UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Programa de Doctorado en Ciencia,
Tecnología y Gestión Alimentaria (RD 99/2011)

Reducing postharvest losses in persimmon. Pre and postharvest aspects involved in fruit quality and new strategies for valorization

PhD Thesis

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Valencia, November 2023

Yo no sé nada más que algo bien insignificante: simplemente recibir el argumento de un sabio y tratarlo en su justa medida

Sócrates

Agradecimientos

No es una tarea fácil reflejar en pocas líneas toda mi gratitud a las personas que han contribuido para que esta Tesis Doctoral se haya convertido en una realidad. Primeramente, quiero expresar mi más sincero y profundo agradecimiento a Alejandra Salvador por creer en mi potencial y por haber confiado en mí desde el primer momento. Ha sido un privilegio contar con su enorme dedicación, esfuerzo y cariño a lo largo de estos años. También agradezco a Ana Quiñones por su orientación y apoyo en mi trabajo.

Asimismo, quiero dar las gracias a todos mis compañeros del Centro de Postcosecha del IVIA, que, más que colegas, han sido mi familia. Agradezco especialmente a Rebeca por su apoyo incondicional a lo largo de este trabajo. Es para mí un gran honor contar con tu amistad y compañía dentro y fuera del laboratorio. A Carmen, por todo el cariño y cuidado que siempre me ha ofrecido. A Pilar por mostrarse siempre dispuesta a ayudarme a lo largo de estos cuatro años. Extiendo mis agradecimientos también a Ana Pilar, Francesc y Lourdes, así como a Oria, Paula, Marco y Gaia. Ha sido un placer trabajar con vosotros.

A Almudena, líder del Centro de Citricultura y Producción Vegetal, y a Juani por toda la ayuda y desempeño en el análisis de biocomponentes.

Al equipo del Centro para el Desarrollo de Agricultura Sostenible, Julia, Isabel y Ana, por los conocimientos que me han aportado sobre nutrición de plantas.

Al grupo de Microestructura y Química de Alimentos de la UPV, Isabel, Empar, Gemma, Amparo y Virginia, por su colaboración en partes fundamentales de esta Tesis.

A los investigadores y compañeros de la Universidad de Sao Paulo, ESALQ (Brasil) y de la Universidad de Cranfield (Reino Unido), con los que he compartido mi experiencia en las estancias que he realizado.

A la Cooperativa Agrícola Ntra. Sra. del Oreto Coop. V., por su apoyo técnico y aporte de material vegetal. En especial agradezco a Mario su compromiso directo con la investigación desarrollada.

Agradezco también a la Cooperativa Agrícola Sant Bernat Coop. V., en concreto a Carlos y Octavio por involucrase en la búsqueda, gestión y manejo de las parcelas bajo cultivo convencional y ecológico para los estudios que se han llevado a cabo.

A Anecoop S. Coop. especialmente a Nicolás, Germán y Javier por su iniciativa, empeño y fe en el proceso de secado como herramienta para valorizar el cultivo de caqui.

A AgroFresh Spain S.L.U. y Natural Hand S.L. por el asesoramiento técnico y material vegetal en parte de los ensayos realizados.

Agradezco también a las personas que hicieron que este camino fuera más ligero. Gracias Drielle, Ricardo, Fernanda y Víctor por compartir conmigo momentos de alegría y desesperación. A mi familia y amigos en Brasil que, aun estando lejos, siempre os tengo a mi lado. Especialmente darte las gracias a ti, Lucas, mi compañero en la vida, por tu cariño, paciencia, optimismo y apoyo incondicional.

Finalmente, agradezco al Ministerio de Ciencia e Innovación de España por la beca FPI y al IVIA por el contrato predoctoral.

ABSTRACT

Persimmon is a crop of great relevance in Spain, which has become the world's second producer and the largest exporter. This production centers mainly on cv. Rojo Brillante in the Valencian Community. In recent years however, Spain has experienced overproduction, which has led to important economic losses and increased fruit waste. Moreover, different preharvest factors, which affect fruit quality, and an inadequate postharvest management, can lead to important postharvest losses. In this context, the objective of the present Thesis is to study strategies to increase persimmon crop profitability by reducing postharvest losses and revalorizing discarded fruit and surplus production. For this purpose, the pre- and postharvest aspects involved in persimmon fruit quality are discussed. Besides, whole fruit drying is proposed as a new valorization strategy.

Of the preharvest aspects related to fruit quality parameters, the plant nutritional status is a major factor that can affect plant material composition and fruit characteristics. Thus, **Chapters I and II** addressed the study of macro- and micronutrients concentrations in the leaves and fruit from organic and conventional management systems, and their relation to the main fruit quality parameters in 'Rojo Brillante' persimmon. The main biocomponents in the fruit from both crop systems were also evaluated. The greater macro- and microelements supplied in the conventional vs. the organic system did not imply a higher accumulation of these elements in leaves and fruit. Macroelements concentration in fruit depended on the fruit flesh area evaluated (apical or basal). The correlation between macronutrients and fruit quality parameters revealed that the Ca and Mg, and the N/Ca and Ca/(K+Mg) ratios were closely related to color, firmness, total soluble solids, and soluble tannins content. The influence of crop management on fruit biocomponents concentration was observed only for malic acid, β-Cryptoxanthin and ascorbic acid, which were higher in the organic than the conventional fruit. The greatest agronomic efficiency noted in the organic crop indicates that the lower fertilization rate in such management is adequate for obtaining fruit with optimal nutrient concentrations and reveals the important role of organic matter in favoring nutrient assimilation. The results obtained in this study

provide new information about the nutritional composition of persimmon grown by organic and conventional management and reinforce the need for balanced fertilization to achieve good fruit quality.

Another preharvest aspect to consider in persimmon management is the application of phytoregulators to prolong the harvesting season. For 'Rojo Brillante', ethephon use is normal to advance harvest, as is gibberellic acid (GA3) to delay maturation. These treatments can affect fruit quality at both harvest and postharvest. Therefore, the postharvest 1-methylcyclopropene (1-MCP) treatment is commonly applied to guarantee postharvest fruit quality. However, the pre-harvest 1-MCP application has been shown to be a novel effective treatment for other fruit types, but with very little information for persimmon. In **Chapter III**, the effect of this treatment was evaluated in different scenarios combined with ethephon or GA3. The results showed that preharvest 1-MCP extended the harvest window and prolonged the commercialization period when applied to the ethephon-treated fruit. In the GA3-treated fruit, which are to be cold-stored, the preharvest 1-MCP treatment can replace the 1-MCP postharvest application, which could be a useful tool for optimizing handling operations in packinghouses.

For 'Rojo Brillante', an effect of harvest moment on fruit behavior during cold storage has been commercially documented. To explain these differences, in **Chapter IV** an in-depth physico-chemical and microstructural characterization of fruit (pretreated with GA3) during five commercial harvests (from November to December) is carried out. Moreover, the firmness changes during cold storage at 0 °C up to 90 days are evaluated. During the studied harvest period, fruit presented high firmness values, which are useful for fruit to be cold stored. Nevertheless, the minor differences revealed in persimmon firmness at harvest strongly influenced fruit behavior during cold storage. Accordingly, the fruit harvested in mid-November had the highest storage potential by maintaining firmness high for up to 90 days, which did not happen in the fruit from subsequent harvests. These differences in postharvest behavior were associated with structural parenchyma integrity at harvest.

On the other hand, it is noteworthy that deastringency treatment is one of the main steps of postharvest handling associated with postharvest losses because it directly influences final fruit quality. Although high CO_2 concentrations are the most commercially applied deastringency treatment, ethanol is also employed in some countries like Brazil. With 'Giombo' persimmon, one of the main cultivars in this country, complete astringency loss proves difficult. In this Thesis (**Chapter V**), the 'Giombo' persimmon treated with ethanol or CO_2 to remove astringency is evaluated to know the physico-chemical and microstructural changes that occur during cold storage. The results suggested that, although ethanol is the usual deastringency treatment for 'Giombo', high CO_2 concentrations are recommended to achieve the fastest tannins insolubilization and to maintain greater flesh firmness during cold storage.

In order to valorize discarded persimmon fruit and surplus production, in this Thesis, whole fruit drying is proposed as a new strategy for 'Rojo Brillante'. A first study (**Chapter VI**) approaches the physico-chemical and microstructural changes that occur during the natural-air drying of persimmon. The obtained results revealed that this variety is suitable for being subjected to the natural drying process by taking into account that the maturity stage influences final product characteristics.

To improve the drying process, convective hot-air drying is also evaluated (**Chapter VII**). After evaluating drying at three temperatures (35 ºC, 40 ºC and 45 ºC), the results showed that the higher the drying temperature, the faster the drying process was, and the final product characteristics depended on the drying temperature, especially in texture terms. Drying at 35 ºC resulted in a product with similar physico-chemical attributes to those achieved by the natural drying method, but in a much shorter time, which improves the commercial viability of this treatment.

RESUMEN

El cultivo del caqui en España ha sufrido un incremento exponencial en los últimos años. En la actualidad España se ha convertido en el segundo productor y el primer exportador a nivel mundial, con una producción centrada principalmente en el cv. Rojo Brillante en la Comunidad Valenciana. Sin embargo, en los últimos años se ha experimentado una sobreproducción, provocando importantes pérdidas económicas por la caída de los precios y un aumento del desperdicio de fruta. Además, diferentes factores precosecha, así como un inadecuado manejo tras la cosecha, pueden afectar a la calidad del fruto y conducir a importantes pérdidas postcosecha. En este contexto, esta Tesis aborda diferentes estrategias para aumentar la rentabilidad del cultivo del caqui mediante la reducción de las pérdidas postcosecha y la revalorización de la fruta que se descarta por baja calidad y de los excedentes de producción. Para ello, se estudian aspectos pre y postcosecha implicados en la calidad del fruto y se propone el secado de la fruta entera como una nueva estrategia de valorización de los frutos que no se destinan para fresco.

Entre los aspectos precosecha, el estado nutricional de la planta es un factor clave que puede afectar a la composición del material vegetal y a las características del fruto. Así, los **Capítulos I y II** abordan el estudio de las concentraciones de macro y micronutrientes en hojas y frutos procedentes de parcelas cultivada bajo condiciones de manejo ecológico y convencional, y su relación con los principales parámetros de calidad del caqui 'Rojo Brillante'. También se evaluan los principales biocomponentes en el fruto de ambos sistemas de cultivo. El mayor aporte de macro y microelementos en el sistema convencional frente al ecológico no implicó una mayor concentración de estos elementos en hojas y fruto. La acumulación de macroelementos en el fruto dependió del área de la pulpa evaluada (apical o basal). La correlación entre macronutrientes y parámetros de calidad del fruto reveló que el Ca, el Mg y los ratios N/Ca y Ca/(K+Mg) se relacionaron estrechamente con el color, la firmeza, y el contenido en sólidos solubles totales y taninos solubles. El efecto del manejo del cultivo en la concentración de biocomponentes del fruto solo se observó para el ácido málico, la β-Criptoxantina y el ácido ascórbico, que resultaron mayores en los frutos ecológicos que en los convencionales. La

mayor eficiencia agronómica observada en el cultivo ecológico indicó que la menor tasa de fertilización durante dicho manejo fue adecuada para obtener frutos con una concentración óptima de nutrientes.

Otro aspecto precosecha a tener en cuenta en el manejo del caqui es la aplicación de fitorreguladores con la finalidad de ampliar el periodo de recolección. En 'Rojo Brillante', es habitual el uso de etefón para adelantar la cosecha, así como de ácido giberélico (AG3) para retrasar la maduración. Estos tratamientos pueden afectar a la calidad de la fruta tanto en cosecha como en postcosecha. Por ello, el tratamiento postcosecha con 1-MCP se aplica habitualmente para garantizar la calidad de la fruta tras la cosecha. Sin embargo, de forma novedosa, la aplicación precosecha de 1-MCP se ha demostrado que tiene efecto positivo en diferentes frutos, pero hay poca información de su efecto en caqui. Así, en el **Capítulo III**, se evalúa el efecto de este tratamiento en diferentes escenarios, en fruta tratada con etefón o con AG3. Los resultados mostraron que la aplicación precosecha del 1-MCP permitió ampliar la ventana de recolección y prolongar el periodo de comercialización cuando se aplicó a la fruta tratada con etefón. En los frutos tratados con AG3, destinados a almacenamiento frigorífico, el tratamiento precosecha con 1-MCP tuvo el mismo efecto que su aplicación postcosecha, lo que lo convierte en una herramienta útil para optimizar las operaciones de manipulación en los almacenes de confección.

En el caso del 'Rojo Brillante', se ha observado a nivel comercial un efecto del momento de cosecha sobre el comportamiento del fruto durante el almacenamiento frigorífico. Para explicar estas diferencias, en el **Capítulo IV** se llevó a cabo la caracterización fisicoquímica y microestructural de frutos (pretratados con AG3) en cinco momentos de cosecha (de noviembre a diciembre). Además, se evaluaron los cambios de firmeza durante el almacenamiento a 0 °C hasta 90 días. Durante el periodo de recolección estudiado, los frutos presentaron valores de firmeza suficientemente elevados para un almacenamiento prolongado. Sin embargo, pequeñas diferencias en la firmeza del fruto en el momento de cosecha influyeron de manera importante en el comportamiento del fruto durante el almacenamiento. Así, la fruta recolectada a mediados de noviembre presentó el mayor potencial de

conservación, manteniendo una firmeza elevada hasta 90 días, lo que no se observó en la fruta cosechada posteriormente. Estas diferencias en el comportamiento postcosecha fueron asociadas a la integridad estructural del parénquima de la pulpa en el momento de cosecha.

Por otro lado, hay que tener en cuenta que el tratamiento postcosecha de desastringencia se asocia a importantes pérdidas tras la cosecha, ya que influye en la calidad final del fruto. Aunque las altas concentraciones de CO_2 es el tratamiento de desastringencia más aplicado comercialmente, el etanol también se emplea en algunos países como Brasil. En el caso del caqui 'Giombo', uno de los principales cultivares de este país, la pérdida completa de astringencia tras cosecha resulta dificultosa. En esta Tesis (**Capítulo V**), se evaluó el efecto del tratamiento con etanol o con CO_2 en los cambios fisicoquímicos y microestructurales que ocurren durante el almacenamiento posterior en caqui 'Giombo'. Los resultados sugieren que, aunque el etanol es el tratamiento habitual de desastringencia para esta variedad, se recomiendan las altas concentraciones de CO_2 para conseguir una insolubilización más rápida de los taninos y el mantenimiento de la firmeza de la pulpa durante el posterior almacenamiento en frío.

Con el fin de valorizar los frutos de caqui descartados por baja calidad y los excedentes de producción, en esta Tesis se propone el secado de fruto entero como una nueva estrategia para el 'Rojo Brillante'. En el **Capítulo VI** se estudiaron los cambios fisicoquímicos y microestructurales que se producen en el fruto durante el secado natural. Los resultados obtenidos revelaron que esta variedad es apta para ser sometida al proceso de secado.

Para mejorar el proceso de secado, también se evaluó el secado por aire caliente (**Capítulo VII**). Tras evaluar el secado del fruto a tres temperaturas (35 °C, 40 °C y 45 °C), se observó una mayor rapidez en el proceso a mayor temperatura. Las características finales del producto fueron diferentes en función de la temperatura de secado, especialmente en términos de textura. El secado a 35 °C dio lugar a un producto con atributos fisicoquímicos similares a los conseguidos por el método de secado natural, pero en un tiempo mucho menor, lo que mejora la viabilidad comercial de este tratamiento.

RESUM

El cultiu del caqui a Espanya ha patit un increment exponencial en els últims anys. En l'actualitat Espanya s'ha convertit en el segon productor i el primer exportador a nivell mundial. La producció està centrada principalment en el cv. Rojo Brillante a la Comunitat Valenciana. No obstant això, en els últims anys, s'ha experimentat una sobreproducció, provocant importants pèrdues econòmiques per la caiguda dels preus i un augment del desaprofitament de fruita. D'altra banda, diferents factors precollita, així com un inadequat maneig després de la collita poden afectar la qualitat del fruit i conduir a importants pèrdues postcollita. En aquest context, esta Tesi aborda diferents estratègies per a augmentar la rendibilitat del cultiu del caqui mitjançant la reducció de les pèrdues postcollita i la revaloració de la fruita que es descarta per baixa qualitat i pels excedents de producció. Per a això, s'estudien aspectes pre i postcollita implicats en la qualitat del caqui. A més, es proposa l'assecat de la fruita sencera com una nova estratègia de valorització del fruit que no es destina per a consumir en fresc.

Entre els aspectes precollita, l'estat nutricional de la planta és un factor clau que pot afectar la composició del material vegetal i a les característiques del fruit. Així, els **Capítols I i II** aborden l'estudi de les concentracions de macro i micronutrients en fulles i fruits procedents de parcel·les cultivada sota condicions de maneig ecològic i convencional, i la seua relació amb els principals paràmetres de qualitat del caqui 'Rojo Brillante'. També es van avaluar els principals biocomponentes en el fruit dels dos sistemes de cultiu. La major aportació de macro i microelements en el sistema convencional enfront de l'ecològic no va implicar una major concentració d'estos elements en fulles i fruit. L'acumulació de macroelements en el fruit va dependre de l'àrea de la polpa avaluada (apical o basal). La correlació entre macronutrients i paràmetres de qualitat del fruit va revelar que el Ca, el Mg i els ràtios N/Ca i Ca/(K+Mg) es van relacionar estretament amb el color, la fermesa, el contingut en sòlids solubles totals i tanins solubles. L'efecte del maneig del cultiu en la concentració de biocomponentes del fruit només es va observar per a l'àcid màlic, la β-Criptoxantina i l'àcid ascòrbic, que van resultar majors en els fruits ecològics que en els convencionals. La major eficiència agronòmica observada

en el cultiu ecològic va indicar que la menor taxa de fertilització durant aquest maneig és adequada per a obtindre fruits amb una concentració òptima de nutrients.

Un altre aspecte precollita a tindre en compte en el maneig del caqui, és l'aplicació de fitorreguladors amb la finalitat d'ampliar el període de recol·lecció. En 'Rojo Brillante', és habitual l'ús de etefon per a avançar la collita, així com d'àcid gibberèl·lic (AG3) per a retardar la maduració. Aquests tractaments poden afectar la qualitat de la fruita tant en collita com en postcollita. Per això, el tractament postcollita amb 1-MCP s'aplica habitualment per a garantir la qualitat de la fruita en postcollita. No obstant això, de manera nova, l'aplicació precollita de 1-MCP, s'ha demostrat que té efecte positiu en diferents fruits, però no hi ha informació del seu efecte en caqui. Així, en el **Capítol III**, es va avaluar l'efecte d'aquest tractament en diferents escenaris, en fruita tractada amb etefón o amb AG3. Els resultats van mostrar que l'aplicació precollita del 1-MCP va permetre ampliar la finestra de recol·lecció i prolongar el període de comercialització quan es va aplicar a la fruita tractada amb etefón. En els fruits tractats amb AG3, destinats a emmagatzematge frigorífic, el tractament precollita amb 1-MCP va tindre el mateix efecte que la seua aplicació postcollita el que el converteix en una eina útil per a optimitzar les operacions de manipulació en els magatzems de confecció.

En el cas de 'Rojo Brillante', s'ha observat a nivell comercial un efecte del moment de collita sobre el comportament del fruit durant l'emmagatzematge frigorífic. Per a explicar estes diferències, en el **Capítol IV** es va dur a terme una caracterització fisicoquímica i microestructural de fruits (pretractats amb AG3) en cinc moments de collita (de novembre a desembre). A més, es van avaluar els canvis de fermesa durant l'emmagatzematge a 0 °C fins a 90 dies. Durant el període de recol·lecció estudiat, els fruits van presentar valors de fermesa prou elevats per a ser emmagatzemats en fred. No obstant això, xicotetes diferències en la fermesa del fruit en el moment de collita van influir de manera important en el seu comportament durant l'emmagatzematge. Així, la fruita recol·lectada a mitjan novembre va presentar el major potencial de conservació, mantenint una fermesa elevada fins a 90 dies, la qual cosa no es

va observar en la fruita collida posteriorment. Estes diferències en el comportament postcollita estan associades a la integritat estructural del parènquima de la polpa en el moment de la recol·lecció.

Cal tindre en compte que el tractament de desastringencia és una de les principals etapes en la manipulació postcollita del caqui associat a important pèrdues postcollita, ja que influeix en la qualitat final de la fruita. Encara que el tractament de desastringencia amb elevades concentracions de CO_2 és el més aplicat comercialment, l'etanol també s'utilitza en alguns països com Brasil. En el cas del caqui 'Giombo', un dels principals cultivars d'este país, la pèrdua completa d'astringencia després de collita resulta dificultosa. En esta Tesi (**Capítol V**), es va avaluar l'efecte del tractament amb etanol o amb CO_2 en els canvis fisicoquímics i microestructurals que ocorren durant l'emmagatzematge posterior en caqui 'Giombo'. Els resultats van suggerir que, encara que l'etanol és el tractament habitual de desastringencia per a 'Giombo', es recomanen les altes concentracions de CO_2 per a aconseguir una insolubilización més ràpida dels tanins i el manteniment de la fermesa de la polpa durant l'emmagatzematge en fred.

Amb la finalitat de valorar els fruits de caqui descartats per baixa qualitat i els excedents de producció, en esta Tesi es proposa l'assecat de fruit sencer com una nova estratègia per al 'Rojo Brillante'. En el **Capítol VI** es van estudiar els canvis fisicoquímics i microestructurals que es produeixen en el fruit durant l'assecat natural. Els resultats obtinguts van revelar que esta varietat és apta per a ser sotmesa al procés d'assecat tenint en compte que l'estat de maduresa va influir en les característiques finals del producte.

Per a millorar el procés d'assecat, també es va avaluar l'assecat per aire calent (**Capítol VII**). Després d'avaluar el procés d'assecat a tres temperatures (35 ºC, 40 ºC i 45 ºC), es va observar una major rapidesa en el procés d'assecat a major temperatura. Les característiques finals del producte van ser diferents en funció de la temperatura d'assecat, especialment en termes de textura. L'assecat a 35 ºC va donar lloc a un producte amb atributs fisicoquímics similars als aconseguits pel mètode d'assecat natural, però en un temps molt menor, la qual cosa millora la viabilitat comercial d'este tractament.

INDEX

I. INTRODUCTION

I. INTRODUCTION

I.1. Origin, Botany and Morphology

Persimmon (*Diospyros kaki* Thunb.) is a species from the family Ebenaceae. It was originated from China more than 3,000 years ago and extended to Japan in the 7th century and to Korea in the 9th century (Perucho et al., 2015; Woolf and Ben-Arie, 2011). The first registrations of its cultivation in Europe date back to the 17th century, when it extended throughout the Mediterranean basin. Migratory flows from Asian countries to North America also introduced persimmon cultivation in California (USA) and Brazil (Badenes et al., 2015).

The genus Diospyros grows almost exclusively in tropical and subtropical areas and includes more than 400 species. The best known and most widely cultivated one is *D. kaki* with wide varietal diversification in China, Japan and South Korea, where more than 2,000 varieties have been described. Only species *D. kaki*, *D. lotus* and *D. virginiana* are adapted to be grown in temperate zones, and the last two are used mainly as rootstocks (Badenes et al., 2015).

In Spain, persimmon was initially found as isolated trees on the margins of plots and close to rural buildings in Catalonia, Andalusia and the Valencian Community. From the ancestral genotypes introduced in Europe, it has evolved to current local varieties (Perucho et al., 2015). In the 20th century, small commercial crop growers began to grow the commonest varieties, such as 'Tomatero' in the Alto Palancia region (Castellón), and 'Picudo' ('Costata') and 'Cristalino' in the Ribera Alta region (Valencia). 'Rojo Brillante' appeared in this region and has led to major improvement in persimmon cultivation in the Valencian Community. Persimmon production in this area now centers on this variety. The origin of the cv. Rojo Brillante is not entirely clear, but the most accepted hypothesis is that it comes from a mutation of the cv. Cristalino (Badenes et al., 2015; Perucho et al., 2015).

Persimmon is a deciduous species with well-defined phenological phases similar to other tree fruit species of temperate climate (Figure 1). Fruit are generally parthenocarpic, i.e. they are seedless due to lack of pollination,

which is one of the main reasons for it being well appreciated on the market (Giordani et al., 2015).

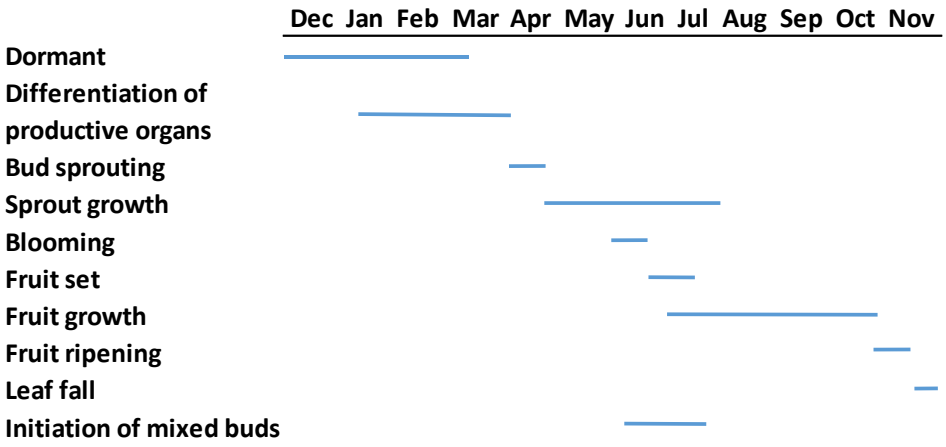

Figure 1. Phenological stages of the 'Rojo Brillante' persimmon in Spain (adapted from Giordani et al., 2015).

Fruit weight varies from a few grams to more than 500 g depending on the cultivar, agroclimatic conditions and tree age. Fruit shape is also very variable, and can be flat, round or elongated. Upon commercial maturity, fruit skin is yellow to orange, and changes to a deep red with ripening. Flesh color varies according to the cultivar, and can be yellow-orange, pink, red-brown or bronze (Giordani et al., 2015; Woolf and Ben-Arie, 2011). Persimmon fruit possess a large green four-lobed calyx around the fruit stem-end. It plays an important role in fruit growth and development for being photosynthetically active and a gas exchange area, since there are no stomata or lenticels on the fruit skin surface (Giordani et al., 2015; Woolf and Ben-Arie, 2011).

One important characteristic of persimmon fruit is the presence of high soluble tannins content, which causes astringency perception by the consumer. Astringency refers to the complex of sensations due to the shrinking, drawing or puckering of the epithelium tissue that covers the tongue as a result of its exposure to tannin acids (ASTM, 1995). The intensity of its perception depends

on the soluble tannins concentration in fruit (Salvador et al., 2020; Tessmer et al., 2014).

Tannins are water-soluble phenolic compounds, and those found in persimmon fruit belong to the group of proanthocyanidins or condensed tannins. They are polymers made of elementary flavan-3-ol units that produce anthocyanidins upon heating in acid media (Rauf et al., 2019; Santos-Buelga and Scalbert, 2000). Soluble tannins in persimmon accumulate in the vacuoles of specialized cells named “tannic cells” (Salvador et al., 2007; Tessmer et al., 2018). When fruit is eaten, soluble tannins are released and react with the salivary proteins in the mouth to form insoluble complexes (Yonemori et al., 2003). These complexes lead to decreased salivary lubrication, which results in perceived astringency.

According to the astringency level at harvest, persimmon cultivars are classified into astringent and non-astringent (Yonemori et al., 2003). Moreover, in both cases, there are cultivars in which fruit astringency is influenced by pollination (pollination variant) and cultivars whose fruit are not affected by pollination (pollination constant). Therefore, persimmon cultivars can be classified into four types (Min et al., 2018): 1) the pollination-constant and non-astringent (PCNA) cultivars present fruit that are always non-astringent in the mature stage because tannins accumulation stops in very early fruit development stages; 2) the pollination-constant and astringent (PCA) cultivars are characterized by always having astringent fruits when mature; 3) the pollination-variant and non-astringent (PVNA) cultivars lose astringency if pollinated and seeds form. Flesh darkens and loses astringency due to the acetaldehyde produced by seeds that insolubilize tannins and trigger oxidation processes; 4) the pollination-variant and astringent (PVA) cultivars, which include the cv. Rojo Brillante. They are characterized by fruit that lose astringency only in the region around seeds, which form when pollinated (Badenes et al., 2015; Besada and Salvador, 2018).

The PCNA persimmon cultivars have low soluble tannins content and can be consumed with high firmness after harvest. However, as the other cultivars present a high soluble tannins content at harvest, they should be subjected to postharvest desastringency treatments before marketing, or otherwise be left

on trees until they overripen and can be consumed accordingly as soft persimmons.

I.2. Production and Economic Value

I.2.1. Worldwide Production

Persimmon is cultivated in many regions of the world, with increasing annual production. According to the FAOSTAT (2022) database, the worldwide persimmon production in 2021 was 4,332,166.55 t on 1,032,183 ha of cultivated area.

The 2019 country ranking indicates that China is the largest producer, with a production of 3,286,620 t that year (Figure 2). Its production is based on a wide range of varieties (Guan et al., 2020; Perucho, 2019). The second most important producer is Spain, with 482,646 t in 2019, mainly centered on the 'Rojo Brillante' cultivar (PVA) in the Valencian Community (eastern Spain), and a minority of the Triumph cultivar (PVA) in Andalusia (southern Spain) (MAPA, 2023; Perucho, 2019). The third largest producer is South Korea, a country with a very long-standing persimmon growing tradition, with production of 258,874 t in 2019. The primary cultivar in this country is the non-astringent 'Fuyu'. Japan, whose persimmon production has continuously decreased in the last six decades, still is the fourth largest producer, with 208,200 t in the same year. Besides 'Fuyu', PVA cultivars 'Hiratanenashi' and 'Tonewase' are the main varieties cultivated in this country (Giordani, 2022). While China, S. Korea and Japan mainly produce processed persimmons, such as dried fruit, juice, teas and powders, Spanish production is marketed mainly as fresh fruit (Sugiura and Taira, 2009; Suh and Kim, 2013).

Azerbaijan, with a growing production, grew 177,129 t in 2019, centered mainly on local astringent varieties. Brazil's production is similar, with 167,721 t on a large production area, mainly used to grow local astringent cultivars, such as 'Rama Forte', 'Giombo' and 'Taubaté' (Perucho et al., 2015). Most of the production in Brazil is destined to its domestic market. However, by having a harvesting season during a period when no fresh fruit is produced in Europe,

in recent years the Brazilian persimmon has filled a gap on the international market and production destined for exportation has increased (Wit, 2019).

Uzbekistan, with an increasing production trend, grew 84,200 t, based on local astringent varieties. In Italy, with 49,675 t and a negative production trend, production centers almost exclusively on the local astringent cultivar Kaki Tipo. Non-astringent cultivars have also been introduced in this country, but with a low commercial impact (Giordani, 2022; Perucho et al., 2015).

The production in Iran and Israel was 29,699 t and 27,000 t, respectively. In Iran, production has considerably increased in the last 10 years and centers mainly on local astringent cultivars. In Israel, production is rising and focuses mostly on the astringent cultivar Triumph (Giordani, 2022; Khademi et al., 2022).

