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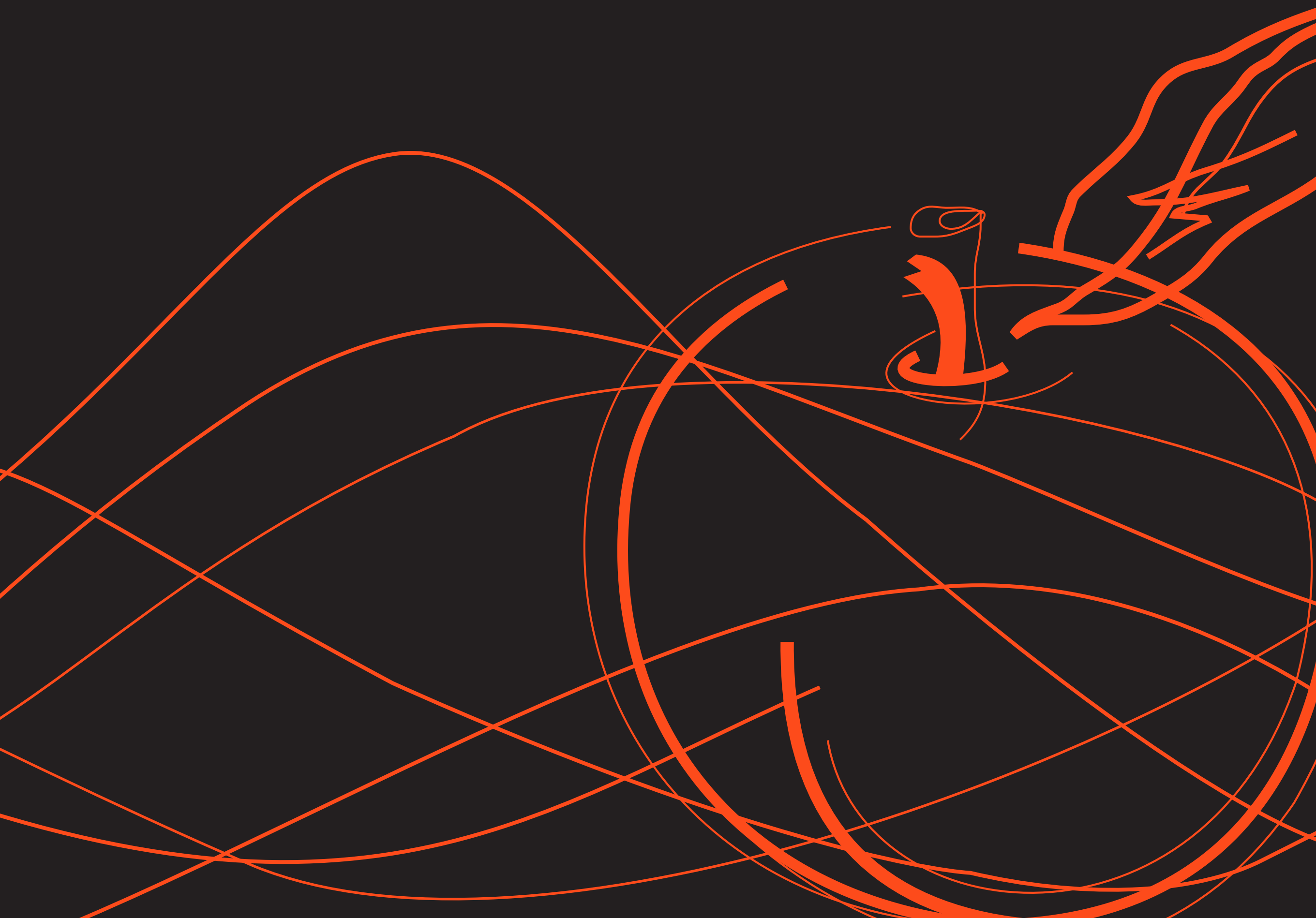

Universitat de Lleida

Escola Tècnica Superior d'Enginyeria Agrària
Departament de Tecnologia d'Aliments

Tratamientos con ácido peracético, radiofrecuencias y microondas para el control de *Monilinia* spp. en poscosecha de fruta de hueso

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Para optar al grado de: **Doctora en Ciencia y Tecnología Agraria y Alimentaria**

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A mis padres y a ti, abuelita

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ÍNDICE

Abreviaturas

AA	Ácido acético (‘Acetic acid’)
ANOVA	Análisis de varianza
ARfD	Dosis de referencia aguda (‘Acute reference dose’)
CE	Comunidad Europea
CFR	‘Code of Federal Regulations’
cfu	Unidades formadores de colonias (‘Colony forming units’)
Dp	Profundidad de penetración (‘Penetration depth’)
EPPO	‘European and Mediterranean Plant Protection Organization’
GRAS	Generalmente reconocido como seguro (‘Generally recognized as safe’)
HP	Peróxido de hidrógeno (‘Hydrogen peroxide’)
HR	Humedad relativa
IFOAM	‘International Federation of Organic Agriculture Movements’
ISM	Industriales, Científicas y Médicas (‘Industrial, scientific and medical)
LMR	Límite Máximo de Residuos
LSD	Mínima diferencia significativa (‘Least significance difference’)
MW	Microondas (‘Microwave’)
PAA	Ácido peracético (‘Peracetic acid’)
PDA	Patata dextrosa agar (‘Potato dextrose agar’)
RH	‘Relative Humidity’
RF	Radiofrecuencias (‘Radio frequency’)
SSC	Contenido de sólidos solubles (‘Soluble solids content’)
US	‘United States’
ε'	Constante dieléctrica (‘Dielectric constant’)
ε''	Factor de pérdida dieléctrica (‘Dielectric loss factor’)

Resumen/Resum/Summary

Resumen

La podredumbre parda causada por *Monilinia* spp. es la enfermedad de poscosecha más importante que afecta a la fruta de hueso. A pesar de que la mayoría de infecciones se producen en el campo, el desarrollo de esta enfermedad ocurre principalmente en poscosecha ocasionando importantes pérdidas económicas al sector. A pesar de que en España, en los años 2012, 2013 y 2014, se ha autorizado de forma temporal y excepcional el uso del producto fitosanitario a base de Fludioxonil 23 % para el control de *Monilinia* spp. en poscosecha de melocotón y nectarina, la aplicación de fungicidas de síntesis en precosecha sigue siendo imprescindible para un buen control de la enfermedad. Sin embargo, en muchas ocasiones los niveles de control son insuficientes y además en los últimos años, la legislación está siendo cada vez más exigente limitando tanto el número de fungicidas que se pueden aplicar como la presencia de residuos en la fruta. Esta situación ha motivado la realización de esta tesis doctoral cuyo principal objetivo fue el estudio de diferentes estrategias de control de la podredumbre parda en poscosecha de melocotón y nectarina. Concretamente, se evaluó el uso del ácido peracético solo o combinado con agua caliente (Capítulo 1) por ser una estrategia que podría implantarse fácilmente en las centrales hortofrutícolas. A continuación, se estudió la mejora del tratamiento de radiofrecuencias estudiado previamente en nuestro laboratorio mediante la inmersión de la fruta en agua durante el tratamiento (Capítulo 2 y 3). Y por último, se estudió el calentamiento por microondas (Capítulo 4) así como la mejora de este tratamiento mediante la inmersión de la fruta en agua (Capítulo 5).

La combinación de 0.25 % de peróxido de hidrógeno, 0.02 % de ácido peracético y 0.075 % de ácido acético correspondiente a 300 mg L^{-1} de ácido peracético (PAA) aplicado mediante baño durante 1 min mostró una elevada eficacia en el control de *M. fructicola* y esta eficacia se mantuvo cuando la misma concentración de PAA se aplicó mediante el producto comercial Proxitane®5:23. La combinación de PAA con agua caliente permitió reducir la concentración eficaz de PAA a 200 mg L^{-1} al aplicarse con agua a 40 ºC durante 40 s sin afectar negativamente a la calidad de la fruta. Esta combinación controló de forma eficaz infecciones recientes de *M. fructicola* independientemente de la concentración de esta, sin embargo, no mostró ningún control de las infecciones de más de 24 h. A la hora de controlar infecciones naturales, el tratamiento solo fue capaz de reducir, aunque ligeramente, la incidencia de podredumbre en 2 de las 3 variedades evaluadas.

La inmersión de la fruta en agua a 20 ºC no solo solucionó la falta de eficacia en nectarinas mostrada por el tratamiento de radiofrecuencias durante 18 min en aire, sino que también, mejoró la eficacia del tratamiento reduciendo de esta forma el tiempo de exposición hasta 9 min. Además, la eficacia del tratamiento con la fruta sumergida en agua no se vio afectada por el tamaño de la fruta, uno de los principales problemas asociados con los tratamientos de radiofrecuencias aplicados en aire. Al aumentar la temperatura del agua a 35 o 40 ºC se consiguió reducir el tiempo de exposición hasta 6 y 4.5 min, respectivamente, observándose reducciones de la enfermedad superiores al 85 % en ambos casos sin afectar a la calidad visual de la fruta.

El tratamiento por radiofrecuencias con la fruta sumergida en agua a 40 ºC durante 4.5 min también mostró una elevada eficacia en el control de infecciones naturales de *Monilinia* spp. y además, la eficacia no dependió del estado de madurez de la fruta. En cambio, la eficacia del tratamiento se redujo, aunque ligeramente, al aumentar el tiempo transcurrido entre la infección y el tratamiento a 48 h o al aumentar la concentración de *M. fructicola* a 10^5 o 10^6 conidios mL^{-1}.

El tratamiento de microondas en aire a 10 kW durante 95 s mostró una elevada eficacia en el control de la podredumbre parda tanto en fruta inoculada artificialmente con *M. fructicola* como en fruta con infecciones naturales. La eficacia de este tratamiento no se vio afectada por el estado de madurez de la fruta, sin embargo, el peso de esta sí que tuvo un efecto significativo, de modo que se observó un mejor control de la enfermedad en la fruta de menor peso en comparación con la fruta de mayor peso. Esta influencia provocó una elevada temperatura en los frutos de menor peso que causó daños térmicos internos.

Al contrario de lo ocurrido en las radiofrecuencias, la inmersión de la fruta en agua a 20 ºC durante el tratamiento de microondas a 10 kW durante 95 s redujo la eficacia mostrada por este tratamiento aplicado en aire. Al aumentar la temperatura del agua a 40 o 45 ºC solo se consiguió incrementar ligeramente la eficacia de este tratamiento, por lo que fue necesario el aumento de la potencia de las microondas hasta 20 kW para obtener un tratamiento eficaz, reduciéndose en un 96 % la incidencia de enfermedad sin provocar daños internos en la fruta cuando el tratamiento de microondas se aplicó durante 60 s con la fruta sumergida en agua a 40 ºC. La eficacia de este tratamiento no dependió ni del peso de la fruta, ni del tiempo transcurrido entre infección y tratamiento (0, 24 o 48 h), ni de la concentración de inóculo de *M. fructicola* (10^3, 10^4 o 10^5 conidios mL^{-1}). Además este tratamiento siguió mostrando un elevado control de la podredumbre parda en fruta con infecciones naturales.

Finalmente, los porcentajes de reducción de la podredumbre parda obtenidos en el estudio de las microondas con la fruta sumergida en agua a 40 ºC y la energía de microondas aplicada se ajustaron a una curva sigmoidea. Según las funciones obtenidas, la mínima energía de microondas necesaria para lograr un 100 % de reducción de la podredumbre parda es de 1287 kJ, de manera que se podría conseguir reducir el tiempo de tratamiento hasta 40 s al aumentar la potencia a 32 kW. Además, también se observó una relación lineal entre la temperatura externa y la energía de microondas aplicada siendo 50.9 ºC la temperatura límite para que no se produzcan daños superficiales en la fruta.

Resum

La podridura marró causada per *Monilinia* spp. és la malaltia de postcollita més important que afecta a la fruita de pinyol. Tot i que la majoria d'infeccions es produeixen a camp, el desenvolupament d'aquesta malaltia succeeix principalment en postcollita ocasionant importants pèrdues econòmiques al sector. Malgrat que a Espanya, en els anys 2012, 2013 i 2014, s'ha autoritzat de forma temporal i excepcional l'ús del producte fitosanitari a base de Fludioxonil 23 % per al control de *Monilinia* spp. en postcollita de préssec i nectarina, l'aplicació de fungicides de síntesi en precollita segueix essent imprescindible per a un bon control de la malaltia. No obstant això, en moltes ocasions els nivells de control són insuficients i a més en els últims anys, la legislació està essent cada cop més exigent limitant tant el nombre de fungicides que es poden aplicar com la presència de residus en la fruita. Aquesta situació ha motivat la realització d'aquesta tesi doctoral amb l'objectiu principal d'estudiar diferents estratègies de control de la podridura marró en postcollita de préssec i nectarina. Concretament, es va avaluar l'ús de l'àcid peracètic aplicat sol o amb aigua calenta (Capítol 1) per tractar-se d'una estratègia que es podria implantar fàcilment a les centrals hortofructícoles. A continuació, es va avaluar la millora del tractament de radiofreqüències estudiat prèviament al nostre laboratori mitjançant la immersió de la fruita en aigua durant el tractament (Capítol 2 i 3). Per últim, es va estudiar l'escalfament per microones (Capítol 4) així com la millora d'aquest tractament mitjançant la immersió de la fruita en aigua (Capítol 5).

La combinació de 0.25 % de peròxid d'hidrogen, 0.02 % d'àcid peracètic i 0.075 % d'àcid acètic corresponent a 300 mg L^{-1} d'àcid peracètic (PAA) aplicat mitjançant bany durant 1 min va mostrar una elevada eficàcia en el control de *M. fructicola* i aquesta eficàcia es va mantindre quan la mateixa concentració de PAA es va aplicar mitjançant el producte comercial Proxitane®5:23. La combinació de PAA amb aigua calenta va permetre reduir la concentració eficaç de PAA a 200 mg L^{-1} quan es va aplicar amb aigua a 40 ºC durant 40 s sense afectar negativament a la qualitat de la fruita. Aquesta combinació va controlar de forma eficaç infeccions recents de *M. fructicola* independentment de la seva concentració, però no va mostrar cap control de les infeccions de més de 24 h. A l'hora de controlar infeccions naturals, el tractament només va ser capaç de reduir, encara que només lleugerament, la incidència de podridura en 2 de les 3 varietats avaluades.

La immersió de la fruita en aigua a 20 ºC no només va solucionar la manca d'eficàcia en nectarines mostrada pel tractament de radiofreqüències durant 18 min en aire, sinó que també, va millorar l'eficàcia del tractament reduint d'aquesta manera el temps d'exposició fins a 9 min. A més, l'eficàcia del tractament amb la fruita submergida en aigua no es va veure afectada pel calibre de la fruita, un dels principals problemes associats amb els tractaments de radiofreqüències aplicats en aire. En augmentar la temperatura de l'aigua a 35 o 40 ºC es va aconseguir reduir el temps d'exposició fins a 6 i 4.5 min, respectivament, observant reduccions de la malaltia superiors al 85 % als dos casos sense afectar a la qualitat visual de la fruita.

El tractament de radiofreqüències amb la fruita submergida en aigua a 40 ºC durant 4.5 min també va mostrar una elevada eficàcia en el control d'infeccions naturals de *Monilinia* spp. i a més, l'eficàcia no va dependre de l'estat de maduresa de la fruita. En canvi, l'eficàcia del tractament es va reduir, encara que lleugerament, en augmentar el temps entre la infecció i el tractament a 48 h o en augmentar la concentració de *M. fructicola* a 10^5 o 10^6 conidis mL^{-1}.

El tractament de microones en aire a 10 kW durant 95 s va mostrar una elevada eficàcia en el control de la podridura marró tant en fruita inoculada artificialment amb *M. fructicola* com en fruita amb infeccions naturals. L'eficàcia d'aquest tractament no es va veure afectada per l'estat de maduresa de la fruita, però el pes d'aquesta sí que va tenir un efecte significatiu, de manera que es va observar un millor control de la malaltia en la fruita de menor pes en comparació amb la fruita de major pes. Aquesta influència va provocar una elevada temperatura en els fruits de menys pes que va causar danys tèrmics interns.

Al contrari de lo que va succeir amb les radiofreqüències, la immersió de la fruita en aigua a 20 ºC durant el tractament de microones a 10 kW durant 95 s va reduir l'eficàcia mostrada per aquest tractament aplicat en aire. En augmentar la temperatura de l'aigua a 40 o 45 ºC només es va aconseguir incrementar lleugerament l'eficàcia d'aquest tractament, pel que va ser necessari l'augment de la potència de les microones fins a 20 kW per obtenir un tractament eficaç, reduint en un 96 % la incidència de malaltia sense provocar danys interns a la fruita quan el tractament de microones es va aplicar durant 60 s amb la fruita submergida en aigua a 40 ºC. L'eficàcia d'aquest tractament no va dependre ni del pes de la fruita, ni del temps entre la infecció i el tractament (0, 24 o 48 h), ni de la concentració d'inòcul de *M. fructicola* (10^3, 10^4 o 10^5 conidis mL^{-1}). A més, aquest tractament va seguir mostrant un elevat control de la podridura marró en fruita amb infeccions naturals.

Finalment, els percentatges de reducció de la podridura marró obtinguts en l'estudi de les microones amb la fruita submergida en aigua a 40 ºC i l'energia de microones aplicada es van ajustar a una corba sigmoide. Segons les funcions obtingudes, la mínima energia de microones necessària per aconseguir un 100 % de reducció de la podridura marró és de 1287 kJ, de manera que es podria aconseguir reduir el temps de tractament fins a 40 s en augmentar la potència a 32 kW. A més, també es va observar una relació lineal entre la temperatura externa i l'energia de microones aplicada essent 50.9 ºC la temperatura límit per a que no es produeixin danys superficials a la fruita.

Summary

Brown rot caused by *Monilinia* spp. is the most important postharvest disease of stone fruit. Fruit are basically infected in the field, but disease development mainly occurs in postharvest where this disease causes significant economic losses. Although in Spain, in 2012, 2013 and 2014, the use of the fungicide based on Fludioxonil 23 % was authorized temporarily and exceptionally to control *Monilinia* spp. in postharvest of peaches and nectarines, but the application of synthetic fungicides in preharvest remains essential for a good control of this disease. However, many times the level of control is insufficient and, besides, in recent years, legislation is being increasingly demanding, since it limits both the number of fungicides that can be applied as the presence of residues in fruit. Therefore, the main objective of this thesis was to study different strategies to control brown rot in postharvest of peaches and nectarines. Specifically, the use of peracetic acid alone or in combination with hot water (Chapter 1) since this method could be easily implemented in a packinghouse. Then, the improvement of the radio frequency treatment previously studied in our laboratory was evaluated by immersing fruit in water during treatment (Chapter 2 and 3). Finally, microwave heating (Chapter 4) as well as the improvement of this treatment by immersing fruit in water were evaluated (Chapter 5).

The combination of 0.25 % hydrogen peroxide, 0.02 % peracetic acid and 0.075 % acetic acid corresponding to 300 mg L^{-1} of peracetic acid (PAA) applied by dipping fruit for 1 min showed high efficacy to control *M. fructicola*, and this efficacy was similar to that observed when the same PAA concentration was applied with the commercial product Proxitane®5:23. The combination of PAA with hot water allowed reducing the effective PAA concentration to 200 mg L^{-1} when it was applied with hot water at 40 ºC for 40 s without negatively affecting fruit quality. This combination effectively controlled recent infections of *M. fructicola* regardless their concentration, however, brown rot was not controlled when time between infection and treatment was over 24 h. In naturally infected fruit, brown rot incidence was slightly but significantly reduced in 2 of the 3 varieties evaluated.

Immersion of fruit in water at 20 ºC not only solved the lack of efficacy observed in nectarines by the radio frequency treatment for 18 min in air, but also improved treatment efficacy since it reduced exposure time to 9 min. Furthermore, the efficacy with fruit immersed in water was not affected by fruit size, one of the

main problems associated with radio frequency treatments applied in air. By raising water temperature to 35 or 40 ºC, exposure time was reduced to 6 and 4.5 min, respectively, in which brown rot reduction was over 85 % for both treatments without affecting visual fruit quality.

Radio frequency treatment with fruit immersed in water at 40 ºC for 4.5 min also showed high efficacy to control natural infections of *Monilinia* spp. and moreover, the efficacy did not depend on fruit maturity level. However, treatment efficacy was slightly reduced by increasing time between infection and treatment to 48 h or concentration of *M. fructicola* to 10^5 o 10^6 conidia mL^{-1}.

Microwave treatment in air at 10 kW for 95 s showed high efficacy to control both natural and artificial infections of *M. fructicola*. The efficacy of this treatment was not affected by fruit maturity level; however, fruit weight had a significant effect, so a better disease control was observed in small fruit in comparison with larger fruit. Internal thermal damages were observed in the smallest fruit due to the high temperatures achieved in these fruit.

Immersion of fruit in water at 20 ºC during microwave treatment at 10 kW for 95 s reduced the efficacy showed by this treatment applied in air. By raising water temperature to 40 or 45 ºC, treatment efficacy was only slightly increased, so an increase of microwave power to 20 kW was necessary for obtain an effective control. Brown rot was reduced by 96 % without causing internal fruit damages when microwave treatment at 20 kW with fruit immersed in water at 40 ºC was applied for 60 s. The efficacy of this treatment was not affected by fruit weight, time between infection and treatment (0, 24 or 48 h) and inoculum concentration of *M. fructicola* (10^3, 10^4 o 10^5 conidia mL^{-1}). Furthermore, this treatment also provided a high brown rot control in naturally infected fruit.

Finally, the percentages of brown rot reduction obtained in the study of microwaves with fruit immersed in water at 40 ºC and the applied microwave energies were fitted to a sigmoid curve. According to obtained functions, complete brown rot reduction could be achieved by applying more than 1287 kJ, thus, a reduction of treatment time to 40 s could be achieved by increasing power level to 32 kW. A linear relationship was observed between external temperature and the applied microwave energy, and 50.9 ºC was the limit temperature to avoid surface damages in the fruit.

Introducción general

1. La podredumbre parda

La podredumbre parda causada por *Monilinia* spp. es la enfermedad más importante que afecta a la fruta de hueso incluyendo melocotones, nectarinas, cerezas, albaricoques y ciruelas. Además de *Monilinia* spp., otros patógenos pueden provocar podredumbres en estos cultivos, aunque en menor medida, como *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill, *Penicillium expansum* Link, *Geotricum candidum* Link, *Botrytis cinerea* Pers.:Fr., *Alternaria alternata* (Fr.:Fr.) y *Mucor piriformis* E. Fischer (Barkai-Golan, 2001). El desarrollo de *Monilinia* spp. no solo se produce en campo sino también en poscosecha llegando a ocasionar hasta un 80 % de pérdidas en años de climatología favorable para la enfermedad (Hong *et al.*, 1998). Estas pérdidas pueden producirse tanto en las centrales hortofrutícolas, en los puntos de venta o en casa de los consumidores, lo que hace que esta enfermedad tenga un gran impacto para el productor ya que a las pérdidas de fruta hay que añadir las reclamaciones y la pérdida de confianza del cliente.

1.1. *Monilinia* spp.

Las principales especies causantes de podredumbre parda en melocotones y nectarinas son *Monilinia laxa* (Aderh. et Rulh.) Honey y *Monilinia fructicola* (G. Wint.) Honey. Existe una tercera especie, *Monilinia fructigena* (Honey in Whetzel), que aunque también puede ser causante de podredumbre parda en fruta de hueso, afecta principalmente a fruta de pepita (Byrde y Willetts, 1977). Además, en 2002, se identificó una cuarta especie, *Monilia polystroma*, cuya presencia se limitaba a Japón (van Leeuwen *et al.*, 2002) pero que en los últimos años también se ha descrito en China, Hungría, República Checa y Suiza (Petróczy y Palkovics, 2009; Zhu y Guo, 2010; EPPO, 2011; Hilber-Bodmer *et al.*, 2012).

A diferencia de *M. laxa* y *M. fructigena*, la presencia de *M. fructicola* es muy reciente en Europa y en España (De Cal *et al.*, 2009) y se encuentra en focos controlados, sin embargo, es la especie más virulenta y ampliamente extendida en otros países productores de fruta de hueso como Estados Unidos, Nueva Zelanda y en algunos países de América del Sur.

Previo a la identificación de *M. fructicola* en España como causante de podredumbre parda en fruta de hueso, los estudios publicados indicaban que *M. laxa* era la principal especie causante de podredumbre en poscosecha de melocotones y nectarinas, identificada en el 85-90 % de los aislamientos realizados a partir de fruta podrida, seguida de *M. fructigena*, identificada en un 10-15 % de los aislamientos (Larena *et al.*, 2005). Sin embargo, estudios recientes indican que en los campos donde *M. fructicola* está presente esta ha desplazado a *M. fructigena* y ahora coexiste con *M. laxa* en niveles similares de frecuencia relativa (Villarino *et al.*, 2013).

El género *Monilinia* se clasificó inicialmente como *Monilia* y se incluyó dentro de los deuteromicetos pero desde el momento en que se conoció su fase sexual, el género pasó a denominarse *Monilinia* y a pertenecer al filo Ascomycota (Eriksson, 2006). En la reproducción asexual, los conidios de las 3 especies se producen en cadenas simples o ramificadas dicotómicamente agrupadas en esporodoquios (Byrde y Willetts, 1977). Estos conidios son unicelulares, hialinos con forma de limón cuyas dimensiones varían entre 11.5-21 x 8-13 μm dependiendo de la temperatura y del medio de cultivo. Por el contrario, en la reproducción sexual, los microconidios, de 2 μm de diámetro, se producen sobre conidióforos y parece que poseen una función espermátida para formar apotecios, de 5-20 mm de diámetro, anaranjados y en forma de copa donde las ascas se encuentran ligeramente pedunculadas. Las ascas tienen dimensiones entre 102-215 x 3-13 μm y están compuestas por 8 ascosporas unicelulares y ovoides de 6-15 x 4-8 μm (Byrde y Willetts, 1977). Los apotecios de *M. fructicola* no han sido encontrados todavía en Europa (Villarino *et al.*, 2010) pero sí que han sido descritos en otros países (Biggs y Northover, 1985). Sin embargo, los apotecios de *M. laxa* y *M. fructigena* se forman rara vez en el campo o en el medio de cultivo y tampoco se han descrito en España (Gell *et al.*, 2009).

Aunque hay diferencias morfológicas entre las tres especies, en algunos casos es difícil distinguirlas, siendo especialmente complicada su identificación por la sintomatología de la enfermedad en fruto (Figura 1). En placa, una de las principales características para la distinción de las tres especies son los márgenes de las colonias ya que estos son lobulados en el caso de *M. laxa* mientras que en *M. fructigena* y *M. fructicola* son enteros. En medio patata dextrosa agar (PDA), *M. fructigena* forma colonias de color crema, con un crecimiento uniforme y continuo y con abundante micelio aéreo. Las colonias de *M. laxa* son de color marrón grisáceo, poco prominentes y con anillos de crecimiento más oscuros (Muñoz *et al.*, 2008). Y *M. fructicola* forma colonias de color marrón grisáceo y de mayor diámetro que las de las otras especies en las mismas condiciones (EPPO, 2009). A pesar de estas diferencias, la identificación de las 3 especies mediante características morfológicas y culturales comporta procedimientos lentos e identificaciones que pueden no ser concluyentes (Lane, 2002). La biología molecular, y en concreto la técnica de la reacción en cadena de la polimerasa ha facilitado de manera importante el análisis de microorganismos y hongos en particular, aportando una capacidad superior para caracterizar, clasificar cepas y estudiar la diversidad genética de las poblaciones (Louws *et al.*, 1999). En este sentido, Gell *et al.* (2007) diseñó una serie de *primer*s específicos que permiten tanto la detección como la identificación de las 3 especies de *Monilinia*.

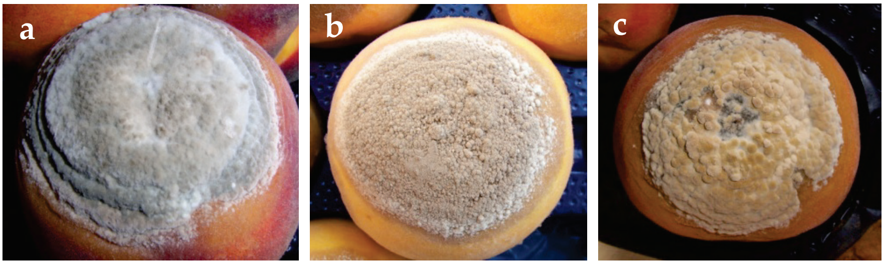

Figura 1. Melocotones afectados por la podredumbre parda causada por *M. laxa* (a), *M. fructicola* (b) y *M. fructigena* (c).

1.2. Ciclo epidemiológico de *Monilinia* spp.

La podredumbre parda se caracteriza por ser una enfermedad policíclica (Figura 2). *Monilinia* spp. sobrevive el invierno principalmente en forma de micelio o conidios en los frutos momificados, restos de pedúnculos, cicatrices, brotes y chancros (Byrde y Willetts, 1977). En primavera, este micelio produce nuevos conidios que, unidos a los ya existentes y a las ascosporas formadas sobre los apotecios, constituyen la fuente de inóculo primario que infecta las flores (Biggs y Northover, 1985). En el Valle del Ebro, la principal fuente de inóculo primario son los frutos momificados de los árboles y además, el número de estos está relacionado positivamente con la incidencia de la podredumbre parda en poscosecha (Villarino *et al.*, 2010).

Tras la infección de las flores, y particularmente en condiciones de humedad, el micelio de *Monilinia* spp. produce hifas cortas que se reúnen ejerciendo presión sobre la epidermis, saliendo al exterior y formando numerosos esporodoquios conidiales sobre los restos florales, desde donde se liberarán nuevos conidios. Al mismo tiempo, el micelio avanza rápidamente hacia los frutos recién formados, produciendo infecciones que permanecerán latentes hasta la madurez del fruto, y hacia el brote formando chancros elípticos con una producción masiva de gomas donde se observan conidios constituyendo así, una fuente de inóculo secundario (Byrde y Willetts, 1977).

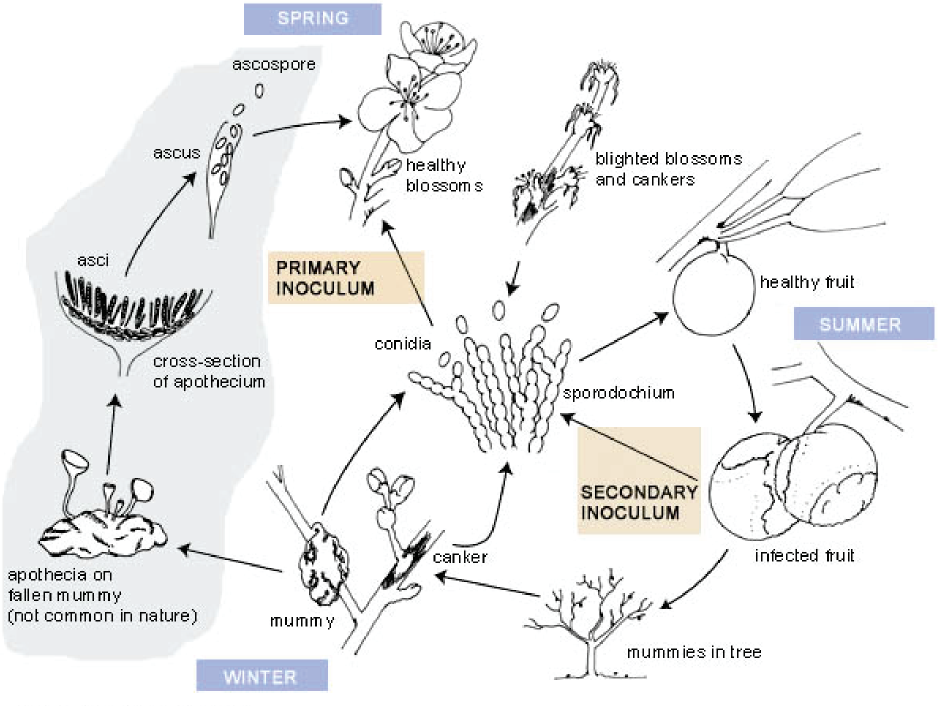

Figura 2. Ciclo epidemiológico de *Monilinia* spp. (Ritchie, 2000).

A partir de los tejidos infectados, los conidios pueden ser dispersados por corrientes de aire, agua de lluvia o también ser transportados por insectos y pájaros (Byrde y Willetts, 1977) y penetran en los frutos sanos a través de heridas o aperturas naturales. Aunque la infección normalmente comienza por la penetración del hongo a través de las heridas, también es posible la sucesiva extensión de la infección por contacto entre frutos adyacentes dando lugar a los denominados nidos (Michailides y Morgan, 1997). Las condiciones climáticas son críticas para la infección, siendo la temperatura y el periodo de humectación, los factores climáticos que más influyen sobre la penetración e infección de *Monilinia* spp. (Gell *et al.*, 2008). Temperaturas superiores a 12 ºC con periodos de humectación superiores a 7 horas diarias o temperaturas superiores a 22 ºC y al menos 4 horas diarias de humectación, son las condiciones favorables para la infección en frutos (Biggs y Northover, 1988).

La infección de los frutos se puede producir en cualquier momento de su desarrollo pero su susceptibilidad a la infección aumenta con la maduración

(Ogawa *et al.*, 1995), siendo el periodo de máxima sensibilidad 2 o 3 semanas antes de la cosecha (Biggs y Northover, 1988). Villarino *et al.* (2011) indicaron que las altas concentraciones de ácido clorogénico y ácido neoclorogénico en los frutos inmaduros podían contribuir a la mayor resistencia de estos frutos a la infección. Por este motivo, si la infección del fruto se produce en los estados iniciales de la formación de este, la infección puede permanecer latente hasta la madurez del fruto, desde donde se puede iniciar el desarrollo de la enfermedad tanto dos semanas antes de la cosecha como después de la misma mientras el fruto madura (De Cal y Usall, 2000). En el Valle del Ebro, la mayor incidencia de infecciones latentes se produce normalmente pocas semanas antes de la cosecha y por el tiempo que tarda en aparecer la primera infección latente se puede llegar a predecir la incidencia de enfermedad en poscosecha con dos meses de antelación (Villarino *et al.*, 2012). En cambio, si la infección se produce en los frutos maduros, los conidios colonizan los tejidos con rapidez apareciendo primero pequeñas manchas de color marrón, que rápidamente muestran podredumbre y se cubren de conidios (De Cal y Melgarejo, 2000). Estos frutos que presentan podredumbre constituyen la fuente de inóculo secundario más importante 2 o 3 semanas antes de la cosecha, cuando los frutos son más susceptibles a la infección. Además, si permanecen en el árbol, estos frutos pierden su contenido en agua dando lugar a las momias características de la enfermedad que servirán como fuente de inóculo primario para el año siguiente (Byrde y Willetts, 1977).

2. Control de la podredumbre parda

2.1. Prácticas culturales

La eliminación del inóculo es uno de los principales métodos culturales recomendados para reducir la incidencia de *Monilinia* spp.. Como se ha comentado anteriormente, el patógeno sobrevive el invierno en frutos momificados, pedúnculos y chancros por lo que la eliminación de estos reduce el inóculo primario que no solo puede infectar a las flores en primavera sino que también, puede infectar posteriormente a los frutos ya que, según estudios nuestros aún no publicados, los frutos momificados pueden seguir produciendo conidios durante todo el verano. Además, también puede ser importante, durante el periodo de floración y el cuajado de los frutos, eliminar las flores infectadas y chancros que ya son una fuente de inóculo secundario para los frutos, así como posteriormente, eliminar los frutos maduros infectados para impedir de este modo, su momificación y la diseminación a otros frutos sanos (Byrde y Willetts, 1977).

Una gestión adecuada del microclima mediante un manejo correcto de la poda es otra práctica cultural que también puede ayudar al control de la enfermedad consiguiendo un mayor grado de insolación y ventilación y reduciendo así, las condiciones de alta humedad relativa que favorecen el desarrollo del patógeno (Michailides y Morgan, 1997). Además, mantener el suelo a una humedad adecuada puede reducir el estrés de la planta y aumentar la tolerancia a la infección floral (Ogawa *et al.*, 1995).

Una fertilización equilibrada con nitrógeno, fosfato y potasio, y una correcta planificación de los riegos, también pueden ser interesante a la hora de reducir la incidencia de podredumbre parda, ya que estos pueden incidir en la resistencia natural que presentan los frutos a desarrollar podredumbres (Usall *et al.*, 2013). Además, aplicaciones foliares con calcio, cuya deficiencia está asociada a la presencia de microfracturas de la cutícula y al incremento de la susceptibilidad de los frutos a los patógenos, pueden reducir no solo el número de frutos infectados por árbol en el momento de la cosecha sino también, reducir la incidencia de podredumbre parda en poscosecha (Elmer *et al.*, 2007).

Por último, también es importante realizar un adecuado manejo durante la cosecha para evitar tanto nuevas infecciones como para reducir la proliferación de la enfermedad durante y después de la cosecha. En este sentido, la utilización de cajas y embalajes limpios, evitar golpes y heridas durante la recolección de los frutos, realizar un correcto transporte hasta la central hortofrutícola y una rápida refrigeración de la fruta recolectada son algunas de las acciones más importantes.

2.2. Fungicidas químicos de síntesis

Actualmente, en la Unión Europea no existe ningún producto químico autorizado para ser aplicado en la poscosecha de fruta de hueso, sin embargo, en España, en los años 2012, 2013 y 2014, se ha autorizado de forma temporal y excepcional, desde junio hasta septiembre, el uso de Scholar® (Fludioxonil 23 %) para el control de *Monilinia* spp. en poscosecha de melocotón, nectarina, cereza y ciruela, en este último caso, solo para exportaciones fuera de la Comunidad Europea. No obstante, aun existiendo dicha autorización, la aplicación de fungicidas de síntesis en precosecha sigue siendo la estrategia de control más importante para controlar la podredumbre parda en fruta de hueso.

La utilización masiva, continuada y en algunos casos poco controlada de los fungicidas químicos de síntesis ha generado una serie de problemas como la aparición de cepas fúngicas resistentes. En las últimas décadas, se han descrito cepas de *M. fructicola* y *M. laxa* resistentes a bencimidazoles, dicarboximidas y triazoles en distintas partes del mundo (Elmer y Gaunt, 1993; Holb y Schnabel, 2007; Thomidis *et al.*, 2009; Chen *et al.*, 2013). En España, hasta el año 2006, no se había detectado ningún aislado resistente de *M. laxa*, sin embargo, recientemente se han descrito cepas de *M. fructicola* y *M. laxa* resistentes a bencimidazoles y a dicarboximidas y de *M. fructicola* a triazoles (Egüen *et al.*, 2012).

Además, la mayor sensibilidad de los consumidores a los problemas que estos productos químicos de síntesis pueden producir en la salud humana y en el medio ambiente, ya supuso, en su momento, la regulación de su uso por parte de los diferentes Estados mediante el establecimiento de una serie de Límites Máximos de Residuos (LMR) bastantes restrictivos. En la actualidad y después de varios años de trabajo, la Comunidad Europea consiguió armonizar los residuos en todos los Estados miembros, y desde el pasado 31 de diciembre de 2008, existe un listado único de materias activas y LMRs común para todos los países de la Unión Europea (Anejo I del Reglamento CE 178/2006 y Anejos II, II y IV del Reglamento CE 149/2008). Según este listado final, de las 917 materias activas que se podían utilizar a principios de los 90 (Directiva CE 91/414) se han pasado a solo 276 a finales del 2008. Desde entonces, la Unión Europea regula la comercialización y el uso de los productos fitosanitarios (Reglamento CE 1107/2009) y establece un marco de actuación para conseguir un uso sostenible de estos productos (Directiva 2009/128/CE y Real Decreto 1311/2012).

A esta situación legislativa hay que añadir las exigencias cada vez más restrictivas de las grandes cadenas de supermercados europeos, que sin un criterio científico claro, restringen el número de productos que admiten, disminuyen el nivel de residuos permitido a un 50 o 75 % del LMR y fijan otros parámetros como la Dosis de Referencia Aguda (ARfD), para exigir así a los productores que disminuyan el uso y la cantidad de fitosanitarios para el control de plagas y enfermedades.

2.3. Métodos alternativos

Toda esta problemática, unida a la creciente preocupación social sobre los riesgos para la salud humana y el medio ambiente relacionados con los altos niveles de fungicidas utilizados, ha incentivado la investigación sobre métodos alternativos a los productos habituales que, por si solos o en combinación, garanticen una eficacia similar o superior a los fungicidas sintéticos en el control de enfermedades, sin los problemas que estos generan y sin afectar negativamente a la calidad.

En los últimos años, numerosos métodos alternativos han sido estudiados para controlar enfermedades de poscosecha en general y la podredumbre parda en particular. Estos métodos alternativos según su naturaleza se pueden clasificar en: métodos biológicos, métodos químicos y métodos físicos.

Entre los métodos biológicos, el uso de microorganismos antagónicos ha sido la técnica más estudiada para el control de enfermedades de poscosecha. Hasta el momento, numerosos microorganismos (bacterias, mohos y levaduras) han demostrado su eficacia para el control de la podredumbre parda en fruta de hueso como *Epicoccum nigrum* (Larena *et al*. 2005; Mari *et al*. 2007), *Penicillium frequentans* (Guijarro *et al.*, 2007), *Bacillus subtilis* (Yánez-Mendizábal *et al.*, 2011), *Bacillus amyloliquefaciens* (Liu *et al.*, 2011), *Aureobasidium pullulans* (Zhang *et al.*, 2010; Mari *et al.*, 2012) y *Phaeosphaeria nodorum* (Pimenta *et al.*, 2012). Actualmente a nivel comercial, el producto Serenade® Max, a base de *Bacillus subtilis*, es el único producto disponible en el mercado para controlar la podredumbre parda en fruta de hueso, sin embargo, su aplicación solo está autorizada en precosecha. Este reducido número de productos comerciales disponibles es debido en parte a la dificultad y al elevado coste que implica su registro, especialmente en la Unión Europea que tiene una normativa muy restrictiva para este tipo de productos (Usall *et al.*, 2013).

