with IP / TP ratio. These parameters were mainly accounted for PC1, while sweetness, presence of strange flavour and translucency, quality loss indices, are mainly accounted for PC2.

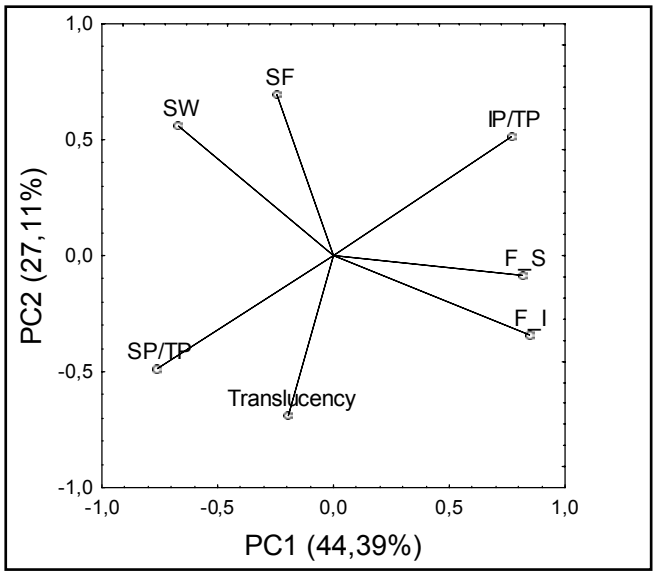

Fig.8. Principal components comparison of variables: Instrumental firmness (F_I), Sensorial firmness (F_S), translucency, strange flavour (SF), sweetness (SW), insoluble pectin / total pectin ratio (IP/TP) and soluble pectin / total pectin ratio (SP/TP).

Fig. 9 represents a projection of the samples onto the space defined by the first two PC's. Analysing geometric distances by cluster analyses four groups can be defined. A separation between calcium and non calcium treated samples is evident, the second one being more correlated with degradation indicators. The first group is composed by non heated and heated fruits without calcium dips at the beginning of storage (NHt_0; Ht_0) and is correlated with higher SP / TP ratio and translucency. The second group was the closest to higher firmness characteristics and is mainly formed by calcium treated samples since the beginning to the 6 day of storage. Calcium treated samples were still correlated with higher firmness at the end of storage period. Non calcium treated samples were those that showed higher quality

deterioration with the presence of strange flavour and an excessive sweet taste, indicative of extensive hydrolysis processes at the end of storage period.

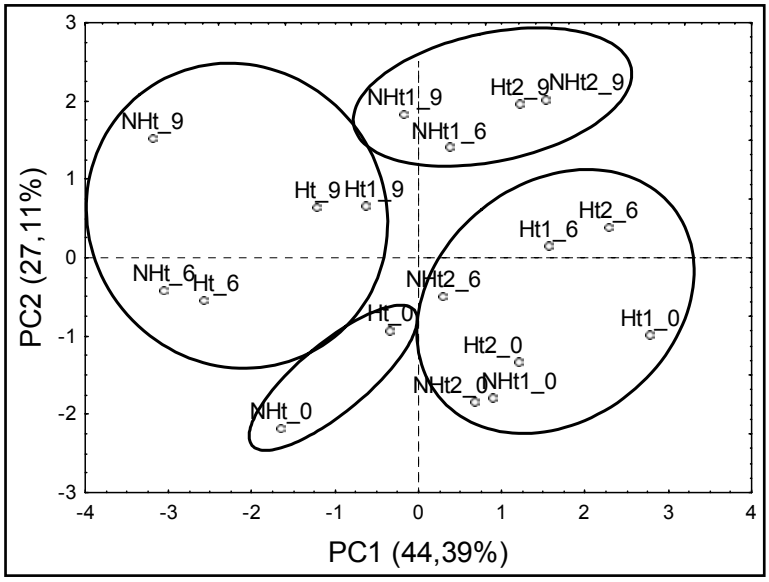

Fig.9. Principal Components comparison of samples. The number after underscore represent the sampling day.

4 Conclusions

Heat treatment at 45 ºC for 25 min, applied to whole kiwifruit followed by post-cut calcium dips, retarded slices' metabolism, lowering respiration rate, as compared to non treated fruits and to fruits subjected to only one of these treatments. Calcium dips were effective in preserving slices' firmness, promoting cross-linkage between Ca^{2+} and pectin chains, as expressed by higher IP/TP ratio. The advantage of heat treatment when provided by itself, as regards the maintenance of fruit firmness, was small, mainly due to low calcium level of raw material. Nevertheless heat treated fruits showed higher IP/TP ratio which indicated a more structured tissue, as observed by SEM. Therefore, the IP/TP ratio is a useful predictor for fruit quality, as a good correlation was observed between this parameter and firmness, measured both instrumentally and sensorially. There was no advantage in using a 2 g/L $CaCl_2$ dip relative to the 1 g/L, as regards fruit quality preservation by maintaining initial fruit

firmness during the 9-day shelf life period, hence the lower concentration may be used.

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Capítulo IV

Tratamentos de descontaminação na produção de kiwi minimamente processado. Efeitos sobre a qualidade.

A importância do processo de descontaminação e o seu impacto na qualidade de kiwi minimamente processado e qualidade do produto são avaliados no presente capítulo. Foram aplicados métodos de descontaminação alternativos ao método convencional. É avaliada a eficácia dos vários processos estudados na redução da carga microbiológica do fruto durante o período de armazenamento, bem como os efeitos na fisiologia e características físicas e químicas.

Alternative sanitizing methods for fresh-cut kiwifruit decontamination. Effects on quality.

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Alternative sanitizing methods for fresh-cut kiwifruit decontamination. Effects on quality.

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Abstract

The effect of different sanitizing methods on CO_2 accumulation, colour, firmness, and microbiological counts of fresh-cut kiwifruit was evaluated during a 10-day storage period. Fruits were subjected, within minimal processing, to water, chlorine, ozonated water, UV-C or heat shock sanitation. Heat shock and UV were effective methods in lowering microbial loads within storage. UV-C treatment was also effective in reducing respiration rate of kiwifruit slices. In general, L^*, c and h^o were not affected by the different sanitizing methods. UV treated fruits showed an increased firmness value but a similar PME activity value was found to that of the water washed (control) fruits.