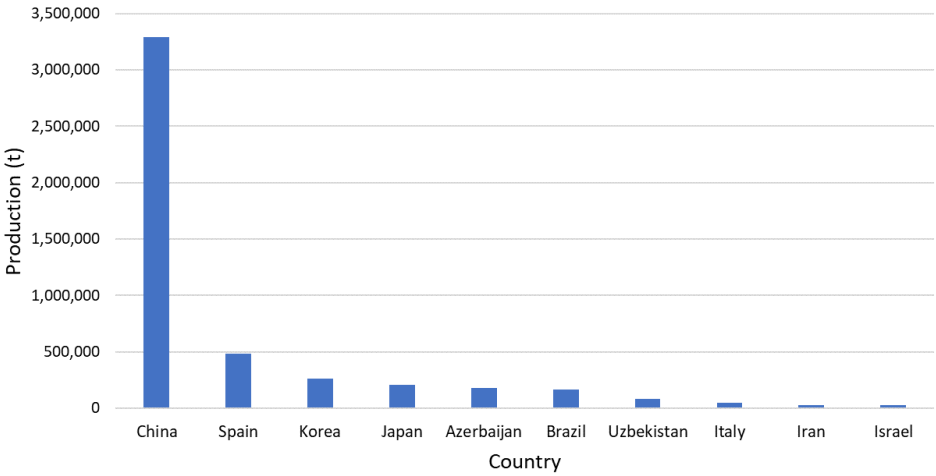

Figure 2. Worldwide persimmon production in 2019. Source: FAOSTAT (2022); Giordani (2022); MAPA (2023).

Of the main exporting countries, the largest persimmon supply worldwide comes from Spain, with a total gain of $238 M in 2021, followed by Azerbaijan ($126 M) and China ($124 M). Together these three countries represent 74 % of global exports (Table 1) (ITC, 2023). This is associated with the introduction

of new postharvest technology for astringency removal and quality maintenance during marketing and fruit storage, which have allowed the commercialization of persimmon on markets far from production areas.

Table 1. Main persimmon exporting countries.

COUNTRY	2019	2020	2021	% of global exports in 2021
	million USD			
Spain	217	243	238	36.1
Azerbaijan	105	92	126	19.0
China	127	206	124	18.7
Uzbekistan	33	49	34	34.4
Lithuania	12	11	10	10.2
Others				19.4

Source: ITC (2023)

I.2.2. Production in Spain

Spain is the main persimmon producer of the countries of the Mediterranean basin and its area of influence. This is due mostly to the emergence of the astringent cultivar Rojo Brillante, which is very productive and of high quality (Perucho et al., 2015).

Between 2004 and 2022, the persimmon cultivation surface area in Spain increased 6-fold and, only in 2022, the Valencian Community had a production area covering 14,430 ha. This represents 90 % of the national persimmon cultivation area, and it produced 407,085 t, which is 93 % of the whole country (Figure 3). Other producer areas in Spain are located mostly in Huelva, a province in southern Spain, based on the cultivar Triumph (Badenes et al., 2022).

In the Valencian Community, persimmon has become an alternative to citrus given its lack of profitability, and together with the improved postharvest technology to eliminate 'Rojo Brillante' astringency, has led to the rapid

development of the production of this cultivar (Fernández-Zamudio et al., 2020; Llácer and Badenes, 2008). This technology has made it possible to obtain some fruit without astringency, while preserving a firm texture, which has, in turn, led to higher market demand and selling to more far-off markets (Perucho, 2019).

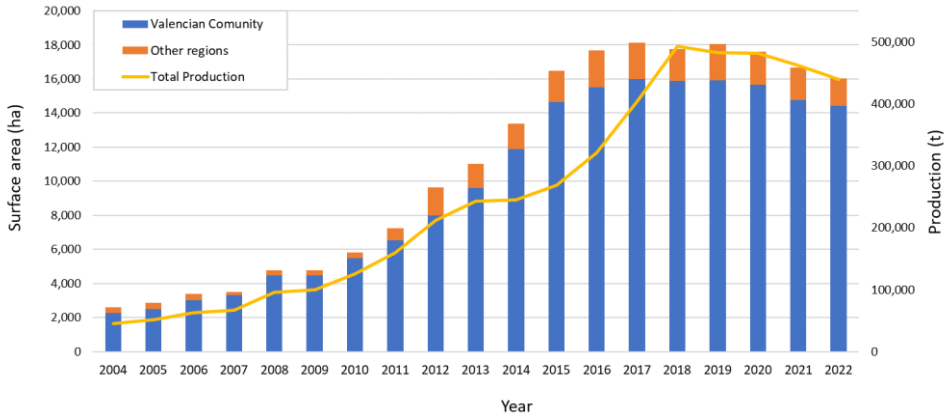

Figure 3. Evolution of the persimmon surface area and production volume in Spain. Source: FAOSTAT (2022); MAPA (2023).

Persimmon production in Spain is largely destined for exports, which account for around 45 % of the total production (Badenes et al., 2022). The main importers of Spanish persimmon are European Union countries, followed by Russia, besides exporting to overseas countries like the United States and China (Mordor Intelligence, 2023). Nevertheless, in recent years, Spain has suffered overproduction, with falling prices associated with adverse weather conditions and the impact of pests. This has led to more plots with a significant volume of unharvested fruit, which brings about important economic losses and a consequent increase in food losses (Fernández-Zamudio et al., 2020). Thus, one of the challenges that the persimmon producing Sector faces is to develop strategies to reduce fruit losses in both pre- and postharvest, and to valorize the discarded product, increasing this crop's profitability.

I.3. Maturation and Fruit Quality

I.3.1. Physiological Changes During Maturation

Based on their respiration and ethylene pattern, persimmon fruit are categorized as climacteric, i.e., they produce a small, but significant, amount of ethylene during ripening and are induced to ripen with autocatalytic ethylene production by exogenously applied ethylene (Nakano et al., 2003; Salvador et al., 2007). Persimmon fruit produces an extremely low peak of ethylene (below 5 nL $g^{-1}h^{-1}$) and, because of this, its climacteric status was initially questioned. However, this fruit is very sensitive to exogenous ethylene even at low concentrations, which not only accelerate softening, but also reduce shelf life and marketability (Besada and Salvador, 2018; Woolf and Ben-Arie, 2011).

As for many other commodities, external color change is the most evident feature to take place during persimmon maturation and is the main maturity index used to decide the persimmon harvest time (Besada and Salvador, 2018). So, persimmon skin color changes from a homogenous green to yellow-orange or orange-reddish tones in the commercial maturity stage as a result of chlorophyll degradation and carotenoid biosynthesis (Giordani et al., 2015; Woolf and Ben-Arie, 2011). In parallel to increased peel color during maturation, flesh color also turns from white to orange. Some persimmon cultivars exhibit a more intense orange flesh color than others (Zhou et al., 2011). Cryptoxanthin has been reported to be the main carotenoid found in the flesh of different persimmon cultivars. Lutein, violaxanthin, zeaxanthin and β-carotene are also carotenoids present in persimmon flesh (Del Bubba et al., 2009; Zhou et al., 2011).

In persimmon, the increase in external color that occurs during ripening is concomitant with flesh firmness loss. Nevertheless, both color evolution and fruit softening during maturation are characteristic of each cultivar (Tessmer et al., 2016). In 'Rojo Brillante', flesh firmness is the most important quality attribute, and a strong negative correlation has been observed between color and firmness values during maturation (Salvador et al., 2007; Tessmer et al., 2016). Gradual fruit softening during ripening is related to microstructural changes in flesh, with progressive parenchyma degradation with less swollen and more deformed cells. As maturity advances, there is an increase in middle

lamella degradation, loss of cell wall material and solutes leakage to intercellular spaces, which lead to overall intercellular adhesion loss in later maturity stages (Salvador et al., 2020; Tessmer et al., 2016; Woolf and Ben-Arie, 2011).

The persimmon's calyx is a very characteristic part of fruit from the commercial point of view. During fruit ripening, desiccation occurs with calyx darkening, which starts from the apical part of sepals and moves towards the base, what is related to chloroplast breakdown and chlorophylls degradation (Lim et al., 2007). In a previous study that used chlorophyll fluorescence imaging (CFI) to assess calyx senescence during ripening in 'Rojo Brillante', a high correlation was found between the CFI parameters and changes in fruit color and firmness during ripening (Fathi-Najafabadi et al., 2021a). In fact, it was indicated that CFI measurements taken on the calyx can act as a potential non-intrusive tool for determining persimmon quality at harvest.

Both astringent and non-astringent persimmon cultivars are highly astringent when fruit is still immature, and differences in astringency between them appear in the growth and ripening stages. While non-astringent cultivars show a gradual decrease in soluble tannins until they become sensorially undetectable in early coloration stages, astringent cultivars also present gradual reduction in soluble tannins, but it is much less marked. Hence the fruit of astringent cultivars have a high soluble tannins content, even when they are completely colored, and astringency is not detectable only in overripe stages when fruit are completely soft (Besada et al., 2015b; Besada and Salvador, 2018).

Early stop of tannic cell development is considered the main cause of natural astringency loss in non-astringent cultivars because it leads to a diluted tannins concentration (Wu et al., 2022). However, some studies have revealed that a tannin insolubilization process occurs during fruit ripening in both astringent and non-astringent cultivars, but it is much faster in the latter (Tessmer et al., 2016; Woolf and Ben-Arie, 2011).

In astringent cultivars, tannins polymerization leads to lowering soluble tannin contents to values around 1 % upon commercial maturity, which corresponds to high sensory astringency values. The level considered to be undetectable by

consumers is around 0.02 %, but this depends on the cultivar (Del Bubba et al., 2009; Tessmer et al., 2014, 2016). In 'Rojo Brillante', sensorial astringency loss occurs when tannin content is around 0.04 %, which takes place when fruit overripens or after postharvest deastringency treatment (Salvador et al., 2007). Nevertheless, when 'Giombo' shows completely soft flesh, a level of soluble tannins is close to 0.5 % and fruit remain slightly astringent (Tessmer et al., 2016).

Persimmon maturation is also accompanied by chemical changes that contribute to the taste of ripe fruit, such as sugar accumulation and acidity. The predominant sugars in persimmon are glucose, fructose and sucrose, which increase throughout fruit development to reach a constant level before harvest maturity (Besada and Salvador, 2018). Acidity is relatively low, even in immature fruit, and does not change with ripening. Malic acid, which is predominant, increases with maturity, and is accompanied by a decline in citric acid. Succinic, fumaric, isocitric, ascorbic, gallic and quinic acids have also been identified (Woolf and Ben-Arie, 2011).

I.3.2. Harvest Time

The determination of harvesting time and proper handling after harvest is a key factor to achieve good persimmon quality and high marketability and profitability (Salvador et al., 2020). The persimmon fruit harvest takes place in autumn, from October to December. As previously mentioned, the maturity index used to decide the persimmon harvest time is external color. For most persimmon cultivars, fruit is ready to harvest when it displays a uniform yellow-orange color (Besada and Salvador, 2018).

Sugar content has been reported as a good maturity index for non-astringent cultivars, but it is not adequate for astringent ones. This is because the measurement of soluble solids includes not only sugar, but also soluble tannins (Besada and Salvador, 2018; Tessmer et al., 2016).

Flesh firmness is the main quality parameter for some cultivars, such as 'Rojo Brillante', which is commercialized with a crispness texture. High firmness

upon harvest also plays a decisive role in protecting fruit from mechanical damage and preserving its quality during postharvest storage and market handling (Besada et al., 2015; Salvador et al., 2020). Optimum persimmon firmness upon harvest is not well-established, and the velocity and severity of postharvest fruit firmness loss depend on fruit conservation time and conditions (Salvador et al., 2005, 2006). The only recommendation is that a minimum of 40 N is necessary upon harvest to guarantee fruit firmness above 20 N after cold storage (Besada et al., 2017). However, for 'Rojo Brillante', it is still necessary to acquire more in-depth knowledge about the effect of harvest moment on postharvest fruit behavior.

I.3.3. Prolonging the Harvest Period. Application of Phytoregulators

Due to the short harvest season, the application of phytoregulators has become a common strategy to advance or delay fruit maturation and to, thus, prolong the harvest period. This has become an important option for producers to avoid market saturation or to meet immediate demand and to reduce production losses. The main treatments employed for persimmon are ethephon (2-Chloroethylphosphonic acid), used to advance maturation, and gibberellic acid (GA3), utilized to delay harvest (Besada and Salvador, 2018).

Preharvest ethephon applications have been commercially employed for different crops to advance maturity. When metabolized by plants, ethephon is converted into ethylene and its effect on advancing persimmon maturation has been extensively observed (Besada and Salvador, 2018). The response to this phytoregulator depends on both its concentration and application time. Agustí et al. (2015) have reported that persimmon treated with a concentration of 15 mg/L or higher presents significantly improved coloration one week after treatment. The best response is achieved when fruit is treated after the growth period and approximately 3.5 weeks before color change occurs (early in September in Spain). Earlier applications (late July) do not have an immediate effect on fruit maturation. The effect of treatment is also observed with increased fruit ethylene production, which parallels color evolution. However, an accelerated softening rate also takes place on trees and during

storage, which negatively affects the postharvest fruit life (Agustí et al., 2015; Besada and Salvador, 2018). So, when ethephon is applied, the harvesting period is short, and, once harvested, this fruit should be rapidly commercialized, given its short shelf-life period. Therefore, a challenge that must be overcome is to improve the storability of this fruit while maintaining its firmness.

GA3 application is, in turn, a well-established treatment to slow persimmon maturation and ripening. It is related to a lowering fruit respiration rate and less ethylene production, and reduces its sensitivity to ethylene (Besada et al., 2008; Woolf and Ben-Arie, 2011). As GA3 also delays tissue senescence, its application to fruit retards color development and fruit softening (Agustí et al., 2015). This growth regulator is usually applied by spraying trees upon fruit color breaking and can delay persimmon maturity by 2 weeks (Salvador et al., 2020). Agustí et al. (2004) found that an GA3 application 25 days before color breaking significantly delayed coloration. If treatment is applied later, the response reduces. The achieved effect also depends on the applied concentration. Increasing concentrations between 0 and 30 mg/L shows a positive response, but higher concentrations do not achieve the same effect. Treatment is usually applied up to three times, every 15-20 days, to enhance the effect (Fathi-Najafabadi et al., 2021b). The delayed persimmon ripening induced by GA3 has been demonstrated to be the result of a delay in the cell wall changes that accompany fruit softening, such as middle lamella dissolution, separation of the plasmalemma from the cell wall and loss of the structural coherence and density of the primary cell wall (Ben-Arie et al., 1996).

Besides the effect on delay fruit maturation, GA3 treatment also has a positive effect on preserving fruit quality during postharvest life because it allows better postharvest performance and a longer fruit storage period. In addition, it has been observed how the combination of the GA3 preharvest treatment with postharvest practices improves fruit quality during prolonged periods (Besada and Salvador, 2018). Indeed, the fruit that undergo prolonged conservation are preharvest-treated with one or multiple applications of GA3.

I.3.4. Relation Between Mineral Nutrition and Fruit Quality

I.3.4.1. Nutrient Fertilization

The relation between tree nutritional status and fruit quality parameters has been documented in several fruit crops, such as apple, pear and citrus (Milošević et al., 2019; Papadakis et al., 2005; Sete et al., 2019). Nevertheless, little information is evaluable about the fertilization requirements and nutritional management to obtain high quality fruit for persimmon. Besides, most of the reported results are affected by a number of variables, such as crop management, fruit maturity stage, analytical methods and evaluated plant organ (Bernacchia et al., 2016; Toselli, 2010).

Some studies have shown that applying nitrogen (N) at high doses can increase fruit weight and delay persimmon maturation, which are reflected as reduced fruit color and total soluble solids, and as high flesh firmness (Choi et al., 2010, 2011; George et al., 2005; Kim et al., 2009). Inversely, less N supply during the growing season leads to better fruit coloration, but decreases fruit growth (Choi et al., 2008, 2012). George et al. (2003) have also reported for 'Fuyu' that N deficit or excess can reduce fruit yield.

On phosphorus (P) fertilization, Da Silva (1992) have noted that it increases persimmon weight and color, which ensures more appreciation on the market. However, a high P supply can diminish fruit production due to a nutritional imbalance in plants (Bellini and Giordani, 2002).

High potassium (K) supply can decrease persimmon total soluble solids and weight, and K deficiency affects fruit set, decreases fruit size, and reduces yields (Pomares et al., 2015). K also shows an uptake antagonism with calcium (Ca) and magnesium (Mg) mobilization, which lead to the deficiency of these two nutrients (Bellini and Giordani, 2002).

Calcium is applied to various fruit crops to increase flesh firmness. In persimmon, Ca deficiency can negatively affect fruit quality, which leads to fruit production with lower firmness values and worse conditions for both cold storage and the application of postharvest treatments (George et al., 2003; Pomares et al., 2015). Ca application can also prevent different disorders, such

as skin cracks, grooves, browning and “top rot” disease (Ferri et al., 2008; Tang et al., 2012).

In a recent study about ‘Jiro’ persimmon, Xu et al. (2020) found that fruit weight correlated positively with N, Ca and Mg, fruit firmness correlated positively with Ca, and total soluble solids correlated negatively with N, Ca, copper (Cu) and zinc (Zn) contents.

For ‘Rojo Brillante’ persimmon, it is necessary to acquire in-depth knowledge about the tree nutritional aspects that affect fruit quality, which can contribute to improve resource use efficiency in the sustainable farming system context.

I.3.4.2. Farming Management

The goal of reducing food loss and waste also includes the concept of promoting sustainable food systems (FAO, 2015). Organic agriculture, as a sustainable alternative to conventional systems, involves using mowed or tilled cover crops, animal manure, composts, and the application of organic fertilizers, which increase soil-organic matter and provide crops with a steady release of nutrients (Martínez-Alcántara et al., 2016).

In Spain, although most persimmon plots are grown following conventional agriculture practices, production by organic farming is increasing (CAECV, 2021). It is known that farm management practices can affect plant material composition and fruit final quality (Bernacchia et al., 2016; Toselli, 2010). However, there is little information available to support the notion that organic farming results in food with higher macro- and micronutrient contents than those conventionally produced. The results of most research works are unclear for various crops, including persimmon (Cardoso et al., 2015; Mditshwa et al., 2017; Rahman et al., 2021). Besides, they do not usually take into account environmental interactions.

The impact of organic matter applied to organic systems on soil chemical properties has profound effects on plant growth and yield. Organic matter from composts and manures are direct sources of slow-release nutrients, and contribute to increase their long-term availability that, in turn, improve plant

health and yield potentials (Reeve et al., 2016). Besides, organic matter can increase the availability of trace elements; nutrient uptake due to an improved cation/anion balance, and the availability of nutrients through improved microbiological activity (Hinsinger, 2001). Lack of synthetic pesticides in organic farming can also lead to positive results in the production of natural defense substances, such as phenolic compounds, due to greater plant exposure to biotic stresses (Faller and Fialho, 2010).

Studies are necessary about the effect of organic and conventional management practices on persimmon quality parameters, as well as its nutrient composition, as a tool to orientate adequate fertilization management to achieve high fruit quality through sustainable management practices.

I.4. Postharvest Technology

I.4.1. Deastringency Treatments

For fresh consumption, astringent persimmon cultivars must be submitted to postharvest treatments to remove astringency before being marketed. For many years, the traditional methods to eliminate fruit astringency have consisted in enhancing the ripening process by treating fruit with ethylene (10 ppm at 20 °C) or ethephon water dipping (50-500 ppm). However, the fruit treated by these methods are commercialized as very soft, which limits their manipulation and shortens their postharvest life (Salvador et al., 2020). Therefore, these treatments have become a minority practice.

The introduction of treatments able to remove astringency while maintaining fruit firmness has been implemented for allowing better postharvest handling, storage and transportation, and also for prolonging the fruit shelf life (Besada et al., 2015a). Indeed, the improvement of these deastringency techniques has been crucial for the marked increase in persimmon production in some countries like Spain, where the production of the astringent cultivar Rojo Brillante has exponentially grown in the last 20 years.

Current widespread deastringency treatments are based on exposing fruit to anaerobic conditions, which trigger acetaldehyde accumulation. This volatile

compound acts as a bridge by connecting proanthocyanidins, which brings about their insolubilization and consequently, astringency loss, but maintains fruit firmness (Besada et al., 2015a; Min et al., 2018). At the microstructural level, during deastringency treatment an insoluble material appears inside the vacuoles of some tannic cells, which are initially filled with soluble material (Salvador et al., 2007).

Of the treatments developed and established based on exposing fruits to anaerobic conditions, some involve exposing fruit to alcohol, carbon dioxide (CO_2), nitrogen (N_2) or warm water (Arnal and Del Rio, 2003; Khademi et al., 2009; Salvador et al., 2007). The most established treatment in the majority of production countries is CO_2 application, which has been commercially adopted for its effectiveness (Besada et al., 2010).

The efficacy of CO_2 treatment depends on different factors, such as CO_2 concentration, temperature, fruit maturity stage and treatment duration (Besada et al., 2010). Concentrations of 95-100 % CO_2 ensure more efficiency and is extremely important guarantee the achievement of a homogeneous distribution of the gas inside chambers. Treatments at lower concentrations need longer exposure times and/or can result in fruit with residual astringency (Besada et al., 2008; Hladnik, 2020). Likewise, low temperatures during treatment slow down the deastringency process and for 'Rojo Brillante', it has been demonstrated that astringency removal is more effective at higher temperatures (Besada et al., 2008).

When fruit are in advanced maturity stages, it is more difficult to remove astringency because, as maturity advances, flesh structure loss occurs at the cell level, which makes CO_2 diffusion through intercellular spaces difficult (Besada et al., 2010; Besada and Salvador, 2018). Therefore, a longer treatment period is necessary to completely remove astringency.

Several studies have dealt with optimizing these parameters. For 'Rojo Brillante', the widely used commercial method involves treating fruit in chambers at 95-100 % CO_2 at 20 °C and at 90 % relative humidity (RH) for 24-36 h (Besada et al., 2008; Munera et al., 2017).

It is important to note that exposing fruit to CO_2 deastringency treatment for excessively long periods may result in internal flesh browning as a response to the oxidative stress triggered by a low oxygen atmosphere (Besada and Salvador, 2018; Novillo et al., 2014). Besides, the intensity of this disorder increases if fruit are stored at low temperature after astringency removal (Besada and Salvador, 2018).

On the other hand, astringency removal performed by applying ethanol vapor is widely used commercially in some countries like Brazil, where it is the main treatment employed for being a less costly alternative with good results (Antoniolli et al., 2000; Vitti, 2009). Thus, 'Giombo' persimmon, one of the main astringent varieties grown in Brazil, is usually treated to remove astringency with ethanol vapor at a concentration of around 1.70 mL ethanol per kg of fruit for 24 h (Tessmer et al., 2018).

It is worth noting that, in addition to insolubilizing soluble tannins, the deastringency treatment causes degradation of cell membranes (Salvador et al., 2007) with consequent fruit softening. This firmness loss is aggravated during cold storage when the fruit have been previously subjected to the deastringency treatment (Besada et al., 2014). For this reason, it is recommended to apply the removal astringency treatment after fruit storage (Salvador et al., 2020).

I.4.2. Cold Storage and Chilling Injury

Persimmon cold storage is a common and necessary practice due to high production and the need to prolong the commercial period. In addition, the use of low temperatures is necessary for overseas commercialization (Besada and Salvador, 2018). However, persimmon cold conservation is a critical process that can trigger major postharvest losses related to chilling injury (CI) development (Pérez-Munuera et al., 2009; Salvador et al., 2004).

CI symptoms, as well as their incidence and severity, depend on the cultivar, storage temperature and duration. These symptoms are generally related to changes in flesh texture, such as softening, gelling and being rubbery, which

render fruit unmarketable (Salvador et al., 2020). In persimmon varieties like 'Fuyu' and 'Suruga', flesh gelling and internal browning have been cited as CI symptoms (Besada et al., 2015a; Salvador et al., 2020). For 'Rojo Brillante', the main CI symptom appears when fruit is exposed to temperatures below 8 ºC, which results in drastic flesh softening. However, they are not usually observed during cold storage, but become apparent when fruit is transferred to commercialization temperatures (Novillo et al., 2015; Salvador et al., 2004).

Other symptoms associated with CI in this cultivar, especially during prolonged storage, are internal browning and the appearance of nodules due to the compaction of localized areas of flesh (Salvador et al., 2020). It has also been observed that after a large storage period at low temperatures, a structural change in flesh can occur without firmness loss but adopting a hard and rubbery texture (Furuta et al., 2021; Salvador et al., 2005).

Many studies have focused on finding solutions to avoid CI in persimmon by means of different postharvest strategies, such as using controlled atmosphere, modified atmosphere packaging and hot water treatment (Rasouli and Khademi, 2018; Zhao et al., 2020). Of the strategies to alleviate CI, the main one is 1-methylcyclopropene (1-MCP) application prior to conservation (Machuca et. al., 2015; Salvador et al., 2004).

1-MCP is a non-toxic compound that acts as a competitive inhibitor of ethylene perception by preventing ethylene binding and eliciting subsequent signal transduction and translation (Baswal and Ramezanian, 2021; Manríquez and Defilippi, 2010; Valero et al., 2016). Postharvest 1-MCP application has become a successful technology for controlling ripening and senescence processes, to maintain fruit quality, and to reduce different postharvest physiological disorders in many horticultural products. 1-MCP is used in combination with proper temperature and relative humidity management and can replace or be utilized in combination with controlled atmospheres (Blankenship and Dole, 2003; Valero et al., 2016). Some commercial names of 1-MCP are EthylBloc®, SmartFresh®, SmartTabs® and EthylBloc® Sachet, which contain different 1-MCP concentrations. When the product is mixed with water or buffer solution, 1-MCP gas is released to the chambers where it is applied.

For persimmon, various studies have reported the effect of 1-MCP on preserving flesh firmness during cold storage (Besada et al., 2008; Harima et al., 2003; Salvador et al., 2004). The effect of 1-MCP on flesh softening control has been associated with cell wall integrity preservation, adhesion between adjacent cells, and the reduction of membrane permeability throughout cold storage and when fruit are transferred to shelf-life temperatures (Pérez-Munuera et al., 2009; Salvador et al., 2020; Zhang et al., 2020).

The response of fruit to 1-MCP treatment is affected by the maturity stage at harvest. It has been observed for different cultivars, such as 'Harbiye' (Öz and Ergun, 2009), 'Hiratanenashi' (Atmadi et al., 2019) 'Saijo' (Kurahashi et al., 2005) and 'Rojo Brillante' (Salvador et al., 2005), that 1-MCP application is able to maintain fruit firmness more effectively in less mature stages than in late-harvested fruits. This has been related to the lower ethylene production rate during conservation.

For 'Rojo Brillante', besides applying postharvest 1-MCP treatment to the fruit destinated for prolonged conservation, fruit are preharvest-treated with GA3, which is a well-established practice by persimmon producers. An improvement to storage capacity with high quality fruit has been reported for the combination of both treatments (Besada et al., 2008).

Despite postharvest 1-MCP application being able to present many benefits, preharvest 1-MCP treatment emerges as a novel option, which has been tested in several crops with different effects, such as delaying color development, flesh softening, ethylene production and ripening, and maintaining fruit quality throughout the postharvest life (Valero et al., 2016; Vilhena et al., 2023). For preharvest 1-MCP applications, a sprayable formulation has been developed, which facilitates its field application by dissolving in water and being applied as a fumigation product. This product, marketed as Harvista® (AgroFresh Solutions Inc., Philadelphia, PA, USA), is the only 1-MCP formulation available today for preharvest use purposes.

Most studies about this treatment have focused mainly on apples and pears because Harvista® application is authorized for these fruits in some countries. Very little information is available on the preharvest 1-MCP effect on

persimmon. Therefore, more studies are needed to evaluate the effect of this novel treatment on persimmon fruit quality.

I.5. Strategies to Valorize Persimmon Production

I.5.1. Postharvest Losses and Current Production Challenges

Food loss is defined by the FAO (2017) as the decrease in quantity or quality of food (agricultural or fisheries products) intended for human consumption, i.e., the production that is not eaten by people or has incurred a reduction in quality, as reflected in its nutritional value, economic value or food safety. One important part of food loss is "food waste", which refers to the discarding or alternative (non-food) use of food that is safe and nutritious for human consumption and that is originated throughout the entire food supply chain.

Growing interest is being shown in food loss mitigation, which is expressly mentioned as a global challenge in the Sustainable Development Goals. Specifically, subtarget 12.3 points out the need to "… by 2030, halve per capita global food waste at the retail and consumer levels and reduce food losses along the production and supply chains, including postharvest losses" (UN, 2015). This objective was incorporated by the European Union by including it in its own goal through the European Green Deal in 2019 (European Commission, 2019; Fernández-Zamudio and Barco, 2021).

Most food loss and waste come from fruit and vegetables, which account for 40-50 %, and result mainly from consuming fresh fruit and its industrial processing (FAO, 2015; Kosseva, 2020). The processes along the supply chain that generate different food loss types can be classified as: 1) agricultural production and harvesting activities, which give rise to on-farm losses; 2) postharvest handling and storage, manufacturing, and distribution processes, which generate postharvest losses; 3) the consumption phase, which generates consumer waste (Xue et al., 2017).

The generation of persimmon losses in Spain is closely related to the overproduction experienced in recent years and to adverse weather conditions, which have led prices to drop. Thus, it is reported that 11.44 % of

the potential persimmon crop has remained unharvested in the field during recent seasons (Fernández-Zamudio et al., 2020). On the other hand, about 20 % of production is lost in warehouses. The quality standards required by the market result in fruit not reaching end consumers. In addition, inadequate postharvest management often leads to significant quality losses (Fernandez-Zamudio et al., 2020; Hosseininejad et al., 2022). In relation to the fruit loss generated in warehouses, apart from fruit waste, loss of economic value occurs due to the investment of resources in pre- and postharvest handling operations. Therefore, one of the current challenges in fruit postharvest technology is to search for strategies to reduce the losses that originate along the whole supply chain.

At present, the alternatives for using the persimmon waste generated in warehouses are minimal. Besides, an additional cost is necessary to remove non-commercial fruit (Fernández-Zamudio and Barco, 2021). Thus, there is great interest shown by the producer Sector in finding procedures that help to add value to not only persimmon surpluses, but also to fruit unacceptable for commercialization purposes. The traditional procedures for using by-products from fruit waste, such as animal feed or agricultural substrates, do not provide the value that fruit producers need to strengthen their competitiveness (García et al., 2018).

Here drying technology could be an interesting strategy to valorize surplus fruit and to increase overall persimmon crop profitability.

I.5.2. Drying Technology

Drying is a simple fruit preservation method and extension of shelf life. It also allows the extension of fruit availability period (Corrêa et al., 2021). Dried persimmons are among the most important processed persimmon products and are very traditional in Asian countries (Zhou et al., 2022).