Los métodos químicos están basados en sustancias presentes de forma natural en plantas, animales o microorganismos o en productos de síntesis que se caracterizan por ser de baja toxicidad para los humanos y la fauna, tener un impacto medioambiental bajo y dejar un bajo contenido de residuos en la fruta (Knight *et al.*, 1997). En los últimos años, los estudios para el control de la podredumbre parda en este campo se han centrado sobre todo en el uso de quitosano (Yang *et al.*, 2012; Ma *et al.*, 2013), aceites esenciales como tomillo, menta y mirto (Lazar-Baker *et al.*, 2011; Sellamuthu *et al.*, 2013), extractos de plantas (Gatto *et al.*, 2011) y aditivos alimentarios (Palou *et al.*, 2009). Aunque en el mercado existen algunos productos de este tipo no hay ningún estudio concluyente donde la eficacia de estos haya sido demostrada para controlar la podredumbre parda en fruta de hueso.

Dentro de los métodos físicos se ha estudiado la radiación ionizante mediante rayos gamma (Kim *et al.*, 2010) y luz ultravioleta (Bassetto *et al.*, 2007), sin embargo, los métodos físicos que presentan mayor interés para el control de enfermedades de poscosecha son los tratamientos térmicos. Casals *et al.* (2010a; 2010b) demostraron la eficacia de un tratamiento de curado a 50 ºC y 95-99 % de humedad relativa durante 2 horas para controlar la podredumbre parda de manera muy satisfactoria en melocotones y nectarinas, incluso en algunos casos eliminando por completo el desarrollo de la enfermedad. Además del curado, otros tratamientos térmicos se han estudiado para controlar *Monilinia* spp. en fruta de hueso como los baños de agua caliente (Karabulut *et al.*, 2010) o la aplicación de radiofrecuencias (Casals *et al.*, 2010d), ambos tratamientos se explicarán con más detalle en el apartado 4 de esta introducción por ser objeto de estudio de la presente tesis. Actualmente, los tratamientos térmicos se utilizan comercialmente en algunos países de América del Sur y en Israel para controlar otras enfermedades de poscosecha principalmente en frutos que se comen con piel o de producción ecológica (Ben-Yehoshua y Porat, 2005). Aunque a nivel comercial el baño de agua caliente se utiliza de forma muy reducida para controlar la podredumbre parda, Spadoni *et al.* (2013) realizaron estudios donde la aplicación de agua a 60 ºC durante 60 s se evaluó a escala comercial obteniéndose buenos resultados.

3. Productos químicos de baja toxicidad: el ácido peracético

El ácido peracético (PAA), también conocido como ácido peroxiacético, es un fuerte agente oxidante y desinfectante disponible comercialmente en forma de mezcla cuaternaria donde se encuentran en equilibrio ácido acético, peróxido de hidrógeno, ácido peracético y agua, tal y como se muestra en la Figura 3 (Kitis, 2004).

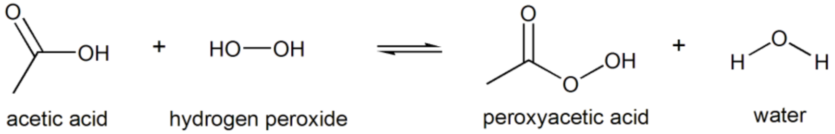

Figura 3. Equilibrio de formación del ácido peracético.

A diferencia de otros desinfectantes ampliamente utilizados en la industria agroalimentaria, como el cloro o el dióxido de cloro, el ácido peracético no genera subproductos tóxicos o mutagénicos después de su reacción con la materia orgánica ya que el ácido peracético se descompone primero en oxígeno y en ácido acético el cual, finalmente, se degrada a dióxido de carbono y agua (Kitis, 2004). Además, la actividad del ácido peracético solo se reduce ligeramente en presencia de materia orgánica y se mantiene en un amplio rango de pH y temperatura (Vandekinderen *et al.*, 2009).

En España, las reglamentaciones no permiten el uso del ácido peracético pero sí del peróxido de hidrógeno y el ácido acético en el agua de bebida y de lavado de frutas y vegetales (Real Decreto 140/2003). En cambio, el *U.S. Code of Federal Regulations* sí que permite el uso de hasta 80 ppm de ácido peracético en el agua de lavado de frutas y vegetales (CFR, 2011). Además, dentro de las Normas IFOAM (*International Federation of Organic Agriculture Movements*) el ácido peracético se encuentra en la lista de desinfectantes de superficies y equipos que pueden estar en contacto directo con productos ecológicos (IFOAM, 2005).

El ácido peracético, debido a su gran poder oxidante, presenta un amplio espectro antimicrobiano y su eficacia ha sido demostrada ante bacterias, mohos, levaduras, virus y endosporas (Block, 2001). Existen pocos trabajos donde se haya investigado el modo de acción del ácido peracético como agente antimicrobiano pero se cree que actúa como otros peróxidos y agentes oxidantes, los cuales oxidan los grupos sulfhidrilo de proteínas, enzimas y otros metabolitos, de forma que se pierde la funcionalidad de muchas de estas macromoléculas y como consecuencia, se produce la ruptura celular por pérdida de funcionalidad de la membrana citoplasmática (Kitis, 2004).

Las propiedades germicidas del ácido peracético fueron descritas por primera vez por Freer y Novy (1902) quienes señalaron la excelente acción de desinfección y esterilización en frío del ácido peracético, pero no fue hasta años más tarde, cuando el desarrollo del proceso comercial para la producción de peróxido de hidrógeno al 90 %, necesario para la fabricación del ácido peracético, permitió disponer de ácido peracético en general (Block, 2001). Desde entonces y debido a las ventajas frente a otros desinfectantes, el ácido peracético ha sido ampliamente estudiado como desinfectante de aguas residuales (Kitis, 2004; Koivunen y Heinonen-Tanski, 2005; Dell'Erba *et al.*, 2007), como desinfectante contra bacterias patógenas de transmisión alimentaria en frutas y vegetales mínimamente procesados (Ölmez y Kretzschmar, 2009; Vandekinderen *et al.*, 2009; Abadias *et al.*, 2011) y como producto higienizante para reducir la concentración de inóculo en el agua de lavado o presente en la superficie de las frutas y vegetales (Alvaro *et al.*, 2009). En este último caso, cabe destacar los resultados obtenidos por Mari *et al.* (1999), quienes observaron, en condiciones semicomerciales, que nectarinas bañadas en una suspensión de conidios de *M. laxa* que habían estado en contacto durante 5 min con 250 mg L^{-1} de ácido peracético no desarrollaban podredumbre.

Por el contrario, los estudios sobre el efecto del ácido peracético en el control de enfermedades de poscosecha han sido limitados. Mari *et al.* (1999) no observaron una reducción significativa del porcentaje de heridas infectadas cuando nectarinas inoculadas artificialmente con *M. laxa* se bañaron durante 1 min en una solución de ácido peracético a 1000 mg L^{-1}. Sin embargo, en un estudio posterior, Mari *et al.* (2004) redujeron la incidencia de *Monilinia* spp. entre un 65 y un 100 % en cerezas, albaricoques, nectarinas y melocotones con infecciones naturales cuando la fruta sin desinfectar y sin heridas se bañó durante 1 min en una solución de 125 mg L^{-1} de ácido peracético y esta eficacia, por lo general, aumentó al incrementar el tiempo de exposición hasta 8 min. Además, en este mismo estudio, el efecto del ácido peracético también se evaluó para controlar infecciones artificiales de *Rhizopus stolonifer*. En este caso, fue necesario una mayor concentración de ácido peracético, 250 mg L^{-1}, para reducir entre un 37 y un 95 % el porcentaje de frutos podridos (Mari *et al.*, 2004).

4. Tratamientos térmicos

Los tratamientos térmicos han sido ampliamente estudiados como método de control tanto de enfermedades como de plagas en poscosecha y constituyen una buena alternativa a los productos químicos de síntesis. Estos tratamientos tienen un amplio abanico de posibilidades ya que pueden ser aplicados directamente a los productos recién recolectados mediante diferentes estrategias. Tradicionalmente, los tratamientos térmicos se han estudiado aplicados mediante agua caliente, aire caliente o vapor caliente, conocido como curado (Lurie, 1998) y más recientemente, mediante la aplicación de radiofrecuencias o microondas, denominado calentamiento dieléctrico (Lu *et al.*, 2007).

Uno de los modos de acción más importantes de los tratamientos térmicos a la hora de controlar enfermedades es el efecto directo sobre el desarrollo de los patógenos mediante la inhibición de la germinación de los conidios, del crecimiento del tubo germinativo o provocando daños sobre las hifas en crecimiento (Ben-Yehoshua y Porat, 2005). Algunos de los efectos directos del calor que han sido observados en *M. fructicola* incluyen cambios en el núcleo, en las pareces celulares, destrucción de mitocondrias y de membranas vacuolares y la pérdida del citoplasma de los conidios (Barkai-Golan y Phillips, 1991). La efectividad de los tratamientos térmicos no solo depende de la temperatura y del tiempo de tratamiento sino que también, factores intrínsecos pueden influir en la eficacia de estos métodos como la especie de patógeno, el contenido de humedad de los conidios, su actividad metabólica, la edad, la carga de inóculo y la posición del patógeno en el huésped (Barkai-Golan y Phillips, 1991).

Además del efecto directo sobre los patógenos, otro modo de acción por el cual los tratamientos térmicos son efectivos a la hora de controlar enfermedades es la inducción de diferentes mecanismos de defensa del huésped (Ben-Yehoshua y Porat, 2005) como la síntesis de materiales antifúngicos constitutivos que actúan como primera línea de defensa contra la invasión de los patógenos. Por ejemplo, la síntesis de enzimas como la fenilalanina amonioliasa, que es un enzima clave en la producción de lignina y de sustancias similares en el sitio de la herida, actuando como barrera física a la penetración de las hifas del patógeno (Brown y Barmore, 1983). Este es un proceso que tiene lugar de forma natural pero que se ve acelerado cuando la temperatura es superior a los 30 ºC. También se ha observado la inducción de la síntesis de compuestos con actividad antifúngica, conocidos genéricamente como fitoalexinas (Ben-Yehoshua *et al.*, 1992) que tienen la habilidad de retardar la germinación de los conidios así como la elongación del tubo germinativo (Kim *et al.*, 1991) y que se sintetizan durante el proceso de cicatrización como respuesta a diversos agentes bióticos o abióticos (Lanza *et al.*, 1994). Además, otro de los mecanismos de inducción de resistencia relacionados con los tratamientos térmicos es el incremento de la biosíntesis de proteínas como la quitinasa o la β-1,3-glucanasa, que son constituyentes de la piel y que juegan un papel muy importante en la degradación de las hifas (Rodov *et al.*, 1996).

4.1. Tratamientos con agua caliente

Los tratamientos con agua caliente fueron descritos por primera vez en 1922 para controlar las enfermedades de poscosecha en cítricos (Fawcett, 1922) y ya en la primeras décadas del siglo XX fue utilizado a escala comercial para el control tanto de enfermedades fúngicas como de plagas. Sin embargo, con el desarrollo de los fungicidas de síntesis selectivos, el uso del tratamiento con agua caliente fue abandonado debido a que los fungicidas presentaban una mayor eficacia, un menor coste y una mayor facilidad de aplicación (Schirra *et al.*, 2000). El uso del tratamiento con agua caliente para controlar *Monilinia* spp. en fruta de hueso fue descrito por

Smith *et al.* (1964) donde lo presentaron como una alternativa efectiva para reducir tanto la mayoría de conidios de *M. fructicola* localizados en la superficie de los melocotones como algunas de las partes vegetativas de este organismo en el interior del fruto.

La aplicación de los tratamientos térmicos mediante baño de agua caliente es un técnica simple que puede ser fácilmente implementada en las centrales hortofrutícolas. En los últimos años, los tratamientos con agua caliente han sido ampliamente estudiados para controlar el desarrollo de podredumbres en numerosas frutas, como cítricos, manzanas y mangos, así como en vegetales y en flores (Fallik, 2004). Respecto al control de *Monilinia* spp., en los últimos años, varios estudios sobre el uso de los baños de agua caliente a diferentes temperaturas y tiempos de exposición han sido publicados. Un tratamiento de agua caliente a 48 ºC durante 12 min fue descrito por Jemric *et al*. (2011) para controlar *M. laxa* tanto en nectarinas como en melocotones. Casals *et al*. (2010c) redujo el tiempo de tratamiento hasta 40 s al aumentar la temperatura del agua hasta 60 ºC reduciendo un 79 % el desarrollo de infecciones naturales de *Monilinia* spp. en melocotones. Karabulut *et al*. (2010) inhibieron por completo el desarrollo de *M. fructicola* en nectarinas, melocotones y ciruelas cuando se bañaron durante 60 s en agua a 60 ºC, sin embargo, esta eficacia se vio reducida tras un periodo de conservación en frío. Y posteriormente, estas mismas condiciones de temperatura y tiempo de tratamiento fueron estudiadas para controlar infecciones naturales a escala comercial (Spadoni *et al.*, 2013).

Aunque los tratamientos con agua caliente tienen una buena actividad contra *Monilinia* spp. y otros patógenos de poscosecha, por lo general, este tipo de alternativas presentan una serie de limitaciones ya que su eficacia depende en gran medida de la edad y concentración de inóculo y de las condiciones fisiológicas de la fruta (Palou *et al.*, 2008), siendo necesario, en ocasiones, su combinación con sustancias químicas para mejorar así su eficacia. Por ejemplo, Margosan *et al*. (1997) demostraron que la mortalidad de los conidios de *M. fructicola* y *R. stolonifer* se producía más rápidamente en soluciones calientes de etanol que en agua caliente y Mari *et al*. (2007) controlaron mejor la podredumbre parda cuando el tratamiento de agua caliente fue combinado con bicarbonato de sodio. Además de para mejorar su eficacia, la combinación del agua caliente también ha sido estudiada con compuestos químicos, especialmente fungicidas, con el fin de reducir las dosis aplicadas del mismo sin disminuir la eficacia de estos en el control de enfermedades (Schirra *et al.*, 2000).

4.2. Calentamiento dieléctrico

El calentamiento dieléctrico es el proceso por el cual las radiaciones electromagnéticas, tanto de radiofrecuencias como de microondas, calientan un material dieléctrico (Rowley, 2004).

En función de cómo los materiales se comportan cuando se someten a un campo electromagnético se pueden clasificar en materiales opacos cuando reflejan totalmente las ondas electromagnéticas, en materiales transparentes cuando las ondas los atraviesa sin ser absorbidas y por último, en materiales dieléctricos, los cuales tienen la habilidad de absorber la energía electromagnética a medida que esta penetra en ellos y de transformarla en energía térmica aumentando así la temperatura en el interior del material al instante (Thostenson y Chou, 1999; Chandrasekaran *et al.*, 2013).

En el calentamiento dieléctrico, la generación de calor es debida principalmente a las fricciones moleculares producidas por el movimiento de iones y de moléculas polares provocado por el continuo cambio de polaridad de los campos eléctricos de las ondas electromagnéticas (Oliveira y Franca, 2002). Los iones tienden a desplazarse hacía la dirección opuesta a su propia polaridad lo que se denomina conducción iónica, en cambio, las moléculas polares rotan para alinearse en función de la polaridad de la onda, tal y como se ilustra en la Figura 4, que es lo que se conoce como rotación dipolar (Marra *et al.*, 2009). Por lo tanto, en el calentamiento dieléctrico el calor es generado directamente en el interior de todo el volumen del producto consiguiéndose así velocidades de calentamiento más rápidas y tiempos de tratamiento más cortos en comparación con los tratamientos térmicos convencionales donde el calor se transporta mediante procesos de conducción y convección desde la superficie hasta el interior del producto.

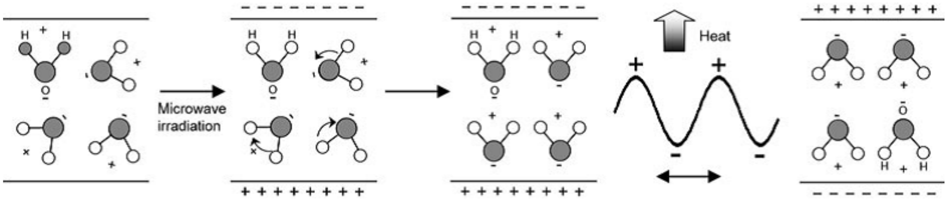

Figura 4. Esquema de la generación de calor mediante el mecanismo de rotación dipolar (Tsuji *et al.*, 2005).

Las propiedades dieléctricas, tanto la constante dieléctrica como el factor de pérdida, son las propiedades físicas más importantes asociadas con el calentamiento dieléctrico (Tang, 2005) ya que describen el comportamiento de un material dieléctrico cuando este se expone a un campo de alta frecuencia (Sosa-Morales *et al.*, 2010). La constante dieléctrica (ε') describe la habilidad de un material para almacenar la energía eléctrica e influye en la distribución del campo eléctrico en el material (Sosa-Morales *et al.*, 2010). En cambio, el factor de pérdida dieléctrica (ε'') influye en la absorción y en la conversión de la energía electromagnética en energía térmica (Vadivambal y Jayas, 2010), de forma que la cantidad de calor generada dentro del material es proporcional al factor de pérdida a una frecuencia y campo eléctrico determinado (Tang *et al.*, 2000). Estas propiedades a su vez dependen de otros muchos factores pero los más importantes son la frecuencia, la temperatura y el contenido de humedad y sales del producto (Lu *et al.*, 2007).

La absorción y la transformación de la energía electromagnética en calor cuando la onda atraviesa un material provoca que la energía de la onda electromagnética disminuya a medida que esta penetra (Marra *et al.*, 2009). Por lo tanto, otro de los factores a tener en cuenta en este tipo de calentamiento es la profundidad de penetración (*Dp*) que se refiere a la distancia por debajo de la superficie a la que la energía de las ondas de microondas o de radiofrecuencias disminuye 1/e (36.8 %) de su valor en la superficie (Sosa-Morales *et al.*, 2010). La profundidad de penetración disminuye al aumentar la frecuencia, por lo tanto, es un parámetro más importante en el calentamiento por microondas (Tang *et al.*, 2000).

La principal desventaja que presenta este tipo de tratamiento es que el calentamiento no es uniforme dando lugar a grandes diferencias de temperatura dentro del producto que pueden causar la aparición de puntos fríos y calientes que no solo pueden afectar a la eficacia del tratamiento sino también a la calidad del producto (Birla *et al.*, 2008; Vadivambal y Jayas, 2010). Esta falta de homogeneidad de las temperaturas no solo es debida a que los productos agrícolas no son uniformes sino que además otros factores como las propiedades dieléctricas, la profundidad de penetración, el medio que rodea al producto y la forma y el tamaño del producto pueden influir (Peyre *et al.*, 1997; Marra *et al.*, 2009). Como muchos son los factores implicados y la falta de uniformidad es la mayor limitación para la implantación del calentamiento dieléctrico en las industrias, en los últimos años las investigaciones se han centrado en el estudio del efecto de estos factores en la distribución de la temperatura tanto para el calentamiento por radiofrecuencias

(Birla *et al.*, 2008) como por microondas (Oliveira y Franca, 2002) para así conocer las principales causas y encontrar medidas que reduzcan la falta de uniformidad. Entre las técnicas propuestas podemos encontrarnos, la combinación del calentamiento dieléctrico con métodos convencionales (Fung y Cunningham, 1980), mantener en movimiento el producto durante el calentamiento (Birla *et al.*, 2004; Geedipalli *et al.*, 2007), equilibrar la temperatura después del calentamiento (Wang *et al.*, 2006) e incorporar un medio absorbente alrededor del producto como podría ser el agua (Ikediala *et al.*, 2002).

El agua, como es un medio absorbente, alrededor del producto ofrece menos resistencia que el aire a los campos eléctricos mejorando de esta forma la distribución de las ondas electromagnéticas dentro del producto (Birla *et al.*, 2008). Sin embargo, también hay que tener en cuenta que al tratarse también de un material dieléctrico, este absorbe energía reduciéndose de esta forma la energía que puede llegar al producto y produciéndose en algunos casos un calentamiento superficial (Ikediala *et al.*, 2002).

4.2.1. Radiofrecuencias

Las radiofrecuencias son ondas electromagnéticas cuya frecuencia varía entre 1 y 300 MHz aunque las frecuencias permitidas para aplicaciones industriales, científicas y medicas (ISM) son 13.56, 27.12 y 40.68 MHz (Marra *et al.*, 2009).

Las radiofrecuencias son generadas por una válvula triodo y son aplicadas mediante electrodos paralelos entre los cuales se coloca el producto. La distancia entre los electrodos puede modificarse para variar así la cantidad de energía de radiofrecuencia transferida al producto. Los equipos industriales de radiofrecuencia pueden trabajar tanto en discontinuo como en continuo, en esta última, el producto es introducido en el interior del equipo mediante una cinta transportadora colocada entre los dos grupos de electrodos. En la Figura 5 se muestra, a modo de ejemplo, el equipo de radiofrecuencias utilizado en los estudios realizados en la presente tesis.

El efecto térmico de las radiofrecuencias fue descubierto por Jacques Arsene d'Ársonval cuando investigaba el efecto de las altas frecuencias (500-1500 kHz) en animales, lo que dio lugar a la creación, en 1895, de la primera unidad de terapia de calor mediante radiación de alta frecuencia en el Hotel Dieu Hospital de Paris. Sin embargo, no fue hasta después de la II Guerra Mundial, cuando esta tecnología se estudió en el procesado de alimentos, en concreto, en la cocción de productos cárnicos elaborados, en la deshidratación y en el escaldado de vegetales. Más tarde, en los años 60, estudios centrados en la aplicación de las radiofrecuencias para la descongelación dieron lugar ya a varias líneas de producción comercial que permitieron, posteriormente, el estudio de las radiofrecuencias en otras aplicaciones como el secado después del horneado de galletas o la pasteurización y esterilización de productos cárnicos (Zhao *et al.*, 2000; Marra *et al.*, 2009).

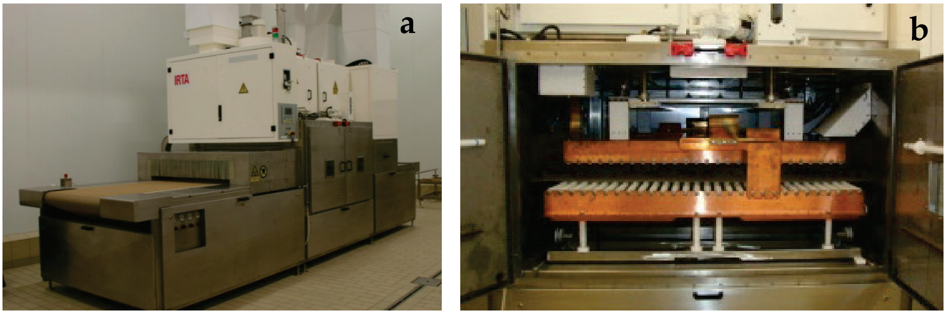

Figura 5. Equipo de radiofrecuencias utilizado en los estudios de la presente tesis (a) y detalle de los grupos de electrodos paralelos (b).

Además de las aplicaciones del calentamiento por radiofrecuencias en diferentes procesos de la industria alimentaria, este tipo de tratamiento también ha sido estudiado como método alternativo para el control de plagas en cerezas (Ikediala *et al.*, 2002), manzanas (Wang *et al.*, 2006), mangos (Sosa-Morales *et al.*, 2009) y nueces (Wang *et al.*, 2001). Por el contrario, pocos estudios se han publicado sobre el uso del calentamiento por radiofrecuencias para controlar enfermedades de poscosecha en fruta. Casals *et al.* (2010d) realizaron los primeros estudios de la aplicación de las radiofrecuencias para controlar la podredumbre parda en fruta de hueso, sin embargo, aunque demostraron la eficacia del tratamiento aplicado durante 18 min en melocotón, el tratamiento no fue efectivo en nectarina.

4.2.2. Microondas

Las microondas son las ondas electromagnéticas cuya frecuencia varía entre 300 MHz a 300 GHz, sin embargo únicamente las frecuencias de 915 y 2450 MHz están autorizadas para aplicaciones industriales, científicas y médicas (Wang y Tang, 2001). La altas frecuencias de las microondas, a diferencia de las de radiofrecuencias, permiten transferir la energía más rápido y por lo tanto, se logran velocidades de calentamiento mayores utilizando menores intensidades de campo (Nelson, 1996; Salazar-González *et al.*, 2012).

En los equipos de microondas, la energía es generada por magnetrones y se transmite a través de una guía de ondas hasta una cámara metálica o cavidad en donde se encuentra el producto. En función del número y potencia de las fuentes generadoras de microondas, los equipos pueden ser de un solo magnetrón de alta potencia o de varios magnetrones de baja potencia. Al igual que los equipos industriales de radiofrecuencia, los de microondas pueden trabajar tanto en discontinuo como en continuo, en este último, el producto es transportado al interior de la cavidad mediante una cinta transportadora (Regier y Schubert, 2004). A modo de ejemplo, en la Figura 6 se muestra el equipo de microondas continuo utilizado en la presente tesis.

Figura 6. Equipo de microondas utilizado en los estudios de la presente tesis.

La energía de microondas no fue descubierta hasta 1940 cuando los científicos John Randall y Henry Boot crearon el primer magnetrón continuo como consecuencia de las investigaciones que estaban realizando sobre el desarrollo de radares para el ejército británico durante la II Guerra Mundial. No fue hasta 1945 cuando, por casualidad, Percy L. Spencer, ingeniero eléctrico de la compañía Raytheon, descubrió el efecto térmico de las microondas cuando trabajaba en el desarrollo de nuevos radares, lo que le llevó a la fabricación, en 1946, del primer horno de microondas. Los primeros estudios realizados se centraron en el uso de las microondas para el tostado del café, el escaldado de vegetales o la liofilización de alimentos. No obstante, los estudios a escala comercial no se empezaron hasta principios de los años 60 cuando se fabricó el primer sistema de microondas industrial en continuo. Desde entonces, el calentamiento por microondas ha sido estudiado como método alternativo para el control de insectos en grano (Nelson, 1996) o en diferentes procesos de la industria alimentaria como secado, pasteurización, esterilización, descongelación y horneado entre otros (Oliveira y Franca, 2002; Chandrasekaran *et al.*, 2013).

Pocos estudios se han publicado sobre el uso del calentamiento por microondas para controlar enfermedades de poscosecha en fruta. Karabulut y Baykal (2002) demostraron la eficacia de las microondas aplicadas durante 2 min con un microondas doméstico a 0.40 kW para controlar tanto *Botrytis cinerea* como *Penicillium expansum* en melocotones. Posteriormente, la combinación de un tratamiento por microondas a 0.45 kW durante 2 minutos con el agente de biocontrol *Cryptococcus laurentii* fue estudiada para controlar *Rhizopus stolonifer* en melocotones (Zhang *et al.*, 2004) y *P. expansum* en pera (Zhang *et al.*, 2006). En ambos estudios, el tratamiento de microondas solo fue capaz de reducir entre un 30 y un 50 % el porcentaje de frutos podridos, sin embargo, esta reducción aumentó hasta un 80 % cuando el agente de biocontrol se aplicó después del tratamiento por microondas.

Las técnicas descritas anteriormente, tanto el ácido peracético como los tratamientos térmicos aplicados mediante agua caliente o por radiofrecuencias y microondas, han sido estudiadas en la presente tesis con el fin de encontrar nuevas alternativas a los fungicidas de síntesis para el control de la podredumbre parda en poscosecha. De este modo se podría proporcionar al sector de la fruta de hueso métodos de control eficaces para hacer frente no solo a las grandes pérdidas ocasionadas por esta enfermedad, sobre todo en años de climatología favorable, sino también a la legislación cada vez más exigente que limita tanto el número de materias activas a utilizar como la presencia de residuos en la fruta.

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Objetivos

El principal objetivo de la presente tesis es el estudio de diferentes estrategias alternativas de control de la podredumbre parda causada por *Monilinia* spp. en poscosecha de melocotón y nectarina. De este modo se pretende proporcionar al sector frutícola de la fruta de hueso métodos de control eficaces para hacer frente no solo a las grandes pérdidas ocasionadas por esta enfermedad sino también a la legislación cada vez más exigente que limita tanto el número de materias activas a utilizar como la presencia de residuos en la fruta.

A continuación se detallan los objetivos específicos de la presente tesis:

1. **Estudiar la eficacia del ácido peracético solo o combinado con agua caliente en el control de la podredumbre parda en melocotón y nectarina**
    - 1.1. Evaluar diferentes concentraciones de peróxido de hidrógeno, ácido peracético y ácido acético aplicados solos o combinados y comparar su eficacia con un producto comercial a base de ácido peracético.
    - 1.2. Reducir la concentración de ácido peracético eficaz mediante la combinación de este con agua caliente.
    - 1.3. Evaluar la eficacia del tratamiento de ácido peracético en soluciones calientes tanto para controlar diferentes tiempos de infección y concentraciones de *M. fructicola* como infecciones naturales de *Monilinia* spp., así como determinar el posible efecto preventivo del tratamiento.
2. **Estudiar la mejora del tratamiento de radiofrecuencias mediante la inmersión de la fruta en agua**
    - 2.1. Determinar el tiempo de tratamiento de radiofrecuencias con la fruta sumergida en agua a 20 ºC necesario para reducir la incidencia de la enfermedad sin causar daños en la fruta.
    - 2.2. Evaluar el efecto del tamaño de la fruta en la eficacia del tratamiento.
    - 2.3. Estudiar la reducción del tiempo de tratamiento mediante el aumento de la temperatura del agua.
    - 2.4. Evaluar el efecto del tratamiento de radiofrecuencias seleccionado en el control de la podredumbre parda a diferentes tiempos después de la inoculación, a diferentes concentraciones de inóculo, en fruta con diferentes estados de madurez y en fruta con infecciones naturales y determinar el efecto de este tratamiento sobre la calidad de la fruta.

3. **Evaluar la eficacia del calentamiento por microondas en el control de la podredumbre parda**
    - 3.1. Determinar la potencia de microondas y el tiempo de tratamiento necesario para reducir la incidencia de la enfermedad sin causar daños en la fruta.
    - 3.2. Estudiar la influencia del peso de la fruta en la eficacia del tratamiento de microondas seleccionado.
    - 3.3. Evaluar la eficacia del tratamiento en fruta con diferentes estados de madurez y con infecciones naturales, así como determinar el efecto del tratamiento en la calidad de la fruta.
4. **Estudiar la mejora del tratamiento de microondas mediante la inmersión de la fruta en agua**
    - 4.1. Evaluar la mejora del tratamiento mediante su aplicación en fruta sumergida en agua a diferentes temperaturas.
    - 4.2. Mejorar la eficacia del tratamiento de microondas con la fruta sumergida en agua mediante el aumento de la potencia de las microondas.
    - 4.3. Estudiar la influencia del peso de la fruta en la eficacia del tratamiento.
    - 4.4. Determinar el efecto del tratamiento de microondas con la fruta sumergida en agua en el control de la podredumbre parda a diferentes tiempos después de la inoculación, a diferentes concentraciones de *M. fructicola* y en fruta con infecciones naturales y evaluar el efecto de este tratamiento sobre la calidad de la fruta.

Capítulo 1

Combination of peracetic acid and hot water treatment to control postharvest brown rot on peaches and nectarines

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Abstract

Brown rot caused by *Monilinia* spp. is the most important postharvest disease of stone fruit. From preliminary studies, the combination of 0.25 % hydrogen peroxide, 0.02 % peracetic acid (PAA) and 0.075 % acetic acid, corresponding to 300 mg L^{-1} of PAA, was selected to control *Monilinia fructicola*. Brown rot control was similarly controlled when the same concentration of PAA was applied with a PAA-based commercial product. In order to reduce PAA concentration, combinations of different concentrations and temperatures were evaluated. A treatment of 200 mg L^{-1} of PAA at 40 ºC for 40 s was selected to control pre-existing and future infections, different inoculum concentrations of *M. fructicola* and to control brown rot on naturally infected fruit. Brown rot was completely controlled with the selected treatment when peaches and nectarines were inoculated 0 h before the treatment but it was not controlled when infection time was increased to 24, 48 and 72 h. Also, the treatment significantly controlled brown rot at all inoculum concentrations evaluated (10^3, 10^4, 10^5 and 10^6 conidia mL^{-1}) in both peaches and nectarines, but no protection against future infections was observed. In naturally infected fruit, brown rot incidence was slightly but significantly reduced to 61 and 36 % in 'Roig d'Albesa' and 'Placido' peaches, respectively, but not in nectarines. Immersion for 40 s in 200 mg L^{-1} of PAA at 40 ºC provides an alternative treatment to control only recent infections of *Monilinia* spp. whatever their concentration without generally affecting fruit quality.

Keywords: *Monilinia* spp., *M. fructicola*, stone fruit, heat treatment

1. Introduction

Brown rot caused by *Monilinia* spp. is the most important postharvest disease of stone fruit. Infection by *Monilinia* spp. mainly occurs in the field at bloom, at the onset of pit hardening, and between 7 and 12 days before harvest (Emery *et al.*, 2000). These infections may remain latent in the fruit until microclimatic conditions are favorable and fruit become mature enough for disease expression (Byrde and Willetts, 1977; Fourie and Holz, 2003). However, postharvest losses by brown rot that routinely occur during storage and transport (Hong *et al.*, 1997) are typically more severe than preharvest losses, sometimes reaching high values (59 %) (Larena *et al.*, 2005). Currently, no chemical fungicides are allowed in the European Union for postharvest application in stone fruits. In addition, public demands to reduce pesticide use and improve environmental and human health, as well as the development of pathogen resistance to widely used synthetic fungicides, limits the preharvest application of chemical products in the field. These concerns, combined with a lack of effective postharvest treatments against *Monilinia* spp. have increased the need to develop new control methods.

In the search for alternatives, substances such as food additives, generally recognized as safe (GRAS) compounds, or low-toxicity chemicals, have been evaluated as alternative control methods for postharvest diseases, either alone or in combination with physical or biological treatments (Gregori *et al.*, 2008; Palou *et al.*, 2009; Casals *et al.*, 2010a).

Peracetic acid (PAA) is commercially available as a quaternary equilibrium mixture containing acetic acid, hydrogen peroxide, peracetic acid and water (Kitis, 2004). PAA breaks down to oxygen and acetic acid, which finally breaks down to carbonic anhydride and water. Spanish regulations allow the use of hydrogen peroxide and acetic acid in drinking and washing water of fruit and vegetables (BOE, 2003). The US Code of Federal Regulations states that the use of peracetic acid in fruit and vegetables is allowed up to 80 ppm in wash water (CFR, 2011). Although peracetic acid is not currently allowed for use in organic processing, at least it is listed among cleansers and disinfectants permitted for direct contact with food in the IFOAM Norms (IFOAM, 2005). Peracetic acid had been widely studied as a disinfectant against spoilage and pathogenic bacteria on fresh-cut fruit and vegetables (Abadias *et al.*, 2011) and as a sanitizing product to reduce conidia in water or present on fruit surfaces (Mari *et al.*, 1999). However, little information is available on the effects of peracetic acid to control postharvest diseases. Mari *et al.*

(2004) reported that immersion of fruit in 125 mg L^{-1} of PAA solution for 1 min controlled brown rot in naturally infected nectarines and peaches and PAA at 250 mg L^{-1} was required to reduce disease caused by *Rhizopus stolonifer* in artificially inoculated stone fruit.

The use of physical treatments, such as hot water, is a simple technique that can be easily used in packinghouses to reduce postharvest diseases of stone fruit. Casals *et al.* (2010a) reported a hot water treatment at 60 ºC for 40 s for controlling brown rot on peaches and nectarines. A briefer exposure time, 20 s, at the same temperature reduced brown rot by 80 % when fruit were passed through a water drench over rotating brushes (Karabulut *et al.*, 2002).

Generally, these alternative treatments have some limitations because their effectiveness is more influenced by environmental factors. For this reason, recent research has focused on the enhancement of the efficacy of the postharvest alternative treatments by the combination of two or more treatments. Over the years, combining chemicals with hot water has been developed in order to improve the effectiveness of treatments alone. For instance, Margosan *et al.* (1997) reported that mortality of conidia of *Monilinia fructicola* and *R. stolonifer* occurred much more quickly in heated ethanol than in hot water. Mari *et al.* (2007) showed similar results when hot water treatments were used in combination with sodium bicarbonate to control brown rot caused by *Monilinia* spp. on stone fruit resulting in better control than either hot water or sodium bicarbonate alone.

The first objective of the present work was evaluate the effectiveness of different concentrations of hydrogen peroxide, peracetic acid and acetic acid either alone or in combination for the control of *M. fructicola*, and then to compare the selected combination with a PAA-based commercial product. In order to reduce the applied peracetic acid concentration, combinations of different PAA concentrations at different temperatures were evaluated in *in vitro* and *in vivo* tests. Finally, a PAA and water temperature combination was selected for evaluating efficacy against brown rot development in naturally infected fruit and in fruit inoculated with *M. fructicola*.

2. Materials and methods

2.1. Fruit

Experiments were conducted with 'Placido', 'Mountain Gold', 'Rome Star', 'Baby Gold 9' and 'Roig d'Albesa' peaches (*Prunus persica* (L) Batch) and 'Albared' and 'Autumn Free' nectarines (*P. persica* var. Nectarine (Ait.) Maxim.). Fruit were grown in commercial orchards located in Lleida (Catalonia) following standard cultural practices and chemical spray programs in the field for pest and disease control. Fruit free of visible wounds and rots and similar visual maturity was selected by hand from fruit bins immediately after harvest. Fruit not used at the time of harvest was stored at 0 ºC not more than 5 days until use.

2.2. Pathogen culture

The isolate of *M. fructicola* (CPMC1) used in this study was from the collection of the Postharvest Pathology Unit, Centre IRTA, Lleida (Catalonia). This strain was isolated from an infected stone fruit, identified by the Department of Plant Protection, INIA, Madrid (Spain), and was maintained on potato dextrose agar (PDA) medium (Biokar Diagnostics, 39 g L^{-1}) amended with acetone (J.T. Baker, 1 %) at 4 ºC in the dark.

2.3. Pathogen production and inoculation methodology

The isolate of *M. fructicola* (CPMC1) was subcultured onto PDA and incubated in the dark at 25 ºC for approximately two weeks. To ensure sufficient conidia production for experimentation, the isolate was inoculated onto peaches or nectarines by wounding the fruit with a sterilized steel rod (1 mm wide and 2 mm long) and transferring conidia and mycelium from the PDA culture to the wound site with a sterile pipette tip. Fruit were then incubated at 25 ºC and 85 % RH in the dark for 5-7 days. Conidia were scraped from infected fruit using a sterile loop and transferred to a test tube containing 5 mL of sterile distilled water and a drop of Tween-80 per liter, and then were sonicated for 5 min. Conidia concentration was measured with a hemocytometer and the suspension diluted to the desired concentration. Fruit to be artificially inoculated were wounded once per fruit with the sterile steel rod and, then, the wound site was inoculated with 15 µL of conidial suspension.

2.4. Chemical products

In preliminary studies, peracetic acid (PAA) solution (~39 % in acetic acid; Sigma-Aldrich, Austria), hydrogen peroxide (HP) 33 % (w/v) stabilized (Panreac Química, S.A.U., Barcelona, Catalonia) and acetic acid (AA) 96 % (Panreac Química, S.A.U., Barcelona, Catalonia) were used. In subsequent studies Proxitane®5:23 (Solvay Chemicals, Barcelona, Catalonia) was used as a PAA-based product. Proxitane®5:23 is a stabilized mixture of 5 % peracetic acid, 23 % hydrogen peroxide and 10 % acetic acid.

2.5. Preliminary screening of hydrogen peroxide, peracetic acid and acetic acid

The effectiveness of different concentrations and combinations of HP, PAA and AA was evaluated to control *M. fructicola* (Table 1). 'Baby Gold 9' peaches were artificially inoculated with *M. fructicola* at 10^3 conidia mL^{-1} as described above. About 2 h later, fruit were treated by applying 15 µL of the chemical solution at the pathogen inoculation site. Control fruit were treated with 15 µL of sterile distilled water. All treatments were conducted with four replicates and ten fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH brown rot incidence was recorded.

Table 1. Chemical composition of treatment solutions used in a preliminary screen to control brown rot in 'Baby Gold 9' peaches.

Treatment solutions	Chemical composition
HP	0.125 % of hydrogen peroxide
2HP	0.25 % of hydrogen peroxide
AA	0.0375 % of acetic acid
2AA	0.075 % of acetic acid
PAA	0.01 % of peracetic acid
2PAA	0.02 % of peracetic acid
3PAA	0.03 % of peracetic acid
HP+AA	0.125 % of hydrogen peroxide + 0.0375 % of acetic acid
2HP+2AA	0.25 % of hydrogen peroxide + 0.075 % of acetic acid
HP+PAA	0.125 % of hydrogen peroxide + 0.01 % of peracetic acid
2HP+2PAA	0.25 % of hydrogen peroxide + 0.02 % of peracetic acid
PAA+AA	0.01 % of peracetic acid + 0.0375 % of acetic acid
2PAA+2AA	0.02 % of peracetic acid + 0.075 % of acetic acid
HP+PAA+AA	0.125 % of hydrogen peroxide + 0.01 % of peracetic acid + 0.0375 % of acetic acid
2HP+2PAA+2AA	0.25 % of hydrogen peroxide + 0.02 % of peracetic acid + 0.075 % of acetic acid

In order to know if the addition of hydrogen peroxide plus acetic acid could react to form peracetic acid, the concentration of PAA of each combination was measured by a colorimetric test (Merck KGaA, Germany).