Keywords: Kiwifruit; minimal processing; sanitizing; quality

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1 Introduction

Decontamination is an important unit operation in minimal processing of fresh-cut fruits and vegetables. Some of the key characteristics of minimally processed fruits and vegetables influencing the microbial quality are the increased surface area or damaged plant tissues which supply nutrients for microbiological growth and tissues that remain metabolically active, together with a degree of processing which is less than that required to ensure microbiological stability (Nguyen-the & Carlin, 1994).

The non-existence of further processing prior to consumption implies that the packaged product must meet both quality and safety criteria, especially as several outbreaks already occurred in the past associated with fresh–cut fruits and vegetables (National Advisory Committee on Microbiological Criteria for Foods, 1999).

The most commonly used decontaminant in fresh-cut fruits and vegetables is chlorine supplied as liquid chlorine, hypochlorites and chlorine dioxide, its microbicidal activity depending on the amount of free available chlorine, as hypochlorous acid (Beuchat, 1998; Beuchat, 2000; Sapers, 2001). However, chlorine shows a low sanitizing efficacy as its availability is dependent on: (1) the presence of organic matter; (2) pH and (3) temperature (Parish *et al.*, 2003). Besides, it is known that it can be harmful due to formation of carcinogenic chlorinated by-products, such as trihalomethanes and chloramines (Sapers, 2001). Therefore, alternative sanitizing methods are needed with improved efficiency and safety.

Heat shock by hot water immersion blanching (80 – 95 ºC for 1 – 2 min) proved to be an effective methodology in reducing microbial growth in apples (Fleischman, 2001) and oranges (Pao, Davis & Parrish, 2001) but the feasibility of the treatment is dependent of the absence of heat injury (Sapers, 2001). A washing treatment at 50 ºC for 60 sec was also effective in maintaining low counts of bacteria during storage of ready-to-use lettuce (Baur, Klaiber, Wei, Hammes & Carle, 2005).

The use of non-ionizing germicidal UV-C radiation seems to be another alternative sanitizing methodology for fresh-cut products. The exposure of bacteria, viruses and spores to UV rays alters bonds within the DNA double helix, yielding

either mutation or lethal effects on cells (Morgan, 1989). The effectiveness of UV-C treatments in reducing microbial loads and improving quality has been reported for lettuce (Allende & Artés, 2003; Allende, McEvoy, Luo, Artés & Wang, 2006), pepper (Vicente, Pineda, Lemoine, Civello, Martinez & Chaves, 2005), cantaloupe melon (Lamikanra, Kueneman, Ukuku & Bett-Garber, 2005), pomegranate arils (López-Rubira, Conesa, Allende & Artés, 2005) and watermelon (Fonseca & Rushing, 2006). In addition, the use of this treatment was approved by Food and Drug Administration (FDA) which alleged that it does not leave any residue in the final product (Allende *et al.*, 2006).

The FDA also approved the use of ozone as an antimicrobial agent for the treatment, storage and processing of foods, either as ozone gas or as an aqueous solution, in direct contact with minimally processed fruits and vegetables. Ozone is a strong oxidizing agent with antimicrobial properties, that rapidly attacks bacterial cell walls with lethal effects (Beuchat, 2000; Suslow, 2003; Mahapatra, Muthukumarappan & Julson, 2005). The effects of ozone as sanitizing agent have been reported, among others, on fresh-cut celery (Zhang, Lu, Yu & Gao, 2005), lettuce (Baur, Klaiber, Hammes & Carle, 2004; Beltrán, Selma, Marín & Gil, 2005) and rocket leaves (Martínez- Sánchez, Allende, Bennett, Ferreres & Gil, 2006).

The objective of the present work is to study the efficiency of sanitizing methods alternative to chlorine for the decontamination of fresh-cut kiwifruit and to evaluate the effects of the applied treatments on the main quality parameters of fruit slices.

2 Materials and methods

2.1 Raw material

Kiwifruit (*Actinidia deliciosa* (A Chev) Liang et Ferguson var *deliciosa* cv Hayward) from the centre of Portugal (Bairrada region), were purchased at a local market, transported to the laboratory and selected for uniform size and absence of wound signals. Fruits were stored at 4 ºC prior to minimal processing operations.

2.2 Fruit preparation

Fruits were sanitized by one of four different methods: (1) 125 ppm free chlorine supplied as sodium hypochlorite solution at pH 6.5; (2) 0.2 ppm ozone supplied as an ozone solution in water; (3) UV radiation at a dose of 1 kJ/m^2 and (4) heat shock at 95 ºC / 30 sec. Fruits washed in water were used as control. Experimental scheme of fruit preparation is summarized in Figure 1.