Many types of drying methods can be applied to persimmon, such as air drying, solar, hot air, spray drying, microwave, osmotic dehydration and freeze drying (Karaman et al., 2014). Natural-air drying of whole persimmon has been traditionally used in China, Japan and South Korea as a way to obtain a product

with good sensory and nutritional attributes. The standard method for drying involves removing calyx sepals and skin, followed by hanging fruit on strings or hooks (Hosseininejad et al., 2022).

In China and Japan, dried fruit can be manually kneaded near the end of the drying process to equally distribute moisture in fruit and to develop the shape of the finished product, besides facilitating sugars to exude out of fruit, which appear as white powder (Sugiura and Taira, 2009). The drying process also favors the insolubilization of the soluble tannins responsible for the astringency character of persimmon and is an interesting option to commercialize astringent cultivars without having to previously summit them to deastringency treatment (González et al., 2022).

After being dried, the period that persimmon can be stored is up to 6 months (Senadeera et al., 2020), what depends on factors like its initial properties, packaging method, storage temperature and final moisture. Fruit with a moisture content of around 50 % (semi-dried) can be preserved only for a couple of months, even in a refrigerator. So, it is recommended to keep them in a freezer for storage or later shipping (Sugiura and Taira, 2009).

Another commonly used method is hot-air drying because it involves little technological complexity and low costs compared to other industrial methods (Karaman et al., 2014). It also allows the drying process to shorten and results to be standardized, which permit processing fruit on an industrial scale. However, the drying temperature under which fruit must remain should be determined according to the cultivar's characteristics to obtain high quality products without losing nutritional characteristics.

Despite being a consolidated technique in several countries, the whole persimmon drying technology is not yet implemented in Spain. This strategy could have a major economic impact on the agri-food sector by increasing the profitability and availability of this seasonal fruit, and diversifying the offer to consumers, thus, open up new trade possibilities for persimmon production.

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II. OBJECTIVES

GENERAL OBJECTIVE

To study strategies to increase persimmon crop profitability by reducing postharvest losses and revalorizing discarded fruit and production surplus.

SPECIFIC OBJECTIVES

To evaluate pre- and postharvest aspects involved in persimmon fruit quality to reduce food loss

- To analyze macro- and micronutrients concentrations in leaf and fruit flesh of persimmon grown under different management systems (organic and conventional) and their relation to fruit quality.

- To investigate the effect of 1-MCP application at preharvest as a strategy to enhance persimmon postharvest quality.

- To study the effect of harvest moment on fruit behavior during cold storage by an in-depth physico-chemical and microstructural study.

- To evaluate the effect of high CO_2 and ethanol concentrations as deastringency treatments on persimmon fruit quality during cold storage.

To study the drying treatment as a strategy to valorize discarded persimmon fruit

- To study 'Rojo Brillante' persimmon's suitability to natural-air drying treatment by the physico-chemical and microstructural characterization of the changes that occur during this process.

- To evaluate the influence of drying temperature on dried 'Rojo Brillante' persimmon quality characteristics.

III. RESULTS

III.1. PRE- AND POSTHARVEST ASPECTS RELATED TO THE PERSIMMON FRUIT QUALITY

CHAPTER I

Leaf and Fruit Nutrient Concentration in 'Rojo Brillante' Persimmon Grown under Conventional and Organic Management, and Its Correlation with Fruit Quality Parameters

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Reference: *Agronomy*, 2022, 12(2), 237.
https://doi.org/10.3390/agronomy12020237

Abstract

This study aimed to evaluate the concentrations of the main macroelements in leaves and fruit grown following organic and conventional practices, and to relate them to physico-chemical parameters during commercial fruit harvests. Three samplings were carried out during fruit maturation. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were determined in leaves and in two fruit flesh areas: basal and apical. Weight, color, firmness, soluble tannins (ST), and total soluble solids (TSS) were also evaluated in fruit. During the study period, the lowering leaf N concentration was accompanied by its increment in flesh. Leaf P and K lowered but did not imply changes in these concentrations in fruit. N, P, and K concentrations were higher in the apical area than in the basal flesh. No changes in Ca concentration occurred in leaf, but Ca translocation from the basal to the apical area was detected in fruit. Management affected the concentrations of leaf K and Mg and the fruit N, P and Ca. The agronomic efficiency of the macronutrients in the organic crops was superior to that in the conventional crops. The Ca and Mg and the N/Ca and Ca/(K+Mg) ratios were closely related to color, firmness, TSS, and ST content.

Keywords: *Diospyros kaki* Thunb.; leaf and fruit nutrients; macronutrients; organic farming; fruit quality attributes.

1. Introduction

In Spain, persimmon production has increased exponentially in the last 20 years from 2474 to 17,601 ha (MAPA, 2021). Nowadays with production exceeding 400,000 tons, Spain has become the world's second largest persimmon producer after China and is the largest persimmon supplier worldwide with 46 % of global exports (FAOSTAT, 2021; MAPA, 2021). The main persimmon production area lies in eastern Spain, the Valencian Community, where production centers on the Rojo Brillante cultivar.

Persimmon grows in temperate zones and the fruits reach their full color about four months after the end of flowering in June (Arnal and Del Río, 2004). The commercial maturation period for persimmon is relatively limited, and can vary from September to December, depending on the stage of the fruit, location, and market demands. This variety presents a fall of leaves from the end of November (Giordani et al., 2015). The fact that the 'Rojo Brillante' persimmon is harvested without having undergone over-ripening, which eliminates its astringency in a natural way, requires it to be subjected to a postharvest deastringency treatment with a high CO_2 concentration, which enables astringency to be eliminated while maintaining fruit firmness. The introduction of this treatment has allowed a quality product to be obtained that is in high demand by national and international markets (Salvador et al., 2004).

The relation between leaf and flesh fruit mineral concentrations and fruit quality has been well documented in some species like apple, orange, and cherry (El-Gioushy, 2016; Jivan and Sala, 2014; Milošević et al., 2015). Nevertheless, information about nutritional persimmon requirements and nutritional management to obtain high quality fruit is available, but scarce. Most studies have been carried out about non-astringent 'Fuyu'. Clark and Smith (1990) investigated the seasonal changes in the composition and distribution of mineral nutrients in this cultivar. George et al. (2000) reported some information about 'Fuyu' growth requirements in Australia. The effect of different nitrogen applications on fruit characteristics and tree production in 'Fuyu' has been studied in Korea (Choi et al., 2008, 2010, 2011; Kim et al., 2009).

Previous reports for persimmon and other fruits indicate that some nutrient concentrations can vary between different areas of the fruit (Clark and Smith, 1990; Liebisch et al., 2009). The influence of some macronutrients on the browning of blossom ends that affects 'Fuyu' persimmon has been related to the Ca/(K+Mg) ratio in this flesh fruit area (Kim et al., 2002). Ferri et al. (2008) found a beneficial effect of applying calcium nitrate and calcium chloride on preventing skin cracks, grooves, and browning of 'Fuyu' persimmon. The "top rot" disease, which affects some cultivars like Gongcheng in China, has been correlated with calcium (Ca) deficiency (Tang et al., 2012). In 'Rojo Brillante', there are no references about the relation between the concentration of the main nutrients and fruit quality parameters. Evaluating nutrition accumulation dynamics in fruit is an interesting strategy for improving nutrient balances and fruit quality (Casero et al., 2017).

Moreover, it is well-known that applying a different fertilizer type with organic or conventional management will affect plant material composition (Bourn and Prescott, 2002). Organic agriculture, as an alternative to conventional crop systems, involves the use of mowed or tilled cover crops, animal manures, composts, and the application of organic fertilizers that increase soil-organic matter and provide a steady release of nutrients to the crops as the organic matter breaks down (Martínez-Alcántara et al., 2016). Conversely, excessive fertilizer rates on conventional crops can led to environmental pollution and biodiversity loss (Puig-Montserrat et al., 2017).

The effect of these fertilizers on the nutritional composition of several species has been addressed in some studies (Bourn and Prescott, 2002). Research has compared the nutrient status of fruit and vegetables from organic and conventional production (Bernacchia et al., 2016; Toselli, 2018; Yu et al., 2018). The most widely evaluated produces are carrots, green-leaf vegetables, potatoes, and apples, but the obtained results were not consistent in many cases. In 'Rojo Brillante' persimmon, no studies have been carried out to compare fruit nutritional concentrations from organic and conventional farms. Nevertheless, in Spain, although most persimmon plots respond to conventional agriculture practices, persimmon production performed by

organic farming practices has increased the total organic production in the Valencian Community up to 16 % in recent years (CAECV, 2021).

In this context, the objective of this study was to analyze the concentrations of the main macroelements in the leaf and flesh of the fruit grown following organic and conventional practices, and to relate them to the physico-chemical parameters associated with fruit quality during commercial fruit harvests. This information could help to carry out adequate nutrition management to achieve better fruit quality.

2. Materials and Methods

2.1. Experimental Conditions and Plant Material

The study was conducted on 'Rojo Brillante' persimmon in six orchards located in Alcudia (Valencia), Spain (lat. 39º11'18.7'' N, long. 0º32'6.2'' W) at an altitude of 42 m above sea level. Three plots were organically managed in the last 10 years, and three plots were conventionally managed.

The climate is semi-arid Mediterranean with average rainfall of 499 mm yr^{-1} that concentrates in autumn and spring. The soil characteristics of both growth management systems are shown in Table 1.

The plantation was established with spacing of 5 m x 4 m in the conventional and organic orchards. Trees were drip-irrigated with four commercial emitters per tree (4 L h^{-1}) to obtain an approximate 33 % wetting area at a depth of 20 cm, according to Keller and Karmelli (1974). The amount of water applied to each tree was equivalent to the total seasonal crop evapotranspiration (ETc) (Doorenbos and Pruitt, 1977). The volume of water applied weekly to each tree was calculated using the expression: ETc = ETo × Kc, where ETc is crop evapotranspiration, ETo is the reference crop evapotranspiration under standard conditions, and Kc is a crop coefficient. ETo was determined using the Penman– Monteith method, as described by Allen et al. (1998). Kc was based on the information described by Castel (1994), which accounts for crop-specific effects on overall crop water requirements in accordance with canopy size and

leaf properties. The fertilization plans of the evaluated orchards are described in Table 2.

Table 1. Soil physico-chemical characteristics.

Parameter	Conventional Orchards	Organic Orchards
Sand (%)	28.1	22.4
Silt (%)	37.1	54.9
Clay (%)	34.8	22.7
USDA Classification	Clay Loam	Silty Loam
pH	8.4	7.6
OM (%)	0.94	3.14
Norg (%)	0.05	0.14
C/N	11.47	13.05
Soluble P_{Olsen}[1] (ppm)	15.2	18.0
Ca_{sse}[2] (meq/L)	6.81	7.43
Mg_{sse}[2] (meq/L)	2.89	2.43
K_{sse}[2] (meq/L)	0.31	0.35

[1]P_{Olsen}: available phosphorus; [2]sse: soils saturation extract.

Table 2. Nutrient fertilization on the studied crops.

Management	Chemical compound (kg year^{-1} ha^{-1})					
	N	P_2O_5	K_2O	CaO	MgO	FLUIDOGAMA®[1]
Conventional	170	74	155	0.0	0.0	0.0
Organic	27	1	27	0.5	0.0	840

[1]Organic product with 2.8 % organic N, 3.1 % K_2O, and 40 % OM.

2.2. Plant Sampling and Sample Preparation

In each plot, 12 trees, four trees as one replicate, were previously marked to subsequent foliar samplings and fruit collection.

During the 2019 commercial harvest period, three leaf and fruit samples were taken from each replicate on 16 October, 7 November, and 12 December.

Foliar sampling was carried out by taking eight leaves from the summer flush leaves of each tree, with the third or fourth leaf from the axilla of reproductive sprouting, placed in the four orientations. In addition, 36 fruit per repetition were collected by taking nine fruits from each tree from the same reproductive sprouting.

Samples were taken to the Valencian Institute for Agricultural Research (IVIA), where leaves were cleaned with non-surfactant soap to eliminate any possible residue that was still attached. Next, they were rinsed with deionized water, dried, chopped, and placed in an oven for two to three days at 60–65 °C to dry. They were then ground in a water-refrigerated mill (IKA M20, IKA-Werke GmbH & Co. KG, Staufen, German) and stored in falcon tubes (Sarstedt, Numbrecht, Germany) at 4 °C pending further analyses. On fruit, physico-chemical determinations were made, and samples were taken to determine the macroelements in pulp. For this purpose, fruit were cut longitudinally into four parts. Two opposite parts were taken, which were subdivided into two zones: basal and apical half (Figure 1). These fractions were peeled and cut into smaller pieces to facilitate drying. They were placed inside an oven at 60–65 °C for three to four days.

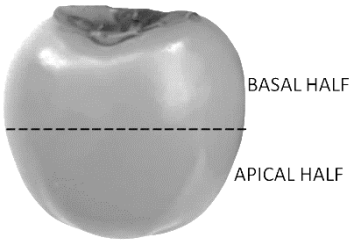

Figure 1. Sketch of the two persimmon fruit parts used for sampling.

2.3. Nutrient Determinations

The total nitrogen (N) concentration of the leaf and fruit flesh fractions was determined by the semi-micro Kjeldahl method (Bremner, 1996) with a Tekator still (Tecator Kjeltec 8200, FOSS IBERIA, S.A., Barcelona, Spain). The

determination of the phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) concentrations was made in the vegetal material obtained by an open-vessel process. This involved the overnight predigestion of half a gram of dried plant material with 10 mL of concentrated HNO_3, followed by digestion at 120 ºC, and then previously cooling to add 2 mL of ultratrace metal-grade 70 % $HClO_4$, and a final digestion at 220 ºC until white fumes were produced. The thus obtained digest was diluted with 25 mL of ultrapure water prior to nutrient determination by simultaneous inductively coupled plasma-atomic emission spectrometry (ICP-AES 6000, Thermo Scientific, Cambridge, UK). The results were expressed as a percentage of dry weight (DW).

2.4. Determination of Physico-Chemical Parameters

The weight, external color, and firmness of the pulp were individually measured on 20 fruits. Each fruit's individual weight was determined on a digital scale (model PB3002- S/FACT, Mettler 85 Toledo, Switzerland). External color was evaluated with a portable colorimeter (Minolta, mod. CR-400, Ramsey, NY, USA) by taking two measurements per fruit on the opposite sides of the equatorial zone. The results were expressed as Color Index (CI = 1000.a/L.b) (Jiménez-Cuesta et al., 1981).

Firmness was evaluated with a texturometer (Instron Corp., mod. 4301, Canton, MA, USA), provided with an 8 mm diameter punch. Two measurements per fruit were taken on the opposite sides of the equatorial zone without skin. The results were expressed as the force in Newtons (N) needed to break pulp.

Soluble tannins (ST) were determined on lots of 15 fruit per treatment, divided into three samples of five fruit. Samples were cut into four longitudinal parts, and two opposite parts were peeled, sliced and frozen at 20 ºC to be later evaluated by the Folin–Denis method described by Arnal and Del Río (2004). The results were expressed as a percentage of fresh weight (FW).

The other two opposite fruit parts were used to analyze total soluble solids (TSS). Samples were placed in an electric juice extractor and the filtered juice

was used to measure TSS content with a digital refractometer (model PR-1, Atago, Japan). The results were expressed as ºBrix.

2.5. Agronomic efficiency

To calculate the agronomic efficiency of the main macronutrients, fruit yield per hectare was divided by the amount of each nutrient in kg ha^{-1} applied to each management system. The values were expressed as kg of fruit yield per kg of applied nutrient.

2.6. Statistical analysis

The statistical analysis was carried out using the Statgraphics Centurion XVII.I software application (Manugistics Inc., Rockville, MD, USA). Data of the macronutrients and fruit quality parameters were subjected to analyses of variance (ANOVA) and multiple comparisons between means at $p \leq 0.05$, as determined by the LSD (Least significant difference) test. A two-way ANOVA was performed by analyzing the effect of the harvesting date and crop management for each leaf nutrient concentration and for each fruit physico-chemical parameter. A three-way ANOVA was performed for the fruit nutrient concentration by analyzing the effect of the three factors (harvesting date, crop management, and fruit part) per nutrient.

A correlation matrix was made to evaluate the strength and direction of the relation between nutrients and fruit quality. The correlation matrix showed the Pearson correlation values, which measured the degree of the linear relation between each pair of elements or variables.

3. Results and discussion

3.1. Macronutrients in Leaf and Flesh Fruit under Conventional and Organic Management

3.1.1. Macronutrients Concentration in Leaves and Fruit

The leaf N content obtained values around 1.5 % in October (Figure 2A), and this significantly lowered during harvests with values of 1.1 % in December (Table 3). Although the N concentration was slightly lower in the leaves from the organic vs. the conventional plots, the differences were not significant. In fruit flesh, the N concentration increased in both measured areas as harvest advanced (Figure 2B). In the apical area, N contents were higher than in the basal area for both management systems. The plot management influence was observed mainly in November and December, with a higher N content in the organic vs. the conventional fruit.

The leaf P concentration significantly lowered and ranged from 0.14 % in October to 0.08 % in December (Figure 2C). For this element, there were no significant differences between the leaves from the organic and conventional managements during the three sampling events (Table 3). On the three study dates, the P concentration in flesh fruit was significantly lower in the basal area than in the apical area (Figure 2D). In October, average values of 0.06 % were detected in the basal flesh, while the concentration in the apical flesh came close to 0.10 %, with no differences between the two managements. No major changes were noted during the following harvest. However, in December, the P concentration lowered in the apical area of the conventional cultivation fruit, while it rose in the basal area of the fruit from both management systems.

The K concentration lowered in leaves from October to December, which followed the tendency of the other mobile evaluated macro-elements (Table 3). However, unlike that observed for N and P, the K content in the leaves from the conventional plots was always higher than that from the organic plots (Figure 2E). In conventional crops, the leaf K concentration lowered from 1.3 % in October to 0.9 % in December, while the values in organic plots went from 1.0 % in October to 0.5 % in December. In fruit flesh, a higher K concentration in the apical area was found, with values around 0.7 % in the basal flesh and

1.0 % in the apical flesh in October, which revealed a variation throughout the study period. In both areas, no important differences were observed between managements (Figure 2F).

The Ca concentration in leaves was not affected by management and remained stable during harvests, with average values of 4.45 % (Figure 2G) (Table 3). In the fruit harvested in October, a much higher Ca concentration was observed in the basal area (values close to 0.05 %) than in the apical area (close to 0.01 %) (Figure 2H). In November, the Ca content in the basal flesh significantly lowered, but increased in the apical flesh. Even so, this concentration in the apical area remained lower than in the basal area. In December, the Ca content of the basal flesh dropped to similar values to those for apical flesh, while the apical flesh values were similar to those obtained in November. During harvests, Ca content was higher in the fruit from the conventional plots vs. the organic plots.

The Mg content in leaves gradually increased throughout the study period. The leaves from the organic plots obtained higher values than those from the conventional plots (Figure 2I and Table 3). In October, the Mg concentration values in both fruit flesh parts came close to 0.045 %, with no differences between conventional and organic management (Figure 2J). A marked reduction took place in November, but the concentration remained unchanged until December with average values around 0.021 %.

The values of the macronutrients found in persimmon leaves and fruit flesh fell within the range of those reported in previous analyses for other varieties, such as 'Fuyu', 'Hachiya', and 'Costata' (Choi et al., 2011; Clark and Smith, 1990; Enab et al., 2018; Rehalia and Sandhu, 2003). In the present study, the reduction in the leaf N, P, and K concentrations from October to December would indicate a progressive stop in nutrient uptake from soil, while the remobilization of the nutrients of this organ would continue before leaf senescence (Pomares et al., 2015).

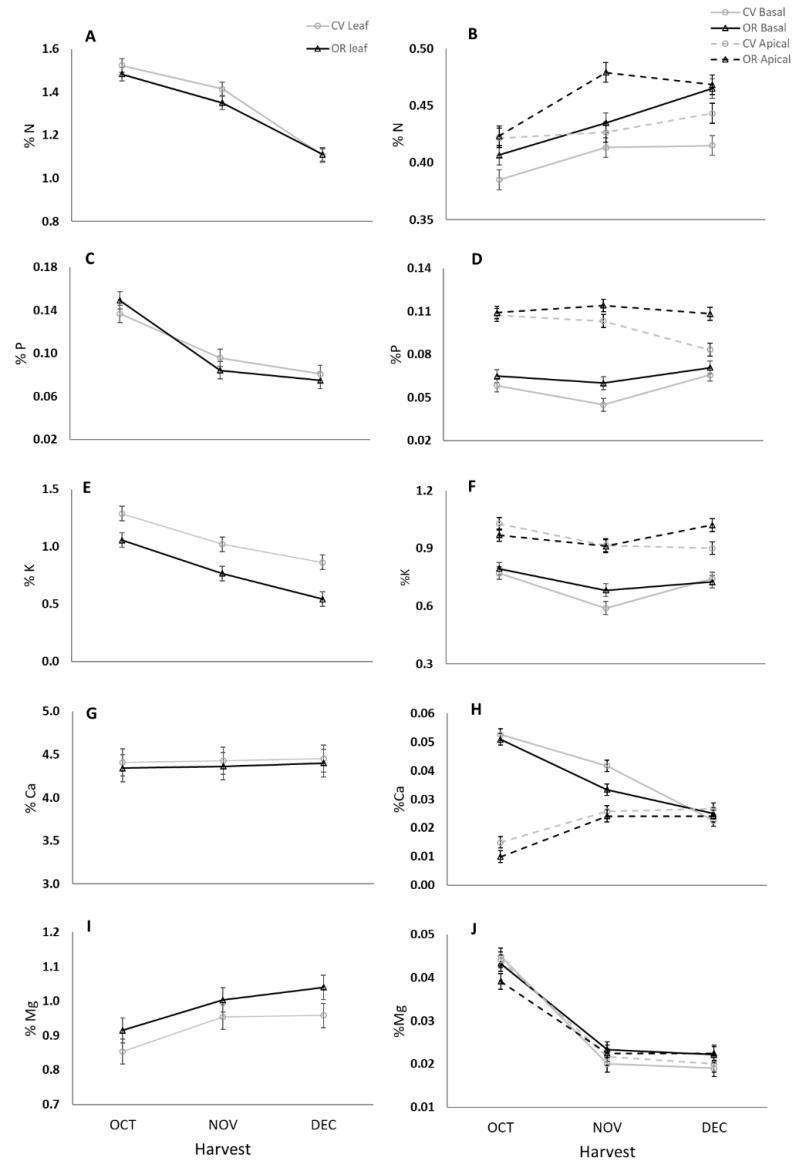

Figure 2. Macronutrient concentration in leaves **(A,C,E,G,I)** and in two fruit flesh areas (basal half and apical half) **(B,D,F,H,J)** during commercial 'Rojo Brillante' persimmon maturation (October to December) cultivated under conventional (CV) or organic (OR) management. Vertical bars represent the Least Significant Difference (LSD) intervals ($p \leq 0.05$).

Table 3. Analysis of variance (ANOVA) results for each factor in macronutrient concentration in leaf and fruit.

Significance						
		N	P	K	Ca	Mg
Leaf	A: Harvest	0.000*	0.000*	0.000*	ns	0.004*
	B: Management	ns	ns	0.000*	ns	0.030*
	AB:	ns	ns	ns	ns	ns
Fruit	A: Harvest	0.000*	ns	0.000*	0.000*	0.000*
	B: Management	0.000*	0.000*	ns	0.019*	ns
	C: Fruit Part	0.000*	0.000*	0.000*	0.000*	ns
	AB:	ns	ns	ns	ns	0.034*
	AC:	ns	0.000*	ns	ns	ns
	BC:	ns	ns	ns	ns	ns
	ABC:	ns	ns	0.018*	ns	ns

Factor A: Harvest moment (October, November and December); Factor B: Crop Management (Conventional and Organic); Factor C: Fruit Part (basal half and apical half); AB, AC, BC, and ABC represent the interaction of the factors. No significance (ns) or the actual P-value when significant (*) ($p \leq 0.05$).

In the conventional crops, as many minerals like N, P, and K are widely used in the form of soluble chemical fertilizers, a higher quantity of the available minerals can be expected in conventional products than in organic alternatives (Bernacchia et al., 2016). Although the input of these three elements was higher in the conventional plots in the present study (Table 2), in leaves only a higher K concentration was exhibited from the conventional plots compared to the organic ones. Regarding P, organic material application can lower the rhizosphere pH to favor the most available form of P (Karami et al., 2011; Pomares et al., 2015), which is explained by the similar P concentrations in the leaves from both studied crop systems (Figure 2C) despite the lack of this nutrient's fertilization in organic cultivation.

The influence of crop management on nutrient concentration proved to depend on different factors, such as evaluated species, cultivar, and the nutrient (Bernacchia et al., 2016; Strik, 2015). Moreover, the effect of

management was studied mainly on the leaf nutrient status, and very few works can corroborate the results obtained in the fruit.

Leaf N, P, and K mobility would not imply increased macronutrient concentration in fruit because these elements possibly move to other reserve organs, such as stems, branches, and roots, before leaves fall (Mei et al., 2015). Therefore, in this study, only an increase in the N concentration was observed in fruit, while a decrease occurred in leaves.

The differences found in leaf N, P, and K between the two management systems were not reflected in fruit. Indeed, the fruit N and P concentrations were higher in the organic than in conventional plots, and no differences appeared for the fruit K concentration.

N and K are the two nutrients that fruit accumulate in the largest amounts, which indicates a higher demand for these nutrients, as shown by other research into crops like orange, kiwifruit, and tamarillo (Clark and Smith, 1988; Clark et al., 1989; Liu et al., 2020). According to Tagliavini and Scandellari (2012), K is often the most represented mineral nutrient in well-developed flesh fruit, where it acts as osmoticum for the transport and storage of sugars and water in fruit.

Very few studies have investigated nutrient distribution in different persimmon fruit parts, and those that have focused mostly on seeking a relation between nutrient distribution and fruit disorders (Ben-Arie et al., 2008; Clark and Smith, 1990; Kim et al., 2002). In the present study, significant differences were found in the N, P, K, and Ca concentrations between fruit basal and apical parts (Figure 2B,D,F,H). These differences were also observed for N and K by Clark and Smith (1990), who found higher concentrations for these elements in the apical part of persimmon cv. Fuyu. However, they also noted that these differences in the K concentration decreased over time, an effect that was not herein observed.

On average, Ca and Mg presented much higher concentrations in leaves than in fruit parts. The Ca concentration was approximately 130-fold higher, and the Mg concentration was up to 30-fold higher in leaves than in fruit. Both nutrients abound more in leaves in most crops because they are not very

mobile elements (Tagliavini and Scandellari, 2012; Zanotelli et al., 2014). Ca movement in trees takes place mainly through the xylem, a circumstance for which Ca is not easily mobilized from old leaves during senescence (Pomares et al., 2015). Although the leaf Ca concentrations remained stable during the sampling period in the present study, a translocation of this element from basal to apical flesh occurred during the study period, which indicates the mobility of this element inside fruit.

The general drop in the Mg concentration in fruit flesh recorded during harvest period has also been reported in some studies for 'Fuyu' persimmon (Clark and Smith, 1990) and has been observed in other fruit, such as pomegranate and medlar (Glew et al., 2003; Mirdehghan and Rahemi, 2007).

3.1.2. Correlation between Macroelements

A simple linear correlation among the nutrients measured in both leaves and fruit was performed (Table 4). Leaf N, P, and K concentrations positively correlated with one another, while a negative correlation appeared between K and Mg. In several crops, a positive interaction between N and P has been described (Aulakh and Malhi, 2005; Fageria and Baligar, 2001; Rietra et al., 2017; Wilkinson et al., 2000). The implicated mechanisms are not well understood, although Wilkinson et al. (2000) suggest that N can increase P uptake in plants by increasing root growth and their capacity to uptake and translocate P. In a comprehensive review of the effect of antagonisms and synergism between nutrients, Rietra et al. (2017) showed a synergism between N and K fertilization that depended on the applied dose of N and K fertilization.

The depressing effect of K on plant Mg uptake has been frequently reported and can be explained as a result of competition for metabolically produced binding compounds (Grunes et al., 1992; Rietra et al., 2017). The negative correlation herein found between leaf Mg and K concentrations has been previously explained by the antagonism that exists between those cations (George et al., 2005).

Table 4. Simple correlation coefficient between the macronutrients in leaves and fruit parts under conventional and organic management.

Organ	Element	Leaf					Fruit basal area					Fruit apical area				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg	N	P	K	Ca	Mg
Leaf	N															
	P	**0.701***														
	K	**0.777****	**0.670***													
	Ca	-0.413	0.202	-0.164												
	Mg	-0.515	-0.432	**-0.772****	0.329											
Fruit basal area	N	-0.458	-0.477	**-0.815****	0.237	**0.919*****										
	P	-0.404	-0.036	-0.439	0.633	0.457	0.554									
	K	0.277	0.203	0.205	0.157	0.308	0.201	-0.198								
	Ca	**0.827*****	**0.805****	**0.844*****	-0.127	**-0.749****	**-0.733***	-0.306	-0.034							
	Mg	0.385	0.706	0.566	0.431	-0.468	-0.455	0.214	-0.066	**0.712*****						
Fruit apical area	N	-0.523	**-0.614***	**-0.808****	-0.116	**0.617****	**0.679***	0.353	-0.448	**-0.612***	-0.454					
	P	0.475	0.29	0.122	-0.292	-0.072	-0.112	-0.287	-0.090	0.509	0.198	0.554				
	K	0.120	0.264	0.168	0.515	-0.038	0.062	0.434	0.088	0.301	0.521	0.201	-0.198			
	Ca	-0.542	**-0.812****	-0.285	-0.346	-0.088	-0.048	-0.152	-0.417	-0.505	-0.568	**-0.733***	-0.306	-0.034		
	Mg	0.599	0.723	0.659	0.247	-0.520	-0.511	-0.005	-0.010	**0.878***	**0.894****	-0.455	0.214	-0.066	**0.712***	

Significant correlation at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***).

For fruit flesh, no correlations were found among N, P, and K measured in the apical or basal flesh areas. However, the fruit Ca concentration appeared to correlate negatively with the N concentration and positively with the Mg concentration in both these parts. The different mobility of these elements in plants from leaves to fruit could lead to an antagonistic effect between Ca translocation in fruit with high N concentrations. However, as Mg and Ca present similar mobilization, the greater absorption of one of them could increase the assimilation of the other in fruit without causing any significant interactions of these nutrients in leaves.

Some significant correlations were found among the nutrients of the flesh basal and apical areas. The Mg of the apical area and the Ca and Mg concentrations of the basal area positively correlated.

While considering the correlations among the nutrient concentrations in leaves and fruit flesh, a negative correlation between K in leaves and N in flesh of both areas was shown, while a positive correlation appeared between Mg in leaves and N in both fruit parts. Moreover, a positive correlation between Ca in the fruit basal area and N, P, and K in leaves was detected. Mg in leaves was negatively correlated with Ca in the basal flesh area. These same correlations were also observed in the fruit apical area, but inversely so.