2.6. Effect of exposure time on PAA efficacy

'Autumn Free' nectarines artificially inoculated with *M. fructicola* at 10^3 conidia mL^{-1} as described above were dipped for 1 or 2 min in a stainless steel tank containing 15 L of the combination of 0.25 % hydrogen peroxide, 0.02 % peracetic acid and 0.075 % acetic acid (2HP+2PAA+2AA). A set of artificially inoculated fruit was immersed in water at 20 ºC for 1 min and was used as a control. All treatments were conducted with four replicates and ten fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH, brown rot incidence was recorded.

2.7. Comparison of the combination of HP, PAA and AA with PAA-based commercial product

This study was carried out in order to compare the prior combination of 0.25 % hydrogen peroxide, 0.02 % peracetic acid and 0.075 % acetic acid (2HP+2PAA+2AA) with a commercial product based on peracetic acid (Proxitane®5:23) to control *M. fructicola* infections. 'Placido' peaches artificially inoculated with *M. fructicola* at 10^3 conidia mL^{-1} as described above were dipped for 1 min in a stainless steel tank containing 15 L of the combination 2HP+2PAA+2AA, corresponding to 300 mg L^{-1} of PAA, or in water with different concentrations of peracetic acid, 50, 100 or 300 mg L^{-1}, obtained from the commercial product Proxitane®5:23. A set of artificially inoculated fruit was immersed in water at 20 ºC for 1 min and was used as a control. All treatments were conducted with four replicates and twenty fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH brown rot incidence was recorded.

2.8. Effect of peracetic acid and water temperature on conidia culturability

The culturability of *M. fructicola* conidia was investigated *in vitro* in order to know the possible reduction of peracetic acid concentration by applying PAA with hot water. Sterile screw-capped glass tubes containing 2.7 mL of sterile distilled water were placed in water baths at 20, 40 and 55 ºC and allowed to equilibrate for 30 min. To a set of tubes at each temperature, Proxitane®5:23 was added to reach a final concentration of 100 mg L^{-1} of peracetic acid. In another set of tubes at each temperature, PAA was not added. Then, in all tubes, 0.3 mL of a *M. fructicola* conidia suspension were added to achieve a final concentration of 10^6 conidia mL^{-1}. After 40 s of exposure time, 0.5 mL were removed from each tube and added to other tubes placed in ice containing 4.5 mL of sterile distilled water. There were three replicates test tubes for each treatment. Aliquots of 100 μL of each suspension were plated onto two PDA Petri dishes. After 72 h of incubation at 25 ºC, the colonies were counted and the results were expressed as percentage of culturable conidia.

2.9. Effect of PAA concentration and water temperature on *M. fructicola* infections

'Mountain Gold' and 'Rome Star' peaches were artificially inoculated with *M. fructicola* at 10^3 conidia mL^{-1} as described above. A stainless steel tank containing 15 L of tap water was heated at 20, 40, 50 and 60 ºC within a 172 L stainless steel water tank fitted with a 9 kW electric resistance heater and thermostat. For each temperature, Proxitane®5:23 was then added to achieve concentrations of PAA of 0, 100, 200 and 300 mg L^{-1}. Finally, metallic grid baskets containing inoculated fruit were submerged in the tank for 40 s. All treatments were conducted with four replicates and five fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH, brown rot incidence was recorded.

2.10. Effect of PAA treatment as a curative or preventive treatment to control *M. fructicola* infections

From the experiment previously described, the combination of hot water at 40 ºC with 200 mg L^{-1} of PAA, obtained from the commercial product Proxitane®5:23, was selected for controlling *M. fructicola* infections.

'Albared' nectarines and 'Baby Gold 9' peaches were artificially inoculated with *M. fructicola* at 10^3 conidia mL^{-1} as described above and were maintained for 0, 24, 48 and 72 h at 20 ºC and 85 % RH. Then, the fruit was dipped for 40 s in 15 L of a solution of 200 mg L^{-1} of PAA at 40 ºC. A set of artificially inoculated fruit of each infection time was immersed in water at 20 ºC for 40 s and was used as a control. All treatments were conducted with four replicates and five fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH brown rot incidence was recorded.

The preventive effect of PAA was evaluated in 'Autumn Free' nectarines and 'Baby Gold 9' peaches. Wounded fruit was dipped for 40 s in a tank containing 15 L of water at 20 ºC or a solution of 200 mg L^{-1} of PAA at 40 ºC. Then, a set of fruit of each treatment was artificially inoculated 0, 24, 48 and 72 h after treatment with *M. fructicola* at 10^3 conidia mL^{-1}. All treatments were conducted with four replicates and five fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH brown rot incidence was recorded.

2.11. Effect of inoculum concentration on PAA treatment efficacy

'Albared' nectarines and 'Baby Gold 9' peaches were artificially inoculated with *M. fructicola* at 10^3, 10^4, 10^5 and 10^6 conidia mL^{-1} as previously described. Fruit were then dipped for 40 s in a tank containing 15 L of a solution at 40 ºC of 200 mg L^{-1} of PAA obtained from the commercial product Proxitane®5:23. A set of artificially inoculated fruit of each inoculum concentration was immersed in water at 20 ºC for 40 s and was used as a control. All treatments were conducted with four replicates and five fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH, brown rot incidence was recorded.

2.12. Effect of PAA treatment on naturally infected fruit

'Placido' and 'Roig d'Albesa' peaches and 'Autumn Free' nectarines with natural infections were immersed for 40 s in a tank containing 15 L of water at 20 or 40 ºC or in a solution at 20 or 40 ºC of 200 mg L^{-1} of PAA obtained from the commercial product Proxitane®5:23. All treatments were conducted with four replicates and twenty fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH brown rot incidence was recorded.

2.13. Effect of PAA treatment on fruit quality

The effect of 200 mg L^{-1} of peracetic acid, hot water at 40 ºC or the combination of both on fruit quality was determined in 'Roig d'Albesa' peaches and 'Autumn Free' nectarines. Fruit were immersed for 40 s in a tank containing 15 L of water at 20 or 40 ºC or in a solution at 20 or 40 ºC of 200 mg L^{-1} of PAA obtained from the commercial product Proxitane®5:23. All treatments were conducted with four replicates and five fruit per replicate. After 2 days of incubation at 20 ºC and 85 % RH, standard quality parameters including firmness, soluble solids content and acidity were determined.

Fruit firmness was measured on two opposite peeled sides using a penetrometer (Effegi, Milan, Italy) fitted with an 8 mm diameter flat tip. The average of those two measurements was considered as one replicate. Twenty fruit per treatment were evaluated and data are expressed in Newtons (N).

Soluble solids content (SSC) was determined with a digital refractometer (Atago PR-100, Tokyo, Japan) by measuring the juice refractive index and data are expressed as percentage of soluble solids. Acidity was measured by mixing 10 mL of juice with 10 mL distilled H_2O and adding three drops of phenolphthalein, which was then titrated with 0.1 N NaOH. Acidity was expressed in grams of malic acid per liter of juice (g m.a. L^{-1}). Juices used to determine SSC and acidity were extracted from five fruit that had been used to determine firmness, thus measurements were conducted with four replicates and five fruit per replicate.

2.14. Statistical analysis

The incidence of brown rot, quality parameters and the percentage of culturable conidia were analyzed by general linear model analysis with JMP®8 statistical software (SAS Institute, Cary, NC, USA). Statistical significance was judged at the level $P<0.05$. When the analysis was statistically significant, the least significance difference (LSD) test for separation of means was used.

3. Results

3.1. Preliminary screening of hydrogen peroxide, peracetic acid and acetic acid

Brown rot was not controlled with hydrogen peroxide (HP and 2HP) and acetic acid (AA and 2AA) applied alone to inoculated wounds. When these products were applied together only the combination of 0.25 % of HP plus 0.075 % AA (2HP+2AA) significantly reduced brown rot incidence to 63 % in comparison with untreated fruit where brown rot incidence was 100 % (Fig. 1).

Brown rot incidence was significantly reduced to 65 % when 0.01 % PAA (PAA) was applied. Brown rot control did not significantly increase when the same PAA concentration was applied in combination with HP, AA or both. In contrast, by increasing PAA concentration to 0.02 and 0.03 %, brown rot incidence was significantly decreased to 38 and 26 %, respectively, in comparison with the lowest concentration applied. In addition, when 0.02 % PAA was applied with 0.25 % HP (2HP+2PAA) or 0.075 % AA (2PAA+2AA) brown rot incidence was significantly reduced to 20 and 18 % of the incidence in the untreated fruit. The best brown rot control was achieved when 0.02 % PAA was combined with both (2HP+2PAA+2AA), where brown rot incidence was reduced to 5 % (Fig. 1).

Generally, in all treatment solutions, the peracetic acid concentration measured by the colorimetric test was the same which was added in the solution. However, between 0.0005 and 0.001 % PAA was measured in HP+AA and 2HP+2AA solutions and 0.03 % PAA in the combination 2HP+2PAA+2AA.

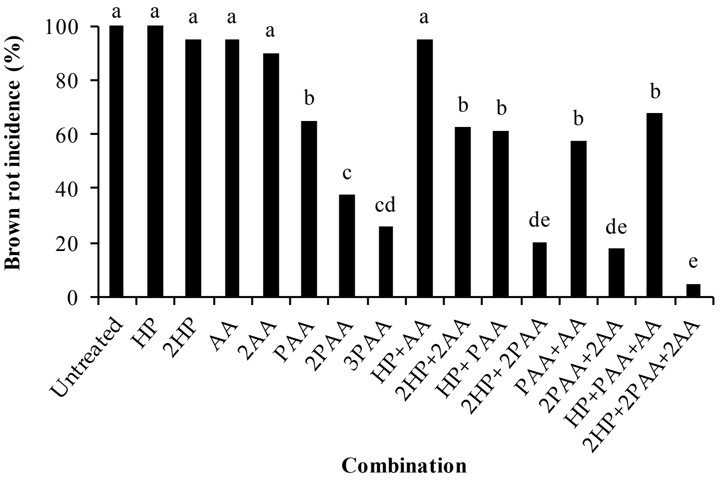

Fig. 1. Brown rot incidence in 'Baby Gold 9' peaches artificially inoculated with *Monilinia fructicola* at 10^3 conidia mL^{-1} untreated or treated by applying 15 µl of hydrogen peroxide (HP=0.125 %; 2HP=0.25 %), peracetic acid (PAA=0.01 %; 2PAA=0.02 %; 3PAA=0.03 %) and acetic acid (AA=0.0375 %; 2AA=0.075 %) applied alone or in combination. After treatments, fruit were incubated for 5 days at 20 °C and 85 % RH. Means with the same letter are not significantly different (*P*<0.05) according to LSD test.

3.2. Effect of exposure time on PAA efficacy

Brown rot incidence was significantly reduced to 20 % in 'Autumn Free' nectarines artificially inoculated and immersed for 1 min in the combination of 0.25 % hydrogen peroxide, 0.02 % peracetic acid and 0.075 % acetic acid (2HP+2PAA+2AA) compared with untreated fruit (100 %). Although brown rot incidence was significantly reduced to 10 % when exposure time was increased to 2 min, phytotoxicity on the skin of nectarines was observed (data not shown).

3.3. Comparison of the combination of HP, PAA and AA with a PAA-based commercial product

Brown rot incidence was significantly reduced to 6 % in 'Placido' peaches immersed for 1 min in the combination of 0.25 % hydrogen peroxide, 0.02 % peracetic acid and 0.075 % acetic acid (2HP+2PAA+2AA) corresponding to 300 mg L^{-1} of peracetic acid in comparison with untreated fruit where brown rot incidence was 53 %. When the same PAA concentration was applied with the commercial product Proxitane®5:23, the percentage of infected fruit was 10 % and no significant differences were observed between both treatments. However, brown rot was not controlled when PAA applied was applied at concentrations of 50 or 100 mg L^{-1} (Fig. 2).

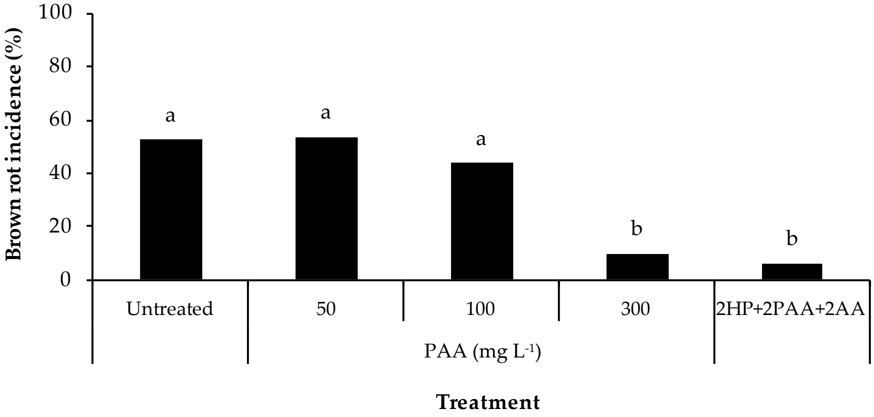

Fig. 2. Brown rot incidence in 'Placido' peaches artificially inoculated with *Monilinia fructicola* at 10^3 conidia mL $^{-1}$ and immersed for 1 min in 50, 100 and 300 mg L^{-1} of peracetic acid solutions obtained from the commercial product Proxitane®5:23 or in the combination of 0.25 % of HP, 0.02 % of PAA and 0.075 % of AA (2HP+2PAA+2AA). After treatments, fruit were incubated for 5 days at 20 ºC and 85 % RH. Means with the same letter are not significantly different (P<0.05) according to LSD test.

3.4. Effect of peracetic acid and water temperature on conidia culturability

Culturable conidia of *M. fructicola* observed in control treatment (20 ºC) were 2.7×10^4 cfu mL^{-1}. When the conidial suspension was incubated for 40 s in 100 mg L^{-1} of PAA, obtained from the commercial product Proxitane®5:23, the percentage of culturable conidia was significantly reduced to 7 % and this, was significantly reduced to 0 % when 100 mg L^{-1} of PAA was applied at 40 ºC for 40 s. A complete inhibition of *M. fructicola* conidia was achieved when conidial suspension was incubated for 40 s at 55 ºC with or without PAA (data not shown).

3.5. Effect of PAA concentration and water temperature on *M. fructicola* infections

In 'Mountain Gold' peaches, brown rot incidence was significantly reduced to 65 % when 100 mg L^{-1} PAA was applied at 20 ºC for 40 s in comparison with untreated fruit (100 %) (Fig. 3A).

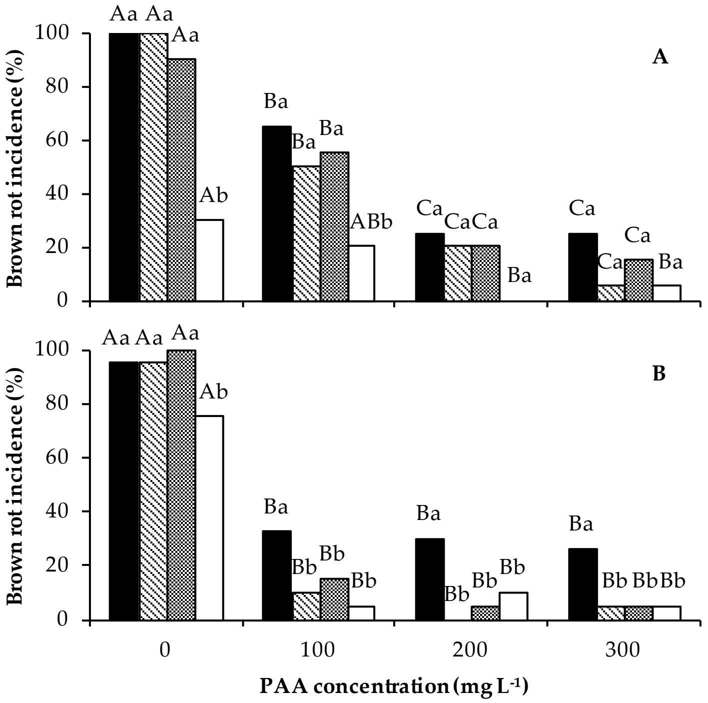

Fig. 3. Brown rot incidence in 'Mountain Gold' (A) and 'Rome Star' (B) peaches artificially inoculated with *Monilinia fructicola* at 10^3 conidia mL^{-1} and immersed in a solution of 0, 100, 200 and 300 mg L^{-1} of peracetic acid applied at 20 (■), 40 (▧), 50 (▩) or 60 ºC (□) for 40 s. After treatments, fruit were incubated for 5 days at 20 ºC and 85 % RH. Means with the same uppercase letter for each temperature or with the same lowercase letter for each peracetic acid concentration are not significantly different (P<0.05) according to LSD test.

Brown rot control was significantly decreased to 25 % by increasing PAA concentration to 200 and 300 mg L^{-1} and no significant differences were detected between the two concentrations. Similar results were obtained when PAA was applied at 40 and 50 ºC, therefore, for each PAA concentration, brown rot control was not significantly increased by increasing water temperature from 20 ºC to 40 and 50 ºC. However, when water temperature at 60 ºC was evaluated, 200 and 300 mg L^{-1} significantly reduced brown rot incidence to 0 and 5 %, respectively (Fig. 3A).

In 'Rome Star' peaches, when 100 mg L^{-1} of PAA was applied for 40 s in water at 20, 40, 50 and 60 ºC, brown rot incidence was significantly reduced to 33, 10, 15 and 5 %, respectively, in comparison with fruit immersed in water without PAA where percentages of infected fruit were over 75 % for all the water temperatures evaluated. Moreover, treatment efficacy was significantly greater when PAA was applied at 40 ºC or more than when it was applied at 20 ºC. Similar results were obtained when 200 and 300 mg L^{-1} PAA was applied, therefore, for a same water temperature, brown rot control was not significantly increased by increasing PAA concentration from 100 mg L^{-1} to 200 and 300 mg L^{-1} (Fig. 3B). None of the combinations evaluated produced damages in the fruit surface (data not shown).

Based on these results, treatment conditions selected to conduct subsequent experiments were 200 mg L^{-1} of peracetic acid applied for 40 s in water at 40 ºC.

3.6. Effect of PAA treatment as a curative or preventive treatment to control *M. fructicola* infections

Brown rot was completely controlled when 'Albared' nectarines and 'Baby Gold 9' peaches were immersed immediately after inoculation in 200 mg L^{-1} PAA at 40 ºC for 40 s in comparison with control fruit where brown rot incidence was 80 % in both varieties. However, when the time of the PAA treatment after inoculation increased to 24, 48 and 72 h, brown rot incidence was only slightly reduced to 70 % in 'Albared' nectarines inoculated 24 h before treatment compared with control fruit (100 %) (data not shown).

Regarding the effect of peracetic acid as a preventive treatment to control future infections, immersion for 40 s in 200 mg L^{-1} PAA at 40 ºC did not show any protective effect against *M. fructicola* artificially inoculated 0, 24, 48 and 72 h after treatment (data not shown).

3.7. Effect of inoculum concentration on PAA treatment efficacy

Immersion for 40 s in 200 mg L^{-1} PAA at 40 ºC in 'Albared' nectarines significantly reduced brown rot incidence to less than 10 % at all *M. fructicola* inoculum concentrations evaluated (10^3, 10^4, 10^5 and 10^6 conidia mL^{-1}) in comparison with control fruit where the percentage of infected fruit was greater than 80 % and no significant differences in treatment efficacy were detected between *M. fructicola* inoculum concentrations (Fig. 4A).

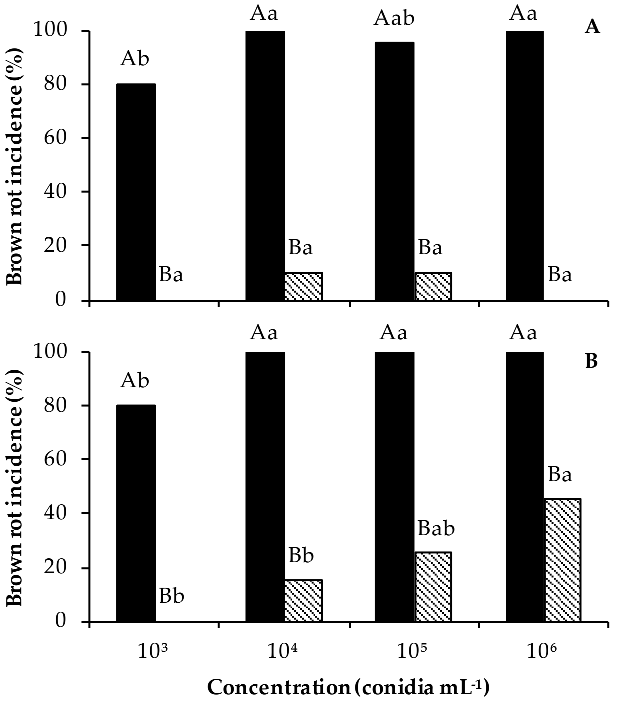

Fig. 4. Brown rot incidence in 'Albared' nectarines (A) and 'Baby Gold 9' peaches (B) artificially inoculated with *Monilinia fructicola* at 10^3, 10^4, 10^5 and 10^6 conidia mL^{-1} and immersed for 40 s in a solution of 200 mg L^{-1} of peracetic acid at 40 ºC (▨) or untreated (■). After treatments, fruit were incubated for 5 days at 20 ºC and 85 % RH. Means with the same uppercase letter for each inoculum concentration or with the same lowercase letter for each treatment are not significantly different ($P<0.05$) according to LSD test.

In ‘Baby gold 9’ peaches, brown rot incidence was significantly reduced to 0, 15, 25 and 45 % when PAA treatment was applied in fruit artificially inoculated with *M. fructicola* at 10^3, 10^4, 10^5 and 10^6 conidia mL^{-1}, respectively, compared with control fruit (over 80 %) but brown rot incidence in treated fruit was significantly lower in fruit inoculated at 10^3 than at 10^6 conidia mL^{-1} (Fig. 4B).

3.8. Effect of PAA treatment on naturally infected fruit

Brown rot incidence was not significantly reduced in ‘Roig d’Albesa’ and ‘Placido’ peaches when fruit was immersed for 40 s in water at 40 °C or in 200 mg L^{-1} peracetic acid. However, the treatment of 200 mg L^{-1} PAA at 40 °C for 40 s significantly reduced the percentage of infected fruit to 61 and 36 %, in ‘Roig d’Albesa’ and ‘Placido’ peaches, respectively, in comparison with untreated fruit where brown rot incidence was 85 and 65 %, respectively, for any studied treatment. In contrast, no brown rot control was observed in ‘Autumn Free’ nectarines (Fig. 5).

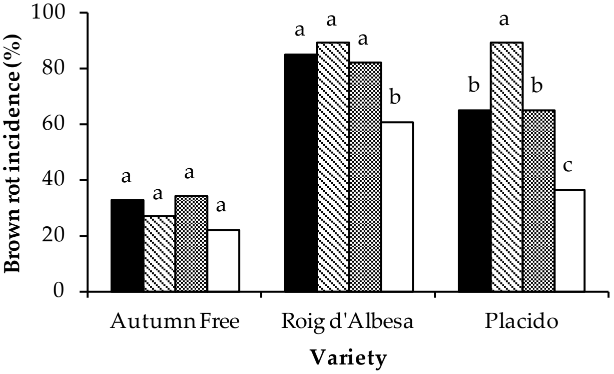

Fig. 5. Brown rot incidence in ‘Autumn Free’ nectarines and ‘Roig d’Albesa’ and ‘Placido’ peaches untreated (■) or immersed for 40 s in water at 40 °C (▧), in 200 mg L^{-1} of peracetic acid at 20 °C (▩) or in 200 mg L^{-1} of peracetic acid at 40 °C (□). After treatments, fruit were incubated for 5 days at 20 °C and 85 % RH. Means with the same letter for each variety are not significantly different ($P<0.05$) according to LSD test.

3.9. Effect of PAA treatment on fruit quality

Soluble solids content was not affected by immersion of 'Roig d'Albesa' peaches for 40 s in water at 40 ºC or in 200 mg L^{-1} PAA, however when fruit was immersed in 200 mg L^{-1} at 40 ºC soluble solids content was significantly lower with 10.4 % in comparison with untreated fruit (10.9 %). Moreover, acidity was not affected for any of the three treatments evaluated (Table 2).

In 'Autumn Free' nectarines, soluble solids and acidity were not affected by immersion for 40 s in 200 mg L^{-1} of PAA and in 200 mg L^{-1} at 40 ºC, however, fruit dipped only in water at 40 ºC showed lower soluble solids content (10.9 %) and more acidity (7.0 g m.a. L^{-1}) in comparison with untreated fruit (Table 2).

Regarding firmness, 'Roig d'Albesa' peaches treated with any of the three treatments showed firmness below 32.2 N and these were significantly lower than untreated fruit (37.8 N). In contrast, firmness in 'Autumn Free' nectarines treated with 200 mg L^{-1} of PAA applied at 20 or at 40 ºC was 16.2 and 18.4 N, respectively, and these firmness were significantly greater than untreated fruit firmness (13.4 N) (Table 2).

Table 2. Standard quality parameters of 'Autumn Free' nectarines and 'Roig d'Albesa' peaches immersed for 40 s in water at 40 ºC or in 200 mg L^{-1} of peracetic acid at 20 or 40 ºC and stored for 2 days at 20 ºC and 85 % RH. Means with the same letter for each variety and quality parameter are not significantly different ($P<0.05$) according to LSD test.

Treatment	'Autumn Free' nectarine			'Roig d'Albesa' peach		
	Firmness (N)	Soluble solids (%)	Acidity (g m.a. L^{-1})	Firmness (N)	Soluble solids (%)	Acidity (g m.a. L^{-1})
Initial	23.4	12.3	7.0	64.7	10.6	2.0
Untreated	13.4 c	11.6 a	6.6 b	37.8 a	10.9 a	2.1 a
40 ºC	14.2 bc	10.9 b	7.0 a	32.2 b	11.3 a	2.0 a
200 mg L^{-1} PAA at 20 ºC	16.2 ab	11.6 a	6.4 b	31.5 b	11.0 a	2.1 a
200 mg L^{-1} PAA at 40 ºC	18.4 a	11.3 ab	6.7 ab	31.8 b	10.4 b	2.2 a

4. Discussion

Alternative treatments to brown rot control on stone fruit have been studied for many years, including hot water (Karabulut *et al.*, 2010) and peracetic acid (Mari *et al.*, 1999; 2004). However, to our knowledge this is the first time that peracetic acid has been investigated in combination with hot water to control postharvest brown rot caused by *Monilinia* spp. on peaches and nectarines.

In the preliminary screening of different concentrations and combinations of hydrogen peroxide (HP), peracetic acid (PAA) and acetic acid (AA), brown rot was not controlled when HP and AA were applied alone to inoculated wounds. Similar results with hydrogen peroxide were obtained by Palou *et al*. (2009) who reported that brown rot was not effectively controlled when higher concentrations of hydrogen peroxide were evaluated in peaches and nectarines artificially inoculated with *M. fructicola*. Hydrogen peroxide was not effective in controlling *Penicillium digitatum* on lemons even with concentrations that injured fruits (Smilanick *et al*., 1995). However, acetic acid controlled *M. fructicola* artificially inoculated in peaches and nectarines when lower acetic acid concentration was applied through fumigation (Sholberg and Gaunce, 1996). Furthermore, contrary to what was observed with hydrogen peroxide and acetic acid, when peracetic acid was applied alone, brown rot incidence was significantly reduced and the treatment efficacy generally increased by increasing PAA concentration. However, the best control of *M. fructicola* was achieved when the combination of 0.25 % hydrogen peroxide, 0.02 % peracetic acid and 0.075 % acetic acid (2HP+2PAA+2AA) was applied.

Peracetic acid solution is produced from the reaction of acetic acid with hydrogen peroxide (Kitis, 2004). The analysis of peracetic acid concentration showed that the real amount of PAA applied with prior combination was 300 mg L^{-1} of peracetic acid. When the combination was applied as an aqueous dip treatment to control *M. fructicola*, 1 min of exposure time was sufficient to control brown rot. Nevertheless, when Mari *et al*. (1999) immersed nectarines artificially inoculated 1 h before treatment with *Monilinia laxa* in PAA at 500 and 1000 mg L^{-1} for 20 s or 1 min the incidence of infected wounds was not reduced. Phytotoxicity on the skin of fruit was observed when exposure time was increased to 2 min. This phytotoxicity could be attributed to the prolonged exposure to hydrogen peroxide applied with the combination evaluated. Palou *et al*. (2009) observed severe skin injury in peaches and nectarines when hydrogen peroxide was applied in inoculated wound. Smilanick *et al*. (1995) also observed rind injury on all lemons after 90 s of treatment in hydrogen peroxide, whereas lemons were not injured when exposure time was decreased.

Commercial products based on PAA are much more stable because a stabilizer or a sequestering agent is employed during the production of PAA solutions (Kitis, 2004). Moreover, commercial formulations containing a lower PAA concentration and no sulfuric acid have fewer problems associated with corrosiveness, making these formulations suitable for use with stainless-steel equipment (Mari *et al.*, 2003). Our results showed that 300 mg L^{-1} PAA obtained from the commercial product Proxitane®5:23 controlled equally that the same concentration applied by the combination (2HP+2PAA+2AA).

Improved effectiveness of chemical treatments to control postharvest diseases using synthetic fungicides as well as alternative chemicals in heated solutions as compared to solutions at room temperature has been reported (Mari *et al.*, 2007; Palou *et al.*, 2009). The results from the *in vitro* study demonstrated that the application of 100 mg L^{-1} at 40 ºC for 40 s reduced significantly more the percentage of culturable conidia than the same PAA concentration at 20 ºC or hot water at 40 ºC alone. Mari *et al.* (1999) achieved a total inhibition of conidial germination of *M. laxa* when conidia were exposed for 5 min to 500 mg L^{-1} PAA. Jemric *et al.* (2011) did not observe a reduction of conidial germination of *M. laxa* heated at 40 ºC for 12 min and Zhang *et al.* (2010) reported that hot water treatment at 50 ºC for more than 40 s was needed to completely inhibit conidia germination of *M. laxa*. Therefore, the combination of peracetic acid and hot water enabled the use of lower temperatures and lower PAA concentrations than if either was used alone.

In the present study, combinations of different PAA concentrations at different temperatures were also conducted under *in vivo* conditions. In 'Mountain Gold' peaches the efficacy of PAA generally increased by increasing peracetic acid concentration from 100 to 200 mg L^{-1}, however, in 'Rome Star' peaches at the same PAA concentration, treatment efficacy increased by increasing water temperature from 20 to 40 ºC. Based on these results, treatment conditions selected were 200 mg L^{-1} of PAA at 40 ºC for 40 s. A synergistic effect of chemical products and heat was also observed by Mari *et al.* (2007) who controlled brown rot on peaches and nectarines better when they combined sodium bicarbonate with hot water. Palou *et al.* (2009) showed that dips in potassium sorbate with hot water increased the efficacy to reduce both brown rot incidence and severity compared with dips applied at room temperature. Margosan *et al.* (1997) reported that mortality of conidia of *M. fructicola* occurred much more quickly in heated ethanol than in heated water and reported that the increase in decay control that occurred when water and ethanol were combined may result from their affecting the same sites in the spore.

Complete brown rot control was achieved when nectarines and peaches were inoculated with *M. fructicola* 0 h before the treatment with 200 mg L^{-1} of PAA at 40 ºC for 40 s but no brown rot reduction was observed when the time between *M. fructicola* inoculation and treatment was increased to 24 h or more. Moreover, our results indicated that treated fruit with 200 mg L^{-1} PAA at 40 ºC for 40 s controlled brown rot at all inoculum concentrations evaluated (10^3, 10^4, 10^5 and 10^6 conidia mL^{-1}) in both peaches and nectarines. Mari *et al.* (2004) also observed a high control of *R. stolonifer* even at the higher inoculum concentration evaluated when 250 mg L^{-1} of PAA were applied for 1 min in stone fruit artificially inoculated. These results suggested that peracetic acid had a high potential to control brown rot whatever the inoculum concentration but it was only effective when the infection had not yet occurred. More than 80 % of viable conidia of *M. fructicola* germinated at 25 ºC and high water activity within 2 h (Casals *et al.*, 2010b). Chlorine dioxide, other sanitizing product as peracetic acid, kills only by contact and is effective only on exposed fungal propagules, such as those suspended in water or on the surface of the fruit, however, it does not kill pathogens after infection has occurred (Mari *et al.*, 1999).

Protection against future infections of *M. fructicola* was not observed by immersing fruit in 200 mg L^{-1} of PAA at 40 ºC for 40 s. Other chemical compounds such as sodium bicarbonate, sodium carbonate or ethanol have been shown to have the same negative effect (Margosan *et al.*, 1997; Smilanick *et al.*, 1999).

The results obtained for naturally infected fruit indicated that brown rot incidence was slightly but significantly reduced in 'Roig d'Albesa' and 'Placido' peaches immersed for 40 s in 200 mg L^{-1} PAA at 40 ºC, however it was not controlled in 'Autumn Free' nectarines. Lower PAA concentrations were needed by Mari *et al.* (2004) who reported a significant reduction of brown rot by dipping fruit in PAA solution at a concentration of 125 mg L^{-1} in naturally infected nectarines and peaches. Natural infections can be not only infections that remain latent in the fruit during the growing season but also conidia on the fruit surface. Therefore, the different efficacy of the peracetic acid observed between both studies to control natural infections could be attributed to differences in the nature of the infections to be controlled. Although synergistic effects of combinations of PAA with hot water were also observed in naturally infected peaches, the level of efficacy achieved in artificial inoculations of *M. fructicola* was greater. Previously in this work we showed that peracetic acid was not effective to control brown rot in fruit artificially inoculated 24 h or more before treatment, thus a possible explanation is that the majority of rots in naturally infected fruit were caused by no recent pre-existing infections and therefore more difficult to control by PAA treatment.

In conclusion, immersion for 40 s in 200 mg L^{-1} of PAA at 40 ºC may provide an alternative treatment to control recent infections of *Monilinia* spp. whatever their concentration, without generally affecting fruit quality. Moreover, under semicommercial conditions brown rot was totally controlled when conidia remained in contact with 250 mg L^{-1} PAA for 5 min (Mari *et al.*, 1999). Water in packing-line dump tanks quickly become heavily contaminated by fungal conidia and may infect dipped fruit. Therefore, peracetic acid not only can control pre-existing recent infections but, as a sanitizer product, may also reduce the level of inoculum in water to prevent infections in healthy fruit.

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Capítulo 2

Immersion of fruit in water to improve radio frequency treatment to control brown rot in stone fruit

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Abstract

Brown rot caused by *Monilinia* spp. is the most important postharvest disease of stone fruit. Currently, no chemical fungicides are allowed in the European Union to be applied to stone fruit after harvest, which has increased the need to develop alternative methods. Radio frequency (RF) treatment at 27.12 MHz with fruit immersed in water was studied to control brown rot in peaches and nectarines artificially inoculated with *M. fructicola*. Additionally, RF treatment in air was also investigated to evaluate the benefit of water immersion to reduce the effect of fruit size on treatment efficacy. RF treatment with fruit immersed in water at 20 ºC applied for 9 min significantly reduced brown rot incidence in both peaches and nectarines and no significant differences in RF efficacy were observed depending on fruit size. However, when RF treatment was applied in air for 18 min, brown rot reduction was significantly higher in large fruit than in small fruit. Finally, the decrease in exposure time of radio frequency treatment with fruit immersed in water with increasing water temperature was also studied. Reduction of treatment time to 6 and 4.5 min was achieved by increasing water temperature at 35 and 40 ºC, respectively, to control brown rot without adverse external and internal damage in both 'Baby Gold 9' peaches and 'Autumn Free' nectarines.

Keywords: *Monilinia fructicola*, heat treatment, postharvest, disease control

1. Introduction

Brown rot caused by *Monilinia* spp. is the most important postharvest disease of stone fruit. Direct yield losses result from infection of flowers (flower and twig blight) and from fruit rot at preharvest, harvest and postharvest. Postharvest losses are typically more severe than preharvest losses, and routinely occur during storage and transport, in some cases even affecting fruit at the processing stage. When conditions are favorable for disease development, postharvest losses may be high, reaching in some cases values of 80-90 % (Hong *et al.*, 1997; Larena *et al.*, 2005). No chemical fungicides are allowed in the European Union to decrease postharvest losses, therefore the current methods to control brown rot include preharvest spraying of fungicides at regular intervals, careful handling of harvested fruit to avoid wounding, good preharvest and postharvest sanitation practices, and rapid cooling and storage after harvest. However, all these strategies are not sufficient to control brown rot in stone fruit, which has increased the need to develop alternative methods.

Several alternative treatments have been investigated to control *Monilinia* spp. in stone fruit in last few years. These include treatments based on biocontrol agents (Yánez-Mendizábal *et al.*, 2011), chemical products with low toxicity (Gregori *et al.*, 2008; Palou *et al.*, 2009) and physical treatments such as hot water (Karabulut *et al.*, 2010) or curing by high temperature and relative humidity (Casals *et al.*, 2010a; 2010b).

Conventional heating is limited by the low thermal conductivity of fruit and thus necessitating prolonged heating in many cases. Casals *et al*. (2010b) reported a curing treatment at 50 ºC for 2 h to control brown rot on peaches and nectarines. However, short treatment times are preferred from the viewpoint of commercial applications (Ikediala *et al.*, 2002). Generally, hot water treatments are shorter where exposure times range between 20 s and 2.5 min and temperatures between 45 and 60 ºC (Margosan *et al.*, 1997; Karabulut *et al.*, 2010). However, their application may need to be combined with an alternative treatment to enhance effectiveness (Karabulut *et al.*, 2002; Casals *et al.*, 2010c).

The need to achieve fast and effective thermal treatment has resulted in the increased use of radio frequency (RF) energy to heat foods. This electromagnetic energy directly interacts with commodities to raise the interior temperature and significantly reduce treatment time as compared to conventional hot water immersion and heated air methods. Dielectric materials, such as most agricultural products, convert electromagnetic energy into heat (Wang *et al.*, 2003). The dielectric properties and specially the loss factor (ε'') of a material, influences both energy absorption and attenuation, and describes the ability to dissipate energy in response to an applied electric field which commonly results in heat generation (Ikediala *et al.*, 2000) so that the amount of heat converted in the food is proportional to the value of the loss factor at a given frequency and electric field (Tang *et al.*, 2000).

Radio frequency heating has been widely studied as a rapid disinfestation treatment for nuts and dry products (Mitcham *et al.*, 2004), cherries (Ikediala *et al.*, 2002), oranges (Birla *et al.*, 2004), apples (Hansen *et al.*, 2006) and mangoes (Sosa-Morales *et al.*, 2009). On the contrary, little information is available about the potential of this treatment to control postharvest diseases. Casals *et al.* (2010d) demonstrated the potential of the use of radio frequency to control brown rot in peaches, however, this treatment was not suitable for control of *Monilinia* spp. in nectarines. Moreover, in previous studies, we observed that RF effectiveness was influenced by fruit size (unpublished data). Therefore, further experiments were needed to address the lack of efficacy in nectarines, the prolonged exposure time and the uneven heating.

Heating uniformity is the most significant problem associated with RF treatment in fresh fruit (Tang *et al.*, 2000). Large temperature variations among and within fruit can affect the effectiveness of the treatment and also fruit quality. Non-uniform heating is not just attributed to the different dielectric properties but also to the different fruit shape and size and the surrounding medium (Birla *et al.*, 2008a). Recently, research with fruit immersed in water has been conducted to overcome non-uniform heating in radio frequency treatments for pest control. Ikediala *et al*. (2002) suggested a saline water immersion technique, Birla *et al*. (2004) kept rotating and moving fruit in the RF field, and Wang *et al*. (2006) studied the application of hot water before, during and after RF heating.

The objectives of this research were: (1) determine the RF conditions with fruit immersed in water that could reduce brown rot in peaches and nectarines without causing visual damage to the fruit, (2) evaluate if the immersion of fruit in water improves the uniformity of the efficacy on different fruit sizes, and (3) determine if the increase in water temperature could reduce the treatment time necessary to control *M. fructicola* artificially inoculated in peaches and nectarines without adverse effects on fruit visual quality.

2. Material and methods

2.1. Fruit

Experiments were conducted with 'Summer Rich', 'Baby Gold 6' and 'Baby Gold 9' peaches (*Prunus persica* (L) Batch) and 'Big Top', 'Fantasia' and 'Autumn Free' nectarines (*P. persica* var. Nectarine (Ait.) Maxim.). Fruit were grown in commercial orchards located in Lleida (Catalonia) following standard cultural practices and chemical spray programs in the field for pest and disease control. Fruit free of visible wounds and rots and similar visual maturity were selected by hand from fruit bins immediately after harvest. Fruit not used at the time of harvest were stored at 0 ºC for a maximum of 5 days until use.

Fruit diameters in all the experiments except in the study of the effect of fruit size were 70 ± 2 mm in 'Big Top', 'Fantasia' and 'Autumn Free' nectarines and 'Baby gold 6' peaches and 75 ± 2 mm in 'Summer Rich' and 'Baby Gold 9' peaches.

2.2. Pathogen culture

The isolate of *M. fructicola* (CPMC1) used in this study was from the collection of the Postharvest Pathology Unit, Center IRTA, Lleida, Catalonia. This strain was isolated from an infected stone fruit and was identified by the Department of Plant Protection, INIA, Madrid (Spain). The strain was maintained on potato dextrose agar (PDA) medium (Biokar Diagnostics, 39 g L^{-1}) amended with acetone (J.T. Baker, 1 %) at 4 ºC in the dark.