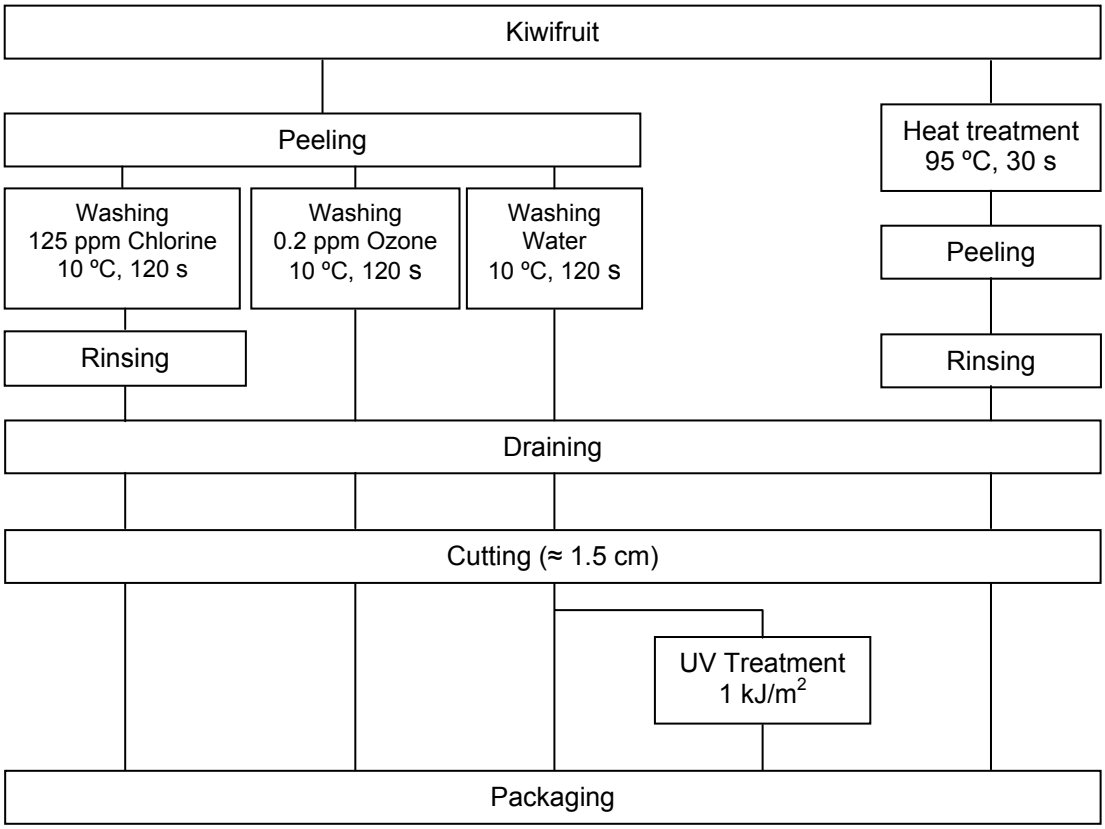

Fig. 1 Fresh cut kiwifruit production diagram according 5 different sanitizing processes.

The UV-C apparatus included a closed box, equipped with germicidal lamps (TUV 15W/G15 T8, Philips) emitting at 254 nm and placed 17 cm above the product, covered with a reflector inner layer to promote homogeneous distribution of light and to avoid shaded areas. UV-C radiation intensity was determined with a photo-radiometer (DELTA OHM LP9021 UVC) as a mean of 16 readings, and the

applied energy (1 kJ/m^2) was obtained multiplying the measured intensity by the exposure time.

The aqueous ozone solution was prepared with an ozone gas generator SPO3 (model OZ5), bubbling the gas through a 120 L deionised water tank. Dissolved ozone concentration was measured spectrophotometrically using the indigo method (American Public Health Association, 2005).

To minimize mechanical damage of the fruits during minimal processing, and as a washing procedure after kiwifruit peeling is needed to remove peel and thrichome residues, all the sanitation procedures were conducted after that operation, with exception to heat shock, in order to avoid outer pericarp vitality loss. Moreover, kiwifruit has a low microbial risk as the crop is grown above ground (Portman, Frankish, & McAlpine, 2002)

Fruit slices were packed in bags made from low-density polyethylene and vinylidene chloride with 3000-4000 and 11000-15000 (mL/m^2/24h/atm) permeability to O_2 and CO_2, respectively, and stored at 4 ºC.

After the applied treatments and at each date of analysis three different bags per treatment, each containing *ca*. 100 g of kiwifruit slices, were evaluated.

2.3 Quality measurements

2.3.1 Microbiological analysis

For microbiological analysis, at each sampling, three bags per treatment were analysed for total aerobic mesophylic and psycrotrophic microorganisms, moulds and yeasts and lactic acid bacteria. Kiwifruit samples (50 g) were homogenised in 450 mL of sterile Ringer solution (SIGMA) with a sterilized homogeniser. Appropriate serial tenfold dilutions were prepared with Ringer solution and used for plating on agar. Total aerobic mesophylic and psycrotrophic microorganisms were counted using a Plate Count Agar (PCA - MERCK) method and incubated at 30 ºC for 72 h and 5 ºC for 10 days respectively. Lactic acid bacteria were enumerated in Lactobacilli De Man, Rogosa and Sharpe agar (MRS - MERCK) and incubated anaerobically in a "moist chamber" at 30 ºC for 5 days. Enumeration of yeasts and moulds was made using yeast glucose chloramphenicol agar (YGC – MERCK) after incubation at 25 ºC for 5 days.

2.3.2 Gas composition

Packages' gas composition was evaluated throughout the storage period. Headspace gas samples were taken with a hypodermic needle through an adhesive septum previously fixed on the bags and were analysed using a checkmate 9900 O_2/CO_2 gas analyzer (PBI-Dansensor, Denmark).

2.3.3 Colour measurement

Colour of minimally processed kiwi was evaluated on the outer pericarp (green section) of slices with a CR 300 Minolta colorimeter (Osaka, Japan) by measuring L*a*b* (CIE) parameters. From those, chroma (c) and hue angle (h^o) were determined, defined as

$$c = \sqrt{a^{*2} + b^{*2}} \qquad (1)$$

$$h^o = \arctan\left(\frac{b}{a}\right) \qquad (2)$$

where a* and b* are the colour readings.

A white tile (L* = 97.46; a* = - 0.02; b* = 1.72) was used for equipment calibration. The C illuminant and a 10º observer were employed. Thirty measures were performed for each of fifteen slices obtained under each condition.

2.3.4 Firmness measurement

Firmness was evaluated by a puncture test on kiwi slices outer pericarp using a TA-XT Plus texture analyser (*Stable Micro Systems*) with a 5 kg load cell. Firmness measurements were taken as the medium force value obtained during the test using a stainless steel probe with 4 mm diameter to penetrate the fruit to a depth of 4 mm, at 1 mm s^{-1}. Mean values were calculated from results of at least 20 measurements in different slices, for each sample.

2.3.5 Pectin methylesterase extraction and assay

About 12 g of kiwifruit outer pericarp (without seeds) was homogenised with 40 mL of cold 1.5 M NaCl in a T25 basic, IKA LABORTECHNIK homogeniser. The homogenate was then stirred for 30 minutes at 4 ºC and centrifuged at 15 000 *g* for 10 minutes, at 4 ºC. The resulting supernatant was used for PME activity assay. PME activity was assayed titrimetrically, using a pH- meter connected to a pH-electrode,

measuring protons from the carboxyl groups obtained from the hydrolysis of methyl esters of pectin, using the method described by Kimball (1991) which was slightly modified. 100 mL of substrate solution (0.25 % (w/v) citric pectin in 0.2 M NaCl) was mixed with 5 mL of PME extract and the pH adjusted to 7.5 with 1 M and 0.05 M NaOH. After the pH reached 7.5, 0.2 mL of 0.02 M NaOH was added and the time required to reach pH 7.5 again was recorded. PME activity was measured at 25 ºC. One unit of PME activity was defined as the amount of enzyme that can cause the release of 1 μmol of COO^- per gram of fresh tissue and per minute (μmol $g^{-1}min^{-1}$).