From an agronomic point of view, the relation between elements can provide more information about crop nutritional status than the concentrations of individual nutrients (Marcelle et al., 1995). Moreover, the ratio among Ca, K, and Mg is often analyzed because it has been reported that both K and Mg can impede Ca accumulation in some fruit species (Madani et al., 2015; Marcelle et al., 1995). Therefore, an imbalance between these elements has been related to some flesh disorders developing in persimmon (Kim et al., 2002). The N/Ca ratio is widely used to predict fruit storage quality in apple and pear (Brunetto et al., 2015). The Ca/(K+Mg) and N/Ca ratios were herein evaluated (Table 5). The Ca/(K+Mg) ratio obtained for flesh fruit was higher in the basal area than in the apical area and fell within the range found for persimmon cv. Fuyu (Kim et al., 2002). In both management systems, a higher N/Ca rate was observed in the apical area than in the basal area of fruit. This ratio in the basal area increased as harvest advanced, while the N/Ca ratio lowered in the apical area, which reveals low Ca mobility in fruit, as well as the importance of N and Ca fertilization for correct fruit postharvest life.

Table 5. Ratios between elements

Organ	Harvest	Ca/(K+Mg)		N/Ca	
		Conventional	Organic	Conventional	Organic
Leaf	October	2.06	2.20	0.35	0.34
	November	2.24	2.47	0.32	0.31
	December	2.44	2.78	0.25	0.25
Fruit basal area	October	0.06	0.06	7.33	8.00
	November	0.07	0.05	9.92	13.05
	December	0.03	0.03	18.44	18.60
Fruit apical area	October	0.01	0.01	28.11	42.33
	November	0.03	0.03	16.52	19.83
	December	0.03	0.02	16.52	19.38

3.1.3. Agronomic Efficiency of the Main Macronutrients

Some of the major critiques made of organic management indicate that organic yields are generally lower than conventional yields (De Ponti et al., 2012). However, better productivity in conventional farming is achieved through higher demand for mineral nutrition. Therefore, comparing crop productivity to its fertilization rate can provide a better understanding of the efficient use of key nutrients for plant productivity. The yield obtained for conventional management in the present study was higher than that achieved in organic crops (Table 6).

Table 6. Yields obtained in different orchards.

Management	Orchard (m^2)	Yield (kg)/Orchard	Yield (kg)/ha
Conventional	30	174 [a]	44,452[a]
Organic	40	97 [b]	33,418[b]

The mean values followed by different letters in a column significantly differ ($p \leq 0.05$).

The agronomic efficiency of the main macronutrients in this study varied considerably between managements and was superior in the organic system (Table 7). This indicates that despite lower organic cultivation yields, these crops need less fertilization compared to the conventional system.

Table 7. Agronomy efficiency of the main macronutrients in each management system ($kg \cdot kg^{-1}$).

Management	N	P	K
Conventional	262^{b}	600	286^{b}
Organic	1243^{a}	∞	1227^{a}

The mean values followed by different letters in a column significantly differ ($p \leq 0.05$).

The agronomic efficiency of a macronutrient can be defined differently according to its application or the variable to be reflected, which can imply a difficulty in comparing the ranks found by different authors. In general, the values observed in China for major conventionally produced fruit crops, including persimmon, are slightly lower than those found herein, with values of 185, 338, and 208 kg kg^{-1} for N, P, and K, respectively (Li et al., 2019). Lin et al. (2016) indicated wide variability for these results depending on site and management conditions. These authors also observed better use efficiency for N in organic crops than conventional crops in different mixed farming systems. Studies into organically managed cropping systems indicate that yields comparable to conventionally managed systems can be achieved, while N losses lower significantly (Balasubramanian et al., 2004). The high mineral fertilization rates applied in conventional systems can reduce use efficiency. This means that when applied N is not completely taken up by plants, it can contribute to environmental problems like air pollution, global warming, groundwater contamination, and eutrophication (Li et al., 2019; Lin et al., 2016).

3.2. Relation between Macroelements and Fruit Quality

'Rojo Brillante' persimmon is a variety characterized by being large in size upon commercial maturity. In October, maximum fruit weight is achieved, with an average value troughout harvests of 248 and 270 g for conventional and organic orchards, respectively (Table 8). This is due mainly to the higher yields obtained under conventional management, which lead to a larger number of lighter fruit (Conti et al., 2014). However, the differences observed in fruit weight were not significant. No increment in size occurred during the following harvests, which agrees with the phenological phases described for this variety in Spain (Giordani et al., 2015).

Table 8. Weight, color index (CI), firmness, soluble tannins (ST), and total soluble solids (TSS) of 'Rojo Brillante' persimmon fruit under conventional and organic managements at different harvest times.

Harvest	Management	Weight (g)	CI	Firmness (N)	ST (%)	TSS (°Brix)
October	Conventional	242.37^{a}	−1.26^{c}	55.05^{a}	0.90^{c}	13.84^{d}
	Organic	257.73^{a}	−1.41^{c}	50.17^{b}	0.85^{c,b}	12.73^{e}
November	Conventional	251.90^{a}	8.54^{b}	42.94^{c}	0.79^{b}	14.42^{d,c}
	Organic	268.88^{a}	9.12^{b}	42.41^{c}	0.69^{b,a}	14.90^{c,b}
December	Conventional	248.98^{a}	14.04^{a}	36.07^{d}	0.61^{a}	16.10^{a}
	Organic	283.96^{a}	13.26^{a}	32.96^{e}	0.58^{a}	15.33^{b}
ANOVA	A: Harvest	ns	0.000 *	0.000 *	0.000 *	0.000 *
	B: Management	ns	ns	0.000 *	0.047 *	0.032 *
	A x B	ns	ns	0.007 *	ns	0.009 *

The mean values followed by the same letter in a column do not significantly differ ($p \leq 0.05$). The table of significance shows the full analysis of variance (ANOVA) results for the evaluated parameters. No significant (ns) or the actual p-value when significant (*).

The external color of fruit is one of the most widely used parameters as a nondestructive harvesting index given the close relation between skin color and the physico-chemical changes that take place during fruit ripening (Salvador et al., 2007). Color increased significantly as harvest advanced from values close to 1.3, which correspond to a homogenous yellow tone hue during the first harvest, to values close to 14, denoting deep orange tones, during the

third harvest, with no differences between managements (Table 8). In most persimmon varieties, harvest takes place when fruit presents a homogeneous external reddish-orange tone with no green background coloration (Besada and Salvador, 2011).

Fruit firmness gradually decreased with maturation advance during the three harvests, as shown by Salvador et al. (2007) for this variety. The fruit firmness from the organic plots was lower than that from the conventional ones (Table 8). In all cases, however, firmness values were optimal from a commercial point of view. Previous reports indicate a strong negative correlation between skin color and flesh firmness, which allows fruit firmness to be predicted from external color as a nondestructive measurement (Salvador et al., 2006; Tessmer et al., 2016).

The soluble tannins (ST) concentration in fruit lowered during all three harvests. In all cases, conventional cultivation obtained higher values than the organic crops (Table 8). This descent was due to ST transformation into the insoluble form (Taira and Ono, 1996), although the observed reduction was not enough to detect complete astringency loss, which coincides with other studies (Salvador et al., 2007; Tessmer et al., 2016). 'Rojo Brillante' persimmon is an astringent cultivar that requires deastringency treatment prior to fruit commercialization, which reduces the ST concentration to levels close to 0.01–0.02 % (Salvador et al., 2007). TSS content increased during the three harvests, with higher values for the conventional management.

The correlation matrix between nutrients and quality parameters is shown in Table 9. The leaf N, P, and K concentrations correlated positively with firmness and ST, and negatively with color and TSS content, but did not correlate with fruit weight. However, in the basal and apical areas, no significant correlation was observed between the N, P, and K and concentrations and most of the evaluated quality parameters.

Previous studies have also reported a negative correlation between leaf N concentration and fruit color and soluble solids, and a positive correlation with flesh firmness, in persimmon (George et al., 2000; 2005). These findings indicate that high N supply could delay fruit maturation. In other fruit like kiwifruit and grape, N and K fertilization influences different fruit quality

parameters, such as flesh firmness and TSS (El-Razek and Treutter, 2011; Pacheco et al., 2008). In 'Fuyu' persimmon, the negative relation between fruit N and soluble solids and color has been related to carbohydrate use during N assimilation (Choi et al., 2012). Hence, fruit would not develop an adequate external color when the carbohydrate supply is limited.

No significant correlation was found between the Ca leaf and fruit quality parameters. Nevertheless, the Mg concentration was positively related to weight fruit and negatively to ST.

Table 9. Simple correlation coefficient between the macronutrients in leaves and fruit parts and fruit quality parameters under conventional and organic management.

Organ	Nutrient	Weight	CI	Firmness	ST	TSS
Leaf	N	−0.404	−0.836***	0.834***	0.733**	−0.781**
	P	−0.204	−0.897***	0.807**	0.704*	−0.818**
	K	−0.634	−0.782**	0.873***	0.859***	−0.582
	Ca	0.689	0.001	−0.197	−0.304	0.113
	Mg	0.901***	0.563	−0.598	−0.794**	0.325
	N/Ca	−0.604	−0.747**	0.751**	0.708**	−0.649
	Ca/K+Mg	0.649	0.702**	−0.776**	−0.716**	0.529
Basal area	N	0.823***	0.588	−0.683	−0.845***	0.346
	P	0.721**	0.237	−0.400	−0.654	0.151
	K	0.192	−0.161	0.188	−0.027	−0.357
	Ca	−0.454	−0.945	0.943***	0.879***	−0.837***
	Mg	−0.293	−0.934	0.898***	0.753**	−0.805**
	N/Ca	0.518	0.881***	−0.869***	−0.876***	0.782**
	Ca/K+Mg	−0.385	−0.769**	0.801**	0.795**	−0.745**
Apical area	N	0.429	0.601	−0.614	−0.652	0.564
	P	0.011	−0.542	0.491	0.410	−0.595
	K	0.274	−0.307	0.226	0.124	−0.361
	Ca	−0.224	0.722**	−0.574	−0.352	0.752**
	Mg	−0.276	−0.888***	0.829***	0.715**	−0.761**
	N/Ca	0.157	−0.716**	0.537	0.349	−0.705*
	Ca/K+Mg	−0.193	0.765**	−0.607	−0.385	0.767**

Significant correlation at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***). CI: color index; ST: soluble tannins; TSS: total soluble solids.

For macronutrient concentrations in fruit, the correlations with the quality parameters differed depending on the measured flesh area. In the basal area, Ca and Mg correlated positively with firmness, ST, and TSS. In the apical area, positive correlations between Ca and color and TSS were found, while Mg correlated with color, firmness, ST, and TSS. Ca is most important in physiological functions, such as cell division, senescence, and the formation of cell membranes, and its deficiency can negatively affect fruit quality by generating less firmness and affecting its conservation capacity (Madani et al., 2015; Rehalia and Sandhu, 2003). In persimmon fruit, flesh firmness is an important quality parameter at harvest time because it determines postharvest management. Changes in fruit firmness have been related to microstructural changes in flesh during ripening (Tessmer et al., 2016).

In other fruit like papaya and apple, a positive relation between Ca and firmness, and a negative correlation between Ca and TSS and color, have been reported (Madani et al., 2015; Marcelle, 1995). Kim et al. (2002) studied the relation between fruit disorders and nutrient concentration and found higher Ca concentrations in the basal area of 'Fuyu' persimmon. These authors reported that the low concentrations of this element in apical flesh, or an imbalance in Ca, K, and Mg distribution, might lead to fruit disorders like blossom-end part browning.

The high correlations between the N/Ca and Ca/(K+Mg) ratios of leaves, or the basal flesh area and color, firmness, and ST, are noteworthy. These results show the importance of a balanced fertilization plan. TSS correlated significantly with N/Ca and Ca/(K+Mg) in fruit. In apples, a high N/Ca is usually linked with high TSS, color, and low flesh firmness values (Marcelle, 1995).

4. Conclusions

This study provides new information about the macronutrient concentration in leaves and different flesh areas during the maturation period of 'Rojo Brillante' persimmon grown under conventional and organic management.

During the study period, the reduction in the leaf N concentration was accompanied by an increase in this element in fruit flesh. Nevertheless, the

descent in P and K observed in leaves did not imply changes in fruit concentration, which indicates that these elements possibly move from leaves to other reserve organs during this period. The flesh apical area accumulates higher N, P, and K concentrations than the basal area.

While no changes were found in the leaf Ca concentration during the maturation period, Ca translocation from the basal to the apical area was evidenced in fruit. No differences in fruit Mg levels were found between the two flesh areas, which lowered during fruit maturation.

Organic management led to a lower K concentration and a higher Mg concentration in leaves compared to conventional production. Nevertheless, management did not affect the concentration of these elements in fruit. Although the management system did not influence leaf N, P, and Ca concentrations, lower Ca and higher N and P were detected in the organic than in the conventional fruit.

An interaction among nutrients was revealed by the correlations performed among them. The correlations between macronutrients and quality parameters highlighted that Ca and Mg, and the N/Ca and Ca/(K+Mg) ratios, were closely related to color, firmness, TSS, and ST content. This result reinforces the relevance of balanced fertilization during the growing season to achieve high fruit quality. Nevertheless, further studies are necessary in subsequent growing seasons to profoundly understand the role of each nutrient in persimmon to improve fruit quality. In addition, the determination of other elements would be important to strengthen the findings of this work.

Acknowledgements

This research was funded by the Ministerio de Ciencia e Innovación of Spain, through Project RTA2017-00045-C02 and the Fondo Europeo de Desarrollo Regional (FEDER). Nariane Q. Vilhena thanks the Ministerio de Ciencia e Innovación of Spain for Grant PRE2018-085833.

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CHAPTER II

Ionomic concentration and metabolomic profile of organically and conventionally produced 'Rojo Brillante' persimmon

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Pending submission

Abstract

This study evaluates mineral concentrations, biocomponents content and fruit quality attributes in 'Rojo Brillante' persimmon grown under organic and conventional management. During two consecutive seasons, leaf and fruit samples were taken at the commercial harvest moment. The concentration of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and boron (B) was determined in both leaves and fruit. Weight, color, firmness, and total soluble solids (TSS) were also evaluated in fruit. Moreover, in the second season, organic acids (citric, succinic and fumaric acids), main sugars (sucrose, glucose and fructose), carotenoids (β-Carotene, β-cryptoxanthin, violaxanthin, lutein and zeaxanthin), phenolic compounds (gallic and ρ-coumaric) and ascorbic acid concentrations were determined in fruit flesh. The crop yield in the conventional plots was bigger than that for organic crops. Nevertheless, the highest agronomic efficiency was found in organic management. In general, the greater nutrient supply in the conventional *versus* the organic system did not result in a higher concentration of macro- and microelements in leaves and fruit. No differences in P, K, Fe, B concentrations were found between crop systems. Mn and Zn appeared at the highest concentrations in the organic plots. This could be explained by the effect of the organic matter applied to the organic crop to favor nutrients assimilation. The organic fruit had higher color values and lower firmness values than the conventional fruit due to the lowest N input in the organic system. Nevertheless, TSS content was not affected by management. The concentration of malic acid, β-Cryptoxanthin and ascorbic acid was higher in the organic vs. the conventional fruit, while no crop system effect was found in the other evaluated biocompounds.

Keywords: *Diospyros kaki*; macronutrients; nutritional compounds; fruit quality, organic crop.

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) is an important fruit crop in Spain, with a production area covering around 17.600 ha and centered mainly on the 'Rojo Brillante' cultivar (MAPA, 2021). Persimmon fruit is highly appreciated for its nutritional quality, and for the presence of bioactive and antioxidant compounds like polyphenols, carotenoids and tannins. The 'Rojo Brillante' cultivar is highly valued for its good quality, and is commercialized with a crunchy texture, a large size and good flavor (Conesa et al., 2020; Munera et al., 2017).

Nowadays in Spain, most plots employed for persimmon cultivation respond to conventional agriculture practices and, therefore, include the use of synthetic pesticides and herbicides, as well as chemical fertilizers (Suciu et al., 2019). Nevertheless, persimmon production performed by organic farming practices has been increasing in recent years. At present, almost 300 ha are used for organic persimmon production (CAECV, 2021). Organic matter from composts and manures is a direct source of slow-release nutrients, and can increase nutrient uptake due to an improved cation/anion balance and nutrients availability through improved microbiological activity (Kumar et al., 2021; Reeve et al., 2016). Lack of synthetic pesticides in organic farming can also lead to positive results in the production of natural defense substances, such as phenolic compounds, due to plants being more exposed to biotic stresses (Faller and Fialho, 2010).

It is noteworthy that final fruit quality can be affected by plant nutrients supply, which is determined by production management (conventional or organic) (Bernacchia et al., 2016). Organic foods are generally perceived as being healthier, tastier and more nutritious than conventionally produced foods. However, there is little evidence to confirm this assumption because comparative data from production systems are inconsistent (Bordeleau et al., 2002; Cardoso et al., 2011). For persimmon, very few studies have compared the nutrient uptake and quality of organic and conventional fruit. A study with the cv. Rama Forte reported that organic fruit contained larger amounts of Zn, while Mg, P, Na, and K concentrations were higher in conventional fruit

(Cardoso et al., 2015). Cardoso et al. (2011) found higher vitamin C content in this cultivar when it was conventionally produced than those organically cultivated. Previous studies on 'Rojo Brillante' have shown that management can affect persimmon macronutrient fruit composition, which depend on both harvest moment and the evaluated flesh area (Vilhena et al., 2022b). However, more information about the mineral and nutritional composition of persimmon fruit grown in different management systems is needed.

Hence the objective of this work is to compare the fruit and leaf mineral concentration (macro- and micronutrient), fruit biocomponents content and quality attributes of organically and conventionally grown 'Rojo Brillante' persimmons.

2. Materials and Methods

2.1. Plant material

This experiment was carried out in six orchards of 'Rojo Brillante' persimmon trees located in Alcudia (Valencia), Spain (lat. 39°11'18.7'' N, long. 0°32'6.2'' W), at an altitude of 42 m above sea level during two growing seasons. This region has a Mediterranean climate with 400–500 mm of mean annual rainfall and average annual temperatures of 15–17 °C.

Three plots had been subjected to organic management in the last 10 years, and the other three to conventional management. The soil in the conventionally managed plots had a clay loam texture (28.1 % sand, 37.1 % silt, 34.8 % clay) with a pH of 8.4 and an organic matter content of 0.94 %. The organic plots had a silt loam soil texture (22.4 % sand, 54.9 % silt, 22.7 % clay) with a pH of 7.6 and 3.14 % organic matter content.

Trees received an annual fertilizer supply by drip irrigation depending on whether management was conventional or organic, as described in Table 1.

Table 1. Nutrient fertilization on the studied crops.

Management	Season	Chemical Compound							
		N	**P_2O_5**	**K_2O**	**Ca**	**Fe**	**Mn**	**Zn**	**B**
		kg year^{-1} ha^{-1}				mg year^{-1} ha^{-1}			
Conventional	1	170	74	155	0.3	2190	1535	1093	120
	2	167	62	113	3.6	720	1650	650	120
Organic	1	27	1	27	0.7	150	120	420	0
	2	20	1	27	0.0	150	31	332	0

In each plot, 12 trees, of which four were one replicate, were previously marked to carry out subsequent leaf and fruit samplings. In two consecutive seasons, leaf and fruit samples were taken when fruit were considered commercially mature (earliest commercial harvest moment): on October 16 and October 28 during the first and second season, respectively. On each sampling date, eight leaves were collected from the summer flush of each tree, with the third or fourth leaf from the axilla of reproductive sprouting, placed in the four orientations, to totalize 32 leaves per replicate. In addition, 36 fruit were collected per replicate by taking nine fruits from each tree from the same reproductive sprouting.

Leaf and fruit samples were transported to the Valencian Institute for Agricultural Research (IVIA). The concentration of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and boron (B) was determined in both leaves and fruit. Weight, color, firmness, and total soluble solids (TSS) were also evaluated in fruit. In the second season, organic acids (citric, succinic and fumaric), main sugars (sucrose, glucose and fructose), carotenoids (β-Carotene, β-cryptoxanthin, violaxanthin, lutein and zeaxanthin), phenolic compounds (gallic and ρ-coumaric) and ascorbic acid concentrations were analyzed in fruit flesh.

The agronomic efficiency of the main applied macronutrients was calculated as fruit yield (kg ha^{-1}) divided by the amount of N, P and K (kg ha^{-1}) applied to each plot.

2.2. Ionomic analysis

Prior to the macro– and microelement determinations, leaves and fruit were washed with deionized water. Five fruit per replicate were longitudinally cut into four parts and two opposite parts were peeled. Samples were dried in a forced air oven at 65 °C for a minimum 72-hour period, ground in a water-refrigerated mill (IKA M20, IKA-Werke GmbH & Co. KG, Staufen, GermanIKA Labortechnick) and stored at 4 °C pending further analyses. Nutrient extraction was performed by wet digestion in a digestor FOSS (Foss Tecator, Sweden). For that purpose, 0.200 g of the pulverized samples was weighed, and 4 mL of Milli-Q, 4 mL of nitric acid (HNO_3) and 2 mL of hydrogen peroxide (H_2O_2) were added to each sample. The tubings were kept at 200 °C for 15 to 20 min. Once digestion had finished, the extracts were diluted in 25 mL beaker to analyzed micronutrient concentration. An aliquot of 0.5 mL was taken from the extraction solution to determine the macronutrients and made up to 10 mL with Milli-Q water. Both tubes were stored until further analysis.

The Kjeldahl method was used for the organic N analysis, performed in a TecatorTM Line distiller (Kjeltec 8200). P, K, Ca, Mg, Fe, Mn, Zn and B were determined by inductively coupled plasma optical emission spectrometry (ICP-OES iCAP 7000, Thermo Scientific, Massachusetts, USA). The results of the macronutrient concentration were expressed as a percentage of dry weight (DW) and the micronutrient concentration was expressed as ppm (DW).

2.3. Determination of the physico-chemical parameters

Weight, external color and flesh firmness were individually measured on 20 fruit per replicate. Fruit weight was determined on a digital scale (model PB3002-S/FACT, Mettler 85 Toledo, Switzerland). External color was evaluated by taking two measurements per fruit on the opposite sides of the equatorial zone using a portable colorimeter (Minolta, mod. CR-400, Ramsey, NY, USA). The results were expressed as Color Index (CI = 1000a/Lb; 'L', 'a', 'b' are Hunter color parameters) (Salvador et al, 2006).

Flesh firmness was evaluated on the equatorial zone of each fruit after removing skin by a texturometer (Instron Corp., mod. 4301, Canton, Mass.,

USA) provided with an 8 mm diameter punch. The results were expressed as the force in Newtons (N) needed to break flesh.

For the TSS determination, samples of five fruit per replicate were taken. Fruits were cut into four longitudinal parts. Two opposite parts were peeled and placed inside an electric juice extractor and the filtered juice was used to determine TSS content by a digital refractometer (model PR-1, Atago, Japan). To avoid tannins interfering with TSS measurements, their insolubilization was previously done following the method of Sugiura et al. (1983). The results were expressed as °Brix.

2.4. Biocomponents analysis

The extraction and determination of sugars, organic acids, vitamin C, phenolic compounds and carotenoids were conducted from the same juice samples used for the TSS determination.

Individual sugars extraction was carried out following the procedure as previously described by Bermejo et al. (2016) and Morales et al. (2021a). Compounds were analyzed by an HPLC device equipped with a refractive index detector (Waters, Barcelona, Spain) using a 5-µm Tracer Carbohydrate column (250 mm × 4.5 mm) (Teknokroma, Barcelona, Spain). The mobile phase was acetonitrile:water (75:25) at a flow rate of 1 mL/min. Compounds were identified by comparing their retention time to standards, and were quantified using an external calibration curve with fructose (RT = 10.60 min), glucose (RT = 12.66 min) and sucrose (RT = 18.54 min). Sugars were obtained from Sigma (Sigma Co., Barcelona, Spain). The results were expressed as g kg^{-1}.

Organic acids extraction was done according to the method described by Bermejo et al. (2016) and Morales et al. (2021a). Compounds were analyzed by HPLC-DAD and HPLC-MS under electrospray ion negative conditions. An ICSep ICE-COREGEL 87H3 column (Transgenomic) was used with an isocratic mobile phase of 0.1 % H_2SO_4 solution at a flow rate of 0.6 mL/min, and the injection volume was 5 µL. The temperature of samples was 5 °C, the column temperature was maintained at 35 °C and UV-Vis spectra were detected at

280-400 nm. Compounds were identified by comparing their retention times and UV-Vis and mass spectral characteristics to the corresponding standards. Concentrations were determined using an external calibration curve with citric acid (RT = 8.01 min; [M-H]+ 191 m/z), malic acid (RT = 9.41 min; [M-H]+ 133 m/z), succinic acid (RT = 11.43 min; [M-H]+ 117 m/z) and fumaric acid (RT = 13.60 min; [M-H]+ 115 m/z). The standard compounds came from Sigma-Aldrich (Sigma Co., Barcelona, Spain). The results were expressed as mg $100g^{-1}$.

Ascorbic acid was extracted according to the method reported by Bermejo et al. (2016). DL-dithiothreitol (DTT) was used as the reducing reagent of dehydroascorbic acid to ascorbic acid as described by Morales et al. (2021a). Ascorbic acid quantification was performed by HPLC-DAD (Waters, Barcelona, Spain) with a reverse-phase column C18 Tracer Excel 5 µm 120 OSDB (250 mm x 4.6 mm) (Teknokroma, Barcelona, Spain) and an isocratic mobile phase of methanol: 0.6 % acetic acid (5:95, v/v). Quantification was carried out at 245 nm by external standard calibration. L-ascorbic acid was obtained from Sigma (Sigma Co., Barcelona, Spain) and DTT from Fluka (Sigma Co., Barcelona, Spain). The results were expressed as mg 100 g^{-1}.

Phenolic compounds were extracted according to the procedure described by Gómez-Martínez et al. (2021) and Morales et al. (2021b). They were analyzed by HPLC-DAD and HPLC-MS in a reverse-phase column C18 Tracer Excel 5 µm 120 OSDB (250 mm x 4.6 mm) (Teknokroma, Barcelona, Spain) under electrospray ion positive and negative conditions, with a gradient mobile phase consisting of acetonitrile (solvent A) and 0.6 % acetic acid (solvent B) at a flow rate of 1 mL/min. The gradient change was as follows: 10 % 2 min, 10-75 % 28 min, 75-10 % 1 min, held at 10 % for 5 min. Compounds were identified by comparing their retention times, UV-vis spectra and mass spectrum data to authentic standards from Sigma-Aldrich, and all the employed solvents were of LC–MS grade. Concentrations were determined using an external calibration curve with gallic acid (RT = 4.61 min; [M-H]+ 169 m/z) and p-coumaric acid (RT = 13.40 min; [M-H]+ 163 m/z). The results were expressed as mg 100 g^{-1}.

Carotenoid extractions were carried out following the procedure described by González et al. (2021) and were analyzed by HPLC-DAD and HPLC-MS in a

reverse-phase column Agilent ZORBAX Eclipse XDB-C18 5 µm. The mobile phase was of water (A): acetonitrile-water-triethylamine (900:99:1) and (B) ethyl acetate at a flow rate of 1 mL/min. The gradient elution was: 0-5 min, 100 % to 75 % A; 5-10 min, 75 % to 30 % A; 10-13 min, 75 % to 0 % A; 13-14 min, 0 % to 100 % A; 14-15 min, 100 % A, with a total run time of 15 min. Compounds were identified by comparing their retention times and absorption spectrum characteristics. The quantification of carotenoids was achieved using calibration curves with commercially available authentic standards: violaxanthin, lutein, zeaxanthin, β-cryptoxanthin and β-carotene from CymitQuimica (Barcelona, Spain). Their quantity was corrected for extraction efficiency based on the β-apo-8'-carotenal internal recovery standard. Concentrations were determined using an external calibration curve with violaxantin (RT = 4.60 min), lutein (RT = 6.65 min), zeaxanthin (RT = 6.80 min), β-cryptoxanthin (RT = 10.40 min) and β-carotene (RT = 12.50 min). The results were expressed as µg 100 g^{-1}.

2.5. Statistical Analysis

The statistical analysis was carried out using the Statgraphics Centurion XVII.I software application (Manugistics Inc., Rockville, MD, USA). Data were subjected to analyses of variance (ANOVA) and multiple comparisons between means at $p \leq 0.05$, with management and season as factors, determined by the LSD (least significant difference) test.

3. Results and Discussion

3.1. Crop yield and agronomic efficiency

One of the drawbacks that is most often pointed out when comparing organic and conventional management is the large difference in crop yield between both agriculture systems. Organic cultivation is often associated with poor performance in fruit productivity terms (De Ponti et al., 2012). The higher productivity of conventional agriculture is associated mainly with high fertilization rates (Seleiman et al., 2020).

In the present study, the yield obtained for conventional management was similar for both seasons and was higher than those achieved with organic crops (Table 2). In organic farming, a decreased yield was recorded during the second season. It is known that crop yield variability among seasons can be influenced by a wide range of factors. In organic farming, one of the main yield-limiting factors is N availability (Seufert, 2019). Thus, in the present study, the lesser N input in organic management in the second year could be related to lower production.

Table 2. Average yields in conventional and organic crops in two harvest seasons.

Season	Yield (kg ha^{-1})	
	Conventional	Organic
1	44,452^{a}	33,418^{b}
2	44,174^{a}	23,914^{c}

The mean values followed by different letters significantly differ when comparing both factors ($p \leq 0.05$).

Nevertheless, when comparing crop productivity to the fertilization rate of the main macronutrients, the agronomic efficiency of the organic crop was much higher than for the conventional agriculture in both study years (Table 3). These results indicate a better use efficiency of the nutrients applied in organic systems. Lin et al. (2016), also found a better nitrogen use efficiency under organic farming systems (arable farming, improved arable farming and agroforestry) when compared to conventional system.

Table 3. Agronomic efficiency of the main macronutrients in conventional and organic managements in two harvest seasons (kg kg^{-1}).

Management	Season	N	P_2O_5	K_2O
Conventional	1	261^{b}	600^{d}	286^{d}
	2	264^{b}	712^{c}	391^{c}
Organic	1	1,237^{a}	33,418^{a}	1,238^{a}
	2	1,195^{a}	23,914^{b}	886^{b}

The mean values followed by different letters in a column significantly differ ($p \leq 0.05$).

3.2. Macro- and micronutrients concentration

In both studied seasons, a higher N input in conventional management was applied, up to six times more than in organic farming (Table 1). However, differences in the leaf N concentrations were observed between the conventional and organic managements, but only in the second year, when the lowest values were detected in the organic plots (Table 4). Nevertheless, the leaf N concentrations obtained for both managements fell within the optimal range established for 'Rojo Brillante' persimmon (1.33-1.50 %) (Morales et al., 2022). The N concentration in the conventional plots was slightly higher than 1.50 %, which indicates that the amount of N added by fertilization could be reduced. The lower leaf N in the organic crop in the second season could be due to lesser N input, which would result in an N concentration being at the threshold of the optimum values. These results corroborate more efficient N use in organic than in conventional fertilization (Vilhena et al., 2022b). Similarly to that observed in leaves, no differences in the N concentration of the conventional fruit were observed between seasons, and values were similar to those of the organic fruit from the first season.