2.3. Pathogen production and inoculation methodology

The isolate of *M. fructicola* (CPMC1) was subcultured onto PDA and incubated in dark at 25 ºC for approximately two weeks. The isolate was inoculated onto peaches or nectarines by wounding the fruit with a sterilized steel rod (1 mm wide and 2 mm long) and transferring conidia and mycelium from the PDA culture to the wound site with a sterile pipette tip. Then, the fruit were incubated at 25 ºC and 85 % RH in the dark for 5-7 days. Conidia were scraped from infected fruit using a sterile loop and transferred to a test tube containing 5 mL of sterile distilled water and a drop of Tween-80 per liter. Conidia concentration was measured with a haemocytometer and the suspension diluted to 10^3 conidia mL^{-1}. Then, the fruit were wounded once per fruit with the sterile steel rod and inoculated with 15 µL of conidial suspension. All radio frequency treatments were performed with artificially inoculated fruit incubated at 20 ºC and 85 % RH for 48 h before the treatment.

2.4. Radio frequency heating system and suitable treatment conditions

Semi-industrial RF equipment (STALAM S.p.A, Nove, Vicenza, Italy) with 15 kW nominal maximum power and a frequency of 27.12 MHz was used to perform the experiments. The RF electric field was generated between two parallel electrodes of 150 cm x 100 cm. The electrode gap was adjustable over a range of 65-205 mm. The fruit were placed on a continuous conveyer belt (velocity from 0.1 to 0.7 m min^{-1}) based on the bottom electrode (tray base), and acted as the capacitor in the electromagnetic field. Height of the top electrode was adjusted to change the effective capacitance and the amount of RF power coupled to the sample. In general, the system was tuned in such a manner that a reduction in distance between the electrodes allowed the coupling of more power into the sample, therefore enhancing the heating efficiency. However, the gap needed to be controlled above a critical minimum value to prevent arcing. The absorbed RF power per unit volume (P_v, W m^{-3}) in a dielectric material can be expressed as (Rowley, 2004):

$$P_v = 2\pi f \varepsilon_0 \varepsilon_r'' E^2$$

where f is the frequency (Hz) of the applied field, E is the electric field intensity (V m^{-1}), ε_0 is the permittivity of the free space (8.85 x 10^{-12} F m^{-1}) and ε_r'' is the dielectric loss factor. In the case of the peach pulp, for a frequency of 27.12 MHz and a temperature of 20 ºC, was ε_r'' = 269.5 ± 46 (Birla *et al.*, 2008b).

The electrode voltage was set at 5800 V and the electrode gap was adjusted at 112 mm for all radio frequency treatments. In RF treatment with the fruit immersed in water, the fruit at room temperature were introduced into a container (260 mm x 260 mm x 105 mm) with 2 L of water, so that the fruit were completely submerged in the water. Then, the containers were placed on the conveyor belt so that the distance between fruit and upper electrode ranged between 40 and 45 mm. For radio frequency treatment in air, the fruit at room temperature were placed on the conveyor belt with the artificial inoculum site of each fruit aligned with the upper electrode and RF treatment was applied for 18 min, exposure time reported by Casals *et al*. (2010d).

In all experiments, the increase of internal temperature during RF treatment was measured with inside-optical fiber temperature probes (FOT-L/10 m; FISO Technologies Inc., Canada) with an accuracy of ± 0.5 ºC. Temperature sensor was placed 10 mm inside the fruit. Immediately after RF treatment the external temperature of each fruit was recorded by a portable infrared thermometer (Testo 831, Testo AG, USA) with an accuracy of ± 1.5 ºC.

2.5. Effect of exposure time on radio frequency efficacy with fruit immersed in water

Peaches and nectarines artificially inoculated with *M. fructicola* as described above were used to perform the experiment to investigate the belt speed related to the exposition time that could reduce brown rot without causing visual fruit damage during RF treatment with fruit immersed in water at 20 ºC. The fruit were immersed in water at 20 ºC and then, RF treatment was applied for 6.4, 7.5 and 9 min in 'Baby Gold 6' peaches and 'Fantasia' nectarines and later, in other experiment, RF treatment was applied for 9, 12 and 18 min in 'Summer Rich' peaches and 'Big Top' nectarines. A set of artificially inoculated fruit immersed in water at 20 ºC was not treated and was used as a control. All the treatments were conducted with four replicates and eight fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH the number of brown rot infected fruit was recorded.

2.6. Effect of fruit size on radio frequency efficacy

Improving the heating uniformity between fruit of different sizes by applying RF treatment with fruit immersed in water was studied in 'Baby Gold 6' peaches artificially inoculated as previously described. The fruit diameters evaluated were 65 ± 2, 70 ± 2 and 75 ± 2 mm. In RF treatment with water immersion, peaches were immersed in water at 20 ºC and then, RF heating was applied for 9 min. In contrast, for RF treatment in air, peaches were placed on the conveyor belt with the artificial inoculum site aligned with the upper electrode and processed during 18 min. In both treatments, the distance between fruit and upper electrode was the same. A set of fruit of each diameter artificially inoculated and immersed in water at 20 ºC or maintained at 20 ºC was not treated and was used as control to RF treatment with water immersion or in air, respectively. All the treatments were conducted with four replicates and eight fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH brown rot reduction was recorded.

2.7. Effect of water temperature on radio frequency efficacy

The decrease in exposure time of radio frequency treatment in fruit immersed in water with increasing water temperature was studied in 'Baby Gold 9' peaches and 'Autumn Free' nectarines artificially inoculated as described above. The fruit were immersed in water at 35 or 40 ºC and then RF treatment was applied for 3.6, 4.5, 6 and 9 min. A set of artificially inoculated fruit immersed in water at 35 or 40 ºC was not treated and was used as control. All the treatments were conducted with four replicates and eight fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH the number of brown rot infected fruit was recorded.

2.8. Visual quality

In all the treatments, external thermal damage, especially changes in the color of the surface, were observed at the end of RF heating. Of those treatments that controlled brown rot without causing external damages to the fruit, internal thermal damages of all the fruit were observed after 5 days of incubation at 20 ºC and 85 % RH once brown rot incidence was recorded. Internal thermal damages, such as internal browning, were determined by cutting each fruit in half.

2.9. Statistical analysis

The incidence of brown rot and brown rot reduction were analyzed using analysis of variance (ANOVA) with JMP®8 statistical software (SAS Institute, Cary, NC, USA). Statistical significance was judged at the level $P<0.05$. When the analysis was statistically significant, the least significance difference (LSD) test for separation of means was used. Arcsine-transformation of the data was performed prior to analysis. Non-transformed means are presented.

3. Results

3.1. Effect of exposure time on radio frequency efficacy with fruit immersed in water

Radio frequency treatment in 'Summer Rich' peaches and 'Big Top' nectarines immersed in water at 20 ºC completely controlled brown rot even at the minimum exposure time evaluated, 9 min, compared with untreated fruit where disease incidence was 16 and 75 %, respectively. At other exposure times, 12 and 18 min, severe thermal damage was observed in peaches and nectarines surface, specifically peel browning (Table 1). The internal 'Summer Rich' peach temperature recorded at the end of the RF process was increased to 41.4, 48.5 and 55.8 ºC with exposure times of 9, 12 and 18 min, respectively (data not shown). The external fruit temperature achieved immediately after RF heating for 9 min was 45.0 and 45.7 ºC in 'Summer Rich' peaches and 'Big Top' nectarines, respectively (data not shown).

Table 1. Brown rot incidence in fruit artificially inoculated with *Monilinia fructicola* 48 h prior to radio frequency treatment with fruit immersed in water at 20 ºC for 9, 12 and 18 min in 'Summer Rich' peaches and 'Big Top' nectarines or for 6.4, 7.5 and 9 min in 'Baby Gold 6' peaches and 'Fantasia' nectarines. After treatment, fruit were incubated at 20 ºC and 85 % RH for 5 days. Arcsine-transformation of the data was performed before statistical analysis. Non-transformed means are shown. Means with the same letter for each variety are not significantly different (*P*<0.05) according to LSD test.

Variety	Time (min)	Brown rot (%)	External[a]/internal damage (%)[b]
'Summer Rich' peach	Untreated	16 a	
	18	0 b	+/nd
	12	0 b	+/nd
	9	0 b	-/0
'Big Top' nectarine	Untreated	75 a	
	18	0 b	+/nd
	12	0 b	+/nd
	9	0 b	-/0
'Baby Gold 6' peach	Untreated	100 a	
	9	19 b	-/0
	7.5	100 a	-/nd
	6.4	97 a	-/nd
'Fantasia' nectarine	Untreated	94 a	
	9	35 b	-/41
	7.5	84 a	-/nd
	6.4	69 ab	-/nd

nd: not determined because fruit suffered external damage or radio frequency treatment was not effective

[a] Presence (+) or no presence (-) of visual thermal damage in fruit surface

[b] Percentage of fruit with visual internal damage

In 'Baby Gold 6' peaches and 'Fantasia' nectarines brown rot was reduced to 19 and 35 %, respectively, when RF treatment was applied for 9 min in comparison with untreated fruit, where brown rot was 100 and 94 %, respectively. When exposure time was decreased to 7.5 and 6.4 min, RF treatment was not sufficient to control *M. fructicola* (Table 1). The internal fruit temperature achieved when RF treatment was applied for 6.4, 7.5 and 9 min was 29.5, 36.8 and 45.5 ºC, respectively in 'Baby Gold 6' peaches and 28.0, 30.0 and 38.1 ºC, respectively in 'Fantasia' nectarines (data not shown). The external fruit temperature achieved immediately after RF heating for 9 min was 42.6 and 42.1 ºC in 'Baby Gold 6' peaches and 'Fantasia' nectarines, respectively (data not shown).

When internal visual quality was evaluated in fruit treated by RF for 9 min, no internal thermal damage was observed in 'Summer Rich' and 'Baby Gold 6' peaches and 'Big Top' nectarines. However, in 'Fantasia' nectarines internal browning around the stone was observed in 41 % of fruit (Table 1).

Based on these results, the exposure time selected to conduct radio frequency treatment with fruit immersed in water with elevated efficacy without causing external fruit damage was 9 min for both peaches and nectarines.

3.2. Effect of fruit size on radio frequency efficacy

In RF treatment with fruit immersed in water at 20 ºC applied for 9 min, the fruit size had not a significant effect on RF efficacy to control *M. fructicola* artificially inoculated in 'Baby Gold 6' peaches and brown rot reduction was 59, 68 and 90 % in 65 ± 2, 70 ± 2 and 75 ± 2 mm of fruit diameter, respectively (Fig. 1). Brown rot incidence for control treatments ranged between 94 and 100 %.

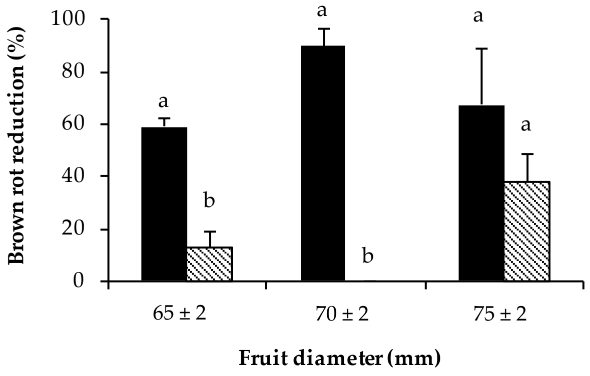

Fig. 1. Brown rot reduction in 'Baby Gold 6' peaches of different diameters, 65 ± 2, 70 ± 2 and 75 ± 2 mm artificially inoculated with *Monilinia fructicola* 48 h prior to radio frequency treatment with fruit immersed in water at 20 ºC for 9 min (■) or in air for 18 min (▨) with the same distance between the top of the fruit and upper electrode. After treatment, fruit were incubated at 20 ºC and 85 % RH for 5 days. Brown rot incidence in untreated fruit ranged between 94 and 100 %. Arcsine-transformation of the data was performed before statistical analysis. Non-transformed means are shown. Means with the same letter for each treatment are not significantly different (P<0.05) according to LSD test. Vertical bars represent ± SE of means.
]

The internal fruit temperature achieved during the RF treatment was 43.1, 45.0 and 46.4 ºC when fruit diameter was 65 ± 2, 70 ± 2 and 75 ± 2 mm, respectively (Fig. 2).

When RF treatment was applied in air for 18 min with the same distance between fruit and upper electrode that in RF heating with water immersion, brown rot reduction in fruit with 75 ± 2 mm of fruit diameter was 38 % and was significantly higher than that observed with the other fruit diameters evaluated 65 ± 2 and 70 ± 2 mm, where brown rot reduction was 13 and 0 %, respectively (Fig. 1). In this trial brown rot incidence in untreated fruit was 100 % for all the diameters evaluated.

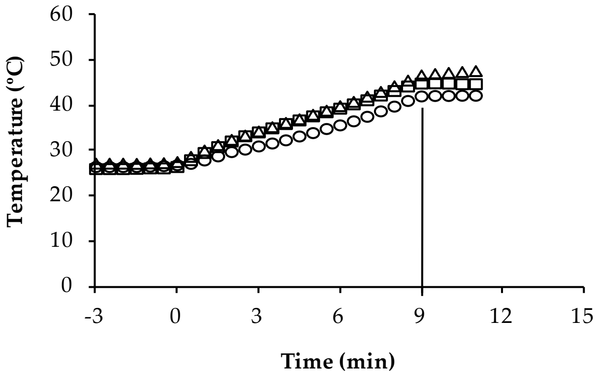

Fig. 2. Evolution of the internal fruit temperature in 'Baby Gold 6' peaches of different diameters, 65 ± 2 (○), 70 ± 2 (□) and 75 ± 2 mm (△) during radio frequency treatment with fruit immersed in water at 20 ºC for 9 min.

3.3. Effect of water temperature on radio frequency efficacy

Radio frequency treatment for 4.5 and 6 min with fruit immersed in water at 35 ºC significantly reduced incidence of brown rot in 'Baby Gold 9' peaches to 38 and 6 %, respectively, compared with untreated fruit (55 %) (Fig. 3A). In 'Autumn Free' nectarines, brown rot was reduced to 6 % when RF treatment was applied at 35 ºC for 6 min in comparison with untreated nectarines where disease incidence was 97 %. When the exposure time was increased to 9 min, complete brown rot control was achieved in both 'Baby gold 9' peaches and 'Autumn Free' nectarines although severe thermal damage as peel browning was observed in fruit (Fig. 3A).

The external fruit temperature achieved when RF treatment was applied for 3.6, 4.5, 6 and 9 min was 39.6, 40.2, 45.6 and 51.3 ºC, respectively in 'Autumn Free' nectarines and 39.9, 41.6, 45.7 and 50.0 ºC, respectively in 'Baby Gold 9' peaches (data not shown).

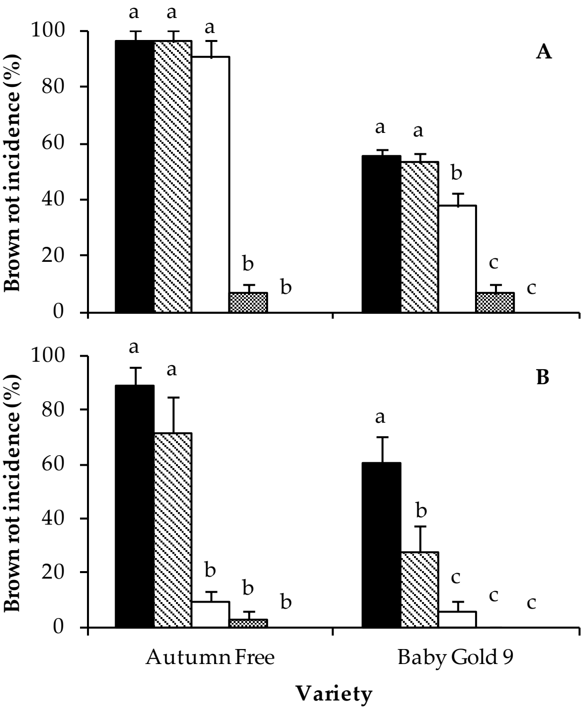

Fig. 3. Incidence of brown rot in 'Autumn Free' nectarines and 'Baby Gold 9' peaches artificially inoculated with *Monilinia fructicola* 48 h prior to immerse them in water at 35 (A) or 40 ºC (B) and heated by radio frequency for 9 (▤), 6 (▩), 4.5 (□) and 3.6 min (▧) or untreated (■). After treatment, fruit were incubated at 20 ºC and 85 % RH for 5 days. Arcsine-transformation of the data was performed before statistical analysis. Non-transformed means are shown. Means with the same letter for each water temperature are not significantly different (P<0.05) according to LSD test. Vertical bars represent ± SE of means.

When RF treatment was applied in water at 40 ºC for 3.6 and 4.5 in 'Baby Gold 9' peaches, brown rot incidence was reduced to 28 and 6 %, respectively, compared with untreated fruit (61 %) (Fig. 3B). When exposure time was increased to 6 and 9 min, RF treatment with water at 40 ºC completely controlled brown rot but causing thermal damage in peaches surface. For 'Autumn Free' nectarines only

4.5 min of exposure time was able to reduce brown rot incidence to 10 % in comparison with untreated fruit (90 %) without causing external visual damage. When exposure time was increased to 6 and 9 min, RF efficacy was not improved and peel browning was observed (Fig. 3B). The external fruit temperature achieved when RF treatment was applied for 3.6, 4.5, 6 and 9 min was 41.6, 45.9, 48.3 and 50.3 ºC, respectively in 'Autumn Free' nectarines and 39.1, 43.4, 47.7 and 52.1 ºC, respectively in 'Baby Gold 9' peaches (data not shown).

When internal visual quality was evaluated in 'Autumn Free' nectarines and 'Baby Gold 9' peaches treated by RF in water at 35 and 40 ºC for 6 and 4.5 min, respectively, internal browning was not observed in any of the fruit evaluated (data not shown).

4. Discussion

Radio frequency treatment has been widely studied to control pest in nuts and fruit but little information is available about the efficacy of this treatment to control postharvest diseases. Casals *et al.* (2010d) were the first to study RF heating to control postharvest brown rot disease in peaches and nectarines and demonstrated the efficacy of RF treatment for 18 min of exposure time for brown rot control in peaches, however, this treatment was not suitable for control *Monilinia* spp. in nectarines. To address the lack of efficacy of RF treatment in nectarines, in the present paper, the fruit were immersed in water during RF treatment.

Our results indicated that RF treatment with fruit immersed in water at 20 ºC applied for 9 min reduced significantly the number of infected fruit in comparison with control in both peaches and nectarines without causing visual damage in the fruit surface. When the exposure time was increased to 12 and 18 min, the fruit suffered severe external thermal damage. On the contrary, decreasing exposure time to 7.5 and 6.4 min, no brown rot control was observed. Therefore, the application of RF treatment with fruit immersed in water at 20 ºC for 9 min not only solved the lack of efficacy of treatment in air in nectarine but also halved the exposure time. The decrease in treatment time when used the water immersion technique may be due to the increase in heating rate. Temperature of fruit surface achieved by Casals *et al.* (2010d) when RF treatment was applied in air for 18 min ranged from 36.3 to 44.9 ºC for 'Summer Rich' peaches and 34 to 40 ºC for 'Big Orange' nectarines. When RF treatment was applied with fruit immersed in water at 20 ºC for 9 min, similar external temperatures were achieved in half time. Similar results were observed by Birla *et al.* (2008a) who, using a computer simulation, found that the presence of water reduced by half the time required for RF heating of fruit in the air to reach the same temperature. Ikediala *et al.* (2002) also observed increased heating rates in the fruit when the water immersion technique was used compared with treatment in air for the same gap of plate electrodes to control codling moth in cherries. The different heating rates can be attributed to differences between the dielectric properties of air and water. Birla *et al.* (2008a) reported that RF heating is influenced by the surrounding medium. Dielectric loss factor influences both energy absorption and attenuation, and describes the ability to dissipate energy in response to an applied electric field which commonly results in heat generation (Ikediala *et al.*, 2000). The dielectric loss factor of tap water with a frequency of 27.12 MHz at room temperature is 19 ± 0.8, however, the dielectric loss factor of the air is approximately 0 (Ikediala *et al.*, 2002), therefore, water offers less resistance to electric field than air.

When RF conditions reported by Casals *et al.* (2010d) were applied in previous studies using fruit with different sizes, complete brown rot reduction was achieved in large fruit unlike just 13 % of reduction obtained in small fruit (unpublished data). Immersion of fresh fruit in water was suggested by Ikediala *et al.* (2002) as a means to overcome the problems associated with non-uniform RF heating. In our study we compared the effect of fruit size on RF treatment efficacy depending on the methodology of RF application (fruit immersed or not in water). In RF treatments in air for 18 min with the same distance between fruit and upper electrode that in RF heating with water immersion, brown rot reduction at 75 ± 2 mm of fruit diameter was significantly higher than that observed with the other fruit diameters evaluated (65 ± 2 and 70 ± 2 mm). Similar results were observed by Ikediala *et al.* (1999), who reported that large cherries heated faster than small ones when the same microwave (another electromagnetic energy) treatment was applied so that the mean temperature achieved by large cherries was higher. Therefore, the differences between RF efficacies depending on fruit size could be caused by differences between heating rates. Birla *et al.* (2008a) reported that RF heating characteristics are not just influenced by the dielectric properties of the fruit but also by fruit shape and size and concluded, based on their results obtained by computer simulation, that uniform RF heating of the fresh fruit in air would not be possible as the uniform heating over the entire fruit section can be expected if the gap between two electrodes is very large. But, maintaining large air gap is no practical for industrial applications because a large gap reduces the electric field strength results in slow heating rates and longer RF treatment. On the contrary, when RF treatment was applied with fruit immersed in water at 20 ºC for 9 min, fruit size had not a significant effect on RF efficacy. The lower influence of fruit size on the effectiveness of RF treatment when it was applied with fruit immersed in water may be due to the similar temperatures achieved, since there were only 3 ºC of difference between the temperature reached by fruit of smaller (65 ± 2 mm) and larger diameter (75 ± 2 mm). The non-uniform heating of fruit may be caused by non-uniform RF fields, for this reason, the better distribution of the electric fields with water immersion resulted in a reduction of the variability in heating rates of the fruit.

In all the experiments with fruit immersed in water, the external and internal visual quality of fruit was observed. Radio frequency treatment with fruit immersed in water at 20 ºC for 9 min was not only the best treatment for brown rot control but it also was the treatment in which the external visual quality of the fruit was not affected. However, Casals *et al.* (2010d) observed thermal damage at the points of contact of peaches with the container when RF treatment in air was applied to control brown rot and suggested that this could be the result of overheating caused by a concentration of electric fields around those contact areas. Similar results were reported by Ikediala *et al.* (2002), who observed in preliminary tests that cherries treated with RF in air suffered thermal damage at the points of contact with the container or with other fruit but when cherries were immersed in water this problem was eliminated. In our work, RF treatment with fruit immersed in water at 20 ºC for 9 min did not affect external quality of peaches and nectarines, however, internal browning was observed in 'Fantasia' nectarines. Birla *et al.* (2008a) by computer simulation showed that immersion of the model fruit in water slightly shifted the hot spot toward the core of the model fruit. Therefore, the internal damage could be the result of core-focused heating caused by dielectric constants differences between water and fruit. Thus, by matching the dielectric properties of the fruit and the immersion water, temperature would increase at a similar rate so that both fruit surface and core temperatures would increase similarly with heating time (Ikediala *et al.*, 2002; Wang *et al.*, 2003).

The results obtained when RF treatment was applied with fruit immersed in hot water indicated that increasing water temperature at 35 and 40 ºC, the treatment time decreased to 6 and 4.5 min, respectively, to control brown rot without adverse external damage in both 'Baby Gold 9' peaches and 'Autumn Free' nectarines. The decrease in treatment time with increasing the water temperature may be due to an increase in heating rate, since the external temperatures achieved when RF treatment was applied with water at 40 ºC for 4.5 min were similar to those obtained by the RF treatment with water at 35 ºC for 6 min. The dielectric loss factor of a material increases with increasing temperature at a fixed frequency (Wang *et al.*, 2008; Sosa-Morales *et al.*, 2009). An increase in the water loss factor provided a conductive media for electromagnetic energy to pass through water as the path of least resistance (Birla *et al.*, 2008a). In addition, hot water immersion technique not only decreased exposure time but also overcame the problem of core-focusing heating. This can be attributed to an increase in water loss factor, which decreased the difference between the dielectric properties of water and fruit and therefore increased the similarity of the heating rate.

In conclusion, our results indicated that RF heating with fruit immersed in water at 20 ºC for 9 min may provide a potential postharvest alternative for brown rot control in peaches and nectarines. The reduction of treatment time to 6 and 4.5 min with increasing water temperature to 35 and 40 ºC, respectively, makes it possible to design continuous equipment to process large quantities of fruit in a short period of time, which would be advantageous in comparison with other heat technologies. Nevertheless, more studies need to be carried out to investigate RF treatments under other conditions, since the response of a pathogen to heat can be influenced by several factors such as time of infection, inoculum concentration, moisture content of spores (Barkai-Golan and Phillips, 1991) and fruit maturity.

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Capítulo 3

Effect of host and *Monilinia* spp. variables on the efficacy of radio frequency treatment on peaches

M. Sisquella, I. Viñas, P. Picouet, R. Torres, J. Usall

Postharvest Biology and Technology (2014), 87, 6-12

Abstract

Brown rot caused by *Monilinia* spp. is the most important postharvest disease of stone fruit. Currently, no chemical fungicides are allowed in the European Union to be applied to stone fruit after harvest. In previous work, radio frequency (RF) treatment for 4.5 min applied with fruit immersed in water at 40 ºC was very promising for the control brown rot on peaches and nectarines. In the present study, the efficacy of this radio frequency treatment was studied employing different infection times, inoculum concentrations, fruit maturity levels and in naturally infected fruit. Generally, infection time and maturity level of fruit did not have a significant effect on the RF treatment efficacy and brown rot incidence was significantly reduced in fruit inoculated 0, 24 or 48 h before treatment and at all maturity levels evaluated in both peaches and nectarines. RF treatment significantly reduced brown rot incidence at all inoculum concentrations evaluated (10^3, 10^4, 10^5 and 10^6 conidia mL^{-1}). However, in peaches, the treatment efficacy was slightly less when the inoculum concentration was increased to 10^5 or 10^6 conidia mL^{-1}. In naturally infected fruit, brown rot incidence was significantly reduced from 92 % among control fruit to less than 26 % in peaches and complete brown rot control was achieved in nectarines. RF treatment did not have an effect on fruit firmness in the varieties tested, and even a delay of fruit softening was observed. Moreover, both external and internal fruit appearance was not affected by the treatment.

Keywords: *Monilinia fructicola*, nectarines, heat treatment, brown rot

1. Introduction

Brown rot is the most important postharvest disease of stone fruit and is essentially caused by two species, *Monilinia laxa* (Aderh.et Rulh.) Honey and *Monilinia fructicola* (G. Wint.) Honey (De Cal *et al.*, 2009). Stone fruit infection by *Monilinia* spp. can take place in the field during the growing season when conditions favor disease development. However, postharvest losses by brown rot that routinely occur during storage and transport (Hong *et al.*, 1997) are typically more severe than preharvest losses, sometimes reaching high levels (59 %) (Larena *et al.*, 2005). Currently, no chemical fungicides are allowed in the European Union to be applied for postharvest treatment of stone fruit. In addition, public demands to reduce pesticide use and improve environmental and human health, as well as the development of resistance to widely-used synthetic fungicides by fungal strains, limits the preharvest application of chemical products in the field. These concerns, combined with a lack of effective postharvest treatments against *Monilinia* spp. have increased the need to develop new control methods.

Heat treatments have been widely studied for many years (Smith *et al.*, 1964), however, heat treatments applied by immersion in hot water, vapor heat, hot air drying, curing or by hot water rinsing and brushing have been also investigated in recent years to control postharvest diseases in peaches (Casals *et al.*, 2010b), oranges (Plaza *et al.*, 2003), apples (Fallik *et al.*, 1995) and lemons (Stange and Eckert, 1994). These conventional heat treatments are limited by the low thermal conductivity of fruit and thus necessitating prolonged heating in many cases. Casals *et al.* (2010b) reported a curing treatment at 50 ºC for 2 h for controlling brown rot in peaches and nectarines. However, short treatment times are preferred from the viewpoint of commercial applications (Ikediala *et al.*, 2002). Generally, hot water treatments are shorter where time exposures range between 20 s and 2.5 min and temperatures between 45 and 60 ºC (Margosan *et al.*, 1997; Karabulut *et al.*, 2010), although their application may need to be combined with an alternative treatment to enhance effectiveness (Casals *et al.*, 2010c; Sisquella *et al.*, 2013b).

The need to achieve fast and effective thermal treatment has resulted in the increased use of radio frequency (RF) energy to heat foods. This electromagnetic energy directly interacts with the fruit interior to quickly raise the center temperature (Tang *et al.*, 2000), because dielectric materials, such as most agricultural products, can store electric energy and convert electric energy into heat (Wang *et al.*, 2001). The dielectric properties and specially the loss factor (ε''), influence both energy absorption and attenuation and describe the ability to dissipate energy in response to an applied electric field, which commonly results in heat generation (Ikediala *et al.*, 2000). The amount of heat converted in the food is proportional to the value of the loss factor (Tang *et al.*, 2000).

Radio frequency heating has been widely studied as a rapid pest control for nuts and dry products (Mitcham *et al.*, 2004), cherries (Ikediala *et al.*, 2002), oranges (Birla *et al.*, 2005), apples (Wang *et al.*, 2006), persimmons (Tiwari *et al.*, 2008) and mangoes (Sosa-Morales *et al.*, 2009). In contrast, little information is available about the possibility of applying RF heating to control postharvest diseases. Casals *et al.* (2010d) demonstrated the potential of the use of RF to control brown rot in peaches. In subsequent work, the improvement of this treatment by applying the RF with fruit immersed in water was investigated by Sisquella *et al.* (2013a) who reported that RF heating for 4.5 min in fruit immersed in water at 40 ºC controlled *M. fructicola* in artificially inoculated peaches and nectarines. The response of a pathogen to heat can be influenced by several factors such as the moisture content of spores, age of the inoculum and inoculum concentration (Barkai-Golan and Phillips, 1991). Therefore, although these new conditions demonstrated high brown rot control, other factors require consideration before RF treatment can be implemented as a commercial treatment.

The aim of this study was to evaluate RF treatment with immersion of peach and nectarine fruit in water at 40 ºC as a control for postharvest brown rot. We examined the effect of RF treatment on brown rot at different times after inoculation of the fruit, on fruit challenged with different inoculum concentrations, and with fruit at different maturity stages. The efficacy of the RF treatment also was tested with naturally infected fruit. In addition, the effect of RF treatment on fruit quality was evaluated in peaches and nectarines.

2. Materials and methods

2.1. Fruit

Experiments were conducted with 'Rome Star', 'Roig d'Albesa' and 'Placido' peaches (*Prunus persica* (L.) Batsch) and 'Fantasia', 'September Red' and 'PP-100' nectarines (*P. persica* (L.) Batsch var. Nectarine (Ait.) Maxim.). Fruit were grown in commercial orchards located in Lleida (Catalonia) following standard cultural practices and chemical spray programs in the field for pest and disease control.

Fruit free of visible wounds and rots and similar visual maturity were selected by hand immediately after harvest. Fruit not used at the time of harvest were stored at 0 ºC until required for experimentation.

2.2. Pathogen culture

The isolate of *M. fructicola* (CPMC1) used in this study was from the collection of the Postharvest Pathology Unit, Centre IRTA, Lleida, Catalonia. This strain was isolated from an infected stone fruit and was identified by the Department of Plant Protection, INIA, Madrid (Spain). The strain was maintained on potato dextrose agar (PDA) medium (Biokar Diagnostics, 39 g L^{-1}) amended with acetone (J.T. Baker, 1 %) at 4 ºC in the dark.

2.3. Pathogen production and inoculation methodology

The isolate of *M. fructicola* (CPMC1) was subcultured onto PDA amended with acetone (J.T. Baker, 1 %) and incubated in the dark at 25 ºC for approximately two weeks. The isolate was inoculated onto peaches or nectarines by wounding the fruit (1 mm diameter and 2 mm depth) with a sterilized steel rod and transferring conidia and mycelium from the PDA culture to the wound site with a sterile pipette tip. Fruit were then incubated at 25 ºC and 85 % RH in the dark for 5-7 days. Conidia were scraped from infected fruit using a sterile loop and transferred to a test-tube containing 5 mL of sterile distilled water and a drop of Tween-80 per liter. Conidia concentration was measured with a haemocytometer and the suspension diluted to the desired concentration. Fruit were wounded once per fruit with the sterile steel rod and inoculated with 15 µL of the desired conidial suspension.

2.4. Radio frequency heating system and suitable treatment conditions

A semi-industrial radio frequency equipment instrument (STALAM S.p.A, Nove, Vicenza, Italy) with 15 kW nominal maximum power and a frequency of 27.12 MHz was used to perform the experiments. The RF equipment is provided with two parallel electrodes of 150 cm x 100 cm. The electrode gap was adjustable over a range of 65-205 mm and the speed of the continuous conveyer belt ranged from 0.1 to 0.7 m min^{-1}.

Radio frequency conditions used in this study were those reported in a previous work (Sisquella *et al.*, 2013a). The electrode voltage was set at 5800 V and the electrode gap was adjusted at 112 mm. Fruit at room temperature were introduced into a container (260 mm x 260 mm x 105 mm) with 2 L of water at 40 ºC, so all fruit were completely submerged in the water. The containers were placed on the conveyor belt and RF treatment was applied for 4.5 min.

2.5. Effect of infection time on radio frequency efficacy

'Roig d'Albesa' peaches and 'PP-100' nectarines were artificially inoculated with *M. fructicola* at 10^3 conidia mL^{-1} as described above and were maintained for 0, 24 or 48 h at 20 ºC and 85 % RH. After this time, fruit were immersed in water at 40 ºC and then, RF treatment was applied for 4.5 min. A set of artificially inoculated fruit of each incubation time was not treated and was used as a control. All treatments were conducted with four replicates and eight fruit per replicate. After treatment, fruit were incubated 5 days at 20 ºC and 85 % RH and then, the number of infected fruit was recorded.

2.6. Effect of inoculum concentration on radio frequency efficacy

'Roig d'Albesa' peaches and 'PP-100' nectarines were artificially inoculated with *M. fructicola* at 10^3, 10^4, 10^5 or 10^6 conidia mL^{-1} as previously described. Once wounds were dry, fruit were immersed in water at 40 ºC and then, RF treatment was applied for 4.5 min. A set of artificially inoculated fruit of each inoculum concentration was not treated and was used as a control. All treatments were conducted with four replicates and eight fruit per replicate. After treatment, fruit were incubated 5 days at 20 ºC and 85 % RH and then the number of infected fruit was recorded.

2.7. Effect of fruit maturity on radio frequency efficacy

In order to achieve different maturity levels, after harvest and before treatment, 'Roig d'Albesa' peaches and 'PP-100' nectarines were maintained for 24, 48 or 72 h at 20 ºC and 85 % RH. After this time, fruit were artificially inoculated with *M. fructicola* at 10^3 conidia mL^{-1} as described above. Once wounds were dry, fruit were immersed in water at 40 ºC and then RF treatment was applied for 4.5 min. A set of artificially inoculated fruit of each maturity level was not treated and was used as a control. All treatments were conducted with four replicates and eight fruit per replicate. After treatment, fruit were incubated 5 days at 20 ºC and 85 % RH and then, the number of infected fruit was recorded.

Prior to the RF treatment the maturities of the three different fruit sets were determined by measuring standard quality parameters including fruit firmness, soluble solids content and acidity. Fruit firmness was measured on two opposite peeled sides using a penetrometer (Effegi, Milan, Italy) fitted with an 8 mm diameter flat tip. The average of those two measurements was considered as one replicate. Sixteen fruit per maturity level were evaluated and data are expressed in Newton (N).

Soluble solids content (SSC) was determined with a digital refractometer (Atago PR-100, Tokyo, Japan) by measuring the juice refractive index and data are expressed as percentage of soluble solids. Acidity was measured by mixing 10 mL of juice with 10 mL distilled H_2O and adding three drops of phenolphthalein, which was then titrated with 0.1 N NaOH. Acidity was expressed in grams of malic acid per liter of juice (g m.a. L^{-1}). Juice used to determine SSC and acidity was extracted from four fruit that had been used to determine firmness, thus measurements were conducted with four replicates and four fruit per replicate.

2.8. Effect of radio frequency treatment on naturally infected fruit

'Rome Star' and 'Roig d'Albesa' peaches and 'PP-100' nectarines with natural infections were immersed in water at 40 ºC and then, RF treatment was applied for 4.5 min. A set of fruit of each variety was not treated and was used as a control. All treatments were conducted with four replicates and twenty-four fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH, the number of infected fruit was recorded.

2.9. Effect of radio frequency treatment on fruit quality

The effect of radio frequency treatment on standard quality parameters was evaluated on 'Roig d'Albesa' peaches and 'September Red' and 'PP-100' nectarines. Fruit were immersed in water at 40 ºC and then, RF treatment was applied for 4.5 min. A set of fruit of each variety was not treated and was used as a control. All treatments were conducted with four replicates and four fruit per replicate. After 2 days of incubation at 20 ºC and 85 %, standard quality parameters including firmness, soluble solids content and acidity were determined as describe above.

Moreover, in all experiments, external and internal fruit appearance was observed. External thermal damage, specifically browning of fruit surface, was evaluated at the end of radiofrequency heating. Internal thermal damages, mainly flesh browning and development of internal cavities, were determined cutting each fruit in half after 5 days of incubation at 20 ºC and 85 % RH once brown rot incidence was recorded.

2.10. Internal and external fruit temperature

The external fruit temperature achieved immediately after RF treatment was measured in 'Rome Star' and 'Roig d'Albesa' peaches and 'September Red', 'Fantasia' and 'PP-100' nectarines by a portable infrared thermometer (Testo 831, Testo AG, USA) with an accuracy of ± 1.5 ºC. All measurements were conducted with eight replicates and eight fruit per replicate. Moreover, internal temperature of one fruit of each replicate during all the RF process was measured in all the varieties evaluated except in 'PP-100' nectarines with an inside-optical fiber temperature probes (FOT-L/10 m; FISO Technologies Inc., Canada) with an accuracy of ± 0.5 ºC. Temperature sensors were placed 10 mm inside the fruit.

2.11. Statistical analysis

The incidence of brown rot and standard quality parameters were analyzed using analysis of variance (ANOVA) with JMP®8 statistical software (SAS Institute, Cary, NC, USA). Statistical significance was judged at the level $P<0.05$. When the analysis was statistically significant, the least significance difference (LSD) test for separation of means was used. Arcsine-transformation of the brown rot incidence data was performed prior to analysis. Non-transformed means are presented.

3. Results

3.1. Effect of infection time on radio frequency efficacy

Brown rot incidence was significantly reduced to less than 10 % in all the infection times studied when radio frequency treatment with fruit immersed in water at 40 ºC for 4.5 min was applied in 'Roig d'Albesa' peaches in comparison with untreated fruit where brown rot incidence was 100 % (Fig. 1A). When RF treatment was applied in 'PP-100' nectarines artificially inoculated 0, 24 and 48 h before treatment, brown rot incidence was significantly reduced to 0, 3 and 19 %, respectively, compared with untreated fruit where brown rot incidence was 81, 74 and 57 %, respectively. However, radio frequency efficacy was significantly lower in fruit inoculated 48 h before treatment than in fruit inoculated 0 and 24 h before (Fig. 1B).

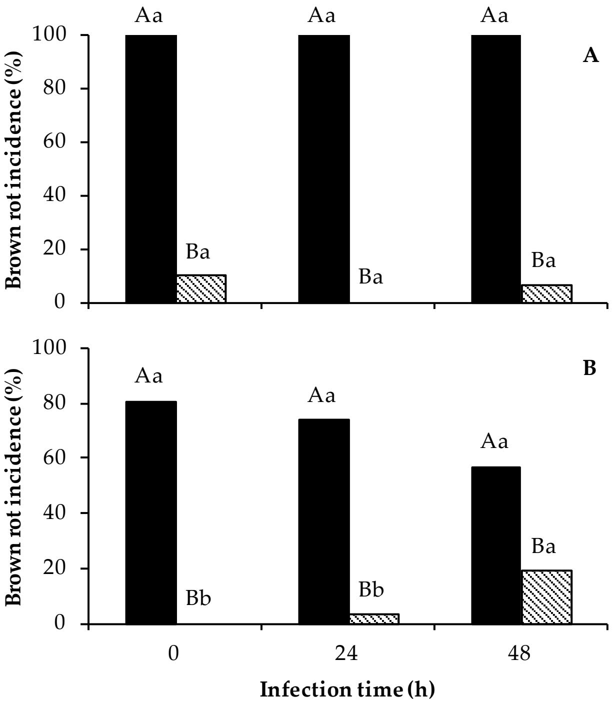

Fig. 1. Brown rot incidence in 'Roig d'Albesa' peaches (A) and 'PP-100' nectarines (B) artificially inoculated with *Monilinia fructicola* 0, 24 and 48 h prior to radio frequency treatment for 4.5 min with fruit immersed in water at 40 ºC (▨) or untreated (■). After treatment, fruit were incubated for 5 days at 20 ºC and 85 % RH. Arcsine-transformation of the data was performed before statistical analysis. Non-transformed means are shown. Means with the same uppercase letter for each infection time or with the same lowercase letter for each treatment are not significantly different ($P<0.05$) according to LSD test.

3.2. Effect of inoculum concentration on radio frequency efficacy

Radio frequency treatment with fruit immersed in water at 40 ºC for 4.5 min significantly reduced brown rot incidence to less than 10 % in 'Roig d'Albesa' peaches artificially inoculated at 10^3 and 10^4 conidia mL^{-1} in comparison with untreated fruit (100 %), however radio frequency efficacy was significantly greater than those observed in the higher inoculum concentrations evaluated, 10^5 and 10^6 conidia mL^{-1}, where brown rot incidence was significantly reduced to 34 and 36 %, respectively (Fig. 2A).