2.4 Statistics

Analysis of variance was applied to the results, as well as a mean comparison test (Fisher LSD), to analyse differences between treatments along storage time. "Statistica" v. 6.1 software from Statsoft, Inc.was used.

3 Results and Discussion

3.1 Effect on microbiological quality

Microbiological loads of fresh-cut kiwifruit subjected to the different sanitizing methods were analysed, during the 10-day storage (Figure 2). No growth of moulds was detected in any of the samples, the data just being accounted for due to yeasts.

After minimal processing operations, water rinsed fruits showed a microbial load of about 3.5 log units, for all studied groups of microorganisms. Neither ozone nor chlorine treatment was effective in reducing initial population, yielding similar figures. Sanitation with UV-C light and heat shock yielded a reduction of the initial population of 1 log unit for total mesophylic and lactic acid bacteria. For yeast the observed reduction was 1.1 and 1.6 log units for UV-C light and heat shock, respectively. No differences were observed between the initial psycrotrophic populations of kiwifruit slices subjected to different sanitizing methods.

During storage, microbial counts of fresh-cut kiwifruit increased. Total mesophylic population is similar in water-rinsed and chlorine or ozone containing

water, throughout storage but, at the end of 10-day storage, UV treated fruits showed a population reduced by 1.6 log units while heat-shocked fruits maintained the initial total mesophylic counts. The same pattern was found for the growth of total psycrotrophic microorganisms. There was no Portuguese regulation establishing microbial limits to fresh-cut fruits and vegetables. However, the Spanish legislation (BOE, 2001) establishes 6 log cfu/g and 7 log cfu/g as the limit of total aerobic mesophiles at processing day and the end of the shelf life, respectively. In the present work, no sample ever exceeded these limits during the storage period. Debevere (2006) point out as indicative values 5 log units and 8 log units after processing and at consumption date respectively, for psycrotrophic microorganisms, these values being also met in this study for kiwifruit slices.

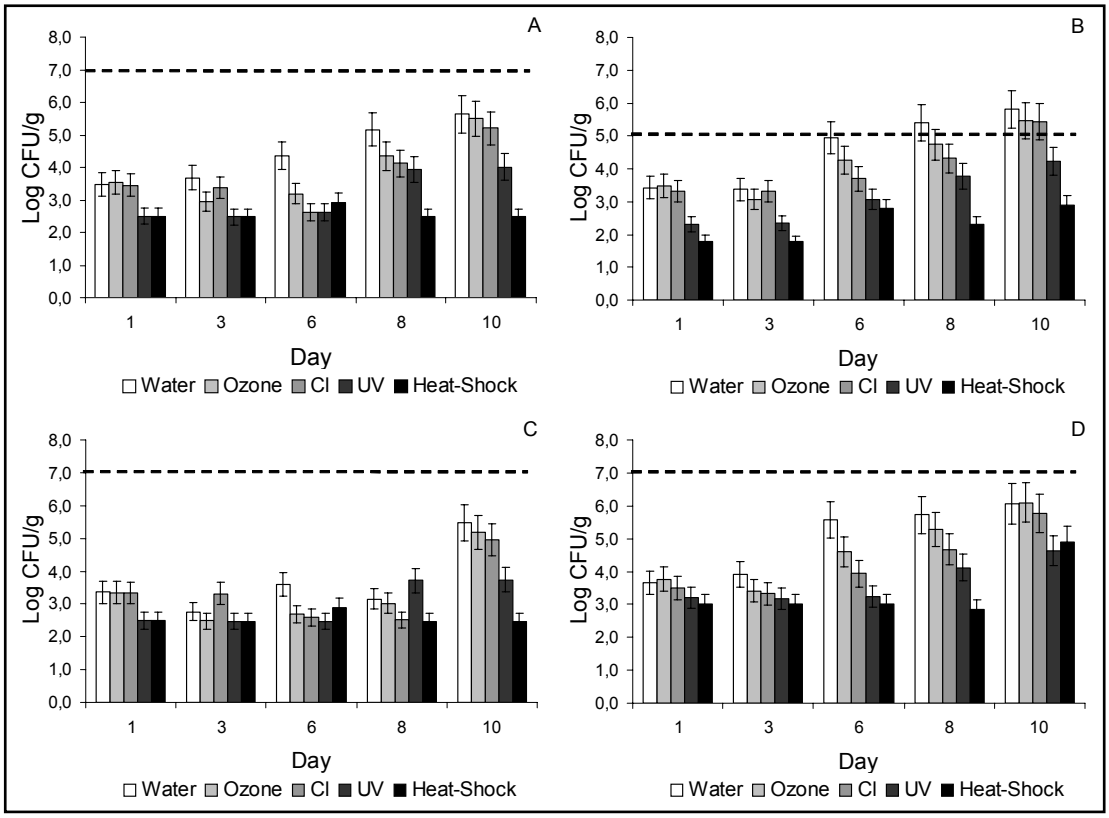

Fig. 2 Microbiological growth on fresh-cut kiwifruit slices, subjected to different sanitizing methods, stored at 4 ºC for up to 10 days. A – Total aerobic mesophylic; B – Moulds and Yeasts; C – Lactic acid bacteria; D – Total psycrotrophic. The horizontal line indicates the Spanish legal limit (BOE, 2000) (A) or indicative values (B, C and D) from Debevere (2006). Error bars indicate de *standard deviation*, n=3.

O'Conner- Shaw, Roberts, Ford & Nottingham (1994) reported that microbial growth did not appear to contribute to spoilage in diced kiwifruit. Nevertheless, water washed fruits achieved the recommended limit for yeasts (5 log unit) after six days of storage and exceeded them after eight days. Fruits sanitized with chlorine and with ozonated water exceeded the recommended limits but only at the end of the analysis period, while yeast loads in UV and heat shock treated fruits was always maintained below the recommended value. Yeast growth often affects product shelf-life since yeasts were responsible for the development of off-flavours due to production of CO_2, ethanol, organic acids and volatile esters (Fleet, 1992; Jacxsens, Devlieghere, Ragaert, Vanneste & Debevere, 2003; Ragaert, Devlieghere & Debevere, 2007).