As shown in Table 1, the P and K inputs in the conventional plots were much higher than in the organic ones. Nevertheless, the concentration of these macronutrients in both leaves and fruit was similar in the two management systems, with no differences between seasons (Table 4). The similar P concentrations in both crop systems could be explained by the effect of the applied organic matter, which reduces the rhizosphere's pH to favor the most available form of P (Karami et al., 2011; Pomares et al., 2015). According to Morales et al. (2022), K leaf concentration was in the range considered optimal for this variety, showing the correct fertilization carried out in both managements, whereas P concentrations were higher, therefore the P applications could be reduced under conventional management. Despite lack of Mg supply, the concentrations detected in leaves and fruit were also similar in both the conventional and organic crops, with values within the optimal range (Morales et al., 2022).

Table 4. Macronutrient concentration in the leaves and fruit of the 'Rojo Brillante' persimmon cultivated by conventional or organic management in two harvest seasons.

		N (%)		P (%)	K (%)	Ca (%)		Mg (%)
LEAVES								
Management (M)								
Conventional		-		0.14	1.27	3.77		0.87
Organic		-						
Season (S)								
1		-		0.14	1.27	4.21^{a}		0.87
2		-				3.33^{b}		
		CV	**OR**					
M x S	**S1**	1.52^{a}	1.48^{a}	-	-	-		-
	S2	1.52^{a}	1.32^{b}					
Significance								
M		**		*NS*	*NS*	*NS*		*NS*
S		*		*NS*	*NS*	***		*NS*
M x S		*		*NS*	*NS*	*NS*		*NS*
FRUIT								
Management (M)								
Conventional		-		0.08	0.90	-		0.04
Organic		-				-		
Season (S)								
1		-		0.08	0.90	-		0.04
2		-				-		
		CV	**OR**			**CV**	**OR**	
M x S	**S1**	0.39^{a}	0.42^{a}	-	-	**S1** 0.05^{a}	0.03^{b}	-
	S2	0.39^{a}	0.31^{b}			**S2** 0.03^{b}	0.03^{b}	
Significance								
M		*		*NS*	*NS*	*		*NS*
S		***		*NS*	*NS*	*NS*		*NS*
M x S		**		*NS*	*NS*	*		*NS*

Significant correlation at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***). *NS*: not significant.

M x S represents the interaction of factors. The means followed by different letters significantly differ ($p \leq 0.05$).

The Ca in leaves was not affected by crop system, although this concentration was lower in the second season (Table 4). It should be noted that the Ca input in the conventional plots was higher in the second season (Table 1). Nevertheless, in both seasons, Ca concentrations were higher than 2.7 %. This value is considered optimum in leaves at harvest (Morales et al., 2022). In the fruit harvested in the first season, the Ca concentration was higher in the conventional plots than in the organic ones. In the second season however, no effect of crop system was found, which was related to a lower Ca concentration only in the conventional fruit. It has been reported that Ca application does not guarantee Ca assimilation by plants because this depends on the applied Ca form (Frossard et al., 2000; Karthika et al., 2018).

Although Fe inputs were much higher in the conventional than in the organic plots, the Fe concentration in both leaves and fruit was similar for both managements (Table 5). However, differences in the leaf Fe concentration were detected between seasons, which may be related to the lesser Fe application in the second season with conventional management, but this did not affect the fruit concentration.

Despite the lower Mn and Zn applications in the organic plots than in the conventional ones, a higher concentration of these elements in leaves was found for organic management (Table 5). It has been reported that higher organic matter content in organic farming can lead to better micronutrients assimilation compared to conventional cultivation (Dhaliwal et al., 2021; Martínez-Alcántara et al., 2016). Moreover, in the organic crops, fungicide products based on Mn and Zn were applied at three different times in the warmest months (July-September). Despite the main purpose not being fertilization here, these applications could have also increased the concentration of these micronutrients in organic leaves. The leaf Mn concentrations came close to the optimal values range in all cases (168.32-338.54 ppm), according to Morales et al. (2022), while the Zn concentration in the conventional leaves was below the established optimal values (28.75-44.10 ppm). In fruit, no influence of crop system on the Mn concentration was observed, while a lower Zn concentration was obtained in conventional vs. organic farming. Cardoso et al. (2015) also reported higher Zn content in the

organic 'Rama Forte' persimmon fruit than its conventional counterpart. In their case however, Zn sulfate was added to the organic plantation.

Table 5. Micronutrient concentration in the leaves and fruit of the 'Rojo Brillante' persimmon cultivated by conventional or organic management in two harvest seasons.

	Fe (ppm)	Mn (ppm)	Zn (ppm)			B (ppm)
LEAVES						
Management (M)						
Conventional	55.97	271.09[b]	-			53.87
Organic		340.46[a]	-			
Season (S)						
1	67.88[a]	305.78	-			53.87
2	44.06[b]		-			
				CV	**OR**	
M X S	-	-	**S1**	9.69[c]	58.6[a]	-
			S2	11.46[c]	47.58[b]	
Significance						
M	*NS*	***	***			*NS*
S	***	*NS*	*NS*			*NS*
M x S	*NS*	*NS*	**			*NS*
FRUIT						
Management (M)						
Conventional	4.64	5.10	3.50[b]			15.17
Organic			5.08[a]			
Season (S)						
1	4.64	5.10	5.05[a]			11.74[b]
2			3.53[b]			18.60[a]
M x S	-	-	-			-
Significance						
M	*NS*	*NS*	**			*NS*
S	*NS*	*NS*	**			*
M x S	*NS*	*NS*	*NS*			*NS*

Significant correlation at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***). *NS*: not significant.

M x S represents the interaction of factors. The means followed by different letters significantly differ ($p \leq 0.05$).

B was applied only to the conventional crops. However, a similar B concentration was found for the two management systems, and also for both leaves and fruit (Table 5), which indicates a better assimilation of this micronutrient in the organic plots. Moreover, differences in the fruit B concentration were detected between seasons despite the similar amount of B applied in both years.

3.3. Fruit physico-chemical parameters

In both seasons, fruit were harvested in October when they reached their maximum weight and in the commercial maturity stage with homogeneous coloration. The crop system did not affect fruit weight during either season, although fruit were lighter in the second season (Table 6). Regarding the external color for both studied seasons, the fruit from organic farms showed higher coloration values, and differences between seasons were also observed (Table 6). Low N availability during the growing season often results in small-sized fruit, but with better coloration (Choi et al., 2008; George et al., 1997). For some persimmon cultivars, high N supply is reported to delay external fruit coloration (Agustí et al., 2004; Choi et al., 2012; Park, 2002). Thus, in the present study, the smaller fruit size and their more advanced coloration in the second season could be related to lower N supply (Table 1). Besides, the differences in fruit color between crop systems could also be explained by the lower N supply in the organic vs. the conventional crop.

Fruit firmness is one of the most important quality parameters for 'Rojo Brillante', which is commercialized with a crisp texture (Besada et al., 2015). A strong negative correlation is known to exist between persimmon skin color and flesh firmness. This fact allows fruit firmness to be predicted from external color as a nondestructive measurement (Salvador et al., 2006; Tessmer et al., 2016). Thus, according to the obtained results of fruit external color, the firmness of the second season was slightly lower than for the first one. Besides, the organic fruit firmness was lower than for the fruit from the conventional plots during both seasons (Table 6). For both management types however, firmness values were considered high enough to be commercialized (Besada et al., 2017). Fruit softening is generally associated with the

dissolution of the middle lamella, and with changes in the composition, structure and linkages between cell wall polysaccharides (Goulao & Oliveira, 2008). Ca seems to be associated with flesh firmness because it is related to cell-wall consistency by interacting with pectins to form a cross-linked polymer network that increases mechanical strength (Liu et al., 2023; Ziogas et al., 2020). Accordingly, the higher firmness values observed in the present study for the conventional crop in the first season were accompanied by a higher Ca concentration in those fruit (Table 3). These results agree with our previous studies, in which a positive correlation of the Ca concentration and fruit firmness appeared for 'Rojo brillante' persimmon (Vilhena et al., 2022b). Liu et al. (2023) found positive effects on Ca treatment for maintaining cell wall structure integrity and for, thus, maintaining high 'Youhou' persimmon fruit firmness values.

Table 6. Weight, color index (CI), firmness and total soluble solids content (TSS) of the 'Rojo Brillante' persimmon grown by conventional and organic management in two harvest seasons.

		Weight (g)	CI	Firmness (N)	TSS (°Brix)
Management	Conventional	216.94	0.54^{b}	51.92^{a}	15.05
	Organic		0.85^{a}	47.31^{b}	
Season	1	250.05^{a}	0.42^{b}	52.42^{a}	15.05
	2	183.83^{b}	0.96^{a}	46.80^{b}	
Significance	*Management (M)*	*NS*	****	*****	*NS*
	Season (S)	*****	*****	*****	*****
	MxS	*NS*	*NS*	*NS*	*NS*

Significant correlation at $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***). NS: not significant.

M x S represents the interaction of factors. The means followed by different letters significantly differ ($p \leq 0.05$).

The TSS content was not affected by either management system (Table 6). With persimmon, it is known that changes in fruit color and firmness occur during fruit maturation and are accompanied by slight variations in TSS content

(Novillo et al., 2016; Vilhena et al., 2022a). Indeed, TSS content in not considered a good maturity index for 'Rojo Brillante' persimmon (Novillo et al., 2016).

3.4. Fruit biocomponents characterization

Different studies have been carried out to address the effect of production management on final fruit quality in different crops, however, the information available to support the idea that organic farming results in fruit with higher bioactive compounds than those conventionally produced is inconsistent (Cardoso et al., 2015; Mditshwa et al., 2017; Rahman et al., 2021).

In the present study, biocomponents characterization in the fruit grown according to conventional and organic practices was made in the second year. Of the studied organic acids, only malic acid, which is predominant in persimmon, was influenced by crop management, with a higher concentration in the fruit from the organic than the conventional plots (Table 7). The data available in previous reports on conventional and organic fruit do not allow us to conclude about a clear effect of cultivation system on organic acids (Lopez-Bucio et al., 2000).

Sugars are one of the main constituents of taste in fruit (Mditshwa et al., 2017). The predominant sugar in this study was sucrose, followed by glucose and fructose, with values falling within the range previously reported for 'Rojo Brillante' persimmon (Del Bubba et al., 2009; Novillo et al., 2015) (Table 7). A marked variability of the relative sucrose abundance in relation to other sugars has been reported in persimmon (Del Bubba et al., 2009). No influence of crop management on sugars was found, although slightly lower values were obtained in the conventional plots than in the organic ones.

Carotenoids are the major pigments present in persimmon fruit and contribute to both color and nutritional value (Yaqub et al., 2016). The individual carotenoids detected in persimmon flesh were β-carotene, β-cryptoxanthin, violaxanthin, lutein and zeaxanthin (Table 7). β-cryptoxanthin was the main carotenoid, while violaxanthin came at the lowest concentration. This falls in line with the findings reported for most persimmon cultivars (Novillo et al.,

2015; Yaqub et al., 2016). β-cryptoxanthin displayed higher concentrations in the fruit from the organic crop than from the conventional one, but no differences were observed for the other identified carotenoids between farming systems. In 'Rama Forte' persimmon, no differences were reported in the content of certain carotenes (β-carotene and lycopene) between organic and conventional fruit (Cardoso et al., 2011). For mandarin juice, Navarro et al. (2011) revealed that organic farming had a positive effect on the total carotenoids content, with significantly higher concentrations for most of the identified carotenoids. However, this effect of organic farming on the different carotenoid biosynthesis was not proposed.

Phenolic compounds have been paid considerable attention for their physiological functions, such as antioxidant, antimutagenic and antitumor activities (Gao et al., 2014). In the present study, gallic acid and ρ-coumaric acid were the main detected phenolic compounds (Table 7), which agrees with previous reports on persimmon (Gao et al., 2014; Yaqub et al., 2016). Crop management did not influence the content of these metabolites. Some studies have reported a higher concentration of phenolic compounds in organic fruit vs. conventional fruit. This has been related to lack of synthetic pesticide application in organic systems, which would induce an increase in plant defense mechanisms, such as the synthesis of phenolic compounds (Mditshwa et al. 2017; Paoletti, 2015). However, this aspect was not considered in the herein evaluated plots.

Ascorbic acid (vitamin C) is one of the essential nutritional attributes in fruit crops that is linked with several fundamental metabolic functions in the human body, including the stimulation of the immune system (Paoletti, 2015). Many studies have indicated that fruit from organic crops have higher vitamin C contents than those from conventional crops. Other research works have found minor or no differences in vitamin C in the fruit grown according to these two systems (Bordeleau et al., 2002). In the present study, the organic crop fruit obtained higher ascorbic acid values than the conventional fruit (Table 7). Similarly, strawberry, tomato and mandarin (Hallmann, 2012; Navarro et al., 2011; Reganold et al., 2010) grown by organic management have exhibited higher vitamin C contents than those grown by conventional practices. Nevertheless in 'Rama Forte' persimmon, Cardoso et al. (2011)

found more vitamin C content in conventionally produced fruit than in those organically cultivated.

In the present study, the highest vitamin C values detected in the organic crops could be related to a small amount of N applied with this management. Previous studies have reported that N fertilizers applied at low rates tend to increase the vitamin C content in many fruits and vegetables. This is because low N availability induces the biosynthesis of nonnitrogen-containing compounds, such as ascorbic acid (Lee and Kader, 2000; Mditshwa et al., 2017).

Table 7. Biocomponents concentration in the 'Rojo Brillante' persimmon fruit cultivated by conventional or organic management.

Group	Compound	Management	
		Conventional	Organic
Organic acids (mg 100 g^{-1})	Malic	177.5[b]	239.1[a]
	Citric	164.8[a]	161.7[a]
	Succinic	97.4[a]	87.5[a]
	Fumaric	8.3[a]	8.0[a]
Individual sugars (g kg^{-1})	Sucrose	99.8[a]	100.2[a]
	Glucose	31.0[a]	37.3[a]
	Fructose	22.3[a]	27.9[a]
Carotenoids (µg 100 g^{-1})	β-Carotene	91.0[a]	115.0[a]
	β -Cryptoxanthin	373.6[b]	581.3[a]
	Violaxanthin	0.6[a]	0.6[a]
	Lutein	4.2[a]	4.7[a]
	Zeaxanthin	37.1[a]	43.5[a]
Phenolics (mg 100 g^{-1})	Gallic	2.2[a]	2.2[a]
	ρ-Coumaric	0.32[a]	0.34[a]
Ascorbic acid (mg 100 g^{-1})		8.5[b]	10.4[a]

The means followed by different letters significantly differ ($p \leq 0.05$).

4. Conclusions

This study provides new information about the mineral and nutritional composition of 'Rojo Brillante' persimmon grown by organic and conventional management systems. The biggest yield was obtained in the conventional crop and was attributed to the higher fertilization rate in this system. Nevertheless, the evaluation of the agronomic efficiency of the main macronutrients indicated that organic management offered higher use efficiency of the applied fertilizers than the conventional crop, which is very interesting for the more sustainable use of key nutrients for plant production.

Although greater N input was supplied in the conventional than in the organic plots, the leaf N concentrations fell within the optimal range established for 'Rojo Brillante' persimmon in both crop systems, which corroborate more efficient N use in organic vs. conventional fertilization.

In general, the greater macro- and microelements supplied in the conventional vs. the organic system did not imply a higher concentration of these elements in leaves and fruit. A similar concentration was observed for P, K, Fe and B in both management systems and the highest concentrations for Mn and Zn in the organic plots. In addition, the optimum range for the leaf concentrations detected in general, in both crop systems, indicates that adequate fertilization was performed for both managements, as well as the important role of organic matter in favoring nutrient assimilation.

More advanced fruit color and lower firmness values were obtained in the organic fruit compared to those conventionally grown, which is related to the lowest N input in the organic crop. Besides, the higher Ca concentration in the conventional fruit flesh was linked with the highest firmness values for this fruit. The influence of crop management on the fruit biocomponents concentration was noted only for malic acid, β-Cryptoxanthin and ascorbic acid, which were higher in the organic than the conventional fruit.

Acknowledgements

This research was funded by the Ministerio de Ciencia e Innovación of Spain, through Project RTA2017-00045-C02 and the Fondo Europeo de Desarrollo Regional (FEDER). The authors thank the Cooperativa Agrícola Sant Bernat Coop. V. for technical support and for supplying the plots used in this study. Nariane Q. Vilhena thanks the Ministerio de Ciencia e Innovación of Spain for Grant PRE2018-085833.

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CHAPTER III

Effect of Preharvest 1-MCP Treatment on the Flesh Firmness of 'Rojo Brillante' Persimmon

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Reference: *Horticulturae*, 2022, 8(5), 350.
https://doi.org/10.3390/horticulturae8050350

Abstract

This study investigated the effect of preharvest 1-MCP treatment on maintaining 'Rojo Brillante' persimmon firmness. Early in the season, preharvest 1-MCP was applied 1, 7 and 10 days after ethephon treatment. The fruit firmness was evaluated during three different harvests and after the commercialization period of 3 d at 3 ºC, plus 6 d at 20 ºC. Late in the season, 1-MCP was applied 3 days before harvest in the fruit treated with gibberellic acid (GA) and then cold-stored for up to 60 days, plus a 6-day shelf life at 20 ºC. The results showed that preharvest 1-MCP delayed the fruit softening induced by ethephon during the harvest period and was the most effective treatment when performed 1 day after ethephon application. Therefore, preharvest 1-MCP extended the harvest period of ethephon-treated fruit. At the end of the season, preharvest 1-MCP had the same effect on maintaining the fruit firmness as the commercial postharvest application.

Keywords: *Diospyros kaki* Thunb.; 1-methylcyclopropene; ethylene; fruit firmness.

1. Introduction

'Rojo Brillante' persimmon (*Diospyros kaki* Thunb.) is the main cultivar produced in the Mediterranean region and is commercialized as fruit with high firmness values after being subjected to deastringency treatment at high CO_2 concentrations (Novillo et al., 2015). Therefore, flesh firmness is the main attribute that must be maintained during the postharvest period (Salvador et al., 2007).

As the persimmon maturation period is short, fruit cold storage is necessary to allow for exportation and to destine part of production to cover commercial demands at the end of the season. However, persimmon is sensitive to low temperature and develops chilling injury (CI) symptoms, such as flesh gelling and firmness loss (Arnal and Del Río, 2004). Previous studies have reported how the postharvest application of 1-methylcyclopropene (1-MCP), an innocuous gas used at very low concentrations, inhibits ethylene action by binding to ethylene receptors, which alleviates CI symptoms in most persimmon varieties (Harima et al., 2003; Luo, 2007). Therefore, 1-MCP is routinely applied in industry for cold-stored persimmon fruit.

To date, 1-MCP is applied to persimmon as a postharvest treatment prior to cold storage. However, preharvest 1-MCP (Harvista®, Philadelphia, PA, USA) treatment, applied as a liquid spray to trees, is a reported novel option for maintaining fruit quality throughout the postharvest and replaces postharvest treatment in some crops, such as apples or pears (Li et al., 2021; Sakaldas and Gundogdu, 2015; Tomala et al., 2020; Varanasi et al., 2013). In persimmon, information about the effect of preharvest 1-MCP application is scarce. Only one study about the 'Fuyu' cultivar has reported a positive effect of this treatment on retarding fruit maturity on trees (Vieira et al., 2016).

In the specific case of 'Rojo Brillante', it would be interesting to know the effect of applying preharvest 1-MCP in different scenarios; on the one hand, at the end of the season to the fruit destined for cold storage. In this case, fruit are usually treated with gibberellic acid (GA) during the preharvest to delay fruit ripening (Besada et al., 2008), whereas postharvest 1-MCP is applied prior to cold storage. On the other hand, the effect of preharvest 1-MCP should be tested early in the season, in fruit treated with ethephon to advance maturity.

When ethephon is applied, the fruit harvesting period is short because they begin to ripen with a consequent firmness loss. Moreover, ethephon-treated fruit must be marketed quickly after harvest. Thus applying 1-MCP is often necessary to maintain commercial firmness values during the marketing period.

In this context, the present study investigated the effect of preharvest 1-MCP application on maintaining the firmness of 'Rojo Brillante' persimmon in these two scenarios: (1) fruit treated with ethephon early in the season; (2) fruit treated with GA late in the season.

2. Materials and Methods

2.1. Fruit Material

2.1.1. Experiment 1: Applying Preharvest 1-MCP to Ethephon-Treated Fruit

This study was conducted in two commercial 'Rojo Brillante' persimmon orchards located in Alcudia (Valencia, Spain) (lat. 39°11'25.5'' N, long. 0°32'43.0'' W and lat. 39°11'25.5'' N and long. 0°28'28.2'' W). Average temperature and relative humidity during the experiment period were taken from the IVIA weather station and ranged from 13.5 ^{o}C to 22.01 ^{o}C and 43.1 % to 94.8 %, respectively.

In each orchard, four rows of six trees were randomly taken for subsequent treatments. All of the trees in both orchards were ethephon-treated (0.08 cm^3 L^{-1}) (Fruitel®, Bayer Cropscience S.L., Leverkusen, Germany) under commercial conditions on October 5, when the fruit color index was -1.75 (CI = 1000 a/Lb, 'L', 'a', 'b' Hunter parameters). The trees of three rows were sprayed with preharvest 1-MCP (pre-MCP, 12 g L^{-1}) (Harvista®, Agrofresh Inc., Philadelphia, PA, USA) on days 1 (pre-MCP-1d), 7 (pre-MCP-7d) and 10 (pre-MCP-10d) after ethephon application, respectively. The fourth row was not treated with preharvest 1-MCP (CTL).

Three harvests took place: the first one the day after the last pre-MCP application (16 October), and the following harvests on 30 October and 10 November. On each harvest date, 150 fruit per treatment were picked. One lot

of 50 fruit was characterized at harvest. The other two lots of 50 fruits were submitted to a simulated 3-day commercialization period at 3 ºC, plus 6 days at 20 ºC, with or without the postharvest 1-MCP treatment (post-MCP). Postharvest 1-MCP (Smartfresh®, Agrofresh Inc.) was applied under commercial conditions (0.5 µL L^{-1} for 24 h) in cold chambers (Salvador et al., 2004).

2.1.2. Experiment 2: Applying Preharvest 1-MCP to Gibberellic Acid-Treated Fruit

This study was performed in the other two commercial 'Rojo Brillante' orchards in Alcudia (Valencia, Spain) (lat. 39°10'51.2'' N, long. 0°30'05.3'' W and lat. 39°10'51.1'' N, long. 0°30'05.9'' W). Average temperature and relative humidity during the experiment period were taken from the IVIA weather station and ranged from 10.1 ºC to 23 ºC and 35 % to 94.8 %, respectively.

In each orchard, four rows of six trees were randomly taken for subsequent treatments. The trees of two rows were sprayed with GA (30 µL L^{-1}) (Berelex® 40 SG, Kenogard S.A., Barcelona, Spain) on September 25, when the fruit skin color index came close to –6 (one GA treatment (GA1)). The trees of the other two rows were sprayed with two GA treatments (GA2) on September 25 and October 15. Three days before harvesting, one row of GA1 and one row of GA2 were sprayed with pre-MCP (22 g L^{-1}). In accordance with commercial criteria, the fruit from GA1 and GA2 were harvested on November 16 and 23 November, respectively.

After harvesting, lots of 50 fruits were formed. One lot per treatment was evaluated at harvest. In order to compare the effect of 1-MCP applied at pre- or postharvest, part of the lots was treated with post-MCP (0.5 µL L^{-1} for 24 h) prior to cold storage. This gave six different treatments:

(1) GA1 (fruit treated once with GA)
(2) GA1 + pre-MCP (fruit treated once with GA + preharvest 1-MCP)
(3) GA1 + post-MCP (fruit treated once with GA + postharvest 1-MCP)
(4) GA1 (fruit treated twice with GA)
(5) GA1 + pre-MCP (fruit treated twice with GA + preharvest 1-MCP)
(6) GA1 + post-MCP (fruit treated twice with GA + postharvest 1-MCP)

One lot of each treatment was evaluated after 20, 40 or 60 days at 0 ºC, plus 6 days at 20 ºC, to simulate the shelf-life period.

2.3. Determinations

At harvest and after the different storage periods, flesh firmness was determined by a texturometer (Instron Corp., mod. 4301, Canton, MA, USA) using an 8 mm diameter punch. The results were expressed as the force (N) needed to break the pulp in the equatorial zone, from which, skin had been previously removed.

Data were subjected to analyses of variance (ANOVA). The multiple comparisons between means were determined by the LSD test ($p \leq 0.05$) with the Statgraphics Centurion XVII.I software application (Manugistics, Inc., Rockville, MD, USA

3. Results

3.1. Effect of Applying Preharvest 1-MCP on Ethephon-Treated Persimmon

On the three harvest dates, an effect of pre-MCP treatments was found on the flesh firmness. No differences between the orchards were observed. The lowest firmness values were for the control fruit (CTL) on all of the harvest dates, and no large differences appeared among the three pre-MCP treatments (Figure 1). Only in the second harvest (30 October) did the pre-MCP-10d fruit have slightly lower values than the pre-MCP-1d and pre-MCP-7d fruit. On the third harvest date, the CTL fruit obtained values of 23.2 N, and the pre-MCP-treated fruit still had values close to 35 N regardless of the date when the treatment was applied.

After the commercialization period, in the fruit harvested on 16 October, the pre-MCP-1d and pre-MCP-7d fruit without post-MCP had the highest values, which were close to 40 N (Table 1). The pre-MCP-10d fruit obtained lower firmness values of 36 N, which were higher than those of the CTL treatment (29.29 N). A similar effect was observed for the following harvests: while the

CTL fruit presented firmness values close to 13 N, the pre-MCP-treated fruit had values above 20 N in all cases.

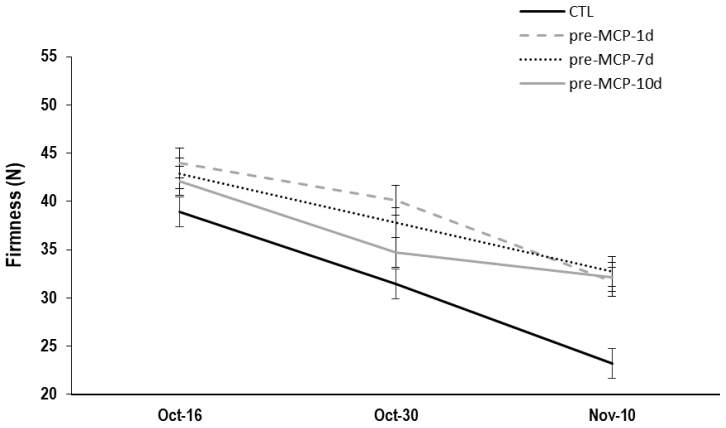

Figure 1. Effect of applying preharvest 1-MCP on days 1 (pre-MCP-1d), 7 (pre-MCP-7d) or 10 (pre-MCP-10d) after ethephon treatment on 'Rojo Brillante' persimmon flesh firmness on three harvest dates. CTL is the fruit not treated with preharvest 1-MCP. Vertical bars represent the least significant differences (LSD) intervals ($p \leq 0.05$).

Table 1. Effect of applying preharvest 1-MCP on days 1 (pre-MCP-1d), 7 (pre-MCP-7d) or 10 (pre-MCP-10d) after ethephon treatment on 'Rojo Brillante' persimmon flesh firmness on three harvest dates. CTL is the fruit not treated with preharvest 1-MCP. Vertical bars represent the least significant differences (LSD) intervals ($p \leq 0.05$).

	Harvest date					
	16 October		**30 October**		**10 November**	
	No Post-MCP	With Post-MCP	No Post-MCP	With Post-MCP	No Post-MCP	With Post-MCP
CTL	29.29^{cB}	36.18^{bA}	13.40^{cB}	24.88^{cA}	13.00^{bA}	18.46^{bA}
pre-MCP-1d	40.59^{aA}	40.68^{aA}	22.63^{bB}	31.46^{bA}	18.88^{aB}	27.32^{aA}
pre-MCP-7d	39.04^{baA}	40.08^{aA}	20.76^{bB}	35.29^{aA}	23.52^{aA}	26.28^{aA}
pre-MCP-10d	35.96^{bA}	34.03^{bA}	29.90^{aA}	32.14^{baA}	23.12^{aA}	21.69^{bA}

The means followed by the same lowercase letter in columns and by the same uppercase letters on lines did not differ from one another according to the ANOVA test ($p \leq 0.05$).

As expected, the postharvest 1-MCP application reduced softening in the CTL fruit. Even so, only the fruit from the first and second harvests had values above 20 N after the commercialization period. In the pre-MCP-treated fruit, the postharvest 1-MCP application did not improve the firmness of the fruit harvested on 16 October (Figure 2). Nevertheless, in the fruit harvested later, a higher firmness was shown when the post-MCP treatment was applied.

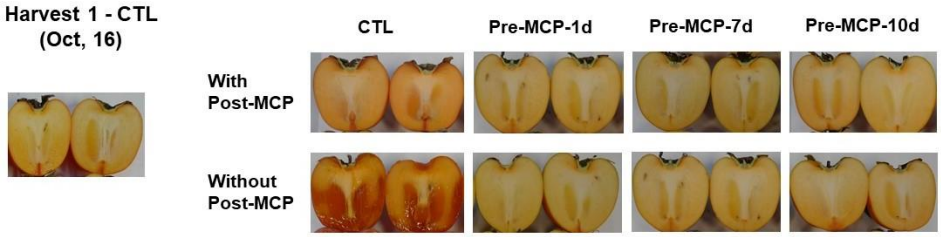

Figure 2. Captured images of 'Rojo Brillante' persimmon at harvest (on 16 October) and after the commercialization period (3d at 3 °C plus 6d at 20 °C). Fruit was preharvest treated with 1-MCP applied on days 1 (pre-MCP-1d), 7 (pre-MCP-7d) or 10 (pre-MCP-10d) after ethephon treatment and postharvest treated or not with 1-MCP (post-MCP). CTL is the fruit without preharvest 1-MCP treatment.

3.2. Effect of Applying Harvista® after GA on Fruit Quality

The fruit treated once with GA (GA1) without the pre- or postharvest 1-MCP treatments lost firmness throughout storage, with values close to 0 N after 40 days (Figure 3A). As expected, the post-MCP treatment maintained high firmness values, similarly to those of the harvest lasting up to 40 days. After 60 days, a slight decrease in values of 31.5 N was observed. It was noteworthy that the fruit treated with pre-MCP 3 days before harvesting had the same firmness values as the fruit treated with post-MCP throughout the storage period (Figure 4).

Regarding the fruit treated twice with GA (GA2), although those not treated with pre- or post-MCP had higher firmness values than GA1 after 20 days, their values were also close to 0 N after 40 days. The pre-MCP-treated fruit obtained

slightly lower firmness values than the post-MCP fruit. After 60 days, both treatments obtained similar values, close to 32 N (Figure 3B).