In 'PP-100' nectarines, complete brown rot control was achieved when RF treatment was applied in fruit inoculated at 10^3 conidia mL^{-1} compared with untreated fruit (81 %) and no significant differences were detected with the other inoculum concentrations evaluated of 10^4, 10^5 and 10^6 conidia mL^{-1} (Fig. 2B).

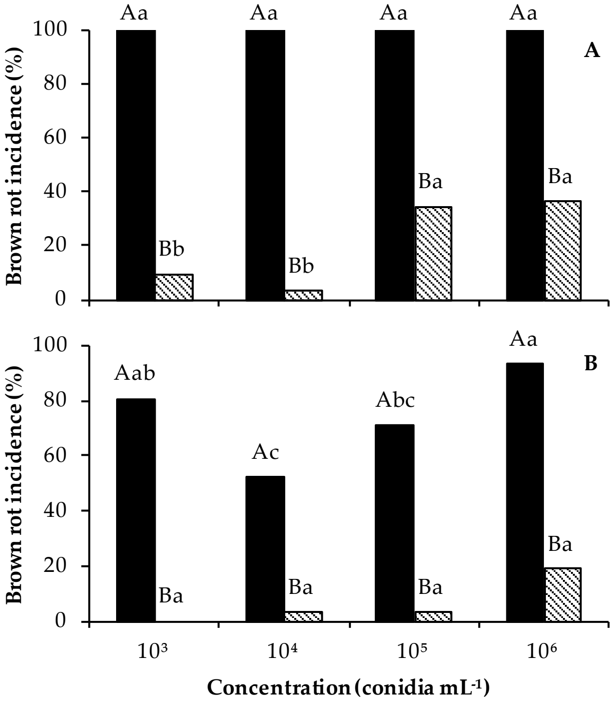

Fig. 2. Brown rot incidence in 'Roig d'Albesa' peaches (A) and 'PP-100' nectarines (B) artificially inoculated with *Monilinia fructicola* at 10^3, 10^4, 10^5 and 10^6 conidia mL^{-1} and treated by radio frequency for 4.5 min with fruit immersed in water at 40 °C (▨) or untreated (■). After treatment, fruit were incubated for 5 days at 20 °C and 85 % RH. Arcsine-transformation of the data was performed before statistical analysis. Non-transformed means are shown. Means with the same uppercase letter for each inoculum concentration or with the same lowercase letter for each treatment are not significantly different ($P<0.05$) according to LSD test.

3.3. Effect of fruit maturity on radio frequency efficacy

Radio frequency treatment with fruit immersed in water at 40 ºC for 4.5 min significantly reduced brown rot incidence to 9, 31 and 18 % in 'Roig d'Albesa' peaches maintained 24, 48 and 72 h at 20 ºC prior RF treatment, respectively, in comparison with untreated fruit (100 %) and no significant differences were detected between fruit maintained at 20 ºC for 24, 48 and 72 h (Fig. 3A). Different maturity levels were achieved between fruit maintained at 20 ºC for 24 and 72 h in which fruit firmness was 8.2 and 7.3 N, respectively (data not shown).

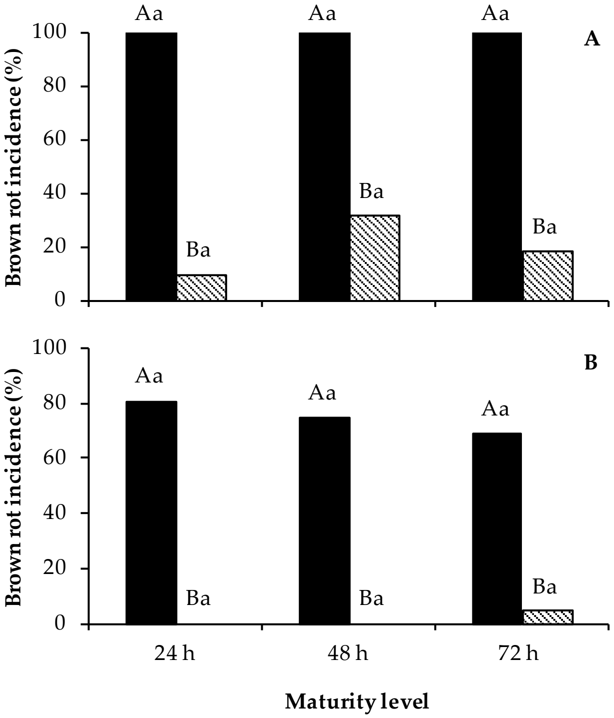

Fig. 3. Brown rot incidence in 'Roig d'Albesa' peaches (A) and 'PP-100' nectarines (B) incubated at 20 ºC and 85 % RH for 24, 48 and 72 h prior to be artificially inoculated with *Monilinia fructicola* at 10^3 conidia mL^{-1} and treated by radio frequency for 4.5 min with fruit immersed in water at 40 ºC (▨) or untreated (■). After treatment, fruit were incubated for 5 days at 20 ºC and 85 % RH. Arcsine-transformation of the data was performed before statistical analysis. Non-transformed means are shown. Means with the same uppercase letter for each maturity level or with the same lowercase letter for each treatment are not significantly different (P<0.05) according to LSD test.

In 'PP-100' nectarines, brown rot incidence was significantly reduced to less than 4 % when fruit were maintained 24, 48 and 72 h at 20 ºC before treatment compared with untreated fruit where brown rot incidence was higher than 69 % (Fig. 3B). Furthermore, different maturity levels were achieved in 'PP-100' nectarines. Firmness in fruit incubated at 20 ºC for 24, 48 and 72 h was 52.4, 36.5 and 14 N, respectively, and significant differences were detected between all of them. Regarding acidity, this was significantly reduced from 8.4 to 7.4 g m.a. L^{-1} with increasing incubation time at 20 ºC from 24 to 72 h. The soluble solids content was not affected for incubation time (data not shown).

3.4. Effect of radio frequency treatment on naturally infected fruit

Radio frequency treatment with fruit immersed in water at 40 ºC for 4.5 min completely controlled brown rot in 'PP-100' nectarines compared with untreated fruit (83 %). When RF treatment was applied in 'Rome Star' and 'Roig d'Albesa' peaches, brown rot incidence was significantly reduced to 13 and 26 %, respectively, compared with untreated fruit where disease incidence was 92 and 100 %, respectively (Fig. 4).

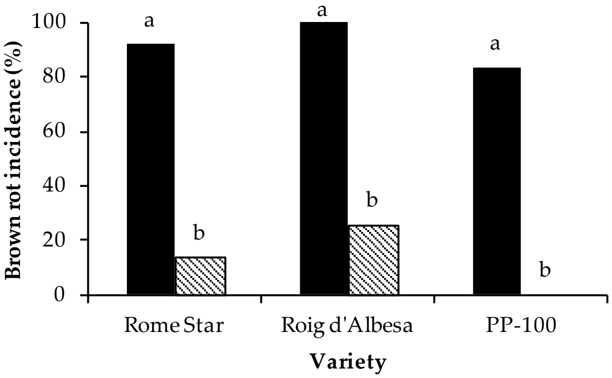

Fig. 4. Brown rot incidence in 'Rome Star' and 'Roig d'Albesa' peaches and 'PP-100' nectarines naturally infected immersed in water at 40 ºC and treated by radio frequency for 4.5 min (▨) or untreated (■). After treatment, fruit were incubated for 5 days at 20 ºC and 85 % RH. Arcsine-transformation of the data was performed before statistical analysis. Non-transformed means are shown. Means with the same letter for each variety are not significantly different ($P<0.05$) according to LSD test.

Table 1. Standard quality parameters of 'Roig d'Albesa' peaches and 'September Red' and 'PP-100' nectarines immersed in water at 40 ºC and treated by radio frequency for 4.5 min or untreated and stored for 2 days at 20 ºC and 85 % RH. Means with the same letter for each variety and quality parameter are not significantly different (P<0.05) according to LSD test.

Treatment	'Roig d'Albesa' peach			'September Red' nectarine			'PP-100' nectarine		
	Firmness (N)	Soluble solids (%)	Acidity (g m.a. L^{-1})	Firmness (N)	Soluble solids (%)	Acidity (g m.a. L^{-1})	Firmness (N)	Soluble solids (%)	Acidity (g m.a. L^{-1})
Initial	67.4	12.9	3.2	47.0	10.9	8.9	52.1	12.1	13.7
Untreated	65.7 a	13.3 a	2.0 a	32.5 a	11.2 a	5.1 a	9.7 b	13.0 a	6.4 a
RF treatment	66.8 a	12.5 b	1.8 b	29.3 a	11.5 a	5.1 a	15.5 a	11.9 a	6.4 a

3.5. Effect of radio frequency treatment on fruit quality

Firmness was not affected by radio frequency treatment with fruit immersed in water at 40 ºC for 4.5 min in 'Roig d'Albesa' peaches and 'September Red' nectarines. However, in 'PP-100' nectarines, firmness in fruit treated by RF, 15.5 N, was significantly higher than in untreated fruit (Table 1). Regarding soluble solids and acidity, only significant differences were detected in 'Roig d'Albesa' peaches, where soluble solids and acidity were significantly reduced in fruit treated by RF from 13.3 to 12.5 % and from 2.0 to 1.8 g m.a. L^{-1}, respectively (Table 1).

In all experiments, when external and internal fruit appearance was evaluated, no thermal damage was observed in either of the varieties studied.

3.6. Internal and external fruit temperature

The changes in internal temperature during RF treatment in 'Rome Star' and 'Roig d'Albesa' peaches and 'September Red' and 'Fantasia' nectarines were very similar in all studied varieties (Fig. 5). The internal fruit temperature achieved immediately after treatment ranged from 34.1 ± 1.7 to 35.8 ± 2.9 ºC obtained in 'Rome Star' and 'Roig d'Albesa' peaches, respectively (Table 2). External temperature recorded at the end of the RF treatment in 'Rome Star' and 'Roig d'Albesa' peaches and 'September Red', 'Fantasia' and 'PP-100' nectarines ranged from 43.3 ± 1.7 to 45.4 ± 1.8 ºC (Table 2).

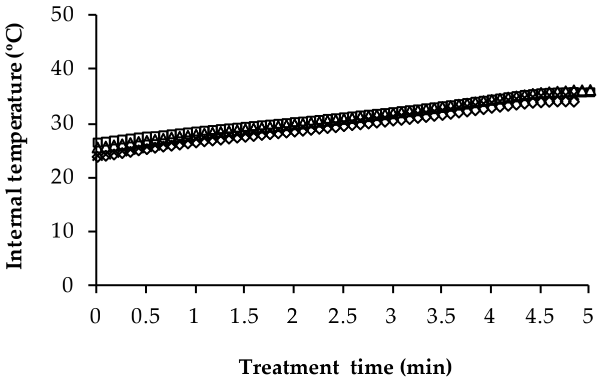

Fig. 5. Evolution of internal fruit temperatures of 'September Red' (△) and 'Fantasia (x) nectarines and 'Rome Star' (◇) and 'Roig d'Albesa' (□) peaches during radio frequency treatment for 4.5 min with fruit immersed in water at 40 ºC.

Table 2. Average internal and external temperature of 'Rome Star' and 'Roig d'Albesa' peaches and 'September Red', 'Fantasia' and 'PP-100' nectarine achieved immediately after radio frequency treatment for 4.5 min with fruit immersed in water at 40 ºC.

Variety	Temperature (ºC ± SD)	
	Internal	External
'Rome Star' peach	34.1 ± 1.7	43.3 ± 1.7
'Roig d'Albesa' peach	35.8 ± 2.9	45.2 ± 0.8
'September Red' nectarine	35.8 ± 2.0	45.1 ± 1.1
'Fantasia' nectarine	35.3 ± 3.3	44.3 ± 1.8
'PP-100' nectarine	nd	45.4 ± 1.8

nd: not determined

4. Discussion

Radio frequency treatment has been widely studied to control pests in nuts and fruit but, little information is available about the efficacy of this treatment to control postharvest diseases. Casals *et al.* (2010d) were the first to study RF heating to control postharvest brown rot disease in peaches and nectarines and demonstrated the efficacy of RF treatment for 18 min for control of *Monilinia* spp. in peaches but not in nectarines. In order to address the lack of efficacy in nectarines and reduce the treatment time, Sisquella *et al.* (2013a) applied the radio frequency treatment with fruit immersed in water at 40 ºC for 4.5 min, achieving a high brown rot control in both, peaches and nectarines. However, there is not information about the influence of fruit and *Monilinia* spp. factors on the efficacy of this radio frequency treatment and this information is very important to evaluate the commercial potential of this treatment.

In the present work, RF treatment with fruit immersed in water at 40 ºC applied for 4.5 min controlled brown rot at all infection times before treatment evaluated in both, peaches and nectarines. However, when this treatment was applied in 'PP-100' nectarines, RF efficacy in fruit inoculated 48 h before treatment was slightly lower in comparison with other infection times studied. Similar results were obtained in peaches by Casals *et al.* (2010d) when RF treatment was applied in air for 18 min. These results suggest that radio frequency treatment would have an advantage over conventional heating, in which different efficacies were observed depending on the infection time of *Monilinia* spp.. Casals *et al.* (2010a) reported that the efficacy of heat treatment conducted with hot wet air at 50 ºC for 2 h and 95-99% RH decreased as the pre-infection time of the established *M. laxa* infection increased.

The incidence of brown rot in 'Roig d'Albesa' peaches and 'PP-100' nectarines was reduced significantly at all inoculum concentration evaluated (10^3, 10^4, 10^5 and 10^6 conidia mL^{-1}), although in 'Roig d'Albesa' peaches, RF efficacy was lower when the inoculum concentration increased to 10^5 or 10^6 conidia mL^{-1}. Similar results were obtained by Casals *et al*. (2010d) when RF treatment in air for 18 min was applied in 'Summer Rich' peaches, however when the same treatment was applied in 'Placido' peaches brown rot only was reduced when fruit were inoculated with *M. fructicola* at 10^3 conidia mL^{-1}. The best efficacy obtained to control different inoculum concentrations in this work could be due to differences in the surrounding medium of RF treatment, air or water. Non-uniform heating is the main problem associated with RF heating and it can reduce the effectiveness of the treatment. Birla *et al*. (2008) reported that RF heating was influenced by the surrounding medium and demonstrated that water offered less resistance to electric field than air. Moreover, immersion water may improve the electric field distribution resulting in reducing the high temperature differential problem among fruit normally associated with treatments in air (Ikediala *et al*., 2002).

Maturity level did not have a significant effect on RF efficacy and the incidence of brown rot was significantly reduced for all maturity levels evaluated in both peaches and nectarines. In contrast, Casals *et al*. (2010a) reported that the efficacy of curing at 50 ºC for 2 h and 95–99% RH decreased when the most mature fruit were tested. A possible explanation for these differences is the heating method used to increase the commodity temperature. In RF heating, the dielectric properties and specially the loss factor (ε'') determine the rate of dielectric heating. Therefore, the lower influence of maturity level on the effectiveness of RF could be due to the loss factor and, consequently, the heating rate and final temperature not being affected by the changes produced by the ripening of the fruit during storage. Nelson *et al*. (1995) demonstrated that the loss factor at lower frequencies showed little dependence on stage of maturity of peaches. Guo *et al*. (2011) reported that with increasing apple maturity, the dielectric properties from measurements on external surface and internal tissues showed no regular changes during the ripening period and no obvious relationships between dielectric properties and firmness were found. However, when Sosa-Morales *et al*. (2009) evaluated the influence of mango ripening on dielectric properties, higher loss factor values were obtained on day 0, in the physiologically mature stage of the fruit, compared with those values determined on 4, 8 and 16 days storage, in the ripen and senescent stages, but this reduction was mainly attributed to the decreased moisture content and the increased pH observed during that period.

Brown rot incidence in naturally infected fruit was significantly reduced to less than 26 % in peaches and complete brown rot control was achieved in nectarines. The lower efficacy observed in peaches could be due to differences in infection times or inoculum concentrations that have to be controlled, since, as we have indicated previously in the present work, 48 h between infection and treatment or high inoculum concentrations could slightly decrease the efficacy of the radio frequency treatment. Even so, it is important to note that brown rot reduction was higher than 74 % in all the varieties studied.

Radio frequency treatment with fruit immersed in water at 40 ºC for 4.5 min did not affect fruit firmness in the varieties tested, and even a delay of the fruit softening was observed in 'PP-100' nectarines. Similar trends have been reported when conventional heat treatments were applied in fruit as moist air heat in nectarines (Anthony *et al.*, 1989), vapor heat in table grapes (Lydakis and Aked, 2003) and curing in peaches (Casals *et al.*, 2010b) and oranges (Nunes *et al.*, 2007). When RF treatments were applied in fruit for pest control, similar results were also reported in apples (Wang *et al.*, 2006), persimmons (Tiwari *et al.*, 2008), mangoes (Sosa-Morales *et al.*, 2009), oranges (Birla *et al.*, 2005) and cherries (Ikediala *et al.*, 2002). The reason for greater firmness in 'PP-100'nectarines could be attributed to heat affects on the ripening-linked softening processes in the fruit. Ethylene is commonly known to be responsible for the acceleration of fruit ripening and it was reported that heat treatments could delay the peak ethylene production, so it could delay the rate of ripening of fruit (Lay-Yee and Rose, 1994; Zhou *et al.*, 2002).

External fruit temperature reached at the end of radio frequency treatment was higher than the internal fruit temperature in all the varieties evaluated. Similar results were observed by Ikediala *et al.* (2002) who observed surface heating when radiofrequency treatment was applied in cherries immersed in 1 % salt water for pest control. With decreasing salt content to 0.15 %, the differences of temperatures between core and fruit surface were reduced. The dielectric loss factor of a material increases with increasing salt content (Sosa-Morales *et al.*, 2010) or temperature (Wang *et al.*, 2008) at a fixed frequency. Therefore, the differences between external and internal temperature could be due to that water at 40 ºC had higher dielectric loss factor than fruit, so most of the radio frequency energy was absorbed by the water resulting in a surface heating. Moreover, the lower internal temperature achieved could explained the absence of internal damage when radio frequency treatment was applied with fruit immersed in water at 40 ºC for 4.5 min, since average temperature at the end of the treatment ranged between 34.1 and 35.8 ºC. These results agree with those reported by Sisquella *et al*. (2013a) when the same radio frequency conditions were used to control brown rot, however, internal thermal damages as a result of core-focusing heating were observed when water temperature was decreased to 20 ºC.

In conclusion, radio frequency treatment for 4.5 min with fruit immersed in water at 40 ºC has attractive commercial potential for control brown rot in stone fruit without impaired fruit quality. However, before the commercial application of this treatment, it is necessary to design specific equipment with water to determine the economic cost of the treatment.

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Capítulo 4

Continuous microwave treatment to control postharvest brown rot in stone fruit

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Abstract

Monilinia spp. are the most important causes of brown rot in stone fruit and no chemical fungicides are allowed in the European Union to be applied to stone fruit after harvest. From preliminary studies, microwave (MW) treatments at 17.5 kW for 50 s and 10 kW for 95 s were selected as effective conditions to control brown rot. Both treatments were investigated to control *Monilinia fructicola* in fruit with different weights and maturity levels and in naturally infected fruit. Fruit weight only had a significant effect on microwave efficacy in 'Placido' peaches treated by MW at 10 kW for 95 s in which better brown rot control was observed in small than large fruit. Maturity level did not have a significant effect on efficacy of MW treatments in any of the varieties evaluated. When both MW treatments were studied in naturally infected peaches and nectarines, brown rot incidence was significantly reduced to less than 14 % compared with untreated fruit where brown rot incidence was higher than 45 %. The effect of both treatments on fruit quality was also evaluated. Fruit firmness was not negatively affected in the varieties tested and even a delay of fruit softening was observed. However, internal damage around the stone was observed, especially in the smallest fruit in which high temperature is achieved at the end of both MW treatments.

Keywords: *Monilinia* spp., *Monilinia fructicola*, peaches, nectarines, heat treatment, disease control

1. Introduction

Brown rot is one of the most important postharvest diseases of stone fruit worldwide and is primarily caused by two species, *Monilinia laxa* (Aderh. et Rulh.) Honey and *Monilinia fructicola* (G. Wint.) Honey. Stone fruit infection by *Monilinia* spp. mainly occurs in the field at bloom, the onset of pit hardening and between 7 and 12 days before harvest (Emery *et al.*, 2000). However, postharvest losses that routinely occur during storage and transport (Hong *et al.*, 1997) are typically more severe than preharvest losses, reaching high values when conditions are favorable for disease development (Larena *et al.*, 2005). No chemical fungicides are registered in the European Union for postharvest treatment of stone fruit, therefore the current methods to control brown rot include preharvest spraying of fungicides at regular intervals, careful handling of harvested fruit to avoid wounding, good preharvest and postharvest sanitation practices, and rapid cooling and storage after harvest. However, all these strategies are not sufficient to control brown rot in stone fruit which has increased the need to develop alternative methods.

Radio frequency (RF) and microwave (MW) heating, also referred to as dielectric heating, could be a potential alternative treatment to control postharvest disease. In the dielectric heating, electromagnetic energy is directly coupled to a dielectric material, such as most agricultural commodities, to generate heat within the material as a result of converting electromagnetic energy into thermal energy. This can significantly increase the heating rates and reduce heating time in comparison with conventional heating (Tang *et al.*, 2000). Dielectric properties and specially the dielectric loss factor (ε'') of a material, influences the conversion of electromagnetic energy into thermal energy, so the amount of heat converted in the food is proportional to the loss factor at a given frequency and electric field (Tang, 2005).

Casals *et al.* (2010d) have already demonstrated the potential of the use of radio frequency to control brown rot in peaches. The improvement of this treatment by applying the radio frequency with fruit immersed in water was investigated by Sisquella *et al.* (2013) who reported that RF heating for 4.5 min in fruit immersed in water at 40 ºC controlled *M. fructicola* in peach and nectarine. However, shorter exposure times are preferred from the viewpoint of commercial applications. With microwaves, because frequency is much greater than for radio frequency, rapid heating can be achieved with much lower field intensities and the problems of arcing in the product are diminished (Nelson, 1996).

Microwaves have been applied to a wide range of products, however, the most predominant effort of current microwave technology has been to control pests of grain and stored products. On the contrary, little information is available about the use of microwaves to control postharvest diseases. Karabulut and Baykal (2002) reported an effective microwave treatment to control *Botrytis cinerea* and *Penicillium expansum* in peaches. Similar results were observed by Zhang *et al*. (2004) for controlling *Rhizopus stolonifer* in peaches. And Zhang *et al*. (2006) combined a microwave treatment with a biocontrol agent to control *P. expansum* on pears. Although microwave heating has been investigated to control different diseases on stone fruit, no information is available about the use of microwave heating to control *Monilinia* spp.. Furthermore, these previous studies were conducted with a household microwave oven, so information about the use of an industrial microwave tunnel to control postharvest disease would make possible to design continuous equipment to be incorporated in the packinghouse handling procedures and process large quantities of fruit.

The main objective of this study was to determine the microwave conditions in a continuous industrial microwave tunnel that could reduce brown rot without causing damage to peaches and nectarines. We also evaluated the efficacy of the selected microwave conditions (10 kW for 95 s and 17.5 kW for 50 s) in (1) fruit with different weights, (2) fruit with different maturity levels, and (3) fruit with natural infections. Furthermore, the effect of MW treatments selected on fruit quality was also evaluated.

2. Material and methods

2.1. Fruit

Experiments were conducted with ‘Roig d’Albesa’ and ‘Placido’ peaches (*Prunus persica* (L) Batch) and ‘Big Top’, ‘September Red’ and ‘PP-100’ nectarines (*P. persica* var. Nectarine (Ait.) Maxim.). Fruit were grown in commercial orchards located in Lleida (Catalonia) following standard cultural practices and chemical spray programs in the field for pest and disease control. Fruit free of visible wounds and rots and similar visual maturity were selected by hand from fruit bins immediately after harvest. Fruit not used at the time of harvest were stored at 0 ºC for a maximum of 15 days until use.

Fruit weights in all the experiments except in the study of the effect of fruit size were 170 ± 10 g in 'Big Top' nectarines, 210 ± 10 g in 'September Red' nectarines, 215 ± 10 g in 'Placido' peaches and 'PP-100' nectarines and 245 ± 10 g in 'Roig d'Albesa' peaches.

2.2. Pathogen culture

The isolate of *M. fructicola* (CPMC1) used was from the collection of the Postharvest Pathology Unit, Centre IRTA, Lleida, Catalonia. This strain was isolated from an infected stone fruit and was identified by the Department of Plant Protection, INIA, Madrid (Spain). Then, the isolate was maintained on potato dextrose agar (PDA) medium (Biokar Diagnostics, 39 g L^{-1}) amended with acetone (J.T. Baker, 1 %) at 4 ºC in the dark.

2.3. Pathogen production and inoculation methodology

The isolate of *M. fructicola* (CPMC1) was subcultured onto PDA amended with acetone (J.T. Baker, 1 %) and incubated in the dark at 25 ºC for approximately two weeks. The isolate was then inoculated onto peaches or nectarines by wounding the fruit with a sterilized steel rod (1 mm wide and 2 mm long) and transferring conidia and mycelium from the PDA culture to the wound site with a sterile pipette tip. Fruit were then incubated at 25 ºC and 85 % RH in the dark for 5-7 days. Conidia were scraped from infected fruit using a sterile loop and transferred to a test tube containing 5 mL of sterile distilled water and a drop of Tween-80 per liter. Conidia concentration was measured with a haemocytometer and the suspension diluted to 10^3 conidia mL^{-1}. Then, the fruit were wounded once per fruit with the sterile steel rod and inoculated with 15 µL of conidial suspension. All microwave treatments were performed with artificially inoculated fruit incubated at 20 ºC and 85 % RH for 24 h before the treatment.

2.4. Microwave heating system and suitable treatment conditions

An industrial microwave tunnel (Synarwave, France) at 2450 MHz was used to perform the experiments. The microwave tunnel is provided with 12 magnetrons which theoretical power of each one range from 200 to 2000 W, so the generator can provide microwave power from 0.2 to 24 kW. The speed of the continuous conveyer belt ranged from 40 to 300 cm min^{-1}. For all the experiments, the desired power was achieved using 10 magnetrons with the same theoretical power each one, so maximum microwave power used in this study was 20 kW.

In all the experiments, the increase in internal temperature during MW treatment was measured with inside-optical fiber temperature probes (FOT-L/10 m; FISO Technologies Inc., Canada) with an accuracy of ± 0.5 ºC. Temperature sensor was placed 10 mm inside the fruit. Immediately after MW treatment the external temperature of eight fruit was recorded by a portable infrared thermometer (Testo 831, Testo AG, USA) with an accuracy of ± 1.5 ºC.

In addition, in all the experiments, external and internal fruit appearance was observed. External thermal damages, specifically changes in the color of the surface, were evaluated at the end of MW heating. Internal thermal damages, mainly flesh browning and development of internal cavities, were determined cutting each fruit in half after 5 days of incubation at 20 ºC and 85 % RH once brown rot incidence was recorded.

2.5. Treatment conditions affecting microwave efficacy

'Big Top' nectarines artificially inoculated as described above were used to perform the experiment to investigate the belt speed related to the exposure time at different powers that could reduce brown rot without affecting visual fruit quality. In a first experiment, microwave powers evaluated were 5, 10, 15 and 17.5 kW and treatment time ranged between 34 and 120 s depending on the power. In a second experiment, 10, 15, 17.5 and 20 kW of microwave powers were evaluated and exposure time ranged between 40 and 100 s. A set of artificially inoculated fruit of each experiment was not treated and was used as a control. All treatments were conducted with four replicates and eight fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH, brown rot incidence was recorded.

2.6. Effect of fruit size on microwave efficacy

The effect of fruit size on the efficacy of microwave treatments was evaluated in fruit with different weights artificially inoculated as described above. 'Roig d'Albesa' peaches with 180 ± 10, 245 ± 10 and 310 ± 10 g of weight were treated by MW at 17.5 kW for 50 s. 'Placido' peaches and 'PP-100' nectarines, both with 170 ± 10, 215 ± 10 and 260 ± 10 g of weight, were treated by MW at 10 kW for 95 s. A set of artificially inoculated fruit of each weight was not treated and was used as a control. All treatments were conducted with four replicates and eight fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH, brown rot incidence was recorded.

2.7. Effect of fruit maturity on microwave efficacy

In order to achieve different maturity levels, after harvest and before treatment, fruit were maintained for 24, 48 or 72 h at 20 ºC and 85 % RH. After this time, fruit were artificially inoculated as described above. 'Roig d'Albesa' peaches were treated by MW at 17.5 kW for 50 s and 'Placido' peaches and 'PP-100' nectarines at 10 kW for 95 s. A set of artificially inoculated fruit of each maturity level was not treated and was used as a control. All treatments were conducted with four replicates and eight fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH, brown rot incidence was recorded.

Prior to MW treatments, the maturity of the three different fruit sets was determined by measuring standard quality parameters including fruit firmness, soluble solids content and acidity. Fruit firmness was measured on two opposite peeled sides using a penetrometer (Effegi, Milan, Italy) fitted with an 8 mm diameter flat tip. The average of those two measurements was considered as one replicate. Twenty fruit per maturity level were evaluated and data are expressed in Newton (N).

Soluble solids content (SSC) was determined with a digital refractometer (Atago PR-100, Tokyo, Japan) by measuring the juice refractive index and data are expressed as percentage of soluble solids. Acidity was measured by mixing 10 mL of juice with 10 mL distilled H_2O and adding three drops of phenolphthalein, which was then titrated with 0.1 N NaOH. Acidity was expressed in grams of malic acid per liter of juice (g m.a. L^{-1}). Juice used to determine SSC and acidity was extracted from five fruit that had been used to determine firmness, thus measurements were conducted with four replicates and five fruit per replicate.

2.8. Effect of microwave treatments on naturally infected fruit

Microwave treatments were applied on naturally infected fruit. 'Roig d'Albesa' peaches were treated by MW at 17.5 kW for 50 s and 'Placido' peaches and 'PP-100' nectarines at 10 kW for 95 s. A set of fruit of each variety was not treated and was used as a control. All treatments were conducted with four replicates and twenty-four fruit per replicate. After 5 days of incubation at 20 ºC and 85 % RH, brown rot incidence was recorded.

2.9. Effect of microwave treatments on fruit quality

The effect of microwave treatments on standard quality parameters was evaluated in 'Roig d'Albesa' peaches treated by MW at 17.5 kW for 50 s and 'Placido' peaches and 'PP-100' nectarines treated at 10 kW for 95 s. A set of fruit of each variety was not treated and was used as a control. All treatments were conducted with four replicates and four fruit per replicate. After 2 days of incubation at 20 ºC and 85 % RH, standard quality parameters including firmness, soluble solids content and acidity were determined as describe above.

2.10. Statistical analysis

The incidence of brown rot and quality parameters were analyzed using analysis of variance (ANOVA) with JMP®8 statistical software (SAS Institute, Cary, NC, USA). Statistical significance was judged at the level *P*<0.05. When the analysis was statistically significant, the least significance difference (LSD) test for separation of means was used.

3. Results

3.1. Treatment conditions affecting microwave efficacy

Brown rot reduction and internal and external temperature achieved immediately after microwave treatment at different powers and exposure times is summarized in Table 1. Brown rot incidence in untreated fruit was 86 and 40 % in the first and the second experiment, respectively. No brown rot control was observed even at the maximum exposure time evaluated, 120 s, when microwave treatment was applied at 5 kW. Complete brown rot reduction was achieved when microwave treatment at 10 kW was applied for 100 s but no brown rot control was observed when exposure time was decreased to less than 60 s. When microwave power was increased to 15 and 17.5 kW, generally, lower exposure time was needed, 70 and 50 s, respectively, to reach a similar level of reduction, 73 and 91 %, respectively. However, no significant differences were observed between 17.5 and 20 kW for the same time of exposure.

Table 1. Brown rot reduction, internal and external temperature at the end of microwave treatments at different powers and exposure times in ‘Big Top’ nectarines artificially inoculated with *Monilinia fructicola* at 10^3 conidia mL^{-1} 24 h prior to microwave treatment. Fruit were incubated for 5 days at 20 ºC and 85 % RH. Brown rot incidence in untreated fruit was 86 and 40 % in the first and the second experiment, respectively. External visual appearance was not affected by any microwave treatments. Means with the same letter for each experiment are not significantly different ($P<0.05$) according to LSD test.

Experiment	Power (kW)	Time (s)	Brown rot reduction (%)	Temperature (ºC)		Internal damage[a]
				Internal	External	
1	5	105	0 c	43	38	-
		120	7 c	45	37	-
	10	34	0 c	31	33	-
		50	0 c	37	34	-
		60	4 c	44	39	-
		70	29 b	47	41	-
	15	34	4 c	40	37	-
		60	93 a	55	46	-
	17.5	34	7 c	50	40	-
2	10	80	73 ab	57	42	-
		90	91 ab	52	43	-
		100	100 a	67	46	+
	15	40	0 d	38	36	-
		50	1 cd	51	39	-
		60	63 ab	52	43	-
		70	73 ab	60	45	+
	17.5	40	46 bc	44	40	-
		50	91 ab	49	44	-
	20	40	64 ab	67	42	+
		50	91 ab	63	46	+

[a] Presence (+) or absence (-) of internal thermal damage, specifically internal browning around the stone, after 5 days at 20 ºC and 85 % RH.

External visual appearance was not affected by either MW treatment evaluated, however, internal thermal damage was observed in fruit treated at 10 kW for 100 s, 15 kW for 70 s and 20 kW for 40 and 50 s.

Based on these results, microwave conditions selected to carry out subsequent studies were 17.5 kW for 50 s and 10 kW for 95 s. In the latter conditions, exposure time was reduced from 100 to 95 s in order to reduce internal temperature at the end of the treatment and consequently, reduce the internal damage observed.

3.2. Effect of fruit weight on microwave efficacy

Fruit size did not have a significant effect on MW efficacy either in 'Roig d'Albesa' peaches treated at 17.5 kW for 50 s (Fig. 1) or in'PP-100' nectarines treated at 10 kW for 95 s (Fig. 2A). In both varieties, brown rot incidence was significantly reduced to less than 6 % in all fruit weights evaluated. The internal temperature achieved in 'Roig d'Albesa' peaches during MW treatment was 54.8, 43.4 and 44.9 ºC when fruit weight was 180 ± 10, 245 ± 10 and 310 ± 10 g, respectively, and the external fruit temperature was 52.8, 49.3 and 46.0 ºC, respectively (data not shown). In the case of 'PP-100' nectarines, the internal temperature was 62.6, 50.6 and 47.5 ºC when fruit weight was 170 ± 10, 215 ± 10 and 260 ± 10 g, respectively, and the external temperature was 44.9 and 40.5 ºC for the least and greatest weight, respectively (data not shown).

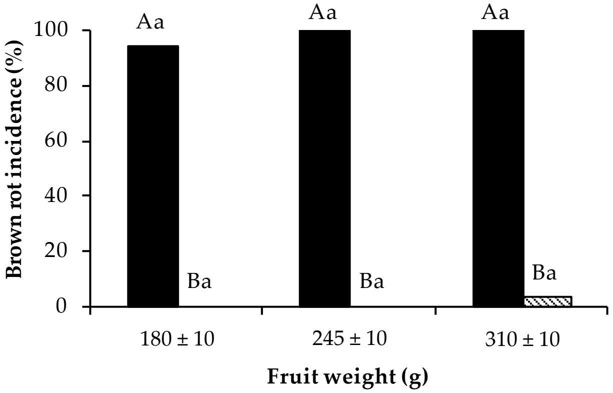

Fig. 1. Brown rot incidence in 'Roig d'Albesa' peaches with different fruit weights, 180 ± 10, 245 ± 10 and 310 ± 10 g, artificially inoculated with *Monilinia fructicola* at 10^3 conidia mL^{-1} 24 h prior to microwave treatment at 17.5 kW for 50 s (▨) or untreated (■). After treatment, fruit were incubated for 5 days at 20 ºC and 85 % RH. Means with the same uppercase letter for each fruit weight or with the same lowercase letter for each treatment are not significantly different (P<0.05) according to LSD test.

In 'Placido' peaches treated at 10 kW for 95 s, brown rot incidence in largest fruit (260 ± 10 g) was significantly reduced to 28 %, however this control was significantly lower than those observed in the other fruit sizes evaluated where brown rot incidence was reduced to 3 % (Fig. 2B). The maximum internal fruit temperature achieved during MW treatment was 54.7, 47.5 and 41.2 ºC when fruit weight was 170 ± 10, 215 ± 10 and 260 ± 10 g, respectively (Fig. 3) and the external fruit temperature immediately after treatment was 45.3, 41.9 and 40.8 ºC, respectively (data not shown).

External visual appearance was not affected by any MW treatment evaluated, however, internal thermal damage, specifically flesh browning around the stone, was observed in all varieties in the smallest size studied. On the contrary, no internal damage was observed when microwave treatments were applied in fruit with the other weights evaluated.

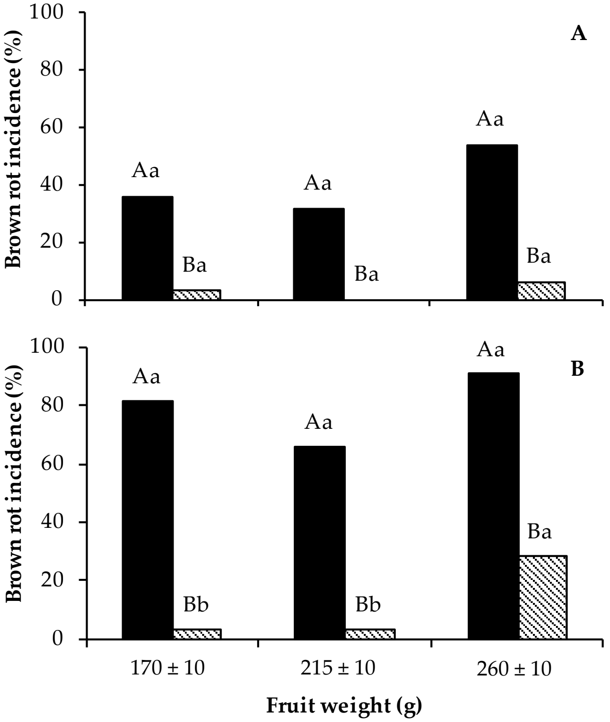

Fig. 2. Brown rot incidence in 'PP-100' nectarines (A) and 'Placido' peaches (B) with different fruit weights, 170 ± 10, 215 ± 10 and 260 ± 10 g, artificially inoculated with *Monilinia fructicola* at 10^3 conidia mL^{-1} 24 h prior to microwave treatment at 10 kW for 95 s (▨) or untreated (■). After treatment, fruit were incubated for 5 days at 20 ºC and 85 % RH. Means with the same uppercase letter for each fruit weight or with the same lowercase letter for each treatment are not significantly different ($P<0.05$) according to LSD test.

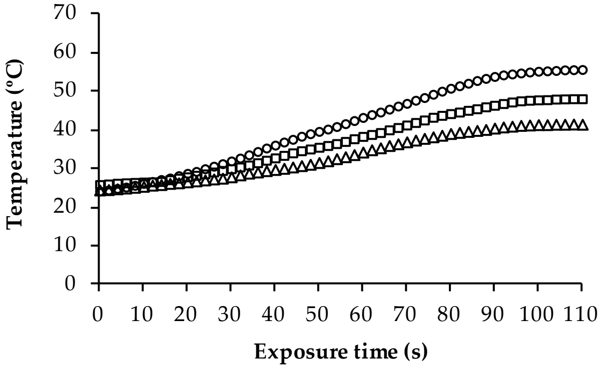

Fig. 3. Evolution of the internal temperature of 'Placido' peaches with 170 ± 10 (○), 215 ± 10 (□) and 260 ± 10 g (△) of weight during microwave treatment at 10 kW for 95 s. Temperature was measured with an optical fiber temperature probe placed 10 mm inside fruit.

3.3. Effect of fruit maturity on microwave efficacy

Microwave treatment at 17.5 kW for 50 s significantly reduced brown rot incidence to 34, 36 and 9 % in 'Roig d'Albesa' peaches maintained 24, 48 and 72 h at 20 ºC prior MW treatment, respectively, in comparison with untreated fruit where brown rot incidence was higher than 96 % and no significant differences were detected between fruit maintained at 20 ºC for 24, 48 and 72 h (data not shown). Different maturity levels were achieved between fruit maintained at 20 ºC for 24 and 72 h in which fruit firmness was 8.2 and 7.3 N, respectively (data not shown).

MW treatment at 10 kW for 95 s completely controlled brown rot in 'PP-100' nectarines maintained 24, 48 and 72 h at 20 ºC before treatment compared with untreated fruit where disease incidence was higher than 69 % (Fig. 4A). Furthermore, different maturity levels were achieved in 'PP-100' nectarines in which firmness in fruit maintained at 20 ºC for 24, 48 and 72 h was 52.4, 36.5 and 14 N, respectively (data not shown). Similar results were observed in 'Placido' peaches treated by MW at 10 kW for 95 s (Fig. 4B) but when standard quality parameters were determined only significant differences were detected in soluble solids content between fruit maintained at 20 ºC for 24 h (15.1 %) and 72 h (14.0 %) (data not shown).

External visual appearance was not affected by either MW treatment evaluated, however, internal thermal damage, specifically flesh browning around the stone, was observed in some 'Roig d'Albesa' peaches treated at 17.5 kW for 50 s. On the contrary, no internal damage was observed in 'Placido' peaches and 'PP-100' nectarines treated by microwave at 10 kW for 95 s.