The initial population of acid lactic bacteria was low, not exceeding the recommended limits (3-4 log units) in all samples (Debevere, 2006), and no significant growth was registered during the 8-days storage period. After 10 days an increase in lactic acid bacteria was observed mainly for the fruits rinsed with water, and with chlorinated and ozonated water; however, the achieved microbial loads still remained 1 log unit lower than indicative values. UV and heat shock samples showed lactic acid bacteria growth 3 and 4.5 log units, respectively, below the indicative limit. Nevertheless Debevere (2006) adverted that those fresh-cut products which exceed the indicative limit should not be considered as inadequate for consumption if no sensorial defects were detected.

The observed inefficiency of chlorine in reducing initial microbial loads and minimizing microorganisms growth within storage of fresh-cut produces was also reported (Parish *et al.*, 2003), namely for rocket leaves, when used at the concentration of 100 mg/L (Martínez-Sánchez, *et al.*, 2006), and for carrots at 200 mg/L (Ruiz-Cruz, Acedo-Félix, Díaz-Cinco, Islas-Osuna & González-Aguilar, 2007). For fresh-cut watermelon the initial reduction of the microbial population by chlorine was only effective for a 5 days long period (Fonseca & Rushing, 2006). The efficiency of the treatment depends on various factors, including pH, temperature and contact with organic material (Beuchat, 2000). In the present work, despite the maintenance of the theoretical optimum conditions of pH and temperature, no advantage was evidenced by chlorine in the sanitizing treatment, when compared to the water treatment. This is in accordance to preliminary results (data not shown) in which a mere 0.2 log units lowering was observed for the chlorine washing

treatment. The similar observed inefficiency of treatment with ozonatated water treatment is not in agreement with several published works. Total bacterial counts on fresh-cut celery treated with 0.03, 0.08 and 0.18 mg/L were significantly lower than those of the same washed with tap water (Zhang *et al.*, 2005). A reduction in total aerobic mesophiles, pseudomonades and *Enterobacteriaceae* on shredded lettuce was observed when comparing ozone treatment (1 mg/L) to tap water wash, but the treatment was not as effective in reducing the initial counts as the chlorine treatment (200 mg/L) (Baur *et al.*, 2004). In addiction, ozonated water (0.5 ppm) was not also effective in reducing mesophylic and yeasts and moulds in fresh cut carrots (Abreu *et al.*, 2005).

UV-C and heat shock were the most effective treatments in microbial population decrease. Martín-Diana, Rico, Barry-Ryan, Frías, Henehan & Barat (2007) reported the effectiveness of a steamer jet-injection treatment as a reduction of 1.8 log units in the mesophylic load of fresh-cut lettuce, while a blanching treatment allows a 3 log cfu/g mesophylic reduction in leafy salads (Rico, Martín-Diana, Barat & Barry-Ryan, 2007). The present results are also in agreement to several authors that report that UV-C treatment is a useful methodology in fresh-cut product decontamination. UV-C radiation on fresh-cut lettuce (2.37, 4.06, 7.11 and 8.14 kJ/m^2) and watermelon cubes (4.1 kJ/m^2) led to lower loads of total aerobic mesophylic and psychrotropic microorganisms when compared to non-treated samples (Allende & Artés, 2003; Allende *et al.*, 2006; Fonseca & Rushing, 2006). However, no benefits were found in the application of 0.56 – 13.62 kJ/m^2 UV-C radiation as regards the microbial population reduction of minimally processed pomegranate, probably due to some protection of the microorganisms by the aril surface composition or topography (López-Rubira *et al.*, 2005). Microbial loads of fresh-cut cantaloupe were significantly reduced when UV-C light was applied after cutting, the effect being more pronounced if the minimal processing operations take place under light exposure (Lamikanra *et al*., 2005).

3.2 Gas compositions within packages

To determine if the different sanitizing methods affect the physiology of kiwifruit slices, gas composition within packages was monitored during storage (Figure 3).

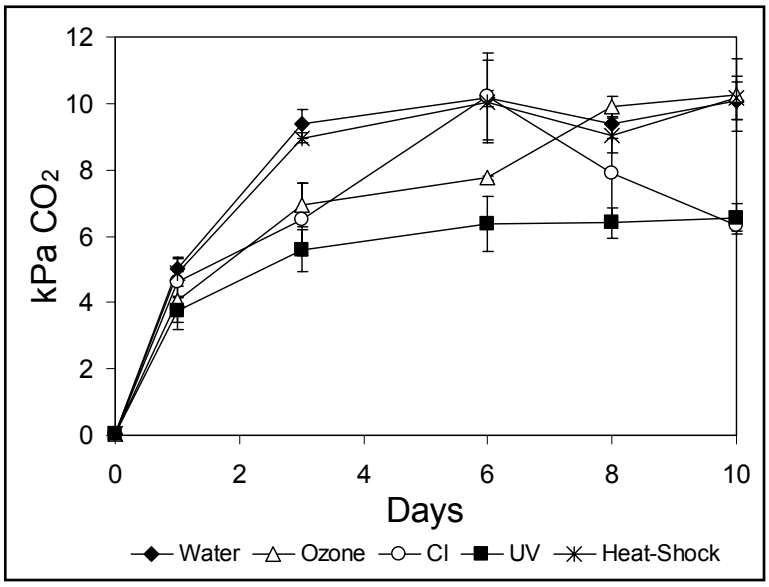

Fig. 3 CO_2 concentration within packages of fresh-cut kiwifruit slices, subjected to different sanitizing methods, stored at 4 ºC for up to 10 days. Error bars indicate de *standard deviation*, n=3.

As expected, CO_2 partial pressure inside the packages increased in all samples, at a higher rate during the first three days of storage, as consequence of incremented metabolic activity due to minimal processing operations. The passive modified atmosphere formed reduced fruit respiration rate and therefore equilibrium levels were achieved. O_2 partial pressure inside the bags decreased, showing an inverse evolution, the differences noted between treatments being similar to those observed for CO_2 (data not shown). The different treatments affect CO_2 production by fresh-cut kiwifruit. The higher CO_2 accumulation occurred within packages of fruits washed in water or subjected to heat-shock. The high CO_2 levels revealed pronounced metabolic activity and are not due to microorganisms CO_2 production because heat shock treated samples showed the lowest microbial loads. Fruits treated with ozone and chlorine showed a lower CO_2 accumulation but slices treated with UV-C non-ionizing radiation displayed the lowest production of CO_2, denoting a decreased respiration rate in comparison to that of other fruit slices.