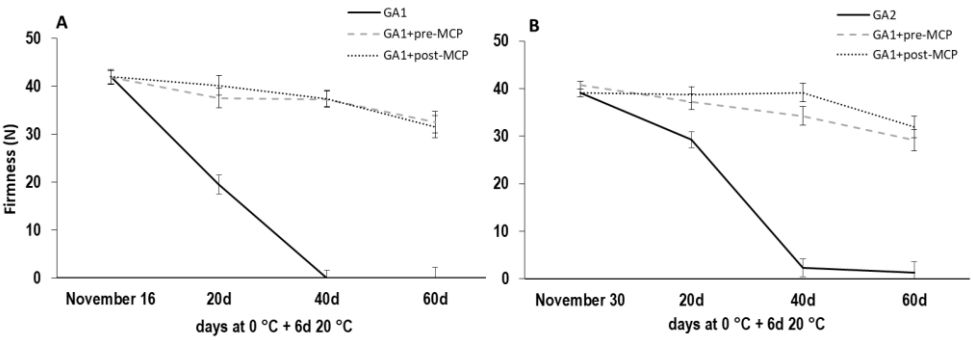

Figure 3. Flesh firmness of the 'Rojo Brillante' persimmon treated with 1-MCP during preharvest (pre-MCP), postharvest (post-MCP) or not treated, during cold storage up to 60 d plus a 6-day shelf life at 20 ºC. GA1 is the fruit treated once with gibberellic acid (A) and GA2 is that treated twice with gibberellic acid (B). Vertical bars denote the least significant differences (LSD) intervals ($p \leq 0.05$).

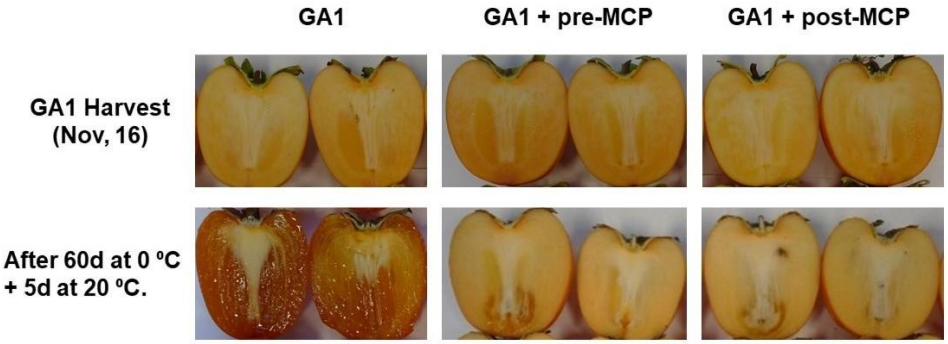

Figure 4. Captured images of 'Rojo Brillante' persimmon treated once with gibberellic acid (GA1) at harvest moment (on 16 November) and after 60 d at 0 ºC plus 6-day shelf life at 20 ºC. Fruit was treated with 1-MCP during preharvest (pre-MCP), postharvest (post-MCP) or not treated.

4. Discussion

Most of the studies that evaluate the effect of pre-MCP are made on apples and pears (Lee et al., 2019; Li et al., 2021; Varanasi et al., 2013). The obtained results show that the combination of pre- and postharvest 1-MCP application optimizes the fruit capacity to retain ripening and reduce the incidence of disorders during cold storage, resulting in a higher fruit firmness as well as longer ethylene suppression. In persimmon, only a study on the cultivar 'Fuyu' compared the effects of pre- and post-MCP treatments on the fruit quality during maturity, and positive results were found with the pre-MCP application (Vieira et al., 2016).

In the present study, we found that the pre-MCP application delayed the 'Rojo Brillante' persimmon fruit firmness loss induced by ethephon during the harvest period and proved to be the most effective treatment when performed 1 day after ethephon application. Therefore, pre-MCP prolonged the harvest period of the ethephon-treated fruit. In papaya, Sañudo-Barajas et al. (2008) found that the application of pre-MCP one day after ethephon was an effective strategy to avoid an excessive softening of the fruit, which allowed for an extension of the shelf-life. It was also reported that the preharvest application of 1-MCP to sweet cherry trees within 3 days of ethephon treatment inhibited ethephon-induced flesh firmness loss (Elfving et al., 2009). In addition, pre-MCP application has been shown to be a good option for maintaining fruit firmness during the posterior marketing period, when fruit are harvested in mid-October without having to apply a postharvest 1-MCP treatment. Moreover, during the subsequent harvests, the pre- and post-MCP combination maintained a greater flesh firmness during the commercialization period than the single post-MCP application.

On the other hand, gibberellic acid is applied in persimmon fruit to delay ripening and to therefore extend the harvest period (Ben-Arie et al., 1985; Besada et al., 2008). 'Rojo Brillante' persimmon destined to cold storage for long periods are those treated on-field with GA and subjected to a post-MCP treatment to avoid the firmness loss. In the present study, a very interesting result is that the application of pre-MCP 3 days before harvesting had the same

effect on maintaining the fruit firmness as the post-MCP application during cold storage for up to 60 days.

5. Conclusion

The pre-MCP application delayed the 'Rojo Brillante' persimmon fruit firmness loss induced by ethephon, prolonging the fruit harvest period, and proved to be the most effective treatment when performed 1 day after ethephon application. In addition, the pre-MCP application maintained the fruit firmness during the marketing period, when fruit were harvested in mid-October without having to apply a postharvest 1-MCP treat- ment. Furthermore, during the subsequent harvests, the pre- and post-MCP combination maintained a greater flesh firmness during the commercialization period than the single post-MCP application.

On fruit treated with GA to delay ripening, the application of pre-MCP three days before harvesting maintained the fruit firmness to the same extent as the post-MCP ap- plication after cold storage. Thus, replacing the post-MCP application with the pre-MCP treatment can be a very useful tool for improving handling operations in packing houses.

Further studies are necessary to elucidate the role of pre-MCP in maintaining quality during the postharvest persimmon fruit period.

Acknowledgements

This research was funded by the European Union through the European Regional Development Fund (FEDER) through Project IVIA-51910 and by the company Agrofresh Spain S.L.U. N.Q.V. thanks the Spanish Ministry of Science and Innovation for Grant FPI-INIA (PRE2018-085833).

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CHAPTER IV

Slight Changes in Fruit Firmness at Harvest Determine the Storage Potential of the 'Rojo Brillante' Persimmon Treated with Gibberellic Acid

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Reference: *Horticulturae*, 2022, 8(2), 140.
https://doi.org/10.3390/horticulturae8020140

Abstract

Today, the 'Rojo Brillante' persimmons undergoing prolonged storage are treated with giberellic acid, which allows the delay of the harvesting to November-December. Although during this period the fruit maintained high commercial firmness, practical experience indicates very different behavior during the posterior cold storage, depending on the harvest moment. To explain what leads to these differences, an in-depth study of the physico-chemical and microstructural changes occurring in the fruit during five commercial harvest times from November to December was carried out. During this period, slight variations in firmness occurred, ranging from 48 to 40 N. Nevertheless, the fruit behavior under cold storage was strongly influenced by the harvest date, which was explained by the degradation of cell wall, cell membrane and tonoplast, mainly noted in fruit from the latest harvests. Therefore, the fruit harvested with firmness close to 48 N had a highly structured cell, which maintained firmness during cold storage for up to 90 days. The fruit harvested with 43 N presented a more degraded structure, while the fruit with initial firmness around 40 N underwent major ultrastructure cell wall and membranes modifications, which led to greater firmness loss. Therefore, the fruit firmness at harvest is decisive for its storage potential.

Keywords: *Diospyros kaki* Thunb; Cryo-FESEM; cold storage; fruit firmness; fruit ultrastructure.

1. Introduction

'Rojo Brillante' is the main persimmon (*Diospyros kaki* Thunb) cultivar produced in Spain, whose production reached 590,000 tons in 2019. It is an astringent variety of the PVA type (pollination variant astringent) (Giordani et al., 2015). Although it is grown mainly in the Mediterranean region, this variety is highly valued commercially in other countries due to its high quality, which leads to the export of a large quantity of its production that is up to 210,000 tons during the 2019 season (IndexBox, 2000). 'Rojo Brillante' maturation takes place between mid-October and the end of November. This short period involves having to introduce techniques that allow an extended harvest period to supply the market with fresh high-quality fruit for a longer period.

Flesh firmness is the most important quality attribute of 'Rojo Brillante' because this cultivar is commercialized as fruit with high firmness values after being subjected to deastringency treatment at high CO_2 concentrations (Besada et al., 2008; Novillo et al., 2014). It is known that flesh firmness at harvest plays a crucial role in fruit quality during the postharvest period (Salvador et al., 2005, 2007; Testoni, 2002). Therefore, introducing pre- and postharvest treatments to prolong commercial periods in order to maintain the fruit firmness during the postharvest period has been a challenge in recent years.

Pre-harvest treatments with gibberellic acid (GA3) at fruit color-break are well established for persimmon; they delay fruit maturity, the green-to-orange color change and also flesh softening on trees by maintaining good fruit firmness at the end of the season (Agustí et al., 2004; Testoni, 2002). Treatment is generally applied up to three times, with a separation of 15–20 days between applications, to enhance delayed fruit maturation.

Cold persimmon storage is also necessary to manage today's large production and to extend the commercial period, especially toward its end. Nevertheless, 'Rojo Brillante' is sensitive to low temperature and develops chilling injury symptoms when exposed to temperatures below 8 ºC (Arnal and Del Río, 2004). The main chilling injury manifestation is firmness loss, which certainly compromises final fruit quality. Therefore, to preserve 'Rojo Brillante', currently it is necessary to apply a treatment with 1-methylcyclopropene

(1-MCP) before cold storage to delay flesh softening (Pérez-Munuera et al., 2009). The combination of GA3 at pre-harvest and 1-MCP at postharvest has been previously reported to prolong low-temperature storability (Besada et al., 2008).

Today, the 'Rojo Brillante' persimmons stored to be commercialized from January onward are the fruit treated with GA3 at pre-harvest, harvested from November to December, and treated with 1-MCP before storage, as this is a well-established practice for persimmon producers. Throughout this harvest period, fruit firmness values remain high, which seems suitable for conservation purposes. Nevertheless, practical experience indicates a very different postharvest behavior between fruit harvested during this period.

Hence, the aim of this study is the physio-chemical and microstructural characterization at different harvest times of fruit destined to prolonged conservation, in order to explain the differences in fruit behavior during cold storage.

2. Materials and Methods

2.1. Fruit Material

This study was conducted in two commercial 'Rojo Brillante' persimmon orchards located in Alcudia (Valencia, Spain). In both orchards, three GA3 treatments (30 µL L^{-1}) were applied under commercial conditions. The first GA3 treatment took place on October 7 and the following applications were performed every 15 days after the first one, on 21 October and 3 November.

Six trees per plot (three trees as one replicate) were previously marked. Five harvests of 140 fruit per replicate were performed, totalizing 280 fruit per orchard at each harvest moment.

The harvests took place from the time skin displayed a commercial uniform greenish-yellow tone, from 11 November to 9 December, with one harvest per week: H1 (11 November); H2 (18 November); H3 (25 November); H4 (2 December); H5 (9 December).

The harvested fruit were transported to the Instituto Valenciano de Investigaciones Agrarias (IVIA, Spain), where one lot of 20 fruit per replicate was characterized at harvest, and three lots of 40 fruit per replicate were stored for 30, 60 or 90 days at 0 ºC, plus 5 more days at 20 ºC, to simulate the shelf-life period. In all cases, fruit were treated with 1-MCP under commercial conditions (500 nL L^{-1} of 1-MCP for 24 h at 1 ºC) before storage (Salvador et al., 2004) and were submitted to the deastringency treatment after storage under standard conditions (95 % CO_2 for 24 h at 20 ºC and 90 % RH). These treatments aimed to reproduce in fruit the standard postharvest practices adopted commercially.

In order to characterize fruit at the different harvest times, the following determinations were made: external color, flesh firmness, total soluble solids content and soluble tannins. Ethylene and CO_2 production were measured in both the fruit and calyx once the calyx was carefully separated from the fruit. A microstructural study of fruit flesh was also performed by electronic cryo-scanning electron microscopy (Cryo-SEM), transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM). Flesh firmness was also determined after each shelf-life period.

2.2. Determinations

Firmness was determined on the equatorial zone, from which skin had been previously removed, on each individual fruit per replicate using a texturometer (Instron Corp., mod. 4301, Canton, MA, USA) by making an 8-mm diameter punch on fruit samples. The results were expressed in Newton units (N) as the force needed to break the flesh (Salvador et al., 2004).

The external color was evaluated with a colorimeter (Minolta, model CR-300, Ramsey, NY, USA) using the L, a, b Hunter parameters. The results were expressed as a color index (CI = 1000 a/Lb) (Jiménez-Cuesta et al., 1981).

Soluble tannins were measured according the Folin-Denis method (Taira and Ono, 1996), as described by Arnal and Del Río (2004), of over 3 samples of 5 fruit per replicate. The results were expressed as a percentage of fresh weight. To determine total soluble solids, fruit juice was obtained from over 3 samples

of 5 fruit per replicate using an electric juice extractor and filtered. To avoid the interference of tannins, their insolubilization was previously carried out using a 5 % polyethyleneglycol solution (w/w) (PEG 6000, Panreac) to precipitate the tannins still present in juice (Sugiura et al., 1983). Measurements were taken by a refractometer (Atagomod. PR1) and the results were expressed as %.

During each harvest, CO_2 and ethylene production were evaluated in four whole fruit per replicate, and also in four calyxes that had been previously separated from fruit according to the method described by Fathi-Najafabadi (2021). The fruit and calyx samples were placed in 1 L and 100 mL jars, respectively. Jars were sealed and placed in a chamber at 20 ºC for 24 h. The concentrations of CO_2 and ethylene inside the jars were measured by taking 1 mL of gas sample from the headspace with a BD Plastic pack syringe, through a silicone septum fitted in the jar and injected into the gas chromatograph with a Poropak QS 80/100 column (model 2000, Perkin-Elmer, Norwalk, CONN, USA). The CO_2 concentration was determined using a thermal conductivity detector. Helium was used as the carrier gas at 63.4 kPa. The injector, oven and detector temperatures were 115 ºC, 35 ºC and 150 ºC, respectively. The ethylene concentration measurement was made using a flame ionization detector. Helium was the carrier gas at 55 kPa. The injector, oven and detector temperatures were 175 ºC, 75 ºC and 175 ºC, respectively. The CO_2 concentration was expressed as mmol kg^{-1} h^{-1}, while ethylene concentration was expressed as nmol kg^{-1} h^{-1}.

Microstructural study was performed on over two fruit per replicate. A cryo-scanning electron microscopy (Cryo-SEM, ZEISS ULTRA 55, Oxford Instruments, Abingdon, UK) was used to observe the flesh samples. They were placed in the holder, frozen by immersion in slush nitrogen and transferred to a cryogenic unit (CT 15,000 C; Oxford Instrument, Oxford, UK) connected to a scanning electron microscope (SEM) (JEOLJSM 5410, JEOL, Tokyo, Japan). After fracturing and being coated with platinum at 5 mA for 20 s, samples were observed at 2 kV at a working distance of 3-5 mm.

Confocal laser scanning microscopy (CLSM) was carried out with a ZEISS 780 microscope coupled to an Axio Observer Z1 inverted microscope (Carl Zeiss,

Germany). A C-Apochromat 40X/1.2 W water immersion objective was used to visualize the samples. Images were obtained at a resolution of 1024 × 1024 pixels using the microscope software (ZEN). Calcofluor White (Fluka, Sigma-Aldrich, MO, USA) was used to stain polysaccharides and was excited with diode line 405 and detected at 410-477 nm. Moreover, carotenoid autofluorescence was observed at 515 nm. To observe and study samples, tissue sections (20 µm thick) were obtained with a cryostat (CM 1950, Leica Biosystems, Nussloch, Germany). The portion of tissue was placed on a slide, and 20 µL of Calcofluor White were added and left to rest for 5 min. Then, the samples were covered with a glass coverslip.

The samples' ultrastructure was observed under a transmission electron microscopy (TEM) (HITACHI HT7800 120 kV, Hitachi, Japan). The fresh flesh samples were cut into cubes (3 mm^3), which were fixed with 25 g L^{-1} glutaraldehyde solution (0.025 M phosphate buffer, pH 6.8, at 4 °C for 24 h), post-fixed with 20 gL^{-1} osmium tetroxide solution (1.5 h), dehydrated using a graded ethanol series (300, 500, 700 and 1000 g kg^{-1}), contrasted with uranyl acetate solution (20 g L^{-1}) and embedded in LR-white resin (Aname, Madrid, Spain). The obtained blocks were cut using a Reichert-Jung ULTRACUT ultramicrotome (Leica Microsystems, Wetzlar, Germany) in ultrathin sections (0.1 µm), collected in copper grids and stained with lead citrate (40 g L^{-1}) to be observed at 100 kV.

2.3. Statistical Analysis

The data were analyzed following the analysis of variance (ANOVA) to evaluate the effect of harvest, orchard and storage time and their interaction. The mean values were compared by the least significant difference test (LSD) at a significance level of 5 %. ($p \leq 0.05$). The analyses were carried out using the Statgraphics Centurion XVII.I software (Manugistics, Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Physico-Chemical Characterization at Harvest

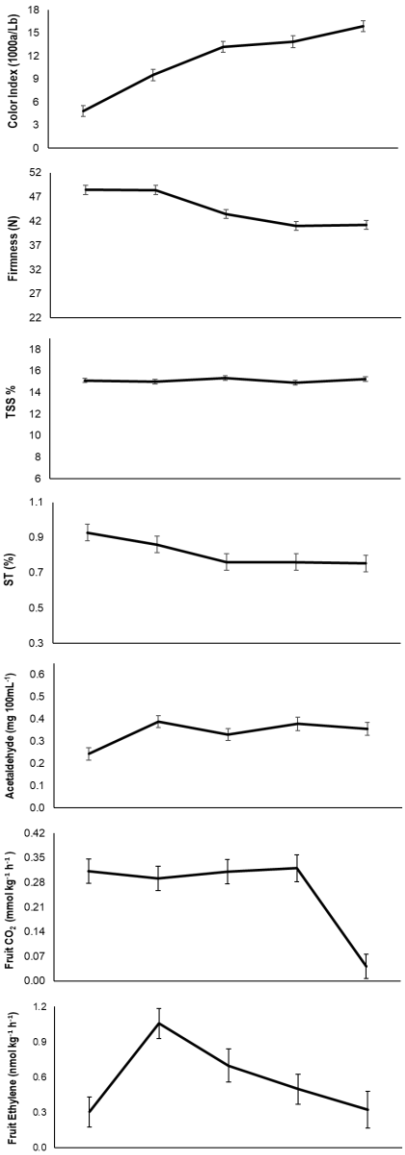
The physico-chemical fruit parameters at 5 harvesting weeks are shown in Figure 1. No influence of the orchard where fruit came from was found for any studied parameter at harvest.

Persimmon fruit are considered ready to harvest when they display a homogeneous orange or red color, depending on the cultivar, with no visible green background (Besada and Salvador, 2018). Therefore, the first harvest took place when fruit displayed the commercial external coloration with a color index close to 5. Over the next 4 weeks, a gradual increase in external coloration was shown until the CI values came close to 17, which corresponded to an orange-red stage.

Firmness is one of the main quality parameters to consider during the 'Rojo Brillante' persimmon commercialization. Nevertheless, the optimum firmness at harvest is not established and largely depends on the storage conditions to which fruit submit (Salvador et al., 2004, 2005). In accordance with company Agrofresh S.A. recommendations, which supplies the 1-MCP application for persimmon, a minimum of 40 N is necessary at harvest to keep fruit at 0– 1 °C, with 1-MCP pretreatment guaranteeing commercial fruit firmness (above 20 N) after 40 storage days (Besada et al., 2017).

In the present study, the fruit demonstrated high flesh firmness values at all the harvests. On 11 November (H1), fruit firmness was 48.4 N. No changes were observed after 1 week (H2), but a decrease occurred at the following harvests to values of 43 N in H3, and of 40 N in H4 and H5.

In 'Rojo Brillante', a strong negative correlation between external color and flesh firmness during fruit maturation has been reported (Salvador et al., 2006; Tessmer et al., 2016; Zhang et al., 2018). Nevertheless, in this study, only a correlation of -0.53 was found between these two parameters. This weak correlation could indicate that an increase in the color of GA3-treated fruit is not always accompanied by diminished firmness. Flesh firmness remaining while external color increases is beneficial from a commercial point of view.



Significance

	CI	Firmness	TSS	ST	CO_2	Ethylene
A: Harvest	0.000	0.000	0.002	ns	0.000	0.000
B: Orchard	ns	ns	ns	ns	ns	ns
AB:	0.027	ns	ns	0.016	ns	ns

Figure 1. Changes in the physico-chemical parameters of 'Rojo Brillante' persimmon fruit at the different harvest times. H: Harvest; H1: 11 November; H2: 18 November; H3: 25 November; H4: 2 December; H5: 9 December. Vertical bars represent the least significant differences (LSD) intervals ($p \leq 0.05$).

During the study period, TSS content was around 15 %, with no changes with harvest advance. Total soluble solids content is not a good maturity index for astringent persimmon cultivars because, when determined at harvest, this measurement includes not only sugar, but also soluble tannins (Besada and Salvador, 2018). In our case, the TSS determination was made after eliminating soluble tannins from samples so that it would indicate only sugar content. The values herein obtained fell within the range of the commercial values reported in previous studies (Conesa et al., 2020; Martínez-Las Heras et al., 2016; Novillo et al., 2016).

At all the harvest times, high soluble tannin (ST) content was observed, which reflects this variety's characteristic astringency (Salvador et al., 2005). Although a reduction in week 3 was detected, it did not suffice to cause astringency loss. The recorded values, between 0.9 % and 0.7 %, fell within the range reported for 'Rojo Brillante' persimmon at harvest throughout the season (Besada and Salvador, 2018; Del Bubba et al., 2009; Tessmer et al., 2016). This high ST content involves having to apply deastringency treatment prior to the commercialization of astringent persimmon varieties, such as 'Rojo Brillante'.

CO_2 fruit production was similar from H1 to H4, with values close to 0.3 mmol kg^{-1} h^{-1}, and drastically dropped at H5 to 0.04 mmol kg^{-1} h^{-1}. Novillo et al. (2016) recorded values within the same range for 'Rojo Brillante' and other cultivars when fruit had homogeneous external coloration.

For fruit ethylene production, very low values were detected at all the harvest times with a maximum peak of 1.06 nmol kg^{-1} h^{-1} at H2, which corresponds to an external CI of 9.5. According to Woolf and Ben-Arie (2011), persimmon produces extremely low ethylene levels with peaks below 2.2 nmol kg^{-1} h^{-1}. Previous studies conducted with 'Rojo Brillante´ have also obtained very low ethylene values during fruit maturation. In those studies, the maximum value came close to 1.76 nmol kg^{-1} h^{-1}, which was observed when fruit had not yet reached commercial coloration (CI of -1) and indicates persimmon's climacteric behavior (Novillo et al., 2016; Salvador et al., 2007). In the present study, the GA3 treatment could have affected ethylene production by fruit by delaying its maximum production peak.

Persimmon calyx status is important from the visual point of view. Persimmon possesses a relatively large calyx compared to other fruit, which is considered a "gas exchange organ" (Kitagawa and Glucina, 1984). The calyx darkens during fruit ripening, which affects external fruit quality. A recent study reports that GA3 treatment delayed calyx senescence, a process that occurs during fruit maturation, and external fruit quality improved (Fathi-Najafabadi et al., 2021). In the present study, the calyx looked green and fresh and showed slight desiccation symptoms only after H3, which coincided with an increase in the CO_2 and ethylene production values (Figure 2).

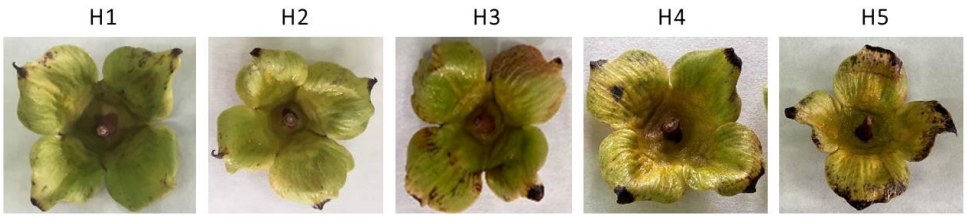
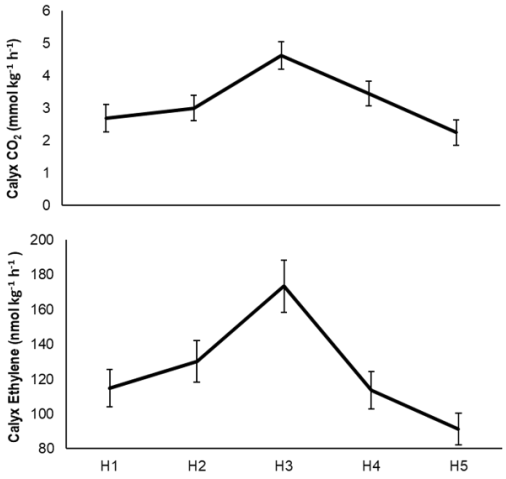


Figure 2. Persimmon calyx aspect at the different harvest times. H: Harvest; H1: 11 November; H2: 18 November; H3: 25 November; H4: 2 December; H5: 9 December.

The maximum calyx CO_2 production was reached at H3, with values of 4.6 mmol kg^{-1} h^{-1} before lowering in following weeks to similar values to those obtained at H1, close to 2.5 mmol kg^{-1} h^{-1} (Figure 3). The calyx ethylene production at H1 was 114.8 nmol kg^{-1} h^{-1} with a maximum value of 173.5 nmol kg^{-1} h^{-1} also at H3, which dropped at the following harvest times (Figure 3). These values are within the range recorded by Besada et al. (2016). Fathi-Najafabadi et al. (2021) found lower ethylene values in the calyxes of the fruit treated with GA3 compared to untreated fruit.



Significance		
	Calyx CO_2	Calyx Ethylene
A: Harvest	0.000	ns
B: Orchard	ns	ns
AB:	ns	ns

Figure 3. Changes in the calyx CO_2 and calyx ethylene productions of 'Rojo Brillante' persimmon fruit at the different harvest times. H: Harvest; H1: 11 November; H2: 18 November; H3: 25 November; H4: 2 December; H5: 9 December. Vertical bars represent the least significant differences (LSD) intervals ($p \leq 0.05$). The table of significance shows the results of a full analysis of variance (ANOVA). No significant (ns) or the actual p-value whenever significant.

3.2. Parenchyma Structure at Harvest

The Cryo-SEM images depict the persimmon parenchyma microstructure of the samples collected from the different harvest times. The tissue of the persimmon harvested at H1 showed high structural integrity (Figure 4A,F). The parenchyma was compact with swollen cells that were turgid and closely bonded to one another. Inside cells, a large vacuole occupied most of the protoplast (Figure 4A). The vacuole was full of soluble material, which looked like a network. This eutectic artifact was generated during the sublimation process when preparing samples (Hernández-Carrión et al., 2015). Some vacuoles are also observed with precipitated solutes, which are insoluble tannins (Salvador et al., 2007). The parenchyma of the fruit harvested at H2

was similar to that of H1. Only in some areas could very slight cell wall and plasmalemma degradation be observed (Figure 4B,G). Both samples from H1 and H2 had a thick homogeneous epidermis formed by small compact cells (Figure 4F,G).

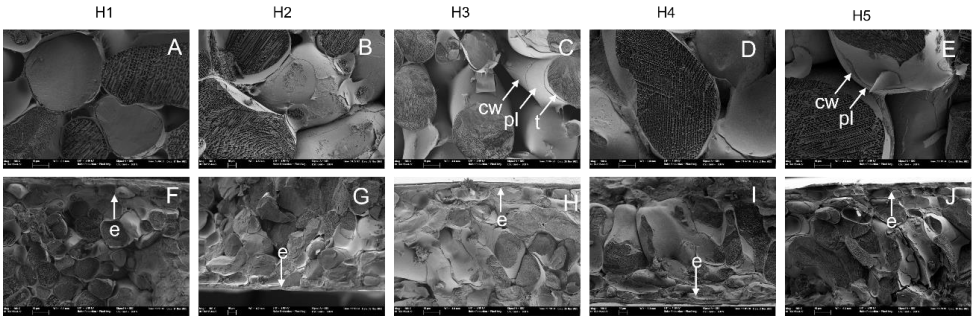


Figure 4. Persimmon images at the different harvest times obtained by cryo-scanning electron microscopy (Cryo-SEM). H: Harvest; H1: 11 November; H2: 18 November; H3: 25 November; H4: 2 December; H5: 9 December. e: epidermis; cw: cell wall; pl: plasmalemma; t: tonoplast. A-E: Details of the innermost area of the flesh; F-J: flesh area closest to the fruit epidermis. Magnification 500x.

The tissue of the flesh fruit from H3 (Figure 4C,H) presented some structural changes compared to the fruit samples harvested at H1 and H2, although the parenchyma was still compact. The plasmalemma and cellular wall were slightly degraded with an increased cell-cell separation in some areas, probably because cellular cement dissolution had taken place. (Figure 4C). The microstructural changes in the samples taken at H4 and H5 were more noticeable (Figure 4D,E) and, although the parenchyma was still structured, cells were slightly deformed and membranes seemed more degraded. Neighbor cells were observed further away from one another than in the other samples. Unlike H1 and H2, the tissue of the H3, H4 and H5 samples displayed a slightly homogeneous epidermis that was thin and constituted by large deformed cells (Figure 4H-J).

When flesh samples were observed by CLSM, major differences among them were noted. The CLSM images (Figure 5) displayed cell walls stained in blue by Calcofluor, a specific staining agent for cell walls (Figure 5A-E), and carotenoids were recorded as green (Figure 5F-J) because of their autofluorescence.

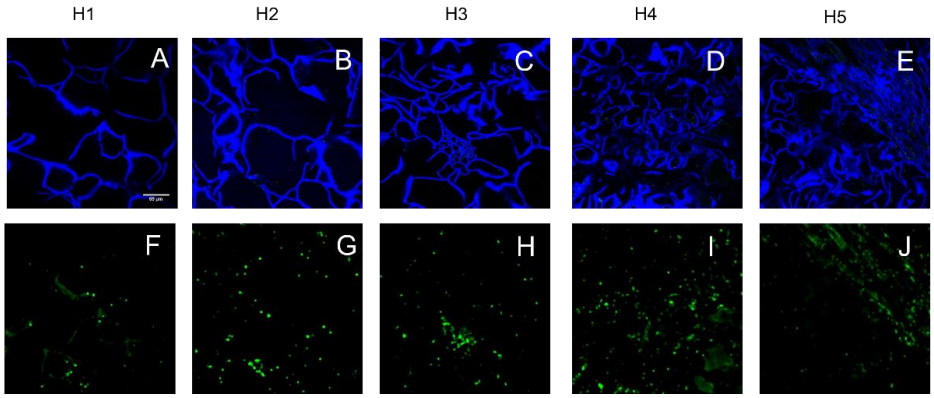


Figure 5. Persimmon images at the different harvest times obtained by confocal laser scanning microscopy. H: Harvest; H1: 11 November; H2: 18 November; H3: 25 November; H4: 2 December; H5: 9 December. Calcofluor-stained cell walls in blue (A-E). Carotenoids are autofluorescens observed in green (F-J). Magnification 40x.