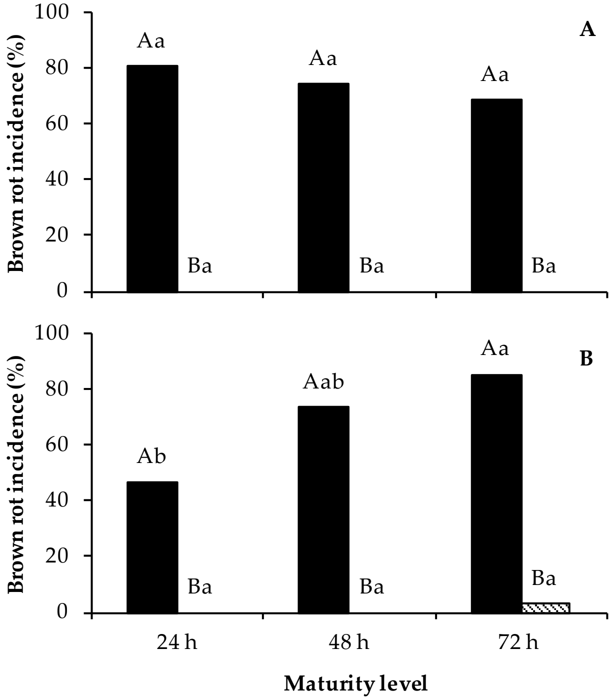

Fig. 4. Brown rot incidence in 'PP-100' nectarines (A) and 'Placido' peaches (B) maintained at 20 ºC and 85 % RH for 24, 48 and 72 h prior to be artificially inoculated with *Monilinia fructicola* at 10^3 conidia mL^{-1} and treated by microwave at 10 kW for 95 s (▨) or untreated (■). After treatment, fruit were incubated for 5 days at 20 ºC and 85 % RH. Means with the same uppercase letter for each maturity level or with the same lowercase letter for each treatment are not significantly different (P<0.05) according to LSD test.

3.4. Effect of microwave treatments on naturally infected fruit

Microwave treatment at 17.5 kW for 50 s significantly reduced brown rot incidence to 14 % in 'Roig d'Albesa' peaches compared with untreated fruit (100 %). When microwave treatment was applied at 10 kW for 95 s, brown rot incidence was significantly reduced to 4 and 12 % in 'Placido' peaches and 'PP-100' nectarines, respectively, in comparison with untreated fruit where brown rot incidence was 45 and 83 %, respectively (Fig. 5). The internal fruit temperature achieved in 'Roig d'Albesa' peaches immediately after microwave treatment at 17.5 kW for 50 s averaged 53.8 ± 8.4 ºC and the external temperature recorded at the end of the treatment was 47.1 ± 4.4 ºC (data not shown). When MW treatment was applied at 10 kW for 95 s the internal fruit temperature averaged 47.2 ± 1.6 and 48.8 ± 2.1 ºC in 'Placido' peaches and 'PP-100' nectarines, respectively, and the external temperature recorded at the end of the treatment was 44.5 ± 1.8 and 43.8 ± 1.9 ºC, respectively (data not shown).

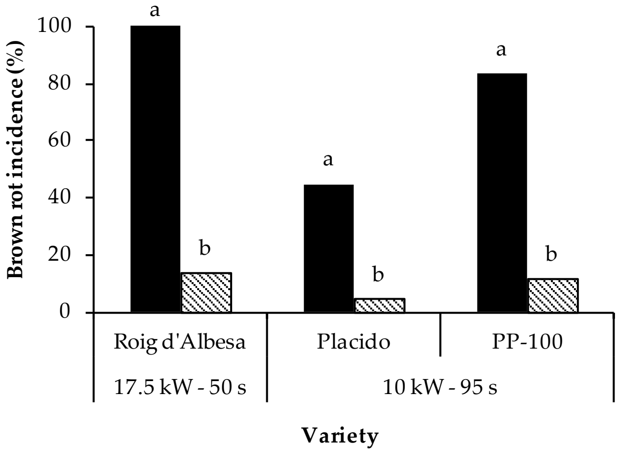

Fig. 5. Brown rot incidence in 'Roig d'Albesa' and 'Placido' peaches and 'PP-100' nectarines naturally infected untreated (■) or treated by microwave (▨) at 17.5 kW for 50 s or at 10 kW for 95 s. After treatment, fruit were incubated at 20 ºC and 85 % RH for 5 days. Means with the same letter for each variety are not significantly different (*P*<0.05) according to LSD test.

External visual appearance was not affected by either MW treatment evaluated, however, internal thermal damage, specifically flesh browning around the stone, was observed in some 'Roig d'Albesa' peaches treated at 17.5 kW for 50 s. On the contrary, no internal damage was observed in 'Placido' peaches and 'PP-100'nectarines treated by microwave at 10 kW for 95 s.

3.5. Effect of microwave treatments on fruit quality

MW treatment at 10 kW for 95 s applied in 'Placido' peaches did not have a significant effect on any quality parameters evaluated. When MW treatment at 17.5 kW for 50 s and 10 kW for 95 s was applied in 'Roig d'Albesa' peaches and 'PP-100' nectarines, respectively, no significant differences were detected in soluble solids content and acidity, however, firmness in fruit treated by MW, 69.3 and 16.7 N, respectively, was significantly higher than in untreated fruit where fruit firmness was 65.7 and 9.7 N, respectively (data not shown).

4. Discussion

Microwave treatments with a 2450 MHz household oven using between 0.4 and 0.45 kW have been previously studied to control postharvest diseases as *B. cinerea*, *P. expansum* and *R. stolonifer* in peaches (Karabulut and Baykal, 2002; Zhang *et al.*, 2004). However, to our knowledge this is the first time that an industrial microwave tunnel was used to apply microwave treatments in continuous for controlling postharvest diseases, including brown rot disease caused by *Monilinia* spp. on peaches and nectarines.

In the preliminary experiment, different microwave powers and exposure times were evaluated, and no brown rot control was observed when microwave treatment at 5 kW was applied even at the maximum exposure time evaluated, 120 s. In the same study, complete brown rot reduction was achieved when MW was applied at 10 kW for 100 s and no significant differences were observed when microwave power was increased to 17.5 kW and the exposure time was reduced to 50 s. A similar pattern was observed by Vadivambal *et al.* (2008) who reported higher pest mortality by increasing power levels and exposure times when microwave treatment was applied in stored grain. In microwave heating, the rate of temperature rise in a commodity depends on the power, frequency, heating time and the material's dielectric loss factor, so higher temperatures in commodities can be achieved by long heating duration and high power (Wang and Tang, 2001). Ikediala *et al.* (1999) obtained higher heating rates with increased microwave power in cherries and Varith and Kiatsiriroat (2004) reported higher temperatures in mangoes treated by microwave at 800 W than in a half power.

Fruit weight only had a significant effect on microwave efficacy in 'Placido' peaches treated by MW at 10 kW for 95 s. Brown rot control in fruit with 260 ± 10 g of weight was significantly lower than that observed with the other fruit weights evaluated (170 ± 10 and 215 ± 10 g). The differences between MW efficacies depending on fruit size could be caused by differences between the final temperatures reached by fruit of different weights, since internal and external temperature of the smallest fruit was, respectively, 13.5 and 4.5 ºC higher than that reached by the largest fruit. Although in the other studied varieties fruit weight did not have a significant effect on MW efficacy, also higher temperatures were reached by small fruit than large fruit. Temperature variation due to size differences were also observed by Ikediala *et al.* (1999) when microwave treatment at 915 MHz was applied to pest control in cherries, however, they reported that large cherries heated faster than small ones so that the mean temperature achieved by large cherries was higher. The different heating pattern observed between both studies could be attributed to not only the different fruit species evaluated but also the different microwave oven used to increase the commodity temperature. The microwave heating rates not only are functions of load characteristic such as size, shape and dielectric properties (Peyre *et al.*, 1997) but also the different design of the microwave cavity significantly affects the pattern of MW distribution and the heat absorption in food stuffs (Varith and Kiatsiriroat, 2004). Heating uniformity is the most significant problem associated with dielectric heating both microwave and radio frequency in fresh fruit (Tang *et al.*, 2000). Influence of fruit size on treatment effectiveness was also observed by Sisquella *et al.* (2013) who reported different efficacies depending on peach diameters when radio frequency treatment was applied in air.

Maturity level did not have a significant effect on efficacy of MW treatments at 17.5 kW for 50 s and 10 kW for 95 s in any varieties evaluated. Similar results were obtained by Casals *et al.* (2010d) in radio frequency treatment to control brown rot in peaches. These results suggest that dielectric heating, microwave or radio frequency treatment, would have an advantage over conventional heating, in which different efficacies were observed depending on the maturity level of fruit. Casals *et al.* (2010a) reported that the efficacy of heat treatment conducted with hot wet air at 50 ºC for 2 h and 95-99 % RH decreased in the most advanced maturity level fruit, incubated at 20 ºC for 72 h.

Brown rot incidence on naturally infected fruit was significantly reduced by both studied microwave treatments in all the varieties evaluated. Similar results were observed when microwave heating with a household oven for 2 min was applied to control natural infections of *P. expansum* and *B. cinerea* in peaches (Karabulut and Baykal, 2002). However, in our study using a microwave tunnel, the treatment time necessary to control natural infections of *Monilinia* spp. was 50 and 95 s depending on the power level used. Postharvest heat treatments to control decay, like hot water, are often applied for a relatively short time because the target pathogens are found on the surface or in the first few layers under the skin of the fruit or vegetable. However, in quiescent or latent infection where fungi are located within the tissue fruit, decay development is not necessarily controlled by conventional heat treatment (Schirra *et al.*, 2000). Casals *et al.* (2010c) observed effective brown rot control when naturally infected fruit were treated with hot water at 60 ºC for 40 s, however, brown rot reduction decreased progressively with increasing storage time and suggested that hot water treatment is effective against conidia of *Monilinia* spp. located on the fruit surface but not against latent infections that probably are placed more deeply in fruit. Therefore, the internal heating effect of microwaves could be an advantage in inhibiting latent infections compared with hot water treatments.

Microwave treatment applied at 17.5 kW for 50 s or at 10 kW for 95 s did not affect negatively fruit firmness in the varieties tested and even a delay of the fruit softening was observed in ‘Roig d’Albesa’ peaches and ‘PP-100’ nectarines. Similar trends have been reported when microwave treatment with a domestic oven at 2450 MHz was applied for 2 min in peaches to control *B. cinerea*, *P. expansum* (Karabulut and Baykal, 2002) and *R. stolonifer* (Zhang *et al.*, 2004). When conventional heat treatments, such as hot wet air treatment, were applied in fruit to disease control, also similar results were reported on peaches and nectarines (Anthony *et al.*, 1989; Casals *et al.*, 2010b). The reason for greater firmness could be attributed to that heat affects the ripening-linked softening processes in the fruit. Ethylene is commonly known to be responsible for the acceleration of fruit ripening and it was reported that heat treatments could delay the peak ethylene production, so it could delay the rate of ripening of fruit (Lay-Yee and Rose, 1994; Zhou *et al.*, 2002).

Internal fruit temperature reached at the end of both MW treatments was higher than the external fruit temperature. Similar pattern was observed by Ikediala *et al.* (1999) who reported cherry pit temperatures about 10 ºC above the surface temperature when heating in a 915 MHz microwave. The higher internal temperature could be attributed to microwave core-focusing energy effect, since spherically and cylindrically shaped foods tend to concentrated MW energy in the center, especially when the diameter is in the range of 20-60 mm (Ikediala *et al.*, 1999). Varith and Kiatsiriroat (2004) also observed that the temperature of the inside layer was hotter than the outer layers in mangoes treated with MW using 400 W of power for 40 s and reported heat damage at the areas where the heat were excessively absorbed. In our study, external visual fruit appearance was not affected by any MW treatments evaluated. However, flesh browning around the stone was observed especially in small fruit. Therefore, these internal damages could be attributed to the excessive internal temperature achieved due to the centre overheating effect, since internal temperature in small fruit with 170 ± 10 and 180 ± 10 g of weight varied between 54.7 and 62.6 ºC. Lower internal temperatures were reached by large fruit where temperature ranged between 41.2 and 44.9 ºC which could explain the different thermal damage depending on fruit size. Internal thermal damage was also observed randomly in fruit when microwave treatment at 17.5 kW for 50 s was applied in 'Roig d'Albesa' peaches with an intermediate weight. This variability in the presence of internal browning could be attributed to the high standard deviation observed in the final temperature, since, 8.4 ºC of standard deviation was recorded in the internal temperature in comparison with the 1.6 and 2.1 ºC recorded, respectively in 'Placido' peaches and 'PP-100' nectarines treated by microwave at 10 kW for 95 s.

In all the experiments, electrical consumption of the overall equipment, including microwaves, conveyor belt and cooling system, was estimated. The energy requirement for treating fruit with 17.5 kW for 50 s was 0.19 kW h kg^{-1} and 0.13 kW h kg^{-1} when treatment was applied at 10 kW for 95 s. Estimating the cost of electricity at 0.18 € kW^{-1} h^{-1} in Spain in 2013, the total cost varied between 0.02 and 0.03 € kg^{-1} of fruit depending on the microwave treatment. This consumption was with a prototype microwave equipment but the real cost would have to be estimated with a commercial equipment specifically designed for this kind of treatment.

In conclusion, our results indicated that microwave treatment at 17.5 kW for 50 s and 10 kW for 95 s may provide an effective treatment to control brown rot in peaches and nectarines. However, the different temperature reached at the end of the treatment depending on fruit weight could affect microwave effectiveness and the internal fruit appearance so further study is needed. Further experiments should investigate if microwave heating could be improved by immersing fruit in water during treatment, by using an intermittent heating or by rotating fruit inside the microwave oven. Thereby, the non-uniformity of electric fields could be reduced and the core focused heating could be altered and therefore, fruit quality would be less affected by MW treatment.

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Capítulo 5

Improvement of microwave treatment with immersion of fruit in water to control brown rot in stone fruit

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Abstract

Monilinia spp. are the most important cause of brown rot in stone fruit. Currently, no chemical fungicides are allowed in the European Union to be applied in stone fruit after harvest. Microwave (MW) treatments at 20 kW with fruit immersed in water at 40 ºC for 50 or 60 s were selected as effective conditions to control brown rot without affecting the appearance of the fruit. The efficacy of the treatments was analyzed on fruit with different weights and at various infection times and inoculum concentrations. When the MW treatment was applied for 50 s, brown rot control was significantly higher for smaller fruit in comparison with larger fruit and MW efficacy decreased with increasing time between inoculation and treatment from 0 or 24 h to 48 h and inoculum concentration from 10^3 to 10^5 conidia mL^{-1}. When the treatment time was increased to 60 s, a better control of brown rot was observed and, in general, none of the studied factors had a significant effect on the efficacy of the treatment. MW treatments were also evaluated on naturally infected fruit; brown rot incidence was significantly reduced to less than 43 % when MW treatment was applied for 50 s and to less than 7 % when applied for 60 s.

Industrial relevance: This study demonstrated the efficacy of MW treatment to control brown rot in postharvest of stone fruit. A relationship between brown rot reduction and the applied MW energy was also provided, which could be useful for designing specific equipment to process large quantities of fruit in less time without affecting the efficacy of the treatment or the quality of the fruit.

Keywords: *Monilinia* spp., *Monilinia fructicola*, postharvest, heat treatment, peaches

1. Introduction

Brown rot is one of the most important postharvest diseases of stone fruit worldwide and is primarily caused by two species, *Monilinia laxa* (Aderh. Et Rulh.) Honey and *Monilinia fructicola* (G. Wint.) Honey. Stone fruit infection by *Monilinia* spp. mainly occurs in the field during the growing season when conditions favor disease development. However, postharvest losses are typically more severe than preharvest losses, sometimes reaching high levels (Hong *et al.*, 1997). Currently, no chemical fungicides are allowed in the European Union to be applied in stone fruit after harvest. In addition, public demands to reduce pesticide use and improve environmental and human health, as well as the risk of pathogens developing resistance to synthetic fungicides, limit the preharvest application of chemical products in the field. The previous aspects, combined with a lack of effective postharvest treatments against *Monilinia* spp. have increased the need to develop new control methods.

Dielectric heating, including radio frequency (RF) and microwave (MW) heating, can be a potential alternative treatment to control postharvest diseases. Dielectric materials, as most agricultural products, convert electric energy at RF and MW frequencies into heat (Wang *et al.*, 2003). Dielectric properties and particularly the dielectric loss factor (ε'') of a material, influence absorption and attenuation, and describes the ability to dissipate energy in response to an applied electric field, which commonly results in heat generation (Ikediala *et al.*, 2002). Thus, the magnitude of heat generation is proportional to the value of the loss factor at a given frequency and electric field (Tang *et al.*, 2000).

The use of MW energy has been widely studied in food processes such as pasteurization and sterilization (Picouet *et al.*, 2009; Chandrasekaran *et al.*, 2013), and for pest control in grain and stored products (Vadivambal *et al.*, 2007). On the other hand, few studies have been published on the use of microwaves to control diseases. The effect of MW treatments to control *Botrytis cinerea, Penicillium expansum*, and *Rhizopus stolonifer* in peaches (Karabulut and Baykal, 2002; Zhang *et al.*, 2004) and *P. expansum* in pears (Zhang *et al.*, 2006) has been assessed in these studies. Moreover, Sisquella *et al.* (2013b) reported a MW treatment to control *Monilinia* spp. in peaches and nectarines; however, the influence of fruit size in the final temperature affected MW effectiveness and internal fruit appearance. Therefore, further experiments should be performed to solve the uneven heating and consequently, the negative effect on quality.

Heating uniformity is the most significant problem associated with dielectric heating. Non-uniform temperature distribution and development of hot and cold spots not only can affect the effectiveness of the treatment but also negatively affect fruit quality. Different dielectric properties between food and the surrounding air cause reflection and refraction phenomena of the microwaves at the interface, resulting in non-uniform electric field distribution (Guan *et al.*, 2002). Immersion of fresh fruit in water was suggested by Ikediala *et al*. (2002) as a means to overcome the problems associated with non-uniform radio frequency heating. Recently, Sisquella *et al*. (2013a) reported that RF effectiveness was influenced by fruit size when RF treatment in air was applied at the conditions reported by Casals *et al*. (2010c). However, lower influence of fruit size on the effectiveness of RF treatment to control brown rot in stone fruit was observed when applied to fruit immersed in water at 20 ºC for 9 min, mainly due to the similar temperatures reached between the different fruit sizes.

The main objective of this study was to determine the MW power and exposure time with fruit immersed in water at different temperatures needed to reduce brown rot without causing external and/or internal damage to the fruit. The efficacy of the selected MW conditions was also evaluated in: (1) fruit with different weights, (2) fruit artificially inoculated with different inoculum concentrations, (3) fruit inoculated at different times before treatment, and (4) naturally infected fruit. Finally, the effect of the selected MW treatments on fruit quality was also evaluated.

2. Materials and methods

2.1. Fruit

'Baby Gold 9', 'Sunlate', 'Pollero', 'Roig d'Albesa', and 'Placido' peaches (*Prunus persica* (L) Batch), and 'Red Jim' and 'Autumn Free' nectarines (*P. persica* var. Nectarine (Ait.) Maxim.) were used for the experiments. Fruit were grown in orchards located in Lleida (Catalonia) and no synthetic fungicide against *Monilinia* spp. was used in the field. Fruit free from visible wounds and rots and similar visual maturity were hand-selected immediately after harvest. The fruit not used at the time of harvest were stored at 0 ºC for a maximum of 15 days until use.

Fruit weight in all the experiments, except in the analysis of fruit size effect, was 185 ± 10 g for 'Pollero' peaches, 190 ± 10 g for 'Autumn Free' nectarines, 200 ± 10 g for 'Placido' peaches, and 215 ± 10 g for 'Red Jim' nectarines and 'Sunlate' and 'Roig d'Albesa' peaches. In the case of 'Baby Gold 9' peaches, fruit weight was 215 ± 10 g, except in the naturally infected fruit for which the weight was 190 ± 10 g.

2.2. Pathogen culture

The isolate of *M. fructicola* (CPMC1) used in this study was from the collection of the Postharvest Pathology Unit, Fruitcentre IRTA, Lleida, Catalonia. This strain was isolated from an infected stone fruit and was identified by the Department of Plant Protection, INIA, Madrid (Spain). The strain was maintained on potato dextrose agar (PDA) medium (Biokar Diagnostics, 39 g L^{-1}) amended with acetone (J.T. Baker, 1 %) at 4 ºC in the dark.

2.3. Pathogen production and inoculation methodology

The isolate of *M. fructicola* (CPMC1) was subcultured onto PDA amended with acetone (J.T. Baker, 1 %) and incubated in the dark at 25 ºC for approximately two weeks. The isolate was then inoculated onto peaches or nectarines by wounding the fruit with a sterilized steel rod (1 mm wide and 2 mm long) and transferring conidia and mycelium from the PDA culture to the wound site with a sterile pipette tip. Next, the fruit were incubated at 25 ºC and 85 % relative humidity (RH) in the dark for 5-7 days. Conidia were scraped from the infected fruit using a sterile loop and transferred to a test tube containing 5 mL of sterile distilled water and a drop of Tween-80 per liter. Conidia concentration was measured with a haemocytometer and the suspension diluted to the desired concentration. For all experiments, fruit were wounded once per fruit with the sterile steel rod and inoculated with 15 μL of the desired conidial suspension.

2.4. Microwave heating system and suitable treatment conditions

An industrial MW tunnel (Synarwave-M.E.S, France) with a frequency of 2450 MHz was used to perform the experiments. The MW tunnel was 6.5 m long with a cavity dimensions of 180 cm in length, 70 cm in width, and 45 cm in height cavity. MW energy was delivered by 12 magnetrons well distributed in a compartment on the top of the cavity. Each magnetron had a theoretical output power range of 0.2 to 2.0 kW, so the total MW output power was between 0.2 and 24.0 kW. The continuous conveyer belt was located at 275 mm from the magnetrons in the cavity and belt speed ranged between 40 and 300 cm min^{-1}.

For all experiments, the desired output power level was achieved using 10 magnetrons, each with the same theoretical output power, so the maximum MW power level used in this study was 20 kW. Fruit at room temperature were placed in a container (285 mm x 235 mm x 90 mm) with 2 L of tap water, so that the fruit were completely submerged in the water. The containers were placed on the conveyor belt and the corresponding MW treatment was applied.

In all experiments, the increase of the internal temperature during MW treatment was measured every second with an inside-optical fiber temperature probe (FOT-L/10 m; FISO Technologies Inc., Canada) placed 10 mm inside the fruit. The temperature measuring range was -40 ºC to +250 ºC with an accuracy of ± 0.5 ºC. The optical fiber probe was connected to a signal conditioner and data were collected by a FISOCommander software (FISO Technologies Inc., Canada). Moreover, immediately after the MW treatment, the external temperature of eight fruit per treatment was also recorded using a portable infrared thermometer (Testo 831, Testo AG, USA) with a 2-point laser marker and a temperature measuring range of -30 to +210 ºC with an accuracy of ± 1.5 ºC.

For all treatments, external and internal fruit appearance was analyzed. External thermal damages, particularly surface color changes, were evaluated at the end of every MW treatment. Internal thermal damages, such as internal browning, were determined cutting each fruit in half after 5 days of incubation at 20 ºC and 85 % RH once brown rot incidence was recorded.

2.5. Effect of water temperature on microwave treatment efficacy

'Baby Gold 9' peaches and 'Red Jim' nectarines were artificially inoculated with 10^3 conidia mL^{-1} as described above and incubated at 20 ºC and 85 % RH for 24 h. Fruit were then immersed in water at 20, 35, 40, or 45 ºC and MW treatment was immediately applied at 10 kW for 95 s. A group of artificially inoculated fruit of each variety was immersed in water at 45 ºC for 95 s and no MW treatment was applied (controls). After the treatment, fruit were stored for 5 days at 20 ºC and 85 % RH and then, brown rot incidence was recorded. Each treatment was applied to four replicates of eight fruit each.

2.6. Effect of power level and exposure time on microwave treatment efficacy

2.6.1. *Efficacy study*

To improve the effectiveness of the microwave treatment with fruit immersed in water at 40 ºC, several MW power levels were evaluated in 'Baby Gold 9' peaches and 'Red Jim' nectarines. Fruit were artificially inoculated with 10^3 conidia mL^{-1} as described above and incubated at 20 ºC and 85 % RH for 24 h. Next, fruit were immersed in water at 40 ºC and MW treatment was immediately applied at 15, 17.5, or 20 kW for 40, 50, 60, or 95 s. A group of artificially inoculated fruit of each variety was immersed in water at 40 ºC for 95 s and no MW treatment was applied (controls). After the treatment, fruit were stored for 5 days at 20 ºC and 85 % RH and then, brown rot incidence was recorded. Each treatment was applied to four replicates of eight fruit each.

2.6.2. *Modeling data*

To obtain useful information that can help design a specific MW equipment to treat peaches immersed in water for brown rot control, the relationship between the percentage of disease reduction and total applied energy was determined using the following four-parametric sigmoid function (Eq. (1)):

$$y = \frac{a-b}{1+e^{-c(E-d)}} + b \qquad (1)$$

where y is the percentage of disease reduction, a is the maximum percentage of disease reduction, b is the minimum percentage of disease reduction, c is related to the slope of a line tangent to the curve at the inflection point (slope=$ac/4$), E is the applied MW energy expressed in kJ, and d is the energy at the inflection point where disease reduction is half of the maximum reduction. The applied MW energy in kJ (E) was calculated by using Eq. (2) where P is the power level (kW) and t is the exposure time (s):

$$E = Pxt \qquad (2)$$

The experimental data were fitted to Eq. (1) using a nonlinear fit using the JMP®8 statistical software (SAS Institute, Cary, NC, USA). The maximum and minimum percentages of disease reduction were fixed to 100 and 0 %, respectively, and the estimate of the parameters c and d was carried out using the Gauss-Newton method.

2.7. Effect of the host and *Monilinia* spp. variables on microwave treatment efficacy

2.7.1. *Effect of fruit weight on microwave treatment efficacy*

The effect of fruit weight on the efficacy of the MW treatment with the fruit immersed in water was studied in fruit with different weights that were artificially inoculated with 10^3 conidia mL^{-1} of *M. fructicola* as previously described, and incubated at 20 ºC and 85 % RH for 24 h until treatment. The fruit weights evaluated were 170 ± 10, 215 ±10, and 260 ± 10 g for 'Sunlate' and 'Roig d'Albesa' peaches and 145 ± 10, 185 ±10, and 225 ± 10 g for 'Pollero' peaches. Fruit were immersed in water at 40 ºC and MW treatment at 20 kW was immediately applied for 50 s in 'Sunlate' and 'Pollero' peaches or 60 s in 'Roig d'Albesa' and 'Pollero' peaches. A group of artificially inoculated fruit of each variety was not treated and was used as a control. In the previous experiment, no effect on *Monilinia fructicola* was observed when fruit were immersed in water at 40 ºC for 95 s without MW treatment, therefore in this and subsequent experiments, control fruit were not immersed in water. After the treatment, fruit were stored for 5 days at 20 ºC and 85 % RH and then, brown rot incidence was recorded. Each treatment was applied to four replicates of eight fruit each.

2.7.2. *Effect of inoculum concentration on microwave treatment efficacy*

'Sunlate', 'Roig d'Albesa,' and 'Pollero' peaches were artificially inoculated with *M. fructicola* with 10^3, 10^4, or 10^5 conidia mL^{-1} as previously described. Once the wounds were dry, fruit were immersed in water at 40 ºC and MW treatment at 20 kW was immediately applied for 50 s in 'Sunlate' and 'Pollero' peaches or 60 s in 'Roig d'Albesa' and 'Pollero' peaches. A group of artificially inoculated fruit of each inoculum concentration was not treated and was used as a control. After the treatment, fruit were stored for 5 days at 20 ºC and 85 % RH and then, brown rot incidence was recorded. Each treatment was applied to four replicates of eight fruit each.

2.7.3. *Effect of infection time before treatment on microwave treatment efficacy*

'Sunlate', 'Roig d'Albesa', and 'Pollero' peaches were artificially inoculated with *M. fructicola* with 10^3 conidia mL^{-1} as previously described and then kept for 0, 24, or 48 h at 20 ºC and 85 % RH. Next, the fruit were immersed in water at 40 ºC and MW treatment at 20 kW was immediately applied for 50 s in 'Sunlate' and

'Pollero' peaches or 60 s in 'Roig d'Albesa' and 'Pollero' peaches. A group of artificially inoculated fruit for each infection time was not treated and was used as a control. After the treatment, fruit were stored for 5 days at 20 ºC and 85 % RH and then, brown rot incidence was recorded. Each treatment was applied to four replicates of eight fruit each.

2.7.4. Effect of microwave treatment on naturally infected fruit

Naturally infected 'Baby Gold 9', 'Roig d'Albesa', and 'Placido' peaches and 'Autumn Free' nectarines were immersed in water at 40 ºC and MW treatment at 20 kW was immediately applied for 50 s in 'Baby Gold 9' peaches and 'Autumn Free' nectarines or 60 s in 'Roig d'Albesa' and 'Placido' peaches. A group of fruit from each variety was left untreated and was used as a control. After the treatment, fruit were stored for 5 days at 20 ºC and 85 % RH and then, brown rot incidence was recorded. Each treatment was applied to four replicates of twenty-four fruit each.

2.8. Effect of microwave treatment on fruit quality

The effect of MW treatment on standard quality parameters was evaluated in 'Sunlate', 'Roig d'Albesa', and 'Pollero' peaches. Fruit were immersed in water at 40 ºC and immediately, MW treatment at 20 kW was applied for 50 s in 'Sunlate' and 'Pollero' peaches or 60 s in 'Roig d'Albesa' and 'Pollero' peaches. A group of fruit from each variety was not treated and was used as a control. After 2 days of incubation at 20 ºC and 85 % RH, standard quality parameters including firmness, soluble solid content, and acidity were determined. Each treatment was applied to four replicates of twenty-four fruit each.

Fruit firmness was measured on two opposite peeled sides using a penetrometer (Effegi, Milan, Italy) fitted with an 8 mm diameter flat tip. The average of those two measurements was considered as one replicate. Sixteen fruit per variety were evaluated and data were expressed in Newton (N).

Soluble solid content (SSC) was determined with a digital refractometer (Atago PR-100, Tokyo, Japan) by measuring refractive index of the juices; data are expressed as percentages of soluble solids. The acidity was measured by mixing 10 mL of juice with 10 mL of distilled H_2O and adding three drops of phenolphthalein and then titrated with 0.1 N NaOH. The acidity was expressed in grams of malic acid per liter of juice (g m.a. L^{-1}). The juices used to determine SSC and acidity were extracted from four fruit that had been used to determine firmness, thus measurements were conducted in four replicates of four fruit each.

2.9. Statistical data analysis

The incidence of brown rot and quality parameters were evaluated using analysis of variance (ANOVA) with the JMP®8 statistical software (SAS Institute, Cary, NC, USA). Statistical significance was judged at the level $P<0.05$. When the analysis was statistically significant, the least significance difference (LSD) test was used for separation of means.

3. Results

3.1. Effect of water temperature on microwave treatment efficacy

Microwave treatment at 10 kW for 95 s with the fruit immersed in water at 20 ºC did not control brown rot in any of the tested varieties. However, when MW treatment was applied with the fruit immersed in water at 40 ºC, brown rot incidence was significantly reduced in both 'Red Jim' nectarines and 'Baby Gold 9' peaches, but brown rot incidence in the treated fruit was over 47 %. When water temperature was further increased from 40 to 45 ºC, brown rot control did not significantly increase in any of the tested varieties (Fig. 1).

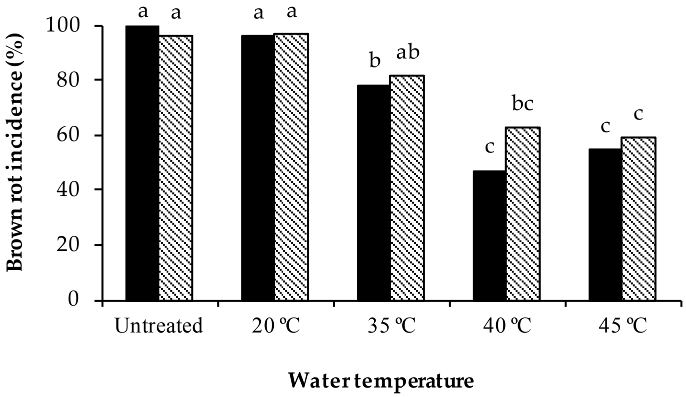

Fig. 1. Brown rot incidence in 'Red Jim' nectarines (■) and 'Baby Gold 9' peaches (▨) artificially inoculated with *Monilinia fructicola* at 10^3 conidia mL^{-1} 24 h before microwave treatment at 10 kW for 95 s with fruit immersed in water at 20, 35, 40 or 45 ºC or immersed in water at 45 ºC for 95 s (untreated). After treatment, fruit were incubated at 20 ºC and 85 % RH for 5 days. Means with the same letter for each variety are not significantly different ($P<0.05$) according to LSD test.

Internal fruit temperature when MW treatment was applied with the fruit immersed in water at 40 ºC was 41.7 and 40.3 ºC in 'Red Jim' nectarines and 'Baby Gold 9' peaches, respectively, while external fruit temperature was 41.2 and 40.5 ºC, respectively under the same treatment conditions (data not shown). No thermal damage was observed in any of the tested varieties regarding internal and external appearance (data not shown).

Based on these observations, microwave treatment water temperature for subsequent experiments was 40 ºC.

3.2. Effect of power level and exposure time on microwave treatment efficacy

3.2.1. Efficacy study

Brown rot was present in 84 and 100 % in untreated 'Red Jim' nectarines and 'Baby Gold 9' peaches, respectively. In general, brown rot reduction increased with increasing exposure time and MW power level for both varieties (Table 1). Complete brown rot control was achieved when MW treatment at all tested power levels was applied for 95 s in 'Baby Gold 9' peaches immersed in water at 40 ºC (Table 1), although external damages were observed when MW treatment was applied at 17.5 and 20 kW (data not shown). When the exposure time was decreased to 60 s, the same level of efficacy was achieved without affecting external and internal appearance when MW treatment was applied at 17.5 and 20 kW in which brown rot reduction was 93 and 96 %, respectively. When MW treatment was applied at 20 kW for 50 s, brown rot reduction was 81 %, being significantly greater than the observed with the other tested power levels (15 or 17.5 kW) using the same exposure time. However, when MW treatment was applied for 40 s, brown rot incidence was only reduced by 33 % even at the maximum MW tested power level, 20 kW (Table 1). Similar results were obtained when the same MW power levels and exposure times were evaluated in 'Red Jim' nectarines immersed in water at 40 ºC (Table 1). In microwave treatments that effectively controlled brown rot, 17.5 kW for 60 s and 20 kW for 50 s and 60 s, internal fruit temperature was below 30 ºC and external fruit temperature was above 44.3 ºC in both 'Red Jim' nectarines and 'Baby Gold 9' peaches (data not shown).

Considering these results, the MW conditions chosen to conduct subsequent experiments with fruit immersed in water at 40 ºC with high efficiency without causing external and internal damages to the fruit were a MW power level of 20 kW for 50 or 60 s.

Table 1. Brown rot reduction (%) in 'Baby Gold 9' peaches and 'Red Jim' nectarines artificially inoculated with *Monilinia fructicola* at 10^3 conidia mL^{-1} 24 h before to immerse them in water at 40 ºC and treated by microwave at 15, 17.5 or 20 kW for 40, 50, 60 or 95 s. After treatment, fruit were incubated at 20 ºC and 85 % RH for 5 days. Brown rot incidence in untreated fruit was 84 and 100 % in 'Red Jim' nectarines and 'Baby Gold 9' peaches, respectively. Means with the same uppercase letter for each microwave power (15, 17.5, 20 kW) or with the same lowercase letter for each exposure time (95, 60, 50, 40 s) are not significantly different (P<0.05) according to LSD test.

	'Baby Gold 9' peaches			'Red Jim' nectarines		
Time (s) / Power (kW)	15	17.5	20	15	17.5	20
95	100[a] Aa	100 Aa	100 Aa	93 Aa	100 Aa	100 Aa
60	76 Bb	93 Aa	96 ABa	63 ABb	85 ABab	96 Aa
50	25 Cb	33 Bb	81 Ba	31 Ba	59 Ba	85 Aa
40	0 Db	21 Bab	33 Ca	0 Cb	0 Cb	33 Ba

[a] Percentage of brown rot reduction

3.2.2. Modeling data

The relationship between the brown rot reduction and the total applied MW energy in fruit immersed in water at 40 ºC is shown in Figure 2. The estimated parameter related with the slope (c) was 0.012 and 0.011 kJ^{-1} in 'Red Jim' nectarines and 'Baby Gold 9' peaches, respectively. The slope of the line tangent to the curve calculated with these parameters was 0.30 and 0.28 for 'Red Jim' nectarines and 'Baby Gold 9' peaches, respectively and therefore, an increase of 100 kJ of the applied energy reduces brown rot incidence in 30 and 28 %, respectively. The MW energy needed to reduce brown rot incidence to 50 % (d) was 855.85 and 862.80 kJ for 'Red Jim' nectarines and 'Baby Gold 9' peaches, respectively and complete brown rot reduction, calculated from Eq. (1), could be achieved by applying 1287 and 1335 kJ in 'Red Jim' nectarines and 'Baby Gold 9' peaches, respectively.

Moreover, a linear relationship was observed between the achieved internal or external temperatures at the end of the MW treatment and the applied MW energy (Fig. 3). A better correlation was observed with external temperatures since the coefficient of determination was higher than 0.95 in both varieties in comparison with the obtained for internal temperatures (under 0.9). External thermal damages were observed when MW treatment was applied for 95 s at 17.5 kW and 20 kW; so the minimum applied MW energy for which external damages were observed was 1662 kJ. Thus, the minimum external temperature that can cause damage to the fruit surface was 50.9 ºC in 'Red Jim' nectarines and 51.7 ºC in 'Baby Gold 9' peaches (Fig. 3).

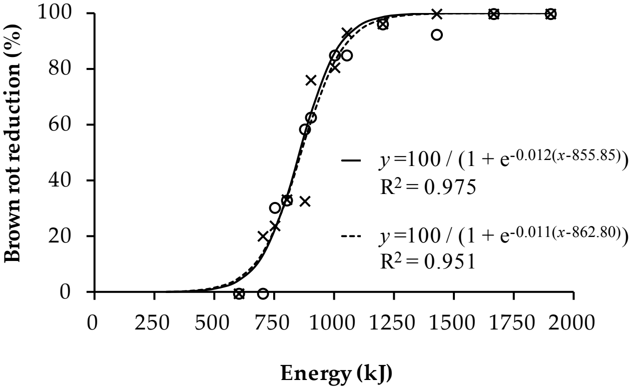

Fig. 2. Relationship between brown rot reduction and applied microwave energy for 'Red Jim' nectarines (○ , —) and 'Baby Gold 9' peaches (x , --).

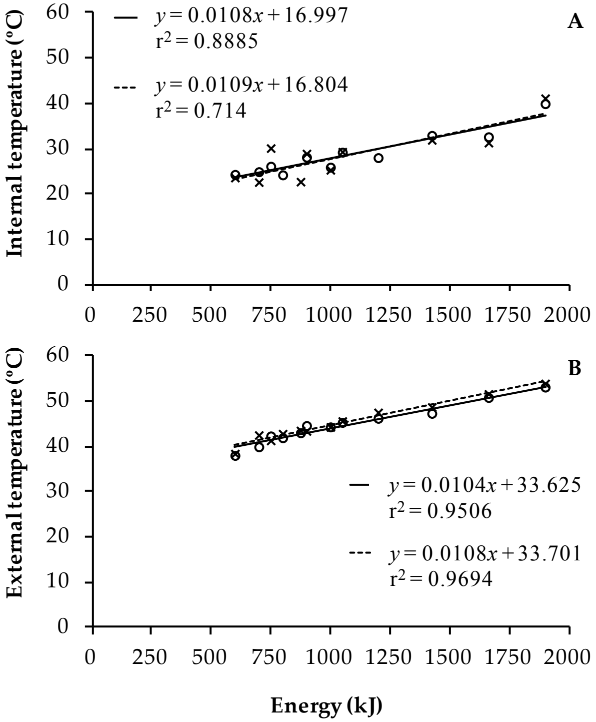

Fig. 3. Relationship between internal (A) and external (B) temperatures achieved at the end of microwave treatment and the applied microwave energy for 'Red Jim' nectarines (○ , —) and 'Baby Gold 9' peaches (x , --).

3.3. Effect of the host and *Monilinia* spp. variables on microwave treatment efficacy

3.3.1. *Effect of fruit weight on microwave treatment efficacy*

Fruit weight had a significant effect on MW treatment efficacy at 20 kW for 50 s with fruit immersed in water at 40 ºC in 'Sunlate' and 'Pollero' peaches. Brown rot was not controlled in 'Sunlate' peaches weighing 260 ± 10 g; however, MW treatment significantly reduced brown rot incidence to 45 and 34 % in fruit weighing 215 ± 10 and 170 ± 10 g, respectively (Table 2). In 'Pollero' peaches, brown rot incidence was significantly reduced to 58 % in larger fruit (225 ± 10 g), but this control was significantly lower than the observed for lower weight fruit where brown rot incidence was reduced to less than 23 % (Table 2).

Table 2. Brown rot incidence and internal and external fruit temperature at the end of microwave treatment at 20 kW on fruit with different weights immersed in water at 40 ºC for 50 or 60 s. 'Sunlate', 'Pollero' and 'Roig d'Albesa' peaches were artificially inoculated with *Monilinia fructicola* at 10^3 conidia mL^{-1} 24 h before treatment. After treatment, fruit were incubated for 5 days at 20 ºC and 85 % RH. Means with the same letter for each variety are not significantly different (P<0.05) according to LSD test.