The effects of UV-C radiation in respiratory activity depend upon the species treated. The results obtained are in accordance with those from other authors reporting a decrease in respiration rate of peppers (Vicente *et al.*, 2005), minimally processed pomegranate arils (López-Rubira *et al.*, 2005), fresh-cut cantaloupe melon (Lamikanra *et al.*, 2005) and broccoli florets (Costa, Vicente, Civello, Chaves &

Martinez, 2006), as a consequence of UV-C treatments. In tomatoes, the climateric peak of respiration is lowered and delayed when fruits were subjected to UV-C radiation, probably due to the maintenance of higher levels of putrescine, an antisenescence agent inhibitory of ethylene synthesis (Maharaj, Arul & Nadeau, 1999). It is known that kiwifruit is highly sensitive to ethylene (Crisosto, Mitcham & Kader, 2000), therefore this pathway could explain the reduction of the respiration rate in UV-C treated slices. Zhang *et al.* (2005) also reported the inhibition of fresh-cut celery respiration rate when subjected to ozonated water treatments, and the efficiency of the inhibition increased with increasing concentration of ozone in water, from 0.08 to 0.18 ppm. Nevertheless, fresh-cut iceberg lettuce respiration rate was not affected by ozonated water wash treatment, as samples washed in 1, 10 and 20 mg/L ozone in water exhibited respiration rates similar to that of water washed samples (Baur *et al.*, 2004; Beltrán *et al.*, 2005).

3.3 Effect on colour

No significant differences in c and hº were observed among slices of the different treatments (Table 1). Analysis of variance showed that only storage time had a significant effect on these parameters.

Small differences where also noted in h^o during storage. A decrease in c values, indicative of some yellowing, can be observed during the storage period in all samples. Fruit slices washed in water or subjected to ozone treatment were those that showed a higher decline in chromaticity.

Both treatment and storage time significantly affected slices' luminosity (L^*) (Table1). A decline in L^* values were observed for all samples. As occurred for h^o, fruit slices treated with UV radiation maintain initial luminosity values within the first six days of storage.

Table 1. Evolution of colour parameters of kiwifruit slices, subjected to different treatments, during storage.

Storage Day	Treatment	*L**	*c*	*h°*
1	Heat-Shock	42.35 ^{a}	18.96 ^{a}	113.65 ^{ab}
	Cl	43.54 ^{aA}	17.10 ^{a}	114.09 ^{a}
	Water	43.12 ^{aA}	18.86 ^{a}	114.78 ^{a}
	Ozone	42.51 ^{aA}	18.58 ^{a}	114.48 ^{a}
	UV	44.19 ^{aA}	19.02 ^{a}	114.93 ^{a}
3	Heat-Shock	41.07 ^{b}	15.22 ^{bc}	114.95 ^{a}
	Cl	41.45 ^{bB}	16.62 ^{a}	114.21^{a}
	Water	41.62 ^{bA}	17.22 ^{a}	114.12 ^{ab}
	Ozone	41.86 ^{abAB}	16.92 ^{ab}	114.72 ^{a}
	UV	43.85 ^{aA}	17.05 ^{ab}	114.07 ^{a}
6	Heat-Shock	40.53 ^{b}	14.29 ^{c}	113.88 ^{ab}
	Cl	39.21 ^{bC}	15.80 ^{ab}	114.56 ^{a}
	Water	39.21 ^{bB}	14.78 ^{b}	115.38 ^{a}
	Ozone	40.51 ^{bB}	14.66 ^{c}	114.48 ^{a}
	UV	42.88 ^{aA}	15.27 ^{bc}	114.20 ^{a}
8	Heat-Shock	38.75 ^{a}	14.04 ^{c}	113.05 ^{b}
	Cl	38.92 ^{aC}	14.29 ^{b}	113.39 ^{a}
	Water	38.37 ^{aB}	12.78 ^{b}	113.03 ^{bc}
	Ozone	40.05 ^{aB}	14.93 ^{bc}	114.06 ^{a}
	UV	39.41 ^{aB}	14.58 ^{c}	114.98 ^{a}
10	Heat-Shock	38.66 ^{bc}	14.46 ^{c}	112.59 ^{b}
	Cl	40.24 ^{abBC}	14.16 ^{b}	114.31 ^{a}
	Water	38.20 ^{cB}	12.98 ^{b}	112.38 ^{c}
	Ozone	37.72 ^{cB}	12.83 ^{c}	112.36 ^{b}
	UV	40.87 ^{aB}	14.20 ^{c}	113.46 ^{a}
Analysis of variance (ANOVA) Effect			***P***	
Treatment		0.000	0.422	0.664
Storage Time		0.000	0.000	0.000
Treament × Storage Time		0.199	0.127	0.409

For *L** column, different lowercase indicate significant differences at each time for different samples and different uppercase indicate significant differences of each sample at different times, at P <0.05, Fisher LSD test. For *c* and *h°* columns, for each sample, different letter indicate significant differences at P <0.05, Fisher LSD test.

3.4 Effect on firmness

The effect of the different sanitizing methods on kiwifruit slices firmness was monitored during the 10-day storage period (Figure 4). After minimal processing UV treated fruits showed a significantly higher firmness, comparing to other samples, value maintained till the third day of storage. The better retention of firmness explain the observed *L** value preservation within the first days of storage as a decrease in the luminosity of kiwifruit slices is due to an induction of translucent water soaked tissue resulting from structure loss instead of browning (Agar, Massantini, Hess-Pierce & Kader, 1999; Beirão-da-Costa, Steiner, Correia, Empis & Moldão-Martins, 2006; Beirão-da-Costa, Steiner, Correia, Leitão, Empis & Moldão-Martins 2007).