In the samples taken at H1 and H2 (Figure 5A,B), cell walls were clearly visible, and cells were relatively spherical and uniformly distributed throughout the parenchyma. In the samples from H3 (Figure 5C), cell walls appeared slightly degraded and tissue was less structured. This degradation was more evident in the samples from H4 and H5 (Figure 5D,E), where cells appeared more disorganized and their shape was more difficult to visualize. Carotenoids were associated mainly with cell walls in all samples. There were more carotenoids and deformed cells as the harvest advanced, which falls in line with Hernández-Carrión et al. (2015).

The ultrastructure of samples can be observed in detail by TEM (Figure 6). The parenchyma tissue of the fruit harvested at H1 was structured and composed of numerous cells and intercellular spaces, which were mainly the result of joining three cells (Figure 6A). Neighboring cells were turgid and closely

bonded to one another by a well-delimited medium lamella. Cellular walls appeared dense and were formed by closely packed cellulose fibrils. Inside cells, a large vacuole occupied most of the protoplast. Both the plasmalemma and tonoplast remained close to the cellular wall, and an intact cytoplasm was observed near the tonoplast and plasmalemma (Figure 6D).

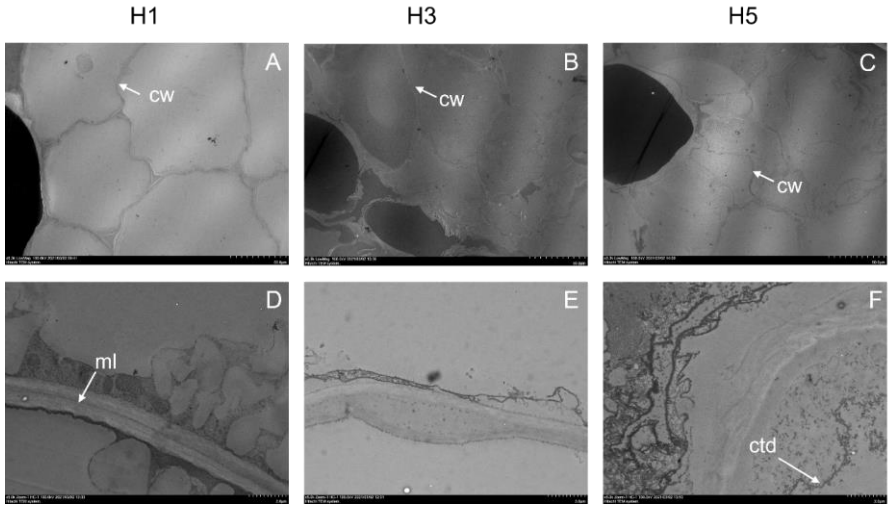


Figure 6. Persimmon images at the different harvest times obtained by transmission electron microscopy (TEM). H: Harvest; H1: 11 November; H3: 25 November; H5: 9 December. cw: cell wall; ml: middle lamella; ctd: degraded cytoplasm. A-C: Magnification 0.3 kx; D-E: Magnification: 5 kx.

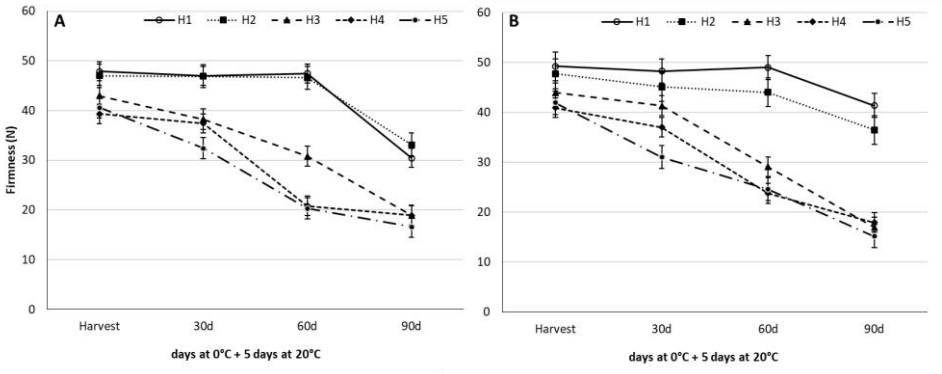
In the samples from H3 (Figure 6B,E), the cell wall appeared swollen, which indicates the solubilization of cell wall components, and cellular cements were degraded and diluted (Figure 6E). Thus, neighboring cells were separated from one another and intercellular spaces increased. Cell walls were irregularly stained, which is related to fibrillar packaging loss. In some areas, cell walls had broken down, and the plasmalemma and tonoplast began to separate from the cell wall (Figure 6B).

The parenchyma of the H5 persimmons (Figure 6C) revealed major ultrastructure modifications. Cells were deformed, contracted and shrunken, and had collapsed in some areas. Intercellular spaces were wider compared to the other samples. The middle lamella had completely degraded. The plasmalemma was destroyed and was not observed in most tissue areas. The tonoplast was drawn away from the cell wall toward the center of the cell. A misstructured and disintegrated cytoplasm was observed inside cells.

3.3. Firmness Loss during Cold Storage Depending on Harvest Time

'Rojo Brillante' persimmon cold storage is a common and necessary practice due to today's high production and the need to extend the commercial period, especially at the end of the season. Commercially speaking, the fruit intended for conservation are those treated with GA3 at pre-harvest because they can be later harvested with good firmness values. This delays harvest until the middle or the end of the season. Fruit still has good firmness values that can be cold-stored for long periods at 0 °C and are combined with the 1-MCP treatment. However, during these periods, the optimal harvest time to allow the longest cold storage periods is not known. In the present study, the fruit collected at the five harvest times were stored at 0 °C for up to 90 days after being treated with 1-MCP. Despite all the fruit demonstrating high firmness values at harvest, the behavior during cold storage between 48 and 40 N was very different depending on the harvest time.

The fruit from the two orchards displayed a similar pattern for the firmness loss of the fruit harvested at the five evaluated times, but with statistical differences due mainly to the different behavior noted between the fruit from H1 and H2 (Figure 7A,B). In orchard 1, the fruit harvested on November 11 (H1) and November 18 (H2) had the highest firmness values that came close to 48 N, whose firmness remained stable up to 60 days before lowering to 36 N after 90 days. In orchard 2, the H2 fruit values were slightly lower than in the H1 fruit; however, after 90 days, they still presented firmness close to 40 N, which was higher than the values recorded for the orchard 1 fruit.



Significance					
A: Storage	0.000	**Orchard 1**		**Orchard 2**	
B: Harvest	0.000	A: Storage	0.000	A: Storage	0.000
C: Orchard	0.018	B: Harvest	0.000	B: Harvest	0.000
AB:	0.000	AB:	0.000	AB:	0.000
AC:	ns				
BC:	0.013				
ABC:	0.012				

Figure 7. Changes in flesh firmness of the 'Rojo Brillante' persimmons from two orchards (orchard 1 (A) and orchard 2 (B)) and harvested at five different harvest times (H) during 30-, 60- and 90-day storage periods at 0 °C, plus a subsequent shelf-life period of 5 days at 20 °C. H1: 11 November; H2: 18 November; H3: 25 November; H4: 2 December; H5: 9 December. Vertical bars represent the least significant differences (LSD) intervals ($p \leq 0.05$). The table of significance shows the results of the full analysis of variance (ANOVA) for the full data and for orchards 1 and 2. No significant (ns) or the actual p-value whenever significant.

The fruit from H3, with 43.5 N at harvest, demonstrated a slight decrease in firmness in orchard 1 after 30 cold storage days, while the firmness in orchard 2 remained stable. From that time onward, a gradual firmness loss took place. After 90 days, firmness values were 17 N and 19 N in orchard 1 and orchard 2, respectively. The fruit harvested at H4 and H5 demonstrated similar values at harvest, close to 41.5 N, but exhibited different firmness loss values during cold storage. The firmness of the fruit from both orchards collected on December 2 (H4) slightly decreased after 30 days. From that point onward, it sharply dropped to average values close to 18 N. Faster gradual softening was demonstrated for the fruit harvested on December 9 (H5), with firmness values close to 32 N after storage 30 days, and the lowest values, close to 16 N,

were obtained after 90 days, with no differences between the fruit from both orchards.

If we bear in mind the high firmness values obtained for fruit at all the harvests, a priori large differences in their behavior during cold storage were not expected. At all the harvest times, fruit obtained firmness values above 40 N, which are required for cold storage. Nevertheless, firmness loss very much depended on harvest date. The microstructural study performed with the fruit flesh at each harvest time could explain the differences in posterior behavior.

Although some changes were observed by Cryo-SEM in the general parenchyma structure of the fruit harvested during the study period, the fruit's capacity to maintain good firmness values during prolonged storage was related mainly to the structural integrity of cell walls and membranes, as evidenced by the TEM ultrastructure study. Therefore, the firmness of the fruit with the highest structural integrity and dense fibrils (H1 and H2) remained above 30 N during storage and over a longer conservation period (up to 90 days). Although the parenchyma appeared well structured in the H3 fruit, an onset of degradation and dissolution of cell walls allowed the firmness to remain stable during storage for a shorter time.

The fastest firmness loss in the fruit harvested in H4 and H5 was related to marked ultrastructure modifications, with the degradation of the cell wall, plasmalemma and tonoplast. However, the parenchyma structure was still visible.

The microstructural changes that occurred concomitantly to firmness loss during persimmon maturation have also been reported in our previous studies (Salvador et al., 2007; Tessmer et al., 2016; Vázquez-Gutiérrez et al., 2011). In these studies, evident changes occurred in the parenchyma structure in the fruit with flesh firmness between 80 and 20 N. Nevertheless, the present study compared the fruit with firmness values within a narrow range, between 48 and 40 N. Very few microstructural differences can be expected in these stages. Nevertheless, according to the results, minor differences in fruit firmness at harvest led to a very different behavior under cold storage, which has been related to the microstructural parenchyma state, especially to the integrity of cell walls and membranes.

Of all the other parameters determined at harvest, it was worth noting the peaks of CO_2 and ethylene production in the calyx observed at H3 (Figure 3), which seemed to coincide with the onset of cell wall and membrane degradation. In a previous study, the firmness loss occurring during the ripening process of 'Rojo Brillante' fruit was correlated with the calyx senescence process by measuring chlorophyll fluorescence (CFI) parameters (Fathi-Najafabadi et al., 2021). This study led to the conclusion that these parameters could act as a potential non-intrusive tool for determining fruit quality at harvest. Accordingly, the results found in the present study reinforce the idea that the state of calyx in persimmon could be an indicator of the flesh structure.

4. Conclusion

Persimmon 'Rojo Brillante' treated with gibberellic acid maintained very high firmness values from 11 November to 9 December. During this period, slight firmness loss was observed, while a relevant increase in coloration took place, which led to a weak correlation between both parameters. The fruit firmness loss observed during cold storage very much depended on fruit firmness in each harvest moment. This is the first work to demonstrate that fruit with small differences in firmness at harvest can present major microstructural differences that significantly influence behavior during cold storage. In the fruit harvested with firmness around 48 N (11 or 18 November), these values remained for 60 cold storage days and presented high commercial values, over 30 N, after 90 days. The fruit harvested a week later (25 November) presented lower firmness values than those of earlier fruit after different cold storage periods. The firmness loss of the fruit harvested with around 40 N (on 2 or 9 December) was faster and demonstrated values close to 30 N only after 30 storage days.

The different postharvest behaviors of the fruit harvested during our study period can be explained by the degradation of cell wall, cell membrane and tonoplast, which came with harvest advance. Knowing these changes is essential for defining the optimum harvest time for prolonged conservation, and for guaranteeing a longer commercialization period with high-quality fruit.

Acknowledgements

This research was funded by Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and Fondo Europeo de Desarrollo Regional (FEDER), through Project RTA2017-00045-C02. Nariane Q. Vilhena thanks the INIA for grant FPI-INIA (PRE2018- 085833).

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CHAPTER V

Structural Changes Caused by CO_2 or Ethanol Deastringency Treatments in Cold-Stored 'Giombo' Persimmon

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Reference: *Agronomy*, 2022, 12(10), 2464.
https://doi.org/10.3390/agronomy12102464

Abstract

Persimmon cv. Giombo is astringent at harvest and must be subjected to astringency removal treatment. To date, the most widespread treatment for this variety involves applying ethanol instead of high CO_2 concentrations, which is the usual treatment with other varieties. This study aims to evaluate the effect of high CO_2 or ethanol concentrations as deastringency treatments on the quality and flesh structure of 'Giombo' persimmon during cold storage. The deastringency process was faster in the fruit treated with CO_2 than with ethanol. One day after treatment, the CO_2-treated fruit showed lower soluble tannin levels than those detected sensorially for this variety, while with the ethanol-treated fruit, these values were obtained after 25 storage days plus the shelf-life period. The tannin insolubilisation process was observed by light microscopy. Loss of flesh firmness during storage was more pronounced when fruit were previously treated with ethanol than with CO_2. This is closely related to greater parenchyma degradation during storage caused by ethanol treatment, which was observed by a microstructural study by cryo-scanning electron microscopy. Therefore, as deastringency treatment for 'Giombo', applying CO_2 instead of ethanol treatment is recommended for better fruit quality, especially when fruit are to be cold-stored.

Keywords: fruit anatomy; astringency; *Diospyros kaki* Thunb.; CO_2; deastringency.

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) cv. Giombo is an astringent cultivar that is produced mostly in Brazil. Nowadays, this country is the fourth persimmon world producer with a planted area of 8,148 ha and a production up to 157,000 tons/year (Andrioli et al., 2021; FAOSTAT, 2021). This cultivar belongs to the pollination-variant and astringent (PVA) group, which develop by parthenocarpy and is completely seedless (Kluge and Tessmer, 2018). Like all PVA cultivars, 'Giombo' fruit contain high levels of soluble tannins (ST), which are responsible for fruit astringency, and thus, applying deastringency postharvest treatment is necessary for fruit to be marketed with high firmness (Tessmer et al., 2014, 2019).

Astringency in persimmon is promoted by condensed tannins, or proanthocyanidins, which are the phenolic oligomers that result from the polymerisation of flavan-3-ol units (Akagi et al., 2010; Dixon et al., 2005) and accumulate in the vacuole of the tannic cells present in fruit parenchyma. Several methods have been used to remove persimmon astringency (Edagi and Kluge, 2009; Monteiro et al., 2017). These treatments stimulate the accumulation of volatile compounds in fruit flesh, such as ethanol and acetaldehyde, while triggering fruit anaerobic respiration. These substances induce ST to polymerise and become insoluble, which is a process that results in loss of astringency (Arnal and del Río, 2004; Vitti, 2009). One of the more commercial treatments involves employing 95 % CO_2 for 24 h at 20 °C.

However, in some producing countries such as Brazil, deastringency treatment by exposing persimmon to ethanol vapour is the most widespread practice because it is a less costly alternative with good results. This treatment is usually applied in a concentration around 1.70 mL per kg of fruit for 24 h at room temperature (Antoniolli et al., 2000; Tessmer et al., 2018; Vitti, 2009), nevertheless, these conditions can vary among producers. It was observed for 'Giombo' that changes in ethanol concentration, duration of the treatment and temperature of the chamber can highly influence the treatment result, affecting fruit final quality (Antoniolli et al., 2000, 2002; Chiou and Kluge, 2006).

'Giombo' maturation is later, which is an advantageous aspect to prolong the persimmon season until cold storage as well as for being a potential variety to be commercialised in other countries when no local fruit is available. Nevertheless, as with other cultivars, 'Giombo' is sensitive to cold storage and develops chilling injury when submitted to low temperatures for a long time. The main chilling injury symptom is flesh softening, which occurs when fruit are transferred from cold storage to shelf-life conditions (Tessmer et al., 2019).

The softening that takes place during storage can be accelerated by the previous deastringency treatment. It is known that high CO_2 concentrations lead to cell wall degradation, which may be associated with firmness loss after treatment application (Salvador et al., 2007). Likewise, deastringency with ethanol can be accompanied by reduced firmness during the postharvest life (Tessmer et al., 2018).

To date, no studies have compared the effect of CO_2 and ethanol deastringency treatment on the flesh structural degradation that occurs during the cold storage of 'Giombo' persimmon. Hence, in the present paper, a microstructure study was carried out to evaluate the effect of high CO_2 and ethanol concentrations as deastringency treatments on 'Giombo' persimmon quality during cold storage.

2. Materials and Methods

2.1. Plant material and Experimental Design

Persimmon cv. Giombo were harvested in the commercial stage from the experimental germplasm bank of ANECOOP Cooperative in Valencia (Spain) and transported to the Instituto Valenciano de Investigaciones Agrarias (IVIA), where they were carefully selected for uniformity of size, colour and lack of defects.

Fruit were divided into 15 lots of 15 fruit and placed in commercial plastic crates. One lot was characterised at harvest. Half the lots were submitted to CO_2 treatment (CO_2 95 %, 20 °C, 90 % RH, 24 h) and the other half were submitted to ethanol treatment (ethanol 1.70 mL kg^{-1}, 20 °C, 24 h). One lot per

treatment was analysed 1 day after the deastringency process. After deastringency treatments, fruit were stored at 1 ºC up to 40 days. One lot of each treatment was evaluated after 15, 25 or 40 cold storage days. At each time point of cold storage, one lot per treatment was transferred to 20 ºC and evaluated after 5 days to simulate a shelf-life period.

The following measurements were taken: external colour, firmness, total soluble solids (TSS), sensory evaluation. Fruit samples were frozen to determine soluble tannins (ST) content. Microstructural analyses of flesh were performed by light microscopy (LM) and cryo-scanning electron microscopy (Cryo-SEM) techniques.

2.2. Skin Colour and Flesh Firmness

Fruit skin colour was evaluated by a Minolta Colorimeter (Model CR-300, Ramsey, NY, USA). The L, a, b Hunter parameters were measured on the skin of each fruit on two opposite zones, and the results were expressed as external colour index (CI = 1000 a/Lb) (Salvador et al., 2007). Flesh firmness was determined by a Texturometer Instron Universal Machine, model 4301 (Instron Corp., Canton, MA, USA) using an 8 mm flat plunger. Fruit firmness values were taken from 15 fruit per lot on opposite sides. The results were expressed as the force in Newtons (N) required to break flesh after removing peel.

2.3. Total Soluble Solids and Soluble Tannins

Fruit were longitudinally cut into four. Two opposite quarters were used to determine total soluble solids and the other two were used to collect samples, which were frozen at -20 ºC to later analyse the ST content. To determine total soluble solids, fruit juice was extracted with an electric juice extractor and filtered. Measurements were recorded by a refractometer (Atagomod. PR1), and the results were expressed as ºBrix.

The ST concentrations were determined using the Folin–Ciocalteu reagent according to the method of Taira and Ono (1996). For quantification purposes, the following were used: 7.5 mL of distilled water, 1 mL aliquot of extract, 0.5

mL of Folin-Ciocalteu reagent (50 %) and 1 mL of supersaturated sodium carbonate solution. The absorbance of the resulting solution was measured at 725 nm by a spectrophotometer (Thermo Scientific Multiskan® Spectrum, Thermo Fisher Scientific Oy, Vantaa, Finland). The results were expressed as a percentage of dry weight (% DW).

2.4. Astringency Sensory Analysis

Fruit's astringency level was sensory evaluated by a semitrained panel made up of 6-8 people who were familiar with persimmon astringency. A 4-point scale was used, where 1 = no astringency and 4 = intense astringency. Samples were presented to panel members on trays labelled with random 3-digit codes and served at room temperature (25 ºC ± 1 ºC). Milk was provided for palate-rinsing between samples.

2.5. Microstructural Study

The microstructural study was performed with two fruit per replicate. For the LM analysis, tissue sections were taken from the equatorial portion of fresh fruit using a stainless blade. Sections were placed on histological slides and stained with vanillin-HCl (1:1, v/v) to identify tannins (Vázquez-Gutiérrez et al., 2011). Tannins react with hydrochloric vanillin to give a red colour. Cutting promotes the extravasate of tannins from tannin cells when they come in a soluble form. Images were taken by LM (Nikon Eclipse E800 V-PS100E, Tokyo, Japan).

For the Cryo-SEM analyses, cubes (3 mm^3) were cut from the equatorial area perpendicularly to the main axis of the persimmon flesh with a stainless-steel cutter. Cubes were then immersed in slush nitrogen (-210 ºC) and transferred to a cryo-trans (CT 15,000 C from Oxford Instruments, Oxford, UK), linked with a JEOLJSM 5410 scanning electron microscope (JEOL, Tokyo, Japan), which operated at a temperature below -130 ºC. Samples were cryofractured at -180 ºC and etched at -90 ºC. Observations under the microscope were made at 15 kV and at a working distance of 15 mm.

2.6. Statistical analysis

Data were subjected to analyses of variance (ANOVA) after testing normality with a Komolgorov–Smirnov test and homoscedasticitywith Levene's test. Themultiple comparisons between means were determined using the least significant difference ($p \leq 0.05$) with the Statgraphics Centurion XVII.I software application (Manugistics Inc., Rockville,MD, USA).

3. Results and Discussion

'Giombo' is an astringent variety with a high ST content, which make applying astringency removal treatment before marketing necessary. In the present study, ST came close to 1.07 % at harvest. One day after deastringency treatment with high CO_2 concentrations, the tannin content drastically dropped to values of 0.08 % (Figure 1). However, this decrease in the ethanol-treated fruit was less marked with values of 0.8 %. The sensorial evaluation revealed that the astringency of the CO_2 fruit was undetectable (sensorial value = 1), while the ethanol-treated fruit were qualified by the sensory panel as astringent (sensorial value = 3.2) (data not shown).

During cold storage and the following shelf-life periods, the CO_2-treated fruit had ST values between 0.07 % and 0.05 %, while the ST content of the ethanol-treated fruit gradually lowered throughout storage. During this treatment, ST dropped to values of 0.4 % and 0.2 % after 15 cold storage days and the posterior shelf life, respectively. At these times, the sensorial panel detected slight-medium astringency (sensorial values between 1.8 and 2.4). After 25 days at 1 ºC, the fruit's ST content was 0.13 % and "residual astringency" was detected. Only after the subsequent shelf life were fruit evaluated by panellists as "absence of astringency" with ST values between 0.07 % and 0.06 %. No changes were observed after 40 days.

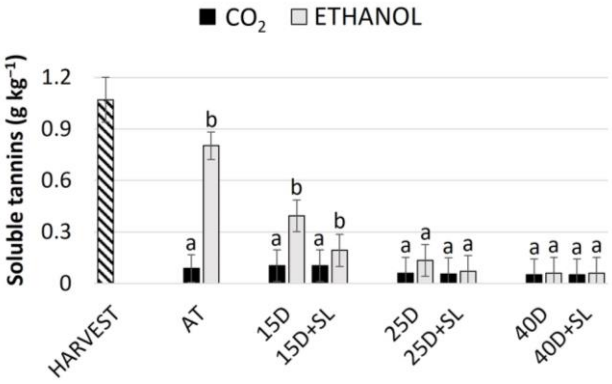

Figure 1. Changes in the soluble tannins of the 'Giombo' persimmon treated with CO_2 or ethanol during cold storage (1 °C) and after shelf-life simulation (5 more days at 20 °C). AT: One day after deastringency treatment, D: Days of cold storage; SL: Shelf-life simulation. The vertical bar represents the least significant difference (LSD) intervals ($p \leq 0.05$) (interaction treatment–storage days). Different letters above bars indicate significant differences among treatments at each moment of evaluation ($p \leq 0.05$).

According to Antoniolli et al. (2000) and Tessmer et al. (2018), the ST content in 'Giombo' should be below 0.1 % so as not to detect astringency. Likewise, in non-astringent cultivars, tannin concentrations have been reported to be below 0.1 % at harvest (Tessmer et al., 2016). Nevertheless, it should be noted that the level of tannins whose astringency is not sensorially detectable depends on cultivars. For example, for the astringent cultivar 'Rojo Brillante', the tannin content must be below 0.04 % to guarantee non-astringent fruit (Besada et al., 2010; Munera et al., 2017; Salvador et al., 2007).

By means of LM and vanillin hydrochloric staining, tannic cells were observed on persimmon flesh. At harvest, tannins had extravasated from the cell vacuole, and they were dispersed throughout flesh and deposited on the whole section with an intense red colour (Figure 2A). One day after CO_2 treatment, most tannins remained inside tannic cells, which indicates the polymerisation of ST (Figure 2B) to non-astringency levels (see Figure 1).

Nevertheless, 1 day after ethanol treatment, a large region with content extravasation was observed (Figure 2C), which is in accordance with the still high ST concentration observed at that time, as shown in Figure 1. After 15 storage days plus shelf life, the ethanol-treated fruit presented lower dispersed content with material accumulation closer to tannic cells (Figure 2D). This finding corresponds to the slight-medium astringency noted with this fruit. After 25 days plus shelf life, tissue presented greater delimitation of the tannic cells dispersed in the degraded parenchyma. Tannic cells were elongated, and content was completely insolubilised with intact walls, which indicate a complete loss of astringency at 40 days plus shelf life (Figure 2E,F).

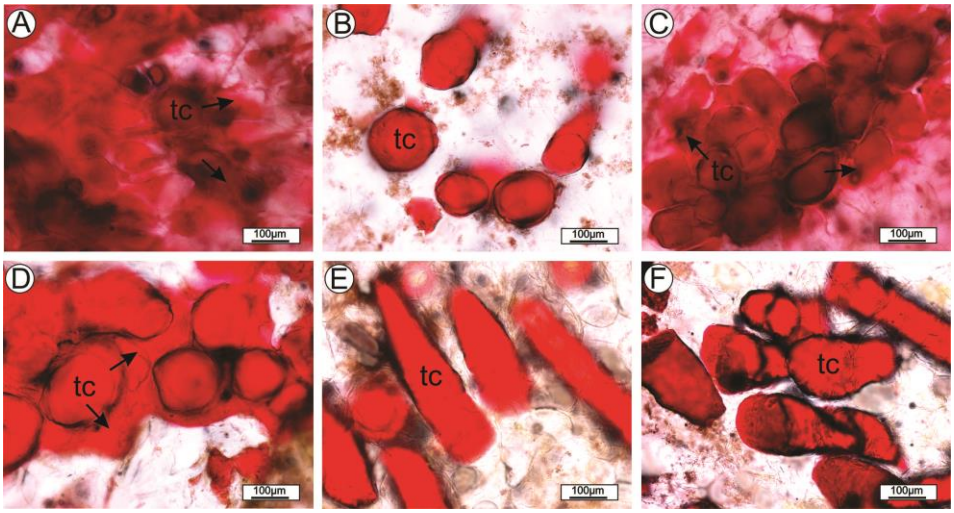

Figure 2. Tissue sections of 'Giombo' persimmon flesh. (A) Harvest, (B) 1 day after CO_2 treatment, (C) 1 day after ethanol treatment, (D) Ethanol-treated fruit after 15 d at 1 °C + 5 d at 20 °C, (E) Ethanol-treated fruit after 25 d at 1 °C + 5 d at 20 °C; (F) Ethanol-treated fruit after 40 d at 1 °C + 5 d at 20 °C. Cells with extravasated tannin content (A,C,D), tannins accumulate near tannic cells (B) and tannic cells intact dispersedly in the degraded parenchyma (E,F). tc: tannic cell.

In both 'Giombo' and 'Rojo Brillante', the tannin insolubilisation process inside tannin cells during fruit maturation has been previously observed by LM (Tessmer et al., 2016).

Flesh firmness is the most important quality attribute to be taken into account after submitting persimmon to deastringency treatment (Salvador et al., 2005). It is also an essential parameter to be maintained during fruit storage and commercialisation.

At harvest, fruit flesh firmness was 35.6 N (Figure 3A). After 15 storage days, fruit firmness obtained an average of 28 N with non-significant differences between the fruit submitted to both deastringency treatments. After the posterior shelf life, this value lowered to 17 N and 13.7 N in the fruit treated with CO_2 and ethanol, respectively. After 25 cold storage days, the CO_2-treated fruit had a firmness of 28 N, but that of the ethanol-treated fruit was 21.4 N. After shelf life, the CO_2 fruit had lower values of 13.4 N, while the ethanol-treated fruit values dropped to 8.7 N, which is a value that is below the commercial limit for persimmon (Besada et al., 2014). After 40 cold storage days, the CO_2-treated fruit firmness was still higher than 15 N, but values came close to 7 N for the ethanol-treated fruit, and the posterior shelf-life period gave a firmness of 5.6 N with no differences between treatments.

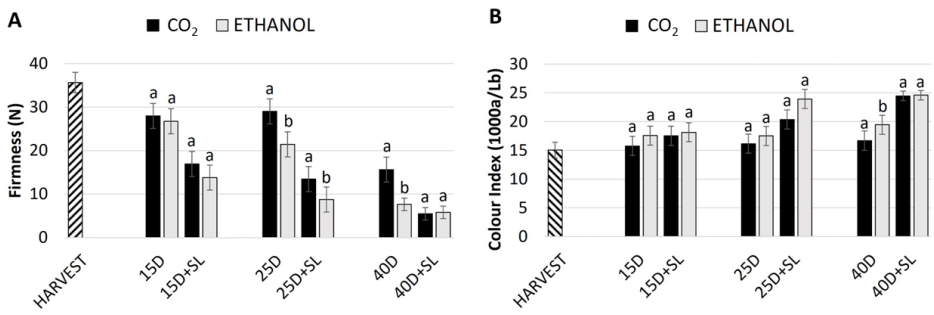

Figure 3. Changes in the (A) firmness and (B) external colour of the 'Giombo' persimmon treated with CO_2 or ethanol after cold storage (1 °C) and after shelf-life simulation (5 more days at 20 °C). D: Days of cold storage; SL: Shelf-life simulation. The vertical bar represents the least significant difference (LSD) intervals ($p \leq 0.05$) (interaction treatment–storage days). Different letters above bars indicate significant differences between treatments at each moment of evaluation ($p \leq 0.05$).

A previous study conducted with Japanese and Chinese varieties noted that the flesh softening caused by deastringency treatment was also more pronounced in the fruit treated with ethanol than in those treated with CO_2 (Yamada et al., 2002). A sharp decrease in flesh firmness was observed when fruit were transferred from 1 °C to shelf-life conditions regardless of the previous deastringency treatment, which is associated with a chilling injury (CI) symptom reported for persimmon fruit (Salvador et al., 2005).

A strong negative correlation between external colour and flesh firmness during fruit maturation has been reported for some persimmon varieties (Salvador et al., 2007; Tessmer et al., 2016). At harvest, fruit had a colour index of 15, with an increase noted during storage (Figure 3B). After 15 storage days, the values slightly increased with values close to 17 but with no large differences between treatments. Only after 25 days at 1 °C plus 5 days at 20 °C did the CO_2-treated fruit obtain lower CI values than those treated with ethanol, with a colour index of 20.3 and 24 for the treatments with CO_2 and ethanol, respectively. After 40 storage days, there was no difference between the CO_2- and ethanol-treated fruit whose average colour was 24.5 after the last shelf-life period.