MW treatment	Variety	Fruit weight (g)	Brown rot (%)	Temperature (ºC) Internal	Temperature (ºC) External
20 kW for 50 s	'Sunlate' peach	Untreated (215 ± 10)	100 a		
		260 ± 10	68 ab	nd[a]	43.9
		215 ± 10	45 bc	25.5	45.7
		170 ± 10	34 c	nd	45.8
	'Pollero' peach	Untreated (185 ± 10)	100 a		
		225 ± 10	58 b	25.0	43.7
		185 ± 10	23 c	nd	45.7
		145 ± 10	16 c	30.4	45.6
20 kW for 60 s	'Roig d'Albesa' peach	Untreated (215 ± 10)	74 a		
		260 ± 10	3 b	33.1	47.6
		215 ± 10	0 b	34.2	45.9
		170 ± 10	0 b	35.0	47.6
	'Pollero' peach	Untreated (185 ± 10)	100 a		
		225 ± 10	3 b	30.4	46.9
		185 ± 10	3 b	32.3	47.0
		145 ± 10	3 b	34.2	48.8

[a] Not determined

When exposure time of MW treatment at 20 kW was increased to 60 s, fruit weight did not have a significant effect on MW efficacy, and brown rot incidence was significantly reduced to below 3 % regardless fruit weight for 'Roig d'Albesa' and 'Pollero' peaches, in comparison with untreated fruit for which brown rot incidence was 74 and 100 %, respectively (Table 2).

Regardless the weight of the tested fruit, no internal or external damage was observed with either MW treatment (data not shown). Internal fruit temperature for all tested weights was below 31 and 35 ºC at the end of MW treatment at 20 kW for 50 and 60 s, respectively (Table 2). External fruit temperature ranged between 43.7 and 45.8 ºC when MW treatment at 20 kW was applied for 50 s and between 45.9 and 48.8 ºC when applied for 60 s (Table 2).

3.3.2. Effect of inoculum concentration on microwave treatment efficacy

MW treatment at 20 kW for 50 s with fruit immersed in water at 40 ºC significantly reduced brown rot incidence to 38 and 23 % in 'Sunlate' and 'Pollero' peaches artificially inoculated with 10^3 conidia mL^{-1}, respectively, in comparison with untreated fruit (100 %); however, MW efficacy was significantly lower when the concentration of the inoculum was increased from 10^3 to 10^5 conidia mL^{-1}, in which case brown rot incidence was 67 and 59 %, respectively (Table 3).

On the contrary, when the exposure time of MW treatment at 20 kW was increased to 60 s, inoculum concentration did not have a significant effect on MW treatment efficacy. Brown rot incidence was significantly reduced to less than 3 % in both 'Roig d'Albesa' and 'Pollero' peaches inoculated with 10^3 conidia mL^{-1} and no significant differences were detected with the other tested inoculum concentrations, 10^4 and 10^5 conidia mL^{-1} (Table 3).

Internal and external damages were not observed in any of the tested varieties with either MW treatments (data not shown).

3.3.3. Effect of infection time before treatment on microwave treatment efficacy

Microwave treatment at 20 kW for 50 s with fruit immersed in water at 40 ºC significantly reduced brown rot incidence in all tested infection times in 'Sunlate' and 'Pollero' peaches; however, MW efficacy was significantly lower in fruit inoculated 48 h before the treatment in comparison with the fruit inoculated at 0 or 24 h (Table 3).

Table 3. Brown rot incidence in 'Sunlate', 'Pollero' and 'Roig d'Albesa' peaches artificially inoculated with *Monilinia fructicola* at different concentrations (10^3, 10^4, 10^5 conidia mL^{-1}) or at 10 ^{3} conidia mL^{-1} 0, 24 and 48 h before MW treatment. Fruit immersed in water at 40 ºC were treated at 20 kW for 50 or 60 s or untreated. After treatment, fruit were incubated at 20 ºC and 85 % RH for 5 days. Means with the same uppercase letter for each inoculum concentration or infection time and the same lowercase letter for each treatment are not significantly different (P<0.05) according to LSD test.

MW conditions	Variety	Treatment	Inoculum concentration (conidia mL^{-1})			Infection time (h)		
			10^3	10^4	10^5	0	24	48
20 kW for 50 s	'Sunlate' peach	Untreated	100[a] Aa	100 Aa	100 Aa	96 Aa	100 Aa	100 Aa
		MW	38 Bb	45 Bb	67 Ba	47 Bb	38 Bb	69 Ba
	'Pollero' peach	Untreated	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa
		MW	23 Bb	66 Ba	59 Ba	29 Bb	23 Bb	60 Ba
20 kW for 60 s	'Roig d'Albesa' peach	Untreated	74 Aa	94 Aa	91 Aa	84 Aa	74 Aa	72 Aa
		MW	0 Ba	9 Ba	19 Ba	6 Ba	0 Ba	9 Ba
	'Pollero' peach	Untreated	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa	100 Aa
		MW	3 Ba	3 Ba	28 Ba	11 Ba	3 Ba	29 Ba

[a] Brown rot incidence expressed as percentage of infected fruit

On the contrary, when MW treatment at 20 kW was applied for 60 s, infection time before treatment did not have a significant effect on treatment efficacy. In 'Roig d'Albesa' peaches, brown rot incidence was significantly reduced to less than 10 % in all tested infection times. Similar results were observed with 'Pollero' peaches (Table 3).

Internal and external damages were not observed in any of the tested varieties with either MW treatment (data not shown).

3.3.4. *Effect of microwave treatment on naturally infected fruit*

Brown rot incidence in naturally infected fruit was significantly reduced to 43 and 23 % when MW treatment at 20 kW for 50 s with fruit immersed in water at 40 ºC was applied to 'Baby Gold 9' peaches and 'Autumn Free' nectarines, respectively, compared with untreated fruit where brown rot incidence was 99 and 92 %, respectively (Fig. 4). When exposure time of MW treatment at 20 kW was increased to 60 s, brown rot incidence was significantly reduced to less than 7 % in 'Roig d'Albesa' and 'Placido' peaches in comparison with untreated fruit where disease incidence was 81 and 20 %, respectively (Fig. 4).

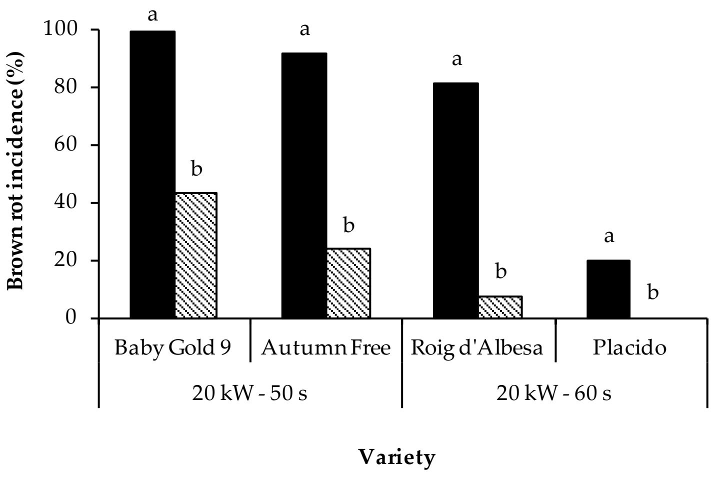

Fig. 4. Brown rot incidence in naturally infected 'Autumn Free' nectarines and 'Baby Gold 9', 'Roig d'Albesa' and 'Placido' peaches. Untreated fruit (■) or fruit immersed in water at 40 ºC and treated by microwave (▨) at 20 kW for 50 or 60 s. After treatment, fruit were incubated at 20 ºC and 85 % RH for 5 days. Means with the same letter for each variety are not significantly different ($P<0.05$) according to LSD test.

Internal and external damage was not observed in any of the tested varieties when either MW treatment was applied (data not shown). The internal fruit temperature immediately after MW treatment at 20 kW for 50 s averaged 28.3 ± 4.2 and 30.9 ± 2.5 ºC in 'Baby Gold 9' peaches and 'Autumn Free' nectarines, respectively, and the external temperature recorded at the end of the treatment was 45.7 ± 1.0 and 45.4 ± 0.5 ºC, respectively (data not shown). When MW treatment at 20 kW was applied for 60 s, the average internal and external temperature was 30.4 ± 0.4 and 46.9 ± 0.5 ºC, respectively, in 'Roig d'Albesa' peaches, and 30.8 ± 2.6 and 47.0 ± 0.5 ºC, for the 'Placido' peaches (data not shown).

3.4. Effect of microwave treatment on fruit quality

No significant differences were determined in any of the assessed quality parameters between untreated and MW treated fruit (MW at 20 kW for 50 s) in 'Sunlate' and 'Pollero peaches or in 'Roig d'Albesa' peaches (MW at 20 kW for 60 s) (data not shown). In 'Pollero' peaches treated by MW at 20 kW for 60 s, no significant effect was observed on soluble solid content and acidity, however, the firmness in the treated fruit, 33.6 N, was significantly higher than that determined in the untreated fruit, 24.7 N (data not shown).

4. Discussion

In a previous work, a microwave treatment at 10 kW for 95 s using a continuous MW tunnel effectively controlled brown rot in peaches and nectarines; however, the influence of fruit size in the final temperature affected the effectiveness of treatment and the internal fruit appearance (Sisquella *et al.*, 2013b). In order to avoid the influence on fruit size on MW effectiveness and internal thermal damage, and to reduce treatment time, several MW treatments with fruit immersed in water have been investigated in the present paper.

Microwave treatment at 10 kW for 95 s with fruit immersed in water at 20 ºC did not control *Monilinia* spp., and brown rot incidence was only slightly reduced to around 45 % with the increase of water temperature to 40 and 45 ºC without causing external or internal damages. However, in a previous work, Sisquella *et al.* (2013b) reduced the incidence of brown rot to less than 15 % when the same MW conditions were applied in air, but internal thermal damage was observed particularly in small fruit due to the high internal temperature reached at the end of the treatment. In the present work, immersion of fruit in water at different temperatures was evaluated to try to reduce the internal damage caused by MW treatment at 10 kW for 95 s. The results indicated that immersion of fruit in water during MW heating solved the problem of internal damage but reduced the efficacy of the MW treatment, probably due to the lower temperatures achieved at the end of the treatment; both internal and external temperatures were under 42 ºC in all the treatments. Many factors can affect MW heating, but the most important are the dielectric properties and the penetration depth (Chandrasekaran *et al.*, 2013). The penetration depth is defined as the depth in a sample at which the MW and radio frequency power drops to 1/*e* (37 %) of its value at the surface (Sosa-Morales *et al.*, 2010). Water is a lossy dielectric material, with small penetration depth causing the field to decay rapidly. The penetration depth of water at 20 ºC with a frequency of 2450 MHZ is 2.4 cm (Cha-um *et al.*, 2009). An increase in water temperature typically reduces the loss factor at high frequencies, accompanied by a slight increase of the penetration depth. Raising water temperature to 40 ºC increases the penetration depth to 4.2 cm (Cha-um *et al.*, 2009). Therefore, the lower temperatures achieved when fruit were immersed in water may be attributed to the limited penetration depth of water, which could indicate that energy was mainly absorbed and converted into heat in the water and thus, a small amount of power was absorbed by the fruit. The improvement of brown rot control by raising water temperature to 40 or 45 ºC could be due to an increase of the amount of MW power absorbed and converted into heat within the fruit as a consequence of a higher penetration depth of water. Lower temperatures were also observed by Koskiniemi *et al.* (2011) in acidified vegetables pasteurized with a MW system when salt was added in the cover solution, which decreased MW penetration.

In order to improve the effectiveness of the MW treatment observed in the previous experiments, MW treatments with the fruit immersed in water at 40 ºC at different power levels were investigated. Brown rot control increased with an increasing exposure time and power level. By increasing MW power level to 20 kW, an effective control of brown rot was achieved when MW treatment was applied for 50 and 60 s, without causing external or internal damage to the fruit. Therefore, the increase in MW power level not only increased treatment effectiveness but it also reduced the treatment time necessary to control brown rot. A similar pattern had been already observed by Sisquella *et al.* (2013b), who reported similar brown rot control in nectarines when the MW power was increased from 10 kW to 17.5 kW, and the exposure time was decreased from 95 to 50 s. However, internal temperatures with MW treatment at 17.5 kW for 50 s were highly variable, leading to internal damages not only in small fruit but also randomly in fruit with intermediate weights. When using MW heating, the temperature rise in a commodity depends on the power level, frequency, heating time, and the material's dielectric loss factor. Thus, the temperature can reach higher values with longer heating and/or higher power level (Wang and Tang, 2001). In the present work, a positive linear relationship was obtained between the internal or external temperatures reached at the end of the treatment and the applied MW energy, so the reduction of the treatment time through the increase of MW power could be attributed to increased heating rates. Cha-um *et al.* (2009) observed that MW power significantly influenced the rate of temperature rise when the water was heated; greater power led to increased heat generation rate within the medium, thereby increasing the rate of temperature rise. Villa-Rojas *et al.* (2010) reported the use of lower heating time to reach an average target temperature through the increase of power level when MW energy was applied to strawberries immersed in water.

Fruit weight had a significant effect on treatment efficacy when MW treatment was applied at 20 kW for 50 s in peaches immersed in water at 40 ºC for which brown rot control was significantly higher in smaller fruit in comparison with larger fruit. Sisquella *et al.* (2013b) also observed different MW efficacies depending on fruit weight when MW treatment at 10 kW for 95 s was applied in air; and the differences were attributed to the internal and external temperatures achieved by small fruit were, respectively, 13.5 and 4.5 ºC higher than those reached by larger fruit. However, when the exposure time of the MW treatment at 20 kW in peaches immersed in water at 40 ºC was increased to 60 s, brown rot control was not affected by fruit size in none of tested varieties. The low influence of fruit size on treatment effectiveness may be due to the fact that similar temperatures were achieved by fruit of different weights, since the higher difference of temperature was observed in 'Pollero' peaches in which the internal and external temperatures of the smaller fruit were, respectively, 3.8 and 1.9 ºC higher than those reached by larger fruit. Different dielectric properties between food and the surrounding air cause reflection and refraction phenomena of the MWs at the interface, resulting in non-uniform electric field distribution (Guan *et al.*, 2002). Therefore, the immersion of fruit in water can improve electric field distribution and reduce the variability of the heating rates. Similar results were observed by Sisquella *et al.* (2013a) when immersion of fruit in water during radio frequency treatment was evaluated in peaches in order to reduce the influence of fruit size observed when RF treatment in air reported by Casals *et al.* (2010c) was applied to peaches with different diameters.

The response of a pathogen to heat can be influenced by several factors such as spore moisture content, age of the inoculum, and inoculum concentration (Barkai-Golan and Phillips, 1991). The results of this study showed that MW treatment at 20 kW for 50 s with fruit immersed in water at 40 ºC significantly reduced brown rot incidence with all tested inoculum concentrations (10^3, 10^4, 10^5, and 10^6 conidia mL^{-1}) and infection times before treatment (0, 24, and 48 h); however, MW efficacy was lower when inoculum concentration was higher than 10^5 conidia mL^{-1} or when the time between the infection and the treatment was 48 h. On the other hand, when treatment time was increased to 60 s, not only MW efficacy was higher but also more uniform results were observed since neither inoculum concentration nor the infection time had a significant effect on treatment efficacy. Similar results were observed by Sisquella *et al.* (2014) when radio frequency treatment was used for treating fruit immersed in water at 40 ºC for 4.5 min to control brown rot in peaches and nectarines. Therefore, in comparison with radio frequency treatment, the use of MW at 20 kW for 60 s for treating fruit immersed in water at 40 ºC effectively controlled brown rot with lower exposure time. The frequency of MW is much greater than radio frequency, thus, rapid heating can be achieved with much lower field intensities (Nelson, 1996). On the contrary, Casals *et al.* (2010a) reported that the efficacy of heat treatment with hot wet air at 50 ºC for 2 h and 95-99 % RH decreased with the increase of the pre-infection time or inoculum concentration of *M. laxa*. Therefore, the results suggest that dielectric heating, either radio frequency or microwave treatment, can be advantageous in comparison with conventional heating.

Brown rot incidence on naturally infected fruit significantly decreased with MW treatment at 20 kW with fruit immersed in water at 40 ºC for 50 s, and this reduction was greater when the time of application was increased to 60 s. Similar results were observed when MW treatment with a household oven at 0.40 kW was applied for 2 min to control natural infections of *P. expansum* in peaches (Karabulut and Baykal, 2002). A combination of MW treatment at 0.45 kW for 2 min with a biocontrol agent was necessary to similarly control natural infections of *R. stolonifer* in peaches (Zhang *et al.*, 2004) and *P. expansum* in pears (Zhang *et al.*, 2006). These studies were performed with household microwave ovens, but in the present study, the use of an industrial microwave tunnel not only allowed working in continuous manner but also using higher power and thus, reducing the treatment time.

A sigmoid relationship was observed between the percentage of brown rot reduction and the applied MW energy, which is linked to the power level and exposure time. Moreover, a linear relationship was observed between the applied MW energy and the external and internal temperatures. Similar relationships were obtained in 'Red Jim' nectarines and 'Baby Gold 9' peaches. Complete brown rot reduction could be achieved by applying more than 1287 kJ, therefore a reduction of treatment time to 40 s could be achieved by increasing power level to approximately 32 kW without reducing MW efficacy. External thermal damage was observed when the applied MW energy was 1662 kJ and consequently when the external temperature was above 50.9 ºC, so a MW treatment at 32 kW for more than 52 s could affect external fruit appearance. All the information obtained with both relationships can be useful to design a specific MW equipment for treating peaches immersed in water to control brown rot, thereby allowing the processing of large quantities of fruit in less time without affecting treatment efficacy and fruit quality.

The results indicated that MW treatment with fruit immersed in water at 40 ºC applied at 20 kW for 50 or 60 s did not negatively affect fruit firmness of the tested varieties. Furthermore, a delay of fruit softening was observed in 'Pollero' peaches treated for 60 s. Similar trends have been reported by Villa-Rojas *et al.* (2010), who observed higher loss of firmness in untreated fruit in comparison with heated fruit when MW treatment with a household oven at 763 W for 1 min 50 s was applied in strawberries immersed in water. Similar results have been also reported with conventional heat treatments applied in peaches and nectarines (Zhou *et al.*, 2002; Casals *et al.*, 2010b). The greater firmness observed in 'Pollero' peaches might be due to the inactivation of cell wall hydrolytic enzymes, mainly polygalacturonase (Malakou and Nanos, 2005) or could be associated to lower ethylene production from heated fruit in comparison to untreated fruit (Budde *et al.*, 2006).

In conclusion, MW treatment at 20 kW for 60 s with fruit immersed in water at 40 ºC may provide an effective treatment to control brown rot in peaches and nectarines without affecting internal and external appearance. However, before this type of treatment can be commercially used to treat fruit immersed in water, a specific MW equipment should be designed, for which the information regarding the relationships between the applied MW energy and brown rot reduction and external and internal temperatures could be useful.

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Discusión general

La presente tesis doctoral evalúa la eficacia de diferentes estrategias alternativas para el control de la podredumbre parda causada por *Monilinia* spp. en poscosecha, principalmente el uso del ácido peracético aplicado en soluciones calientes y la aplicación de tratamientos térmicos mediante radiofrecuencias y microondas. Estas estrategias se estudiaron con el fin de proporcionar al sector de la fruta de hueso nuevos métodos de control para hacer frente a las grandes pérdidas que ocasiona esta enfermedad en poscosecha y a la legislación cada vez más exigente que limita el número de fungicidas que se pueden aplicar.

En la presente tesis se estudió el ácido peracético combinado con agua caliente por tratarse de un tratamiento de tecnología simple, de fácil disponibilidad y de bajo coste que además podría incorporarse fácilmente en las propias líneas de confección ya existentes en las centrales hortofrutícolas lo que representaría, para su aplicación a nivel comercial, una clara ventaja frente a otros métodos alternativos de control.

Por otro lado, el calentamiento dieléctrico ya sea por radiofrecuencias o por microondas, es una tecnología nueva que, a pesar de que para su implantación a nivel comercial sería necesario diseñar un equipo específico, presenta una serie de ventajas frente a los tratamientos térmicos convencionales, como velocidades de calentamiento mayores y tiempos de tratamientos más cortos, que hacen que sea una alternativa atractiva para ser estudiada. En estudios previos realizados en nuestro laboratorio por Casals *et al*. (2010d) ya se observó el gran potencial de las radiofrecuencias para el control de la podredumbre parda en melocotones, sin embargo los resultados en nectarinas no fueron satisfactorios. Por este motivo, en la presente tesis se estudió la mejora del tratamiento de radiofrecuencias mediante la inmersión de la fruta en agua para solucionar las limitaciones que este mostró, así como estudiar, el calentamiento por microondas, cuyo potencial para el control de *Monilinia* spp. se desconoce y que podría permitir obtener tiempos de tratamientos menores que facilitarían su aplicación a nivel comercial.

1. Control de la podredumbre parda mediante el ácido peracético solo o combinado con agua caliente

1.1. Efecto del ácido peracético

La eficacia de diferentes concentraciones de peróxido de hidrógeno (HP), ácido peracético (PAA) y ácido acético (AA) solos o combinados se evaluaron para el control de la podredumbre parda mediante un estudio preliminar (Cap. 1). De las diferentes soluciones evaluadas, se escogió la combinación de 0.25 % de peróxido de hidrógeno, 0.02 % de ácido peracético y 0.075 % de ácido acético (2HP+2PAA+2AA) por ser el tratamiento que mostró una mayor reducción de la podredumbre.

Dado que el ácido peracético se produce por la reacción de ácido acético y peróxido de hidrógeno (Kitis, 2004), la concentración de ácido peracético aplicada mediante la combinación 2HP+2PAA+2AA se midió para comprobar de este modo si la adición de estos dos compuestos podía provocar una reacción entre ellos formando más ácido peracético que se sumaría al ya añadido inicialmente. Los resultados mostraron que la concentración real de ácido peracético aplicada fue de 0.03 % (300 mg L^{-1}), por lo que la concentración de PAA se incrementó de 0.02 hasta 0.03 % al añadir peróxido de hidrógeno y ácido acético a la combinación.

Cuando aplicamos la combinación 2HP+2PAA+2AA mediante baño durante 1 min en fruta inoculada artificialmente, esta siguió mostrando una elevada eficacia reduciendo un 80 % la incidencia de la podredumbre (Figura 1). A pesar de que la eficacia aumentó hasta un 90 % al incrementar el tiempo de exposición hasta 2 min, el tratamiento tuvo un efecto fitotóxico en la piel de las nectarinas. Esta fitotoxicidad puede deberse a una exposición prolongada al peróxido de hidrógeno aplicado mediante la combinación ya que en otros trabajos se han descrito efectos similares. Palou *et al*. (2009) observaron daños en la piel de melocotones y nectarinas cuando 0.1 % de peróxido de hidrógeno se aplicó en heridas previamente inoculadas. Al igual que Smilanick *et al*. (1995) quienes observaron daños superficiales en limones bañados en una solución de 5 % de peróxido de hidrógeno durante 90 s pero no al reducir el tiempo de exposición a 30 s.

El tratamiento con 300 mg L^{-1} de ácido peracético aplicado mediante el producto comercial Proxitane®5:23 durante 1 min redujo un 81 % el porcentaje de frutos podridos por *M. fructicola* y esta eficacia fue igual a la que se observó cuando la misma concentración de PAA se aplicó mediante la combinación 2HP+2PAA+2AA. Estos resultados son importantes ya que los productos comerciales a base de ácido peracético son mucho más estables debido a que durante su producción se emplea un estabilizador o un agente secuestrante (Kitis, 2004). Además, estas formulaciones comerciales no suelen contener ácido sulfúrico y por lo tanto, tienen menos problemas de corrosión haciendo que estas sean adecuadas para su uso en equipos de acero inoxidable (Mari *et al*., 2003).

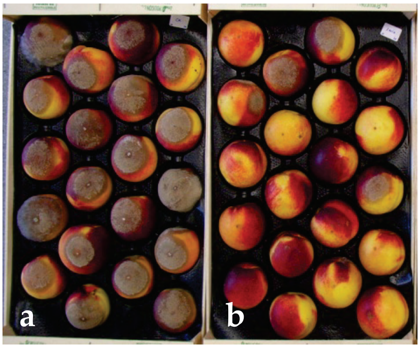

Figura 1. Incidencia de *M. fructicola* en nectarinas 'Autumn Free' sin tratar (a) o tratadas durante 1 min en la combinación 2HP+2PAA+2A (b).

Estudios anteriores de ácido peracético para el control de podredumbre parda en fruta de hueso, determinaron que este no controlaba la enfermedad en nectarinas inoculadas artificialmente con *M. laxa* incluso cuando se bañaron durante 1 min en una solución de ácido peracético a 1000 mg L^{-1} (Mari *et al.*, 1999). No obstante, en un estudio posterior, Mari *et al.* (2004) redujeron la incidencia de *Monilinia* spp. entre un 65 y un 100 % en cerezas, albaricoques, nectarinas y melocotones con infecciones naturales cuando la fruta se bañó durante 1 min en una solución de 125 mg L^{-1} de ácido peracético. Esta mejor eficacia se atribuyó a que las infecciones naturales procedían mayoritariamente de conidios presentes en la superficie de la fruta y por lo tanto más fáciles de controlar por el tratamiento de ácido peracético.

1.2. Efecto de la combinación de ácido peracético con agua caliente

Durante los últimos años, las sustancias químicas combinadas con los tratamientos térmicos se han estudiado para el control de enfermedades de poscosecha con el fin de mejorar la eficacia de estos. De este modo, con este tipo de combinaciones se puede conseguir, por un lado, el uso de temperaturas o tiempos de exposición menores y por otro lado, reducir la concentración eficaz de las sustancias químicas (Barkai-Golan, 2001). En este sentido, Mari *et al.* (2007) controlaron mejor la podredumbre parda en melocotones y nectarinas mediante la combinación de bicarbonato de sodio con agua caliente que los tratamientos aplicados por separado. Palou *et al.* (2009) mostró que los baños de sorbato potásico con agua caliente incrementaban la eficacia del sorbato potásico aplicado a temperatura ambiente para reducir tanto la incidencia como la severidad de

Monilinia spp.. Y Margosan *et al.* (1997) demostró que la mortalidad de los conidios de *M. fructicola* se producía más rápido en soluciones calientes de etanol que en agua caliente sola.

Los resultados del estudio *in vitro* demostraron el efecto sinérgico de la combinación de ácido peracético con agua caliente ya que la aplicación de 100 mg L^{-1} de PAA a 40 ºC durante 40 s redujo significativamente el porcentaje de conidios cultivables en comparación con la misma concentración de PAA o el agua caliente a 40 ºC aplicados por separado. Por este motivo, y con el objetivo de reducir la concentración de ácido peracético eficaz en el control de la podredumbre parda, se evaluó la eficacia de diferentes concentraciones de ácido peracético (0, 100, 200 o 300 mg L^{-1}) a diferentes temperaturas (20, 40, 50 o 60 ºC) durante 40 s en fruta inoculada artificialmente.

Este efecto sinérgico no se observó en los melocotones 'Mountain Gold' ya que para una misma concentración de ácido peracético la eficacia no aumentó al incrementar la temperatura del agua, sin embargo, la eficacia del tratamiento fue mayor al aumentar la concentración de ácido peracético de 100 a 200 o 300 mg L^{-1}. En cambio, en los melocotones 'Rome Star' ocurrió totalmente lo contrario, la eficacia del tratamiento incrementó al aumentar la temperatura del agua de 20 ºC a 40, 50 o 60 ºC pero para una misma temperatura no hubo diferencias entre las concentraciones de ácido peracético evaluadas. En base a estos resultados, las condiciones de tratamiento que se seleccionaron fueron 200 mg L^{-1} de ácido peracético aplicado a 40 ºC ya que además, estas condiciones no afectaron negativamente ni al aspecto visual de la fruta ni a los parámetros estándar de calidad como la firmeza, el contenido de sólidos solubles y la acidez.

El tratamiento con 200 mg L^{-1} de ácido peracético a 40 ºC aplicado durante 40 s redujo significativamente la incidencia de *M. fructicola* en todas las concentraciones de inóculo evaluadas (10^3, 10^4, 10^5 o 10^6 conidios mL^{-1}) y por lo general, esta eficacia no disminuyó con el aumento de la concentración. A pesar de que este tratamiento controló por completo la incidencia de podredumbre parda cuando *M. fructicola* se inóculo el mismo día del tratamiento, no fue eficaz de controlar infecciones de 24, 48 o 72 h. En condiciones *in vitro*, más del 80 % de los conidios viables de *M. fructicola* son capaces de germinar en 2 horas a 25 ºC y con elevada actividad de agua (Casals *et al.*, 2010e). Por lo tanto, estos resultados indicarían que a pesar de que el ácido peracético tiene un elevado potencial para controlar la podredumbre parda independientemente de la concentración de inóculo, este únicamente es eficaz si la infección de *M. fructicola* todavía no ha ocurrido o es muy reciente. Otros tipos de desinfectantes, como el cloro, presentan características similares ya que cómo bien se conoce, el cloro no es un producto sistémico sino que actúa por contacto siendo únicamente eficaz contra conidios suspendidos en agua o presentes en la superficie de fruta pero no contra patógenos que se encuentran por debajo de la piel ni cuando estos ya han infectado (Barkai-Golan, 2001).

Además, el tratamiento con 200 mg L^{-1} de ácido peracetico a 40 ºC durante 40 s no protegió a la fruta de futuras infecciones cuando *M. fructicola* se inoculó artificialmente 0, 24, 48 y 72 h después del tratamiento. Otros compuestos químicos como el bicarbonato de sodio, el carbonato sódico o el etanol también mostraron el mismo efecto negativo cuando se estudiaron para la prevención de enfermedades (Margosan *et al.*, 1997; Smilanick *et al.*, 1999).

Finalmente, el tratamiento con 200 mg L^{-1} de ácido peracético a 40 ºC durante 40 s se evaluó para el control de infecciones naturales de *Monilinia* spp.. En este caso, el tratamiento no mostró ningún control de la podredumbre parda en las nectarinas 'Autumn Free' y solo redujo ligeramente la incidencia de enfermedad en los melocotones 'Roig d'Albesa' y 'Placido'. Las infecciones naturales de *Monilinia* spp. pueden proceder de infecciones latentes producidas en el campo, de infecciones recientes que todavía no se han desarrollado y de inóculo que se encuentra en la superficie de la fruta. Por lo tanto, la menor eficacia observada puede deberse a que las infecciones naturales procedían mayoritariamente de infecciones latentes o de más de 24 h y tal cómo se ha podido observar en los resultados descritos anteriormente, el tratamiento solo es eficaz en el control de infecciones muy recientes. Resultados similares también fueron descritos por Mari *et al.* (2004) quienes observaron una menor eficacia del ácido peracético para controlar infecciones naturales en cerezas 'Nero I' lo que atribuyeron a que la mayoría de estas infecciones probablemente provenían de infecciones latentes.

2. Control de la podredumbre parda mediante el calentamiento por microondas

En el calentamiento dieléctrico, tanto por radiofrecuencias (RF) como por microondas (MW), el calor es generado directamente en el interior de todo el volumen de un material dieléctrico por la interacción directa de este con la energía electromagnética. De este modo, se pueden conseguir velocidades de calentamiento mayores y tiempos de tratamiento más cortos en comparación con los tratamientos térmicos convencionales (Tang *et al.*, 2000). Por este motivo, en los últimos años, el calentamiento dieléctrico se ha estudiado ampliamente en procesos de la industria alimentaria (Marra *et al.*, 2009; Chandrasekaran *et al.*, 2013) y como método alternativo al bromuro de metilo para el control de plagas en cerezas (Ikediala *et al.*, 1999; 2002), mangos (Varith y Kiatsiriroat, 2004; Sosa-Morales *et al.*, 2009) y manzanas (Wang *et al.*, 2006). Sin embargo, existen pocos trabajos que estudien el potencial de este tipo de tratamientos para el control de enfermedades de poscosecha.

En general, las altas frecuencias de las microondas, a diferencia de las de radiofrecuencias, permiten transferir la energía electromagnética más rápido utilizando menores intensidades de campo de modo que se pueden lograr velocidades de calentamiento mayores y por lo tanto, tiempos de tratamiento más cortos (Nelson, 1996; Salazar-González *et al.*, 2012).

Los estudios de microondas de la presente tesis se llevaron a cabo en un equipo de microondas industrial que constaba de una cavidad de 1.70 m donde estaban situados 12 magnetrones con una potencia teórica cada uno de ellos que podía variar entre 0.2 y 2 kW. A causa de problemas técnicos, la potencia de trabajo se consiguió mediante el uso de 10 magnetrones que trabajaban a la misma potencia, por lo que la máxima potencia a la que se podía trabajar fue de 20 kW. Al tratarse de un equipo en continuo, la fruta se introducía dentro de la cavidad mediante una cinta transportadora cuya velocidad máxima era de 3 m min^{-1}.

En el calentamiento por microondas, el aumento de la temperatura en un producto depende principalmente de la potencia, la frecuencia, el tiempo de exposición y del factor de pérdida dieléctrica del producto (Wang y Tang, 2001). Por este motivo, en primer lugar se evaluó la eficacia de diferentes potencias de microondas (5-20 kW) a diferentes tiempos de exposición (34-120 s) para determinar las condiciones óptimas capaces de controlar la podredumbre parda de forma eficaz sin afectar a la calidad de la fruta (Cap. 4). De todas las combinaciones estudiadas se seleccionó el tratamiento de microondas a una potencia de 10 kW durante 95 s ya que mostró una elevada eficacia en el control de *M. fructicola* sin afectar al aspecto visual interno y externo de la fruta.

Una vez determinadas las condiciones óptimas del tratamiento, el siguiente paso fue evaluar la influencia del tamaño de la fruta (170 ± 10, 215 ± 10 y 260 ± 10 g) en la eficacia del tratamiento de microondas. Aunque el peso de la fruta no influyó en la eficacia del tratamiento en las nectarinas, sí que tuvo un efecto significativo en los melocotones en los que la eficacia del tratamiento se redujo en los frutos de mayor tamaño. Las diferentes eficacias observadas en función del peso de los melocotones pueden atribuirse a las diferentes temperaturas alcanzadas por los frutos de diferente peso ya que la temperatura interna y superficial de los frutos de menor peso fue, respectivamente, 13.5 y 4.5 ºC superiores a las alcanzadas por los frutos de mayor peso. Además, en las nectarinas, a pesar de que el tamaño no influyó en la eficacia, también se observaron temperaturas superiores en la fruta de menor tamaño en comparación con la de mayor tamaño.

La apariencia externa de la fruta no se vio afectada en ninguna de las variedades. Sin embargo, la influencia del tamaño en la temperatura interna alcanzada por los frutos durante el tratamiento de microondas provocó daños internos en los frutos de menor tamaño (170 ± 10 g) debido a las elevadas temperaturas internas que se alcanzaron en estos frutos, ya que estas temperaturas incluso superaron los 60 ºC en el caso de las nectarinas ‘PP-100’ (Figura 2). Varith y Kiatsiriroat (2004) también describieron en mangos daños térmicos en las zonas donde el calor se había absorbido en exceso cuando estos se trataron con microondas durante 40 s a una potencia de 400 W.

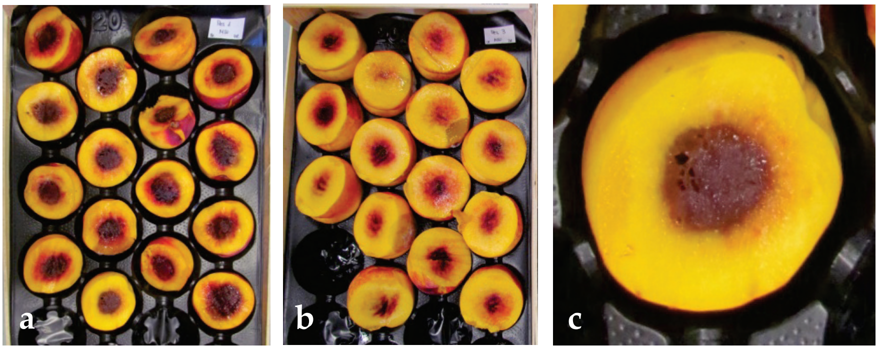

Figura 2. Diferencia entre el aspecto interno de las nectarinas ‘PP-100’ de menor (a) y mayor (b) peso (170 ± 10 y 260 ± 10 g) tratadas por microondas a 10 kW durante 95 s y detalle de los daños internos causados por el tratamiento en la fruta de menor tamaño (c).

La mayor temperatura interna en los frutos de menor tamaño y por lo tanto la aparición de daños internos puede deberse a que en los materiales de forma esférica, como podrían ser los melocotones y las nectarinas, el punto más caliente tiende a encontrarse en el centro del material ya que la superficie curva de estos provoca una convergencia de las ondas en el centro tal y como se muestra en la Figura 3a (Zhang y Datta, 2005). De este modo, en el centro de los materiales esféricos se puede absorber mayor energía y como consecuencia mayor es la temperatura que se alcanza. En el calentamiento por microondas, este efecto ocurre especialmente en materiales esféricos cuyo diámetro varía entre 20 y 60 mm (Ikediala *et al.*, 1999). La ausencia de daños internos en la fruta de mayor peso y por lo tanto de mayor tamaño puede deberse a que la profundidad de penetración de las ondas en la fruta es menor que el radio de esta de modo que la convergencia de las ondas en el centro es menor (Figura 3b) y como consecuencia la temperatura interna alcanzada también es menor.

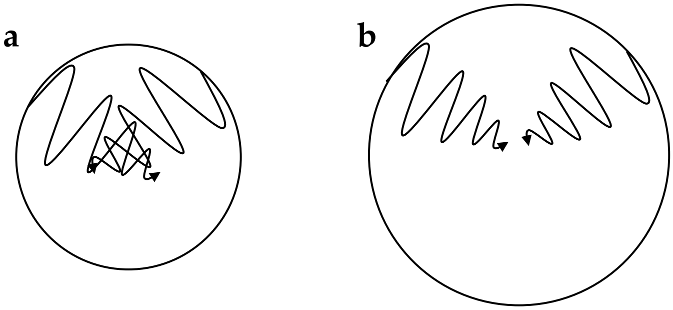

Figura 3. Esquema sobre la convergencia de las ondas en el centro de la fruta de menor tamaño (a) y de mayor tamaño (b). Adaptado de Varith y Kiatsiriroat (2004).

El tratamiento de microondas a 10 kW durante 95 s no afectó negativamente a la calidad de la fruta cuando los parámetros estándar de calidad (firmeza, contenido de sólidos solubles y acidez) se evaluaron después de conservar la fruta 2 días a 20 ºC. Incluso, en las nectarinas ’PP-100’ se observó una disminución de la pérdida de firmeza en comparación con la fruta control. Resultados similares ya fueron descritos en melocotones después de un tratamiento de microondas (Karabulut y Baykal, 2002; Zhang *et al.*, 2004), en manzanas (Wang *et al.*, 2006), naranjas (Birla *et al.*, 2005) y cerezas (Ikediala *et al.*, 2002) después de tratarse por radiofrecuencias y en nectarinas (Anthony *et al.*, 1989), melocotones (Casals *et al.*, 2010b), uvas (Lydakis y Aked, 2003) y naranjas (Nunes *et al.*, 2007) después de aplicar un tratamiento térmico convencional. Este efecto de los tratamientos térmicos podría deberse a que el calor afecta a los procesos de maduración vinculados con el ablandamiento de la fruta, ya que se ha observado que los tratamientos térmicos retrasan el pico de producción de etileno, hormona que regula la maduración, y por lo tanto podrían retrasar la maduración de la fruta (Lay-Yee y Rose, 1994; Zhou *et al.*, 2002).

El nivel de madurez de la fruta, conseguido almacenando la fruta a 20 ºC durante 24, 48 o 72 h previas al tratamiento, no tuvo un efecto significativo en la eficacia del tratamiento de microondas a 10 kW durante 95 s en ninguna de las variedades evaluadas, obteniéndose reducciones superiores al 95 % en todos los casos. Resultados similares fueron descritos por Casals *et al*. (2010d) cuando el tratamiento de radiofrecuencias se aplicó en melocotones para controlar la podredumbre parda. En el calentamiento dieléctrico, las propiedades dieléctricas, en especial el factor de pérdida, determinan la velocidad de calentamiento y por consiguiente la temperatura final. Por lo tanto, estos resultados sugieren que los cambios producidos en la fruta por la maduración durante el almacenamiento no provocan cambios significativos en las propiedades dieléctricas de los melocotones y nectarinas. Este hecho ya fue descrito por Nelson *et al*. (1995) quienes observaron que el factor de pérdida dieléctrica de melocotones mostraba una baja dependencia del estado de madurez de la fruta.

Finalmente, el tratamiento de microondas a 10 kW durante 95 s se evaluó para el control de infecciones naturales de *Monilinia* spp. tanto en melocotón como en nectarina. El tratamiento de microondas mostró una elevada eficacia en el control de estas infecciones reduciendo el porcentaje de frutos podridos un 85 % y un 91 % en nectarinas 'PP-100' y melocotones 'Placido', respectivamente.

Uno de los principales factores limitantes para que un tratamiento de poscosecha pueda adaptarse fácilmente a las líneas de confección presentes actualmente en las centrales hortofrutícolas es el tiempo de tratamiento. Aunque 95 s es un tiempo de exposición relativamente corto, sigue siendo muy elevado para que el tratamiento de microondas pueda llegar a aplicarse a nivel comercial en la línea. En el calentamiento por microondas, el aumento de la temperatura de un producto depende principalmente de la potencia y del tiempo de exposición (Wang y Tang, 2001), de modo que se pueden conseguir velocidades de calentamiento mayores al aumentar la potencia de las microondas (Villa-Rojas *et al*., 2010). En el presente trabajo, al aumentar la potencia a 17.5 kW se redujo hasta 50 s el tiempo de tratamiento eficaz para el control de la podredumbre parda sin causar daños visuales en la fruta por lo que todos los ensayos descritos previamente también se realizaron a las condiciones de tratamiento anteriores.