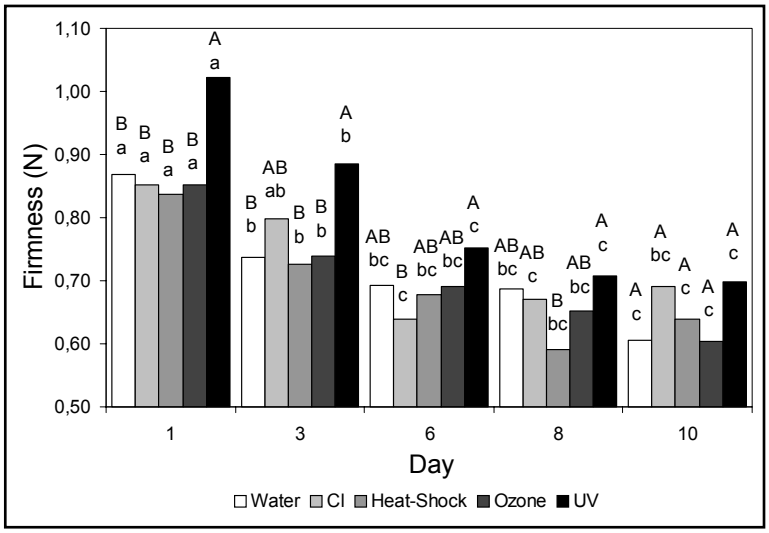

Fig. 4 Firmness evolution of fresh-cut kiwifruit slices, subjected to different sanitizing methods, stored at 4 ºC for up to 10 days. Above each bar: different lowercase indicates significant differences for each sample along storage time; different uppercase indicates significant differences between samples at the same storage time (Fisher LSD test, $P < 0.05$).

The other four treatments did not show any effect on fruit slices firmness as no differences were noted among fruit slices during all storage period. The results are in accordance to previously published works reporting that, wash treatments with tap water, chlorinated and ozonated water also did not influence texture parameters of shredded lettuce (Baur *et al.*, 2004; Beltrán *et al.*, 2005).

Better firmness retention of fruits and vegetables as consequence of UV-C light exposure was reported for fresh-cut cantaloupe melon (Lamikanra *et al.*, 2005), peppers (Vicente *et al.*, 2005), strawberries (Marquenie *et al.*, 2002) and tomato (Maharaj *et al.*, 1999; Barka, Kalantari, Makhlouf & Arul, 2000). The mechanism involved in fruit firmness preservation could be related to the increased level of polyamines observed in UV-C treated fruits (Maharaj *et al.*, 1999), as a correlation was observed between these compounds and the suppression of cell wall softening enzymes polygalacturonase and pectin methylesterase (Barka *et al.*, 2000).

3.5 Effect on PME activity

In order to understand the mechanism underlying the differences found in UV treated and water washed (control) fruits firmness, the activity of PME was measured (Table 2).

Table 2. PME activity of fresh-cut kiwifruit, one day after minimal processing

Treatment	PME activity (μmol COO^- $min^{-1}g^{-1}$)
Water	1.54 ± 0.22 [a]
UV	1.68 ± 0.22 [a]

Mean values ± standard deviation. a indicate no significant differences at $P < 0.01$.

Several works refer to the influence of UV treatments in enzymes activity. Lamikanra *et al.* (2005) found that UV treatment increased peroxidase activity while lowered esterase and lipase activity. Barka *et al.* (2000) reported that 3.6 kJ/m^2 UV-C treatments minimize the activity of cell wall degrading enzymes, inducing their proteolysis or reducing their *de novo* synthesis, delaying softening and tomato firmness decay. Nevertheless a 73.8 mJ/cm^2 UV-C treatment did not influence orange juice pectin methylesterase activity (Tran & Farid, 2004). Our results did not show any significant differences between PME activity of the water washed and the UV treated slices. It is therefore hypothesized that the mechanism involved in kiwifruit firmness preservation was the one described for 'McIntosh' apples and strawberries (Kramer, Wang & Conway, 1989; Ponappa, Scheerens & Miller, 1993). These authors reported that exogenous polyamines effectively suppressed cell wall softening and activity of polygalacturonase. This mechanism is similar to that of

calcium ions, involving the formation of cation cross-links with pectic acid and other polysaccharides, due to their ability to bind to cell walls, thus limiting accessibility of the cell wall to degrading enzymes.

4 Conclusions

UV and heat shock were efficient treatments with respect to microbial quality of fruits, by maintaining the microbial loads below the legal or recommended limits for 10-day storage. Ozone and chlorine treatments were not effective in reducing microbiological growth as microbial counts were similar to the ones of fruits just washed in water.

Fresh cut kiwifruit slices metabolism, assessed as CO_2 production, was affected by sanitizing treatments, being the lowest respiration rate observed in UV-C treated fruits.

When compared with the control treatment, colour parameters of fruit slices were not affected by the different treatments with exception to luminosity of UV treated samples, which was nevertheless maintained for the first six days of storage. L^* value maintenance was due to the lower existence of water-soaked tissues as, in the same 6-day period, UV samples showed improved firmness retention.

Therefore, UV-C non ionizing radiation, even at a low dose (1 kJ/m^2) seems to be an interesting and promising methodology for fresh cut kiwifruit sanitizing, allowing overall quality preservation of fruit slices. Further studies are needed in order to better understand the mechanism behind fruit slices firmness preservation.

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Capítulo V

Conclusões gerais

1 Conclusões gerais

O objectivo principal do presente trabalho consistiu no estudo de metodologias de produção de kiwi minimamente processado que permitissem manter elevados níveis de qualidade durante o período de vida útil, tornando-o compatível com a comercialização. Assim, como principais etapas teve o estudo: (i) dos processos de degradação envolvidos na perda de qualidade de kiwi minimamente processado; (ii) da importância da adequação da matéria-prima ao processamento mínimo; (iii) de novas metodologias capazes de minimizar as perdas de qualidade sofridas por kiwi minimamente processado, durante o processo de armazenamento bem como (iv) o impacto que as metodologias aplicadas apresentaram na qualidade global do produto.

Os resultados obtidos permitiram retirar um conjunto de conclusões como se descrimina de seguida.

- O principal mecanismo de perda de qualidade, envolvido na degradação de kiwi minimamente processado durante o seu armazenamento é o amolecimento excessivo do pericarpo exterior do fruto, o que o torna inaceitável do ponto de vista sensorial.
- O estado de maturação do fruto condiciona a sua adequação como matéria-prima na produção de kiwi minimamente processado bem como, a escolha do tipo de metodologia (s) a aplicar durante o processamento, com vista à conservação e preservação de qualidade do produto minimamente processado.
- Tratamentos térmicos moderados, por imersão em água, mostraram ser uma metodologia eficaz na preservação de qualidade de kiwi fresco cortado. O binómio temperatura / tempo de tratamento que conduziu a melhores resultados foi 45 ºC / 25 minutos, reduzindo a taxa de amolecimento e perda de textura do fruto fatiado ao longo do seu armazenamento a 4 ºC.
- A activação da enzima pectina metilesterase (PME) e subsequente formação de complexos com o ião cálcio, foi o principal mecanismo envolvido na preservação de firmeza do fruto, tendo sido verificado tanto pelo incremento de actividade da enzima em consequência do tratamento térmico como por um maior teor de cálcio ligado nos frutos tratados.