El tratamiento de microondas a 17.5 kW durante 50 s mostró en todos los casos un elevada eficacia en el control de la podredumbre parda y esta además, no se vio influenciada ni por la madurez de la fruta ni por el peso de esta. Sin embargo, este tratamiento no solo causó daños internos en la fruta de menor tamaño (180 ± 10 g) sino que también se observaron daños de forma aleatoria en la fruta de 245 ± 10 g. Este hecho, puede deberse a la variabilidad que presentaron las temperaturas internas alcanzadas por esta fruta al final del tratamiento ya que la desviación estándar de estas fue de 8.4 ºC a diferencia de los 1.6 y 2.1 ºC observados cuando se aplicó en tratamiento de microondas a 10 kW durante 95 s en melocotones 'Placido' y en nectarinas 'PP-100', respectivamente.

El calentamiento por microondas ya se ha estudiado para el control de enfermedades de poscosecha, sin embargo, estos estudios son limitados. Karabulut y Baykal (2002) demostraron la eficacia de un microondas doméstico a 0.4 kW durante 2 min para el control de *Botrytis cinerea* y *Penicillium expansum* en melocotones observando reducciones superiores al 75 % en ambos casos. Posteriormente, las microondas se aplicaron a 0.45 kW durante 2 min para el control de *Rhizopus stolonifer* en melocotones (Zhang *et al.*, 2004) y *P. expansum* en pera (Zhang *et al.*, 2006), sin embargo, en estos estudios las eficacias observadas fueron menores ya que las reducciones no superaron el 50 %.

Nuestros resultados demuestran que el calentamiento por microondas también es efectivo en el control de *Monilinia* spp. en melocotones y nectarinas cuando el tratamiento se aplicó a 10 kW durante 95 s mediante un microondas industrial en continuo. Además, todos los estudios de microondas anteriores se realizaron con un microondas doméstico por lo que los resultados de esta tesis pueden proporcionar una idea más concreta de las condiciones de tratamiento a nivel comercial. No obstante, hay que tener en cuenta que la influencia del tamaño en la temperatura interna alcanzada no solo puede afectar a la eficacia del tratamiento sino que además provoca daños internos en la fruta lo que hace necesario seguir estudiando este tipo de calentamiento con el fin de solucionar los problemas que este presenta.

3. Mejora de los tratamientos de radiofrecuencias y microondas mediante la inmersión de la fruta en agua

La falta de uniformidad en las temperaturas alcanzadas dentro de un producto o entre diferentes productos es el principal problema que presenta el calentamiento dieléctrico tanto por radiofrecuencias como por microondas (Ikediala *et al.*, 2002). En los últimos años, diferentes técnicas se han propuesto con el fin de solucionar los problemas asociados al calentamiento no uniforme (Fung y Cunningham, 1980;

Birla *et al.*, 2004; Wang *et al.*, 2006). En este sentido, Ikediala *et al*. (2002) sugirieron sumergir las cerezas en agua durante el tratamiento de radiofrecuencias aplicado para el control de plagas y así solucionar principalmente la gran diferencia entre la temperatura interna y superficial que observó cuando el tratamiento se aplicó en aire.

En estudios previos realizados en nuestro laboratorio por Casals *et al*. (2010d), el calentamiento por radiofrecuencias a 27.12 MHz aplicado durante 18 min mostró un gran potencial para controlar la podredumbre parda en poscosecha de melocotones, sin embargo, este mismo tratamiento no fue capaz de controlar la enfermedad en nectarinas. Además, en estudios preliminares que se realizaron al inicio de esta tesis, se observó que la eficacia de este tratamiento estaba influenciada por el tamaño de la fruta obteniéndose reducciones significativamente superiores en la fruta de mayor tamaño en comparación con la de menor tamaño (Cap. 2).

Por este motivo, en esta tesis se han dedicado muchos esfuerzos a la mejora del tratamiento de radiofrecuencias descrito por Casals *et al*. (2010d) así como del tratamiento de microondas descrito en el Capítulo 4 y discutido previamente en el apartado 2. Para ello, se estudió la aplicación de ambos tratamientos con la fruta sumergida en agua para mejorar por un lado, tanto la falta de eficacia en las nectarinas como disminuir la influencia del tamaño en la eficacia del tratamiento de radiofrecuencias durante 18 min y por otro lado, evitar la influencia del tamaño de la fruta en la temperatura interna alcanzada después del tratamiento de microondas a 10 kW durante 95 s que es la principal causa de los daños internos observados.

3.1. Efecto de la inmersión de la fruta en agua

El equipo de radiofrecuencias utilizado en la presente tesis para realizar los estudios sobre la mejora del tratamiento de radiofrecuencias, constaba de una cavidad de 1.5 m en el interior de la cual estaban situadas dos líneas de electrodos, una en la parte superior y otra en la inferior. Al tratarse de un equipo en continuo, la fruta se introducía dentro de la cavidad mediante una cinta transportadora situada por encima de la línea de electrodos inferiores cuya velocidad máxima era 0.7 m min^{-1}.

El tratamiento de radiofrecuencias durante 18 min con la fruta sumergida en agua a 20 ºC controló por completo la incidencia de podredumbre parda, sin embargo, la fruta sufrió daños superficiales severos debido a la elevada temperatura externa que se alcanzó al final del tratamiento (56 ºC). Al disminuir el tiempo de exposición hasta 9 min, el tratamiento de radiofrecuencias siguió mostrando una elevada eficacia tanto en melocotón como en nectarina, con reducciones que variaron entre un 63 y un 100 %, sin causar daños superficiales en la fruta. Además, al aumentar la temperatura del agua a 35 y 40 ºC el tiempo de tratamiento disminuyó hasta 6 y 4.5 min, respectivamente, reduciendo la incidencia de *M. fructicola* por debajo de un 10 % en ambos casos (Cap. 2). Estos resultados muestran que la inmersión de la fruta en agua no solo solucionó la falta de eficacia en nectarinas mostrada por el tratamiento de radiofrecuencias descrito por Casals *et al*. (2010d), sino que además mejoró su eficacia reduciendo así el tiempo de tratamiento.

La reducción del tiempo de tratamiento tanto por la inmersión de la fruta en agua a 20 ºC como por el aumento de esta temperatura hasta 35 o 40 ºC puede deberse a un aumento de la velocidad de calentamiento. Las temperaturas externas alcanzadas después del tratamiento de radiofrecuencia durante 9 min con la fruta sumergida en agua a 20 ºC variaron entre 42.1 y 45.7 ºC y estas temperaturas fueron similares a las que se lograron con el tratamiento de RF con agua a 35 ºC durante 6 min (45.6 - 45.7 ºC) y con agua a 40 ºC durante 4.5 min (43.4 - 45.9 ºC). Además, estas temperaturas también fueron similares a las que se obtuvieron cuando el tratamiento de radiofrecuencia se aplicó en aire durante 18 min (Casals *et al.*, 2010d). Birla *et al*. (2008) mediante simulación por ordenador también observó que la presencia del agua reducía a la mitad el tiempo requerido por el tratamiento de radiofrecuencias en aire para llegar a una misma temperatura. Este aumento de la velocidad de calentamiento se atribuyó a que el agua, al ser un material dieléctrico, ofrece menos resistencia al paso de la energía electromagnética que el aire aumentando de esta forma la cantidad de energía que llega a la muestra. Además, la reducción del tiempo de tratamiento al aumentar la temperatura del agua podría deberse a que la resistencia que ofrece el agua al paso de la energía disminuye ya que, por lo general, a bajas frecuencias, como sería el caso de las radiofrecuencias, el factor de pérdida dieléctrica de un material aumenta al incrementar la temperatura (Sosa-Morales *et al.*, 2010).

Por el contrario, cuando la inmersión de la fruta en agua a diferentes temperaturas (20, 35, 40 o 45 ºC) se estudió para mejorar el tratamiento de microondas a 10 kW durante 95 s descrito en el Capítulo 4 de esta tesis, el comportamiento que se observó fue totalmente distinto (Cap. 5). El tratamiento de microondas a 10 kW durante 95 s con la fruta sumergida en agua a 20 ºC no controló

M. fructicola en ninguna de las variedades evaluadas y la incidencia de podredumbre parda solo se redujo, aunque ligeramente, al incrementar la temperatura del agua a 40 y 45 ºC. Por lo tanto, en el caso de las microondas, la inmersión de la fruta en agua redujo la eficacia que el tratamiento mostró cuando se aplicó en aire, probablemente debido a que las temperaturas superficiales e internas fueron inferiores a 42 ºC en todos los tratamientos.

La profundidad de penetración es uno de los parámetros a tener en cuenta en el calentamiento dieléctrico pero es especialmente importante en las microondas ya que su valor disminuye al aumentar la frecuencia (Tang *et al.*, 2000). En el caso del agua a 20 ºC, la profundidad de penetración a la frecuencia utilizada en los tratamientos de radiofrecuencias, 27.20 MHz, es de 77.9 cm (Wang *et al.*, 2008), en cambio, a la frecuencia de microondas, 2450 MHz, es de tan solo 2.4 cm (Cha-um *et al.*, 2009). Esta gran diferencia entre las profundidades de penetración en función de la frecuencia podría explicar el distinto comportamiento observado de los tratamientos de radiofrecuencias y microondas al aplicarse en la fruta sumergida en agua. En el caso de las microondas, la baja profundidad de penetración de las ondas electromagnéticas en el agua probablemente provoca que no llegue suficiente energía a la fruta para aumentar de esta forma su temperatura ya que la energía electromagnética se absorbe y se convierte en calor principalmente en el agua. Por el contrario, en el tratamiento de radiofrecuencias, como la profundidad de penetración es mayor, esta no es un factor limitante y el agua actúa como un medio conductor de la energía electromagnética. A diferencia de lo que ocurre en radiofrecuencias, a altas frecuencias, como las de microondas, el aumento de la temperatura del agua reduce su factor de pérdida dieléctrica y como consecuencia su profundidad de penetración aumenta hasta 4.2 cm (Cha-um *et al.*, 2009). Por lo tanto, la mejora del control de la podredumbre parda mediante el aumento de la temperatura del agua a 40 o 45 ºC puede deberse a un aumento de la cantidad de energía absorbida y convertida en calor por la fruta debido al aumento de la profundidad de penetración del agua.

Uno de las razones por las cuales la inmersión de la fruta en agua se estudió durante el tratamiento de microondas a 10 kW durante 95 s fue la de evitar la producción de daños internos en la fruta, especialmente en la de menor tamaño, debida a la elevada temperatura interna alcanzada en estos casos. En ninguna de las variedades estudiadas se observaron daños internos, sin embargo, hay que tener en cuenta que las temperaturas que se lograron no fueron suficientes como para controlar la podredumbre parda de forma eficaz. No obstante, cabe destacar que probablemente aun aumentando la eficacia del tratamiento de microondas con la fruta sumergida en agua, la baja profundidad de penetración del agua evitaría alcanzar elevadas temperaturas internas y por lo tanto, daños internos. Por este motivo, en base a los resultados se decidió estudiar la mejora de la eficacia del tratamiento de microondas con la fruta sumergida en agua a 40 ºC mediante el aumento de la potencia aplicada a 15, 17.5 o 20 kW (Cap. 5).

El aumento de la potencia a 15 kW mejoró la eficacia del tratamiento de microondas durante 95 s con la fruta sumergida en agua a 40 ºC observándose reducciones superiores al 90 % tanto en melocotón como en nectarina. Además, al aumentar la potencia hasta 20 kW, el tratamiento de microondas redujo un 96 % la incidencia de *Monilinia* spp. en las dos variedades estudiadas al aplicar el tratamiento durante tan solo 60 s sin causar daños internos y externos a la fruta. Por lo tanto, el aumento de la potencia permitió obtener un tratamiento de microondas con la fruta sumergida en agua eficaz y que a su vez no causara daños internos en la fruta.

3.2. Efecto del tamaño de la fruta

Uno de los principales problemas que presentaron los tratamientos de radiofrecuencias y microondas cuando se aplicaron en aire fue la influencia del tamaño de la fruta en la eficacia de los tratamientos (Cap. 2 y 4). Por este motivo, se consideró importante evaluar si la inmersión en agua reducía la influencia del tamaño de la fruta en la eficacia del tratamiento de radiofrecuencias durante 9 min con la fruta sumergida en agua a 20 ºC (Cap. 2) y del tratamiento de microondas a 20 kW durante 60 s con la fruta sumergida en agua a 40 ºC (Cap. 5).

Los resultados mostraron que la inmersión de la fruta en agua eliminaba la influencia del tamaño de la fruta en la eficacia tanto del tratamiento de radiofrecuencias como del de microondas (Figura 4). Esta menor influencia puede deberse a que las temperaturas finales de la fruta de distinto tamaño fueron similares. En el caso de las radiofrecuencias, solo hubo 3 ºC de diferencia entre la temperatura interna de la fruta de menor (65 ± 2 mm) y mayor (75 ± 2 mm) diámetro (Cap. 2). En cambio, cuando el tratamiento de radiofrecuencia se aplicó durante 18 min en aire la diferencia entre las temperaturas internas de la fruta de los mismos diámetros anteriores fue de 9 ºC (datos no mostrados). Por lo que respecta al tratamiento de microondas, la temperatura interna de los melocotones 'Roig d'Albesa' de menor peso (170 ± 10 g) fue tan solo 2 ºC superior a la que se alcanzó en los frutos de mayor peso (260 ± 10 g) (Cap. 5). Esta diferencia contrasta con los 13.5 ºC que se observaron entre los frutos del mismo peso cuando el tratamiento de microondas se aplicó sin agua (Cap. 4).

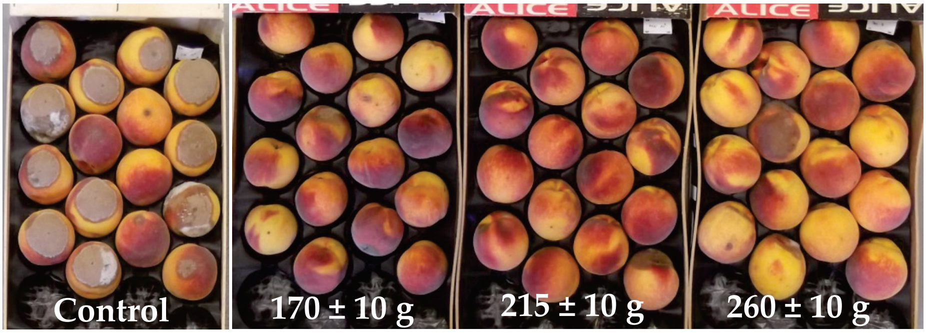

Figura 4. Incidencia de *M. fructicola* en melocotones 'Roig d'Albesa' de 170 ± 10, 215 ± 10 y 260 ± 10 g tratados mediante microondas a 20 kW durante 60 s con la fruta sumergida en agua a 40 ºC o sin tratar (control).

3.3. Otros factores que afectan a la eficacia del tratamiento: madurez del fruto, concentración del hongo y tiempo de infección

La efectividad de los tratamientos térmicos puede verse influenciada por varios factores como la especie de patógeno, el contenido de humedad de los conidios, su actividad metabólica, la edad, la carga de inóculo y la posición del patógeno en el huésped (Barkai-Golan y Phillips, 1991). Por este motivo, aunque los tratamientos de radiofrecuencias y microondas con la fruta sumergida en agua mostraron una elevada eficacia en el control de la podredumbre parda tanto en melocotón como en nectarina, factores como el tiempo entre la infección y el tratamiento o la concentración de inóculo requieren tenerse en consideración antes de que alguno de los tratamientos pueda ser implementado comercialmente. En base a los resultados discutidos anteriormente en este apartado, los tratamientos seleccionados para la realización de estos estudios fueron el tratamiento de radiofrecuencia durante 4.5 min con la fruta sumergida en agua a 40 ºC (Cap. 3) y el tratamiento de microondas a 20 kW durante 60 s con la fruta sumergida en agua a 40 ºC (Cap. 5).

El tratamiento de radiofrecuencias durante 4.5 min con la fruta sumergida en agua a 40 ºC controló la podredumbre parda en todos los tiempos de infección realizados antes del tratamiento (0, 24 y 48 h) y en todas las concentraciones de

M. fructicola (10^3, 10^4, 10^5 y 10^6 conidios mL^{-1}) estudiadas tanto en la variedad de melocotón como en la nectarina evaluadas. Sin embargo, la eficacia de las radiofrecuencias disminuyó ligeramente cuando las nectarinas 'PP-100' se inocularon 48 h antes del tratamiento y cuando los melocotones 'Roig d'Albesa' se inocularon con 10^5 o 10^6 conidios mL^{-1}. Aun así, cabe destacar que en estos últimos casos la reducción de la podredumbre parda fue superior al 64 % (Cap. 3). Con respecto al tratamiento de microondas a 20 kW durante 60 s con la fruta sumergida en agua a 40 ºC, la eficacia del tratamiento no se vio afectada ni por el tiempo transcurrido entre la infección y el tratamiento (0, 24 y 48 h) ni por las concentraciones de inóculo de *M. fructicola* (10^3, 10^4 y 10^5 conidios mL^{-1}) evaluadas (Cap. 5) mostrando en todos los casos una reducción de la podredumbre parda superior al 70 %. En cambio, en otros sistemas térmicos como el curado a 50 ºC durante 2 h y humedad relativa del 95-99 % se observó que la eficacia del tratamiento disminuía al aumentar el tiempo de la infección de *M. laxa* antes del tratamiento o al aumentar la concentración de *M. fructicola*. En este último caso el control de la podredumbre se redujo de un 95 % a un 18 % al aumentar la concentración de inóculo de 10^3 a 10^6 conidios mL^{-1} (Casals *et al.*, 2010a).

En el caso del tratamiento de radiofrecuencias también se evaluó el efecto del nivel de madurez de la fruta sobre la eficacia del tratamiento. Los diferentes estados de madurez de la fruta evaluados no afectaron a la eficacia del tratamiento en ninguna de las variedades estudiadas. Estos resultados están de acuerdo a los observados cuando el tratamiento de microondas a 10 kW fue realizado durante 95 s (Cap. 4) o el tratamiento de radiofrecuencia durante 18 min (Casals *et al.*, 2010d) se aplicaron sin inmersión en agua. Por el contrario, la eficacia del tratamiento de curado a 50 ºC durante 2 h y 95-99 % de HR disminuyó en la fruta más madura (Casals *et al.*, 2010a).

Además, cuando ambos tratamientos, radiofrecuencias y microondas, se estudiaron para el control de infecciones naturales de *Monilinia* spp. (Cap. 3 y 5), ambos tratamientos siguieron mostrando una elevada eficacia en el control de esta enfermedad. La reducción de la incidencia de la podredumbre parda fue superior al 74 % en el tratamiento de radiofrecuencias (Figura 5a) y al 90 % en el de microondas (Figura 5b).

Estos resultados ponen de manifiesto que el calentamiento dieléctrico, ya sea mediante radiofrecuencias o microondas, presenta una serie de ventajas frente a los tratamientos térmicos convencionales, ya que su eficacia, en general, no depende ni de la concentración de inóculo, ni del tiempo transcurrido entre la infección y el tratamiento. Además, a pesar de que se consiguen tiempos de tratamiento más cortos mediante el tratamiento con agua caliente a 60 ºC, donde los tiempo pueden variar entre 40 y 60 s, la eficacia de estos varía en función de la variedad e incluso se reduce tras un periodo de conservación en frío (Casals *et al.*, 2010c; Spadoni *et al.*, 2013).

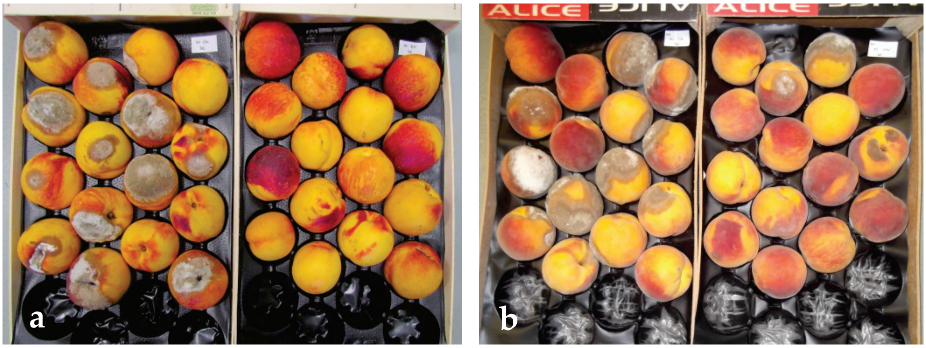

Figura 5. Incidencia de podredumbre parda en nectarinas 'PP-100' con infecciones naturales sin tratar o tratadas mediante radiofrecuencias con la fruta sumergida en agua a 40 ºC durante 4.5 min (a) y en melocotones 'Roig d'Albesa' con infecciones naturales sin tratar o tratados mediante microondas a 20 kW durante 60 s con la fruta sumergida en agua a 40 ºC (b).

Uno de los objetivos por los cuales en esta tesis se estudió el calentamiento por microondas fue el de conseguir tiempos de exposición cortos que facilitarían la aplicación comercial del tratamiento. A pesar de que el tiempo de exposición conseguido con el tratamiento de microondas de 60 s, fue menor que el de las radiofrecuencias de 4.5 min, tiempos de exposición inferiores a 1 min son preferibles desde un punto de vista tecnológico. Por este motivo, los estudios descritos anteriormente también se evaluaron con un tratamiento de microondas a 20 kW durante 50 s con la fruta sumergida en agua a 40 ºC ya que en estudios preliminares, este tratamiento mostró una eficacia superior al 80 % (Cap. 5). Los resultados mostraron que al reducir el tiempo de exposición a 50 s, la eficacia del tratamiento dependió del tamaño de la fruta observándose en la fruta de menor peso reducciones significativamente superiores a las de mayor peso, y además, esta eficacia se redujo al aumentar el tiempo entre la infección y el tratamiento de 0 o 24 h hasta 48 h y al incrementar la concentración de *M. fructicola* de 10^3 a 10^4 o 10^5 conidios mL^{-1}.

3.4. Efecto de los tratamientos con la fruta sumergida en agua en la calidad de la fruta

El aspecto visual tanto interno como externo de la fruta tratada por microondas a 20 kW durante 60 s con agua a 40 ºC no se vio afectado en ninguno de los casos, ni siquiera en la fruta de menor peso evaluado (145 ± 10 g). Este hecho puede deberse a que las temperaturas internas que se lograron al final del tratamiento fueron inferiores a 35 ºC en todos casos (Cap. 5) a diferencia de los 54.7 y 62.6 ºC que se alcanzaron cuando el tratamiento de microondas se aplicó a 10 kW durante 95 s en aire (Cap. 4). Estas menores temperaturas internas pueden atribuirse a que, como se ha comentado anteriormente, las ondas electromagnéticas a la frecuencia de microondas tienen muy baja profundidad de penetración por lo que cuando la energía llega a la fruta esta no penetra hasta el interior produciéndose de esta forma un calentamiento superficial. Este hecho está de acuerdo con las temperaturas externas que se lograron ya que en todos los casos fueron superiores a las internas variando entre 45.9 y 48.8 ºC (Cap. 5).

En el caso de las radiofrecuencias, la inmersión de la fruta en agua a 20 ºC solucionó los problemas de daños térmicos en los puntos de contacto de los melocotones con el contenedor descritos por Casals *et al*. (2010d) cuando el tratamiento de RF se aplicó en aire. Sin embargo, en los estudios de esta tesis se observaron daños internos alrededor del hueso en un 41 % de las nectarinas 'Fantasia' (Cap. 2). Esto puede deberse a que tal y como observó Birla *et al*. (2008) mediante simulación por ordenador, la inmersión de la fruta en agua desplaza ligeramente el punto más caliente hacia el centro de la fruta, de forma que en ese punto se consiguen las temperaturas más altas. Al aumentar la temperatura del agua a 40 ºC y disminuir el tiempo de tratamiento a 4.5 min los problemas de daños internos desaparecieron (Cap. 2). Las temperaturas internas y externas de la fruta después del tratamiento de RF durante 4.5 min y agua a 40 ºC variaron entre 34.1 y 35.8 ºC y entre 43.3 y 45.4 ºC, respectivamente (Cap. 3). Por lo tanto, estos resultados sugieren, que la inmersión de la fruta en agua durante 40 ºC provoca, al igual que ocurrió en las microondas, un calentamiento superficial que además explicaría la ausencia de daños internos en la fruta. Aunque en las radiofrecuencias, la profundidad de penetración no es un factor limitante, al aumentar la temperatura del agua, aumenta su factor de pérdida dieléctrica y por lo tanto, disminuye la profundidad de penetración de las ondas (Wang *et al*., 2008).

El efecto de ambos tratamientos sobre los parámetros estándar de calidad (firmeza, contenido de sólidos solubles y acidez) también se evaluaron después de conservar la fruta durante 2 días a 20 ºC. Por lo general, ninguno de los parámetros se vio afectado por los tratamientos e incluso se observó una firmeza mayor en las nectarinas 'PP-100' tratadas por radiofrecuencias (Cap. 3) y en los melocotones 'Pollero' después del tratamiento por microondas (Cap. 5). Este hecho ya se observó cuando el tratamiento de microondas a 10 kW durante 95 s se aplicó en aire por lo que se ha discutido ampliamente en el apartado 2 de esta discusión.

3.5. Modelización del tratamiento de microondas con la fruta sumergida en agua

Finalmente, con el fin de obtener información que pueda ayudar en el diseño de un equipo de microondas en continuo específico para su aplicación en fruta sumergida en agua, se determinó la relación entre el porcentaje de reducción de la podredumbre parda y la energía total aplicada (potencia x tiempo). Los resultados de los tratamientos de microondas aplicados tanto en nectarinas 'Red Jim' como en melocotones 'Baby Gold 9' sumergidos en agua a 40 ºC se ajustaron a una curva sigmoidea cuyas funciones se calcularon mediante regresión no lineal (Cap. 5).

El parámetro relacionado con la pendiente de la recta tangente a la curva, estimado mediante la regresión, fue de 0.012 kJ^{-1} en las nectarinas 'Red Jim' y 0.011 kJ^{-1} en los melocotones 'Baby Gold 9'. A partir de estos parámetros, se pudo calcular la pendiente de la recta cuyos valores fueron de 0.3 y 0.28, respectivamente, lo que significa que un incremento de 100 kJ puede aumentar el porcentaje de reducción de podredumbre parda un 30 y 28 %, respectivamente. Según las funciones calculadas, la energía de microondas necesaria para reducir un 50 % la incidencia de podredumbre parda es de 855.85 y 862.80 kJ para nectarinas 'Red Jim' y melocotones 'Baby Gold 9', respectivamente. Además, un 100 % de reducción se lograría al aplicar más de 1287 kJ, de modo que si se quiere conseguir un tratamiento de 40 s la potencia de microondas a la que se debería de trabajar es de aproximadamente 32 kW controlando de esta forma la podredumbre parda por completo.

Además, también se observó una relación lineal entre la temperatura interna o externa de la fruta alcanzada al final del tratamiento y la energía total aplicada (Cap. 5). Dado que se observaron daños externos en la fruta cuando el tratamiento de microondas durante 95 s se realizó a 17.5 y 20 kW, la mínima energía total aplicada con la cual se produjeron daños externos en la fruta fue de 1662 kJ. Por lo tanto, según las regresiones lineales, la mínima temperatura externa por encima de la cual se producen daños es 50.9 ºC, de modo que si se aplicara un tratamiento a 32 kW se producirían daños si el tiempo de exposición superara los 52 s.

4. Consideraciones finales

El tratamiento con 200 mg L^{-1} de ácido peracético a 40 ºC durante 40 s es un método de control cuya aplicación comercial sería posible sin grandes inversiones ya que el ácido peracético podría incorporarse en el agua de volcado de las centrales hortofrutícolas utilizada para introducir la fruta a la línea de confección y únicamente sería necesario instalar un sistema en la balsa de volcado para calentar el agua a 40 ºC. Otra opción más sencilla sería la aplicación de un tratamiento de 300 mg L^{-1} de ácido peracético a 20 ºC durante 1 min, que no necesitaría calentamiento y que se podría aplicar en las instalaciones actuales de las centrales. Sin embargo, la mayoría de infecciones con las que llega la fruta a la central son infecciones que se han producido en el campo antes de la cosecha, por lo que en vista a los resultados obtenidos, el tratamiento con ácido peracético no proporcionaría una estrategia que pudiera hacer frente a todas las pérdidas ocasionadas por esta enfermedad, sino que tendría un nivel de control parcial. No obstante, en estudios realizados por Mari *et al*. (1999) bajo condiciones semicomerciales se observó que conidios que habían estado en contacto con soluciones de 250 mg L^{-1} de ácido peracético durante 5 min eran incapaces de producir podredumbre parda en la fruta. Por lo tanto, el ácido peracético podría reducir el nivel de inóculo del agua de volcado y así prevenir que este pueda infectar a la fruta sana. De este modo, el ácido peracético podría sustituir a los desinfectantes comúnmente utilizados en las centrales hortofrutícolas en el agua de volcado, como el cloro, ya que el ácido peracético no solo no genera subproductos tóxicos o mutagénicos sino que además, su actividad es más estable en presencia de materia orgánica y a cambios de pH y temperatura (Kitis, 2004; Vandekinderen *et al.*, 2009).

En cambio, el calentamiento dieléctrico, tanto por radiofrecuencias como por microondas proporcionaron un mejor control de la podredumbre parda tanto en melocotón como en nectarina ya que, por lo general, la eficacia del tratamiento de radiofrecuencias durante 4.5 min con la fruta sumergida en agua a 40 ºC o del tratamiento de microondas durante 60 s con la fruta sumergida en agua a 40 ºC no dependió ni del tiempo de infección, ni de la concentración de inóculo, ni del estado de madurez en la fruta. Además, ambos tratamientos también mostraron una elevada eficacia en el control de infecciones naturales de *Monilinia* spp., una de las grandes limitaciones que presento el tratamiento de ácido peracético con agua caliente.

A pesar de que en el tratamiento de radiofrecuencias la inmersión de la fruta en agua a 40 ºC redujo el tiempo de exposición de 18 min a 4.5 min, este tiempo sigue siendo elevado para su aplicación comercial en continuo durante la confección de la fruta. Un equipo con el que se pudiera tratar la fruta durante ese tiempo tendría una longitud muy grande para que este se pudiera incorporar en la línea de clasificación, y aunque se diseñara un equipo que ocupara menos espacio el tratamiento ralentizaría el proceso de confección. Por lo tanto, su aplicación comercial tendría que ser en discontinuo o en una línea a parte de la de confección y la fruta debería tratarse antes de que esta fuera clasificada. Esto requeriría más trabajo y podría suponer un obstáculo para su aplicación comercial sobre todo para las centrales con gran producción de fruta, pero no para producciones pequeñas.

Por el contrario, el calentamiento por microondas a 20 kW con la fruta sumergida en agua a 40 ºC proporciona un tratamiento eficaz en 60 s que sería un tiempo de exposición más factible para que este tipo de tecnología pudiera aplicarse en continuo incorporándose en la línea de confección de la fruta. Sin embargo, hay que tener en cuenta que para que el tratamiento de microondas pueda utilizarse a nivel comercial primero sería necesario diseñar un equipo de microondas específico que pudiera tratar la fruta sumergida en agua y además permitiera trabajar a mayor potencia, lo que podría reducir el tiempo de tratamiento facilitando de esta forma su aplicación comercial. Además, la incorporación de este equipo a las líneas de confección actuales necesitaría de una fase de puesta a punto del sistema en la que se debería estudiar el efecto de otros factores como la temperatura de entrada del producto, volumen de fruta tratada por hora o la forma del producto por si se quisiera tratar otro tipo de fruta de hueso como podrían ser los paraguayos.

Todos estos aspectos, como el diseño y la modelización de los tratamientos y de su aplicación práctica en la central hortofrutícola pueden ser objeto de estudios posteriores a la realización de la presente tesis.

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Conclusiones/Conclusions/Conclusions

CONCLUSIONES

Control de la podredumbre parda mediante el ácido peracético solo o combinado con agua caliente

1. La combinación de 0.25 % de peróxido de hidrógeno, 0.02 % de ácido peracético y 0.075 % de ácido acético correspondiente a 300 mg L^{-1} de ácido peracético (PAA) aplicado mediante baño durante 1 min, tiene una elevada eficacia en el control de *M. fructicola* y esta eficacia se mantiene cuando la misma concentración de PAA se aplica mediante el producto comercial Proxitane®5:23.
2. La combinación de ácido peracético con agua caliente permite reducir la concentración de ácido peracético a 200 mg L^{-1} al aplicarse con agua a 40 ºC durante 40 s sin afectar negativamente a la calidad de la fruta.

3. La eficacia del tratamiento con 200 mg L^{-1} de ácido peracético a 40 ºC durante 40 s no depende de la concentración de inóculo de *M. fructicola,* pero solo controla infecciones muy recientes, dejando de ser efectivo para infecciones de más de 24 h.

Control de la podredumbre parda mediante el calentamiento por microondas

4. El tratamiento de microondas a 10 kW durante 95 s tiene una elevada eficacia en el control tanto de infecciones artificiales de *M. fructicola* como de infecciones naturales sin afectar negativamente a la calidad de la fruta.

5. La eficacia del tratamiento de microondas a 10 kW durante 95 s no depende del estado de madurez de la fruta, pero sí del peso de esta, lo que provoca una elevada temperatura en los frutos de menor peso que causa daños internos.

Mejora de los tratamientos de radiofrecuencias y microondas mediante la inmersión de la fruta en agua

Tratamiento de radiofrecuencias

6. La inmersión de la fruta en agua a 20 ºC soluciona tanto la falta de eficacia en nectarina como la influencia del tamaño del fruto mostradas por el tratamiento de radiofrecuencias en aire, reduciendo además el tiempo de exposición necesario para el control de *M. fructicola* de 18 a 9 min.

7. El aumento de la temperatura del agua a 35 o 40 ºC reduce el tiempo de exposición necesario hasta 6 y 4.5 min, respectivamente.

8. La eficacia del tratamiento de radiofrecuencias con la fruta sumergida en agua a 40 ºC durante 4.5 min es muy elevada en el control de infecciones naturales de *Monilinia* spp. y además no depende del estado de madurez de la fruta. Sin embargo, la eficacia se ve afectada por la concentración de patógeno y el tiempo entre la infección y el tratamiento.

Tratamiento de microondas

9. El tratamiento de microondas a 10 kW durante 95 s que es eficaz en aire, deja de ser efectivo al aplicarse con la fruta sumergida en agua a 20 ºC pero tiene un cierto control de *M. fructicola* al incrementar la temperatura del agua a 40 ºC.

10. Al aumentar la potencia hasta 20 kW, el tratamiento de microondas con la fruta sumergida en agua a 40 ºC durante 60 s, tiene un elevado control de *M. fructicola* sin afectar a la calidad de la fruta.

11. El tratamiento de microondas a 20 kW durante 60 s con la fruta sumergida en agua a 40 ºC controla las infecciones naturales de *Monilinia* spp. y además, la eficacia no depende ni del peso de la fruta, ni del tiempo entre la infección y el tratamiento, ni de la concentración de *M. fructicola*.

12. La disminución del tiempo de exposición hasta 50 s del tratamiento de microondas a 20 kW con la fruta sumergida en agua a 40 ºC provoca que la eficacia del tratamiento dependa de todos los factores estudiados, peso, tiempo de infección y concentración de inóculo, mostrando niveles de eficacia menores que si se aplica durante 60 s.

CONCLUSIONS

Control de la podridura marró mitjançant l'àcid peracètic sol o combinat amb aigua calenta

1. La combinació de 0.25 % de peròxid d'hidrogen, 0.02 % d'àcid peracètic i 0.075 % d'àcid acètic corresponent a 300 mg L^{-1} d'àcid peracètic (PAA) aplicat mitjançant bany durant 1 min, té una elevada eficàcia en el control de *M. fructicola* i aquesta eficàcia es manté quan la mateixa concentració de PAA s'aplica mitjançant el producte comercial Proxitane®5:23.

2. La combinació d'àcid peracètic amb aigua calenta permet reduir la concentració d'àcid peracètic a 200 mg L^{-1} en aplicar-se amb aigua a 40 ºC durant 40 s sense afectar negativament a la qualitat de la fruita.

3. L'eficàcia del tractament amb 200 mg L^{-1} d'àcid peracètic a 40 ºC durant 40 s no depèn de la concentració d'inòcul de *M. fructicola*, però només controla infeccions molt recents, deixant de ser efectiu per a infeccions de mes de 24 h.

Control de la podridura marró mitjançant l'escalfament per microones

4. El tractament de microones a 10 kW durant 95 s té una elevada eficàcia en el control tant d'infeccions artificials de *M. fructicola* com d'infeccions naturals sense afectar negativament a la qualitat de la fruita.

5. L'eficàcia del tractament de microones a 10 kW durant 95 s no depèn de l'estat de maduresa de la fruita, però sí del pes d'aquesta, la qual cosa provoca una elevada temperatura en els fruits de menor pes que causa danys interns.

Millora dels tractaments de radiofreqüències i microones mitjançant la immersió de la fruita en aigua

Tractament de radiofreqüències

6. La immersió de la fruita en aigua a 20 ºC soluciona tant la manca d'eficàcia en nectarina com la influència del diàmetre del fruit mostrades pel tractament de radiofreqüències en aire, reduint a més el temps d'exposició necessari per al control de *M. fructicola* fins de 18 a 9 min.

7. L'augment de la temperatura de l'aigua a 35 o 40 ºC redueix el temps d'exposició necessari fins a 6 i 4.5 min, respectivament.

8. L'eficàcia del tractament de radiofreqüències amb la fruita submergida en aigua a 40 ºC durant 4.5 min és molt elevada en el control d'infeccions naturals de *Monilinia* spp. i a més, no depèn de l'estat de maduresa de la fruita. No obstant això, l'eficàcia es veu afectada per la concentració de patogen i el temps entre la infecció i el tractament.

Tractament de microones

9. El tractament de microones a 10 kW durant 95 s que és eficaç en aire, deixa de ser efectiu en aplicar-se amb la fruita submergida en aigua a 20 ºC però té un cert control de *M. fructicola* en incrementar la temperatura de l'aigua a 40 ºC.

10. En augmentar la potència fins a 20 kW, el tractament de microones amb la fruita submergida en aigua a 40 ºC durant 60 s, té un elevat control de *M. fructicola* sense afectar a la qualitat de la fruita.

11. El tractament de microones a 20 kW durant 60 s amb la fruita submergida en aigua a 40 ºC controla les infeccions naturals de *Monilinia* spp. i a més, l'eficàcia no depèn ni del pes de la fruita, ni del temps entre la infecció i el tractament, ni de la concentració de *M. fructicola*.

12. La disminució del temps d'exposició fins a 50 s del tractament de microones a 20 kW amb la fruita submergida en aigua a 40 ºC provoca que l'eficàcia del tractament depengui de tots els factors estudiats, pes, temps d'infecció i concentració d'inòcul, mostrant nivells d'eficàcia menors que si s'aplica durant 60 s.

CONCLUSIONS

Peracetic acid alone or in combination with hot water to control brown rot

1. The combination of 0.25 % hydrogen peroxide, 0.02 % peracetic acid and 0.075 % acetic acid corresponding to 300 mg L^{-1} of peracetic acid (PAA) applied by dipping fruit for 1 min effectively controls *M. fructicola* and similar control is observed when the same PAA concentration is applied with the commercial product Proxitane®5:23.

2. The combination of peracetic acid with hot water reduce the effective peracetic acid concentration to 200 mg L^{-1} when it is applied with hot water at 40 ºC for 40 s without negatively affecting fruit quality.

3. The efficacy of 200 mg L^{-1} of peracetic acid at 40 ºC for 40 s does not depend on inoculum concentration of *M. fructicola*, but it only controls recent infections, since it is not effective to control infections of over 24 h.

Microwave heating to control brown rot

4. Microwave treatment at 10 kW for 95 s effectively controls both artificial and natural infections of *Monilinia* spp. without negatively affecting fruit quality.

5. The efficacy of microwave treatment at 10 kW for 95 s does not depend on fruit maturity level, but it depends on fruit weight, which causes internal thermal damages in the smallest fruit due to the high temperatures achieved by these fruit.

Immersion of fruit in water to improve radio frequency and microwave treatment

Radio frequency treatment

6. Immersion of fruit in water at 20 ºC solves both the lack of efficacy in nectarines as the influence of fruit size showed by radio frequency treatment in air and moreover, it reduces exposure time required to control *M. fructicola* from 18 to 9 min.

7. The increase of water temperature to 35 or 40 ºC reduces the required exposure time to 6 and 4.5 min, respectively.

8. Radio frequency treatment for 4.5 min with fruit immersed in water at 40 ºC presents high efficacy to control natural infections of *Monilinia* spp. and besides, treatment efficacy does not depend on fruit maturity level. However, the efficacy is affected by pathogen concentration and time between infection and treatment.

Microwave treatment

9. Microwave treatment at 10 kW for 95 s that is effective in air, ceases to be effective when it is applied with fruit immersed in water at 20 ºC, but it has a certain control of *M. fructicola* by increasing water temperature to 40 ºC.

10. By increasing power to 20 kW, microwave treatment for 60 s with fruit immersed in water at 40 ºC effectively controls *M. fructicola* without affecting fruit quality.

11. Microwave treatment at 20 kW for 60 s with fruit immersed in water at 40 ºC controls natural infections of *Monilinia* spp. and its efficacy is not affected by fruit weight, time between infection and treatment and *M. fructicola* concentration.

12. The reduction of exposure time to 50 s of microwave treatment at 20 kW with fruit immersed in water at 40 ºC causes treatment efficacy to depend on all studied factors, weight, infection time and inoculum concentration, by showing levels of efficacy lower than those observed when it is applied for 60 s.