- A aplicação do tratamento térmico permite ainda incrementar o valor nutricional do fruto pois induz a síntese de ácido ascórbico aumentando o já elevado teor em vitamina C.
- Por outro lado induz também um aumento no teor de açúcares solúveis tornando o fruto mais doce o que apresenta uma importância relevante pois, frutos com melhores características para processamento mínimo, encontram-se num estado de maturação mais incipiente o que os pode inviabilizar do ponto de vista sensorial dado a elevada acidez e baixo teor de sólidos solúveis.
- A eficácia dos tratamentos térmicos na manutenção de qualidade dos frutos cortados depende de vários factores nomeadamente do estado de maturação em que o fruto se encontra. Assim, a eficiência do tratamento é maior quando o fruto se encontra maduro mas numa fase precoce da maturação, da firmeza inicial (*"firm ripe"*) e do teor de cálcio do mesmo.
- Tratamentos de imersão em cálcio apresentam-se como alternativa à aplicação de tratamentos térmicos quando estes, pelo exposto anteriormente, não se adequam mas verificou-se um efeito sinérgico entre as duas metodologias na preservação da integridade dos tecidos durante o armazenamento.
- A sinergia entre os dois tratamentos só ocorre quando estes são aplicados sequencialmente. Tratamentos térmicos moderados em soluções de cloreto de cálcio (1-3 g/L) não apresentaram vantagem interferindo marginalmente na qualidade do kiwi fresco cortado devido ao efeito inibitório da activação da PME que apresentam. Por outro lado, o efeito da aplicação de tratamentos térmicos moderados pode ser incrementado se, durante as operações de processamento mínimo, forem efectuados tratamentos de imersão em soluções de cálcio. Este efeito é sobretudo notado nos primeiros dias de armazenamento mas, ao longo do mesmo os frutos mantêm um maior razão pectina solúvel / pectina total e, pela observação microscópica, tecidos mais estruturados. Contudo, de entre as concentrações de cálcio estudas, a mais baixa (1 g/L) revelou-se suficiente não tendo sido verificada vantagem no seu incremento.
- Dos estudos de descontaminação efectuados foi possível concluir que o kiwi não apresenta um risco elevado de degradação por via microbiológica. Os reduzidos valores de pH em conjunto com os baixos níveis de contaminação inicial que o fruto normalmente apresenta conduzem a que não seja este o principal motivo de degradação do fruto minimamente processado. Contudo,

por questões de segurança, o processo de produção tem que incluir uma fase de descontaminação que garanta a inocuidade do produto.

- Tratamentos de higienização através de água ozonada ou clorada não se mostraram eficazes na descontaminação de kiwi minimamente processado, conseguindo níveis de redução microbiológica semelhantes aos atingidos com a lavagem em água e ultrapassando, ao longo do armazenamento, os valores recomendados pela bibliografia para contagens de leveduras. Contudo também não apresentaram qualquer influência negativa nos parâmetros físicos de qualidade.
- Tratamentos de exposição a radiação ultra-violeta de baixo comprimento de onda (UV-C, 1kJ / m^2) e tratamentos de branqueamento (95 ºC / 30s) revelaram-se eficientes na descontaminação, permitindo manter os níveis microbiológicos de kiwi fresco cortado abaixo dos valores recomendados durante 10 dias de armazenamento.
- O tratamento com radiação UV-C conduziu ainda à alteração da fisiologia do fruto permitindo uma favorável manutenção da textura e cor do mesmo durante o armazenamento.

Assim, como conclusão geral podemos salientar que o kiwi é um fruto que reúne características nutricionais interessantes e, por não se adequar ao consumo sem descasque, apresenta interesse para processamento mínimo. Os problemas de conservação que manifesta são possíveis de minimizar através de metodologias que assentam maioritariamente em processos físicos, mais desejáveis do ponto de vista ambiental e do consumidor. No entanto, é necessário um conhecimento aprofundado da matéria-prima de forma a poder seleccionar, em cada circunstância, qual a metodologia que permite manter o maior índice de qualidade, por um período de tempo adequado.

De salientar ainda que os resultados encontrados permitiram definir metodologias de conservação para aplicação na produção de kiwi minimamente processado a nível industrial.

2 Perspectivas de trabalho futuro

Durante a execução do trabalho que agora se apresenta muitas vezes se verificou que a meta estabelecida constituía também um novo ponto de partida e, as respostas obtidas para as questões formuladas foram o mote para novas dúvidas.

Assim, no final da realização deste trabalho existem ainda alguns tópicos que merecerão ser objecto de trabalhos futuros.

- Estudos intensivos na definição da qualidade de kiwi para adequação ao processamento mínimo seriam interessantes, nomeadamente a montante da indústria, no desenvolvimento de práticas culturais adequadas à obtenção de frutos com as características pretendidas.
- Verificado o efeito benéfico dos tratamentos térmicos seria importante explorar metodologias alternativas ao tratamento clássico, como os tratamentos óhmicos que do ponto de vista industrial poderão merecer interesse.
- A utilização da radiação UV-C revelou-se interessante, não só do ponto de vista microbiológico mas também, como metodologia de conservação ao induzir alterações na fisiologia do fruto. Seria de todo o interesse estudar o impacto da exposição a doses diferentes de radiação no metabolismo de kiwi minimamente processado.
- Por último, pretendeu-se com este trabalho encontrar respostas que permitissem a produção de kiwi minimamente processado com mais qualidade o que só é possível através de um conjunto de acções integradas. Assim, tentou-se actuar em fases diferentes do processo de produção pela aplicação de tratamentos de conservação, antes do corte, depois do corte bem como na fase de descontaminação. Ficou ainda por explorar uma outra etapa de grande relevância, o processo de embalagem. Estudos sobre o impacto da aplicação de embalagens activas na qualidade de kiwi minimamente processado seriam com certeza úteis.