The flesh firmness loss during storage and after deastringency treatments was closely associated with microstructural parenchyma degradation. After 15 days at 1 °C, the fruit from both treatments displayed quite a structured parenchyma, with rounded cells and small air-filled intercellular spaces (Figure 4A,B,E,F). Cells had a compact mass, which indicates the presence of insoluble tannins inside their vacuoles as a consequence of the deastringency process. Nevertheless, in the fruit from the CO_2 treatment (Figure 4A,B), more cells with insolubilised material were observed compared to the ethanol-treated fruit (Figure 4E,F). After the shelf-life period, slight cell compaction loss was observed in the CO_2-treated fruit, and more changes were detected in the flesh structure of the ethanol-treated fruit (Figure 4C,D,G,H). The parenchyma appeared more deteriorated with intercellular spaces invaded by soluble material. The cell membrane was degraded in some cells.

After 25 cold storage days, no major changes were observed in the structure of the CO_2-treated fruit (Figure 4I,J) compared to that observed after 15 days. However, the flesh of the ethanol-treated fruit was more degraded with deformed cells, and there was also clear cell–cell separation in some areas (Figure 4M,N). Flesh degradation was more evident when fruit flesh firmness diminished after the subsequent shelf-life period. Notwithstanding, differences between the CO_2- and the ethanol-treated fruit were found. The flesh integrity of the CO_2-treated fruit was preserved, although some cells were deformed and the spaces between them became bigger (Figure 4K,L). The parenchyma of the ethanol-treated fruit was more deteriorated, and the membranes in some areas were hard to distinguish in the collapsed tissue (Figure 4O,P).

After 40 cold storage days, the flesh structure degraded in the fruit from both treatments, but this effect was stronger in the ethanol-treated fruit (Figure 4Q,R,U,V). More drastic changes occurred after the shelf-life period. The typical cell structure was lost, and the parenchyma was very deteriorated (Figure 4S,T,X,Z). Most cells had lost their integrity, and the cell membrane was indistinguishable in compacted tissue. Although these changes were observed in the fruit from both treatments, they were more evident in the fruit treated with ethanol.

In relation to soluble solids, fruit showed 21.4 °Brix at harvest, which slightly lowered after the astringency removal and subsequent storage periods to values between 18 and 19.2 °Brix. No major differences between treatments were found (data not shown). This reduction may be influenced by the insolubilisation of soluble tannins, which can be quantified together with soluble solids as reported in previous studies (Salvador et al., 2007).

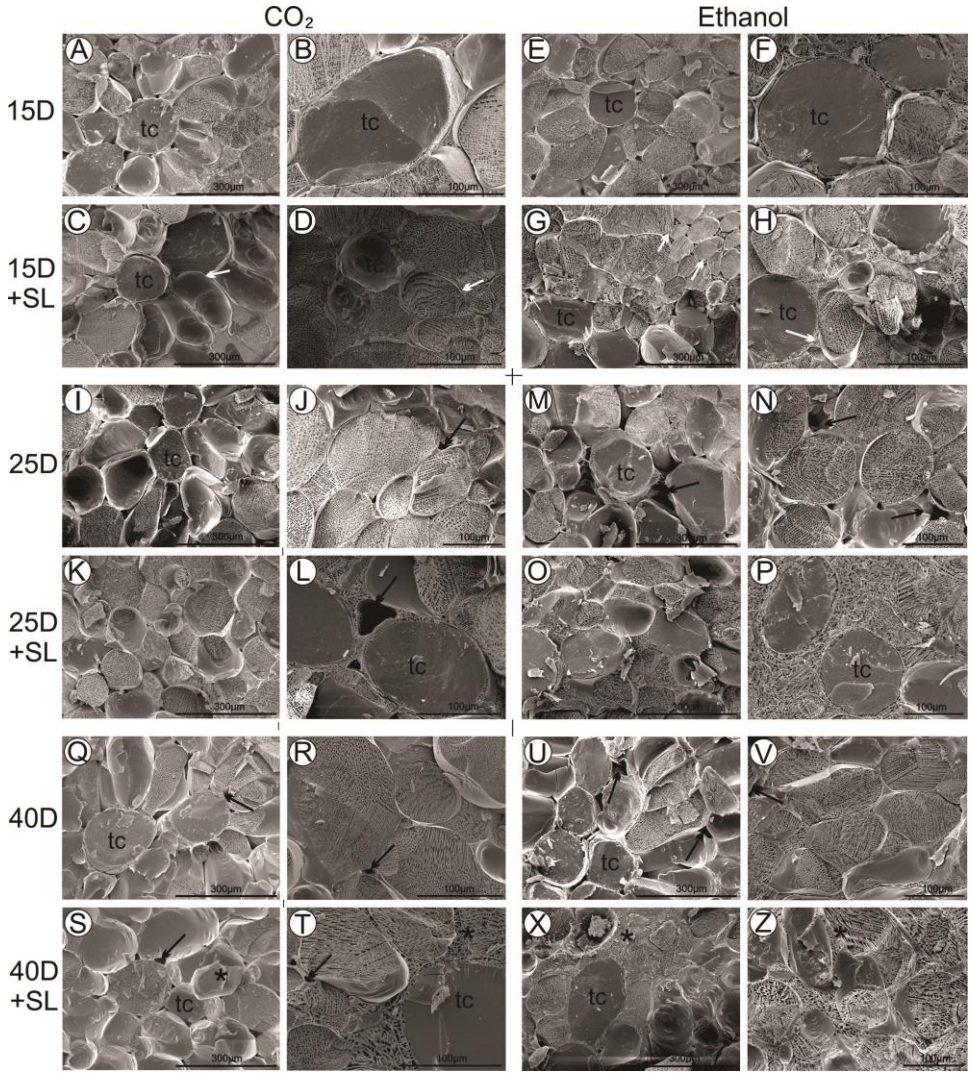

Figure 4. 'Giombo' persimmon images obtained by cryo-scanning electron microscopy (Cryo-SEM) throughout storage after treatment with CO_2 (A–D,I–L,Q–T) or ethanol (E–H,M–P,U,V,X,Z). Black arrow: cell separation and spaces formation. White arrow: soluble material in intercellular spaces. TC: tannin cell. *: cell wall degradation. Vertical columns: Deastringency treatments. Horizontal columns: Days (D) of storage at 0 °C or Days of storage at 0 °C plus shelf life (SL) (5 days at 20 °C).

4. Conclusions

This work shows in 'Giombo' persimmon that deastringency with high CO_2 concentrations results in faster tannins insolubilisation than with ethanol. In addition, the parenchyma degradation during cold storage and the subsequent shelf life is more severe in ethanol-treated fruit, resulting in a faster firmness loss than in CO_2-treated fruit. Therefore, fruit treated with CO_2 maintained high firmness after 25 storage days plus shelf life, while ethanol-treated fruit did not show marketable firmness. These results suggest that in the case of 'Giombo', although ethanol is the commonplace deastringency treatment, it is recommended to introduce a treatment with high CO_2 concentrations to obtain better fruit quality, especially when fruit are to be cold stored.

Acknowledgements

The authors thank the Agrupación Nacional de Exportación de Cooperativas Citrícolas (ANECOOP) for supplying the persimmon cultivar fruit herein used. N.Q.V. thanks the Spanish Ministry of Science and Innovation for Grant FPI-INIA (PRE2018-085833).

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III.2. DRYING AS A NEW STRATEGY TO VALORIZE DISCARDED PERSIMMON FRUIT AND PRODUCTION SURPLUS

CHAPTER VI

Physico-Chemical and Microstructural Changes during the Drying of Persimmon Fruit cv. Rojo Brillante Harvested in Two Maturity Stages

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Reference: *Foods*, 2020, 9(7), 870.
https://doi.org/10.3390/foods9070870

Abstract

The physico-chemical and microstructural changes of 'Rojo Brillante' persimmons in two maturity stages (S1 and S2) were evaluated during air drying. The maturity stage influences moisture loss. A Moisture level of approximately 50 %, a limit at which persimmons are considered semidried, was reached after 21 and 28 days for S1 and S2, respectively. Shrinkage resulting from water removal led to secondary epidermis formation concomitantly to internal flesh gelling, which was related to moisture loss and water activity changes of each fruit part. The thicker epidermis and the lower volume of gelled area inside the S1 fruits led to harder fruit compared to the S2 fruits. The microstructural study revealed parenchyma degradation during drying in both the outermost area (secondary epidermis) and internal flesh, and this process was faster in S1 than in S2. The second peel presented hollows, generated by water outflow, which were bigger in S1 and explained the faster internal dehydration in S1. During drying, slight browning occurred, as reflected in the declining color parameters (L*, h* and C*). Water removal led to soluble tannin reduction to non-astringency values on day 28.

Keywords: *Diospyros kaki* Thunb.; cryo-FESEM; semidried; air drying; quality characteristics.

1. Introduction

Persimmon fruit (*Diospyros kaki* Thunb.) is a species that originates from China and was introduced to Europe in the 17th century (Besada and Salvador, 2018). Spain recently became the second biggest producer of persimmon fruit in the world, with an expected production of 600,000 tons by 2020 centered mainly around the astringent cultivar 'Rojo Brillante' due to its high quality and adaptation to climate conditions (Khademi et al, 2013; Mañez, 2019).

In Europe, the marketing season of persimmon goes from November to January, in late autumn and early winter, with a plentiful supply. However, the accelerated maturation process of persimmon reduces its availability all year round and increases the number of postharvest losses, which can be as high as 20 % of production (Martínez-Las Heras et al., 2016; Senica et al., 2016). In this context, the productive sector faces the challenge of introducing new product options to reduce losses associated with persimmon fruit management and seasonality.

The drying process is a usual and simple technique of which its aim is fruit preservation to extend the period that it is available. The production and consumption of semidried persimmons are traditional in Asian countries like South Korea, China and Japan, with a variety of processing methods to generate a new high quality and stable product, with good sensory attributes (Gardeli et al., 2010; Li, 2012).

Dried persimmon can be classified into dried and semidried based on its water content. Although the moisture limit for this classification varies between countries and varieties, on average, semidried persimmons are processed at around the 50 % moisture level, whereas dried fruits can reach about 35 % (Kang et al., 2004; Kim et al., 2018).

The production of dried persimmons enables the commercialization and exportation period to be prolonged as reduced moisture content and consequently, volume and weight improve shelf life due to low microbial and biochemical degradation, which reduces packaging, storage and transportation costs (Chauhan et al., 2009). Drying also emerges as a management option because this process also reduces astringency in some astringent varieties. In

China, this is the main way to commercialize astringent persimmons, which are more suitable for drying than non-astringent cultivars because they turn brown and tough (Akyildiz et al., 2004; Li, 2012).

Despite being a consolidated technique in several countries and the fact a considerable number of studies have been published about drying methods, changes in quality characteristics, and nutritional benefits in these regions, in Spain, there is still no record of semidried persimmon production or published references about the effects of the drying process on fruit. Therefore, this study aims to provide information on the physico-chemical and microstructural changes that occur during drying 'Rojo Brillante' persimmon fruit harvested in two maturity stages.

2. Materials and Methods

2.1. Fruit Samples and Experimental Procedure

Persimmons fruit cv. Rojo Brillante were harvested from commercial orchards at l'Alcudia (Valencia, Spain) in two maturation stages. The criteria for harvesting each maturity stage were firmness and external color index. In persimmon, the external color is the most common non-destructive index for harvesting and a strong negative correlation between skin color and firmness values during maturation has previously been reported (Salvador et al., 2006, 2007). L, a, b Hunter parameters were measured and results were expressed as a skin color index as in Equation (1):

$$CI = 1000 \times a/Lb \quad (1)$$

where CI is the color index and 'L', 'a' and 'b' are the Hunter Lab parameters of color scale.

The early stage (S1) had firmness values of 49.3 N ± 6.9 and a peel color index of 6.7 ± 2.0 and the late stage (S2) had firmness values of 31.5 N ± 5.8 and a peel color index of 21.6 ± 2.1. Harvests took place throughout November.

After harvests, fruit were transported to the Instituto Valenciano de Investigaciones Agrarias (IVIA; Moncada, Spain) where they were selected according to homogenous color and absence of external damage. Then 120

fruits from each maturity stage were peeled of manually with a peeler to be subsequently immersed for 10 min in 4.5 % sodium metabilsufite ($Na_2S_2O_5$) solution, which is used as a disinfectant, antioxidant, and preservative agent (Akyildiz et al., 2004).

The fruit were placed on hooks and hung by the pedicel for natural drying in the IVIA's pilot plant for up to 63 days. Six fruits were placed on each hook to separate stages 1 and 2. Average temperature and relative humidity during the drying period were taken from the IVIA weather station and ranged from 6 ºC to 17 ºC and 49 % to 83 %, respectively.

2.2. Determinations

The initial fruit characterization was made with 20 fruits from each maturity stage. The same analyses were carried out once weekly with 12 fruit (two hooks) from each stage. Three pieces of fruit were used for the moisture and water activity determinations and the microstructural study. Nine were employed for the other physico-chemical analyses. All the analyses were determined based on individual fruits.

2.2.1. Weight and Diameters

Fruit weight was individually measured on a precision scale (model PB3002-S/FACT, Mettler Toledo, Switzerland). The longitudinal and equatorial diameters, as well as the thickness of the secondary epidermis generated due to the drying process, were measured by a pachymeter (Mitutoyo 500-267V-CDL20CP, Japan).

2.2.2. Moisture and Water Activity

The fruit were cut into two parts. One half was used to measure the moisture and water activity of the whole fruit. In the other half, the outmost area (secondary epidermis) was carefully separated with a scalpel blade from the

rest of the pulp (internal flesh) in order to measure the moisture and water activity in each of these parts.

Samples were ground in a crushing machine, of which its water content (x_w) and water activity (a_w) were measured by a Vaciotem, J.P. Selecta vacuum oven (60 ± 1 ºC, pressure <100 mm Hg) and an Aqualab CX-2 Decagon Device, respectively. Three replicates were measured per fruit.

2.2.3. Firmness and Color

Firmness was measured in a Texturometer Instron Universal Machine (model 4301, Instron Corp., Canton, MA, USA) using a 35 mm flat plunger. It approached fruit in such a way that subjected it to a compression force of 10 N along its equatorial axis. The relation between the deformation produced by the applied force and the initial fruit diameter was expressed as millimeters of deformation.

Fruit color was evaluated after peeling with a Minolta Colorimeter (model CR-400, Ramsey, NY, USA). The lightness (L*), chroma (C*), and hue angle (h*) values of persimmons were measured directly on two opposite equatorial zones of each fruit.

2.2.4. Total Soluble Solids and Tannin Content

These analyses were also carried out with whole fruits and by also separating the secondary epidermis (generated during drying) and internal fruit flesh. To determine total soluble solids (TSS), the samples of each fruit were crushed by a Polytron homogenizer (model PT 3100D, Kinematica, Switzerland). To avoid tannins interfering with total soluble solid measurements, the insolubilization of tannins was previously done following the method of Sugiura et al. (1983). Measurements were recorded with a refractometer (Atagomod. PR1) and the results were expressed as ºBrix.

Soluble tannins (ST) were determined by the Folin-Denis method (Taira, 1995), as described by Arnal and Del Río (2004), and the results were expressed as a percentage of dry weight (DW).

2.2.5. Microstructure Analysis

The microstructural study was performed by Cryo-field emission scanning electron microscopy Ultra 55 FESEM (ZEISS, Oberkochen, Germany) (Cryo-FESEM). Cubes (3 mm^3) were cut from the equatorial area perpendicularly to the main axis of the persimmon flesh with a stainless-steel cutter. These cubes were then immersed into slush nitrogen (-210 °C) and transferred to a cryo-trans GeminiSEM 500 (ZEISS, Oberkochen, Germany) linked with a field emission scanning electron microscope, which operated at a temperature below -130 °C. Samples were cryofractured at -130 °C and etched at -90 °C.

For the light microscopic (LM) analysis, issue sections were taken from the internal flesh using a stainless blade. Sections were placed on histological slides and stained with calcofluor (0.1 %), a specific agent that identifies cell walls. Cell walls react with calcofluor and give a blue color (Vázquez-Gutiérrez et al., 2016). Dyes were detected using a mercury arc lamp as the light source. The excitation and emission filters for calcofluor were 370/36–25 nm and 440/40–25 nm, respectively. A Nikon ECLIPSE 80i (Nikon Co., Ltd., Tokyo, Japan) light microscope was used. Samples were observed at the 4x magnification. Images were captured and stored at 1280 x 1024 pixels using the microscope software (NIS-Elements F, version 4.2, Nikon, Tokyo, Japan).

2.3. Statistical Analysis

Data were subjected to analyses of variance (ANOVA) and multiple comparisons between means at $p \leq 0.05$, determined by the LSD test. The Statgraphics Centurion XVII.I software application was used for the statistical analysis (Manugistics Inc., Rockville, MD, USA).

3. Results and Discussion

3.1. Weight Loss, Changes in Fruit Shape, Shrinkage and Secondary Epidermis Formation

The drying process of foods reduces the total weight as water is removed from the material, which provides an overall volume reduction of whole fruit

(Rahman, 2008). In the present study, the fruit in the first maturity stage (S1) had an initial weight of 195.3 g, which was lower than the fruit in the second one (S2), which came close to 252 g (Figure 1A). During the drying process, the fruit from both stages underwent considerable weight loss until approximately day 28. Then, while fruit weight remained stable for the S1 fruit, it continued to slowly decrease in the S2 fruit, with no significant differences over time. When drying ended, the fruit from both stages had an average weight of 54 g.

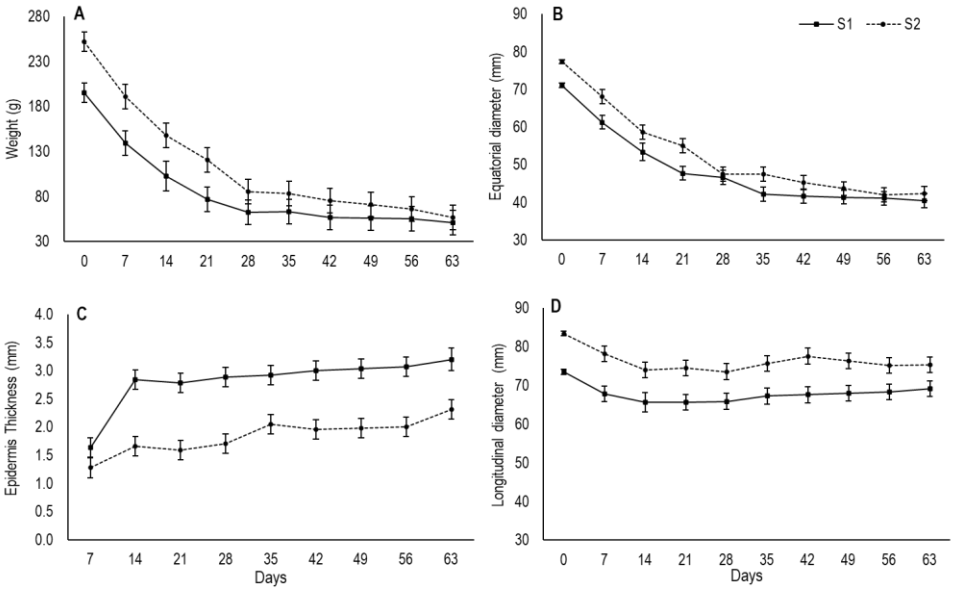

Figure 1. Changes in (A) weight, (B) equatorial diameter, (C) secondary epidermis thickness and (D) longitudinal diameter during the drying process of persimmon cv. Rojo Brillante in two maturity stages (S1 and S2). Vertical bars represent Least Significant Differences (LSD) intervals ($p \leq 0.05$).

Fruit size changes were reflected by the values of longitudinal and equatorial diameter, which were measured during the drying process (Figure 1B,D). Concomitantly to the reduced fruit weight, the equatorial diameter descended gradually until day 28 in both stages and then showed a tendency to stabilize

by reaching values of 40.4 cm and 42.3 cm for S1 and S2, respectively, at the end of the experiment, with no significant differences.

The longitudinal diameter showed slight variation throughout drying and varied from 73.5 cm to 69.2 cm for S1 and from 83.4 cm to 75.4 cm for S2 (Figure 1D). In both stages, the most marked decrease took place on the first 14 days, after which time no changes were observed. The slight variation observed in the longitudinal diameter during the drying process was probably due to the position at which fruit was hung by suffering the influence of gravity.

In this study, S2 had higher initial values for weight and diameters and displayed more weight loss than S1, probably due its larger evaporation surface. According to May and Perré (2002), the surface area of drying products directly influences the drying rate, reporting that the longer the exchange surface of the fruit, the greater the water loss. These results are different to what Cho et al. (2017) observed, who found that smaller sized fruits showed greater weight loss compared to bigger ones during the drying process.

The reduction in the overall fruit volume during drying is usually defined as shrinkage (Rahman, 2008). Dry fruit shrinkage is not always uniform in the material dimension and depends on how uniformly water removal occurs in it. In the present study, shrinkage was accompanied by the outermost fruit zone evidently warping and wrinkling, which became more evident with drying time (Figure 2). This feature is caused by loss of internal volume due to water evaporation, which induces a moisture gradient and fruit display this distinctive quality (Achanta and Okos, 1996; Mayor and Sereno, 2004).

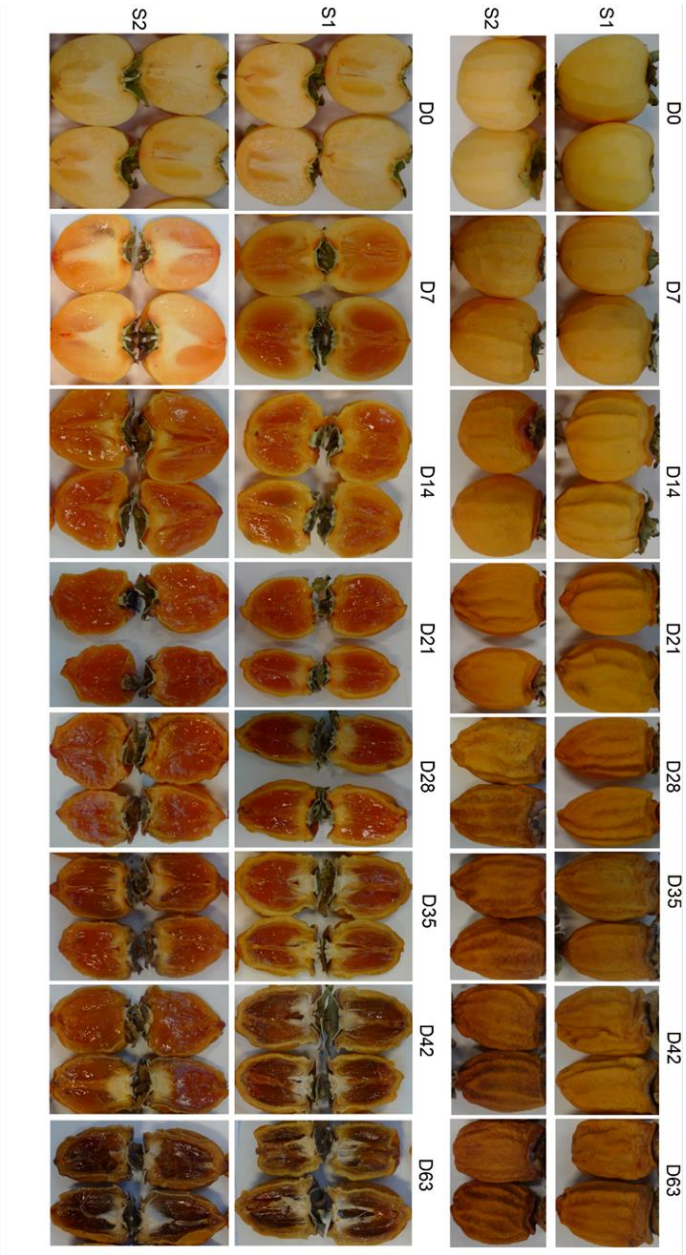

Figure 2. Captured images of persimmon cv. Rojo Brillante in two maturity stages (S1 and S2) during the drying process.

During the drying process, shrinkage is usually concomitant to the formation of a rigid external layer that results from a high water evaporation rate from the external fruit zone, which loses moisture faster than the inner zone. This process is known as “Case Hardening” or “Crust Formation” and is either desirable or not in dried food products. In extreme cases, this process can lead to skin that is practically impervious to moisture, which encloses the volume of the material so that no interior moisture can be removed (Achanta and Okos, 1996; Rahman, 2008; Sosa et al., 2010). In persimmon fruit, Kang et al. (2004) referred to this external rigid structure as a second peel or a secondary epidermis.

In this study, the second peel was already visible on day 7 for S1 and its thickness was 1.64 mm (Figure 1C). After 14 days, drastic thickening had occurred before stabilizing until day 35 and increasing again after day 40. The secondary epidermis had thickness values of 3.2 mm by the end of the assay. In S2, this layer was evident on day 14 and its thickness was 1.65 mm. Afterwards, it underwent no further changes until day 28, when it slightly increased, with values of 2.3 mm on day 63.

As the images reflect (Figure 2), the formation of this second peel was accompanied by a drastic change in the internal fruit structure. Internal flesh appears more gelatinous with the drying progress as the secondary epidermis causes resistance near the fruit surface, which fixes the volume inside the fruit and hinders water loss from the innermost region, which becomes softer and looks rubbery or has a gelatinous aspect (Mayor and Sereno, 2004). In S1 after 7 days, the internal flesh texture drastically changed and displayed gelation symptoms. In S2, this flesh gelation started later and was visible after 14 days.

3.2. Changes in Moisture and Water Activity

In the present study, during the drying period both moisture content and water activity were measured for the whole fruit (Figure 3A,C). Moreover, these parameters were also determined separately in the outer (secondary epidermis) and inner areas (internal flesh) of fruit from day 7 onward (Figure 3B,D). At harvest, moisture content came close to 0.8 gH_2O/g for the fruit from

both maturity stages and steadily lowered over the 63 days that the evaluation lasted (Figure 3A). The decline in moisture was faster in S1 fruit than in S2.

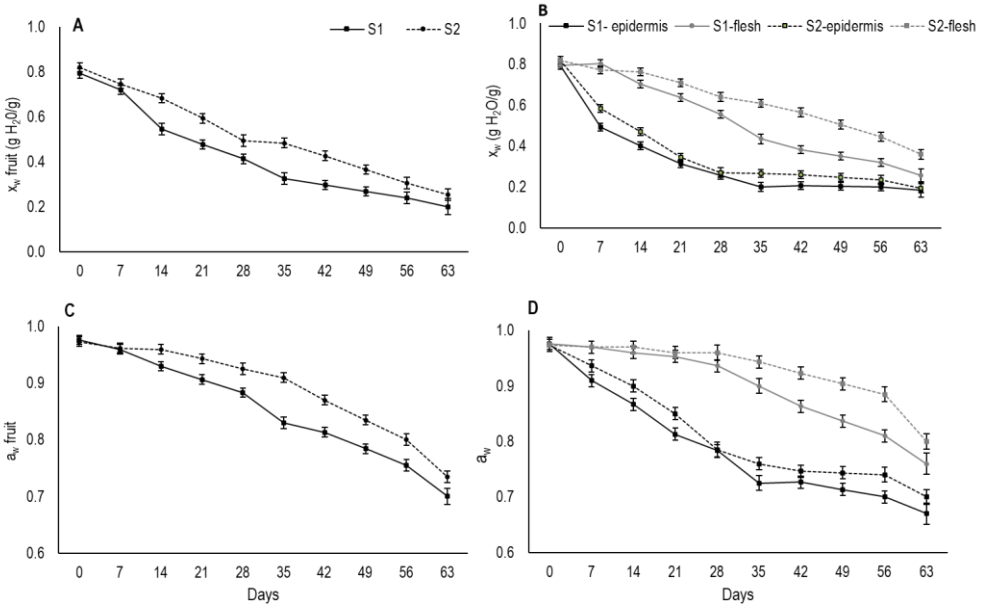

Figure 3. Changes in (A,B) moisture (x_w) and (C,D) water activity (a_w) of whole fruit and by parts (secondary epidermis and internal flesh) during the drying process of persimmon cv. Rojo Brillante in two maturity stages (S1 and S2). Vertical bars represent Least Significant Differences (LSD) intervals ($p \leq 0.05$).

According to Kang et al. (2004), moisture at around 50 %, or 0.5 gH_2O/g, is the limit at which persimmon fruits are traditionally named semidried. For the whole fruit, this value was reached on days 21 and 28 for S1 and S2, respectively. In the Asian market, semidried persimmons usually have a better market value for being characterized by a softer texture, which are more appealing compared to fully-dried fruit (Yamada et al., 2009).

As expected, moisture loss in both maturity stages was significantly higher in the outer area than in the internal flesh (Figure 3B). In the outermost fruit area, moisture content sharply dropped during the first drying week for the fruits from both stages and this drop was more marked in S1. Afterward,

moisture gradually lowered to days 35 and 28 for S1 and S2, respectively, from which time the moisture tended to stabilize and reach values that came close to 0.18 gH_2O/g in S1 and 0.19 gH_2O/g in S2.

The marked reduction in moisture in the outermost area during the first experimental weeks would be the cause of the second peel formation in the fruit. The faster external moisture loss exhibited in S1 fruit would explain the earlier formation of the second peel compared to fruit from S2. It was noteworthy that when second peel formation was observed on day 7 and day 14 in S1 and S2, respectively, the moisture content of this area was similar and came close to 0.48 gH_2O/g.

Moreover, in S1, this structure was thicker throughout drying, which would also be related to the more marked moisture loss in S1 compared to S2. According to Achanta and Okos (1996), if the drying rate is slow enough that moisture lost from the product surface is replenished by moisture from the inside, crust formation may be inhibited. In this research, the slower drying rate in S2 produced a crust, but it was considerably thinner than S1.

Regarding changes in internal flesh moisture during drying, major differences were observed between both maturity stages (Figure 3B). S1 showed stable internal moisture until day 7, then it began to gradually decrease. From 28 days onwards, the internal moisture dropped more sharply until reaching values of 0.26 gH_2O/g on day 63. In S2, a gradual moisture reduction took place from day 14, achieving a moisture value of 0.36 gH_2O/g on day 63. Throughout drying, this stage showed higher moisture content than S1.

When comparing the weight loss dynamics to moisture variation in fruit, it is noteworthy that the most pronounced weight reduction took place during the first 28 days (Figure 1A), then the stabilization tendency followed the same pattern as the outer area moisture loss (Figure 3B). The moisture content in this area remained stable from 28 days onward, while internal flesh, with a higher moisture content, continued to slowly lose moisture. This behavior would result from the reduction in water evaporation and secondary epidermis hardening according to Rahman (2008). In addition, although moisture continued to constantly drop in the inner fruit area until 63 drying days in both

stages, weight loss was not statistically significant because only a small amount of water continued to be lost.

Water activity is the relation between the vapor pressure of food in balance with the surrounding atmosphere and the vapor pressure of water under the same conditions. It represents the efficiency at which the water present in fruit can participate in chemical reactions (Hii et al., 2019; Kim et al., 2014). Initial water activity values were measured and came close to 0.80 for the whole fruit in both stages (Figure 3C). A slight decrease was recorded until day 28 and day 35 in S1 and S2, respectively. From this time, a major decrease took place. Throughout the drying period, the a_w values were always higher in S2 than in S1.

When a_w was measured in the outermost area, a similar pattern to moisture was observed during the drying period (Figure 3D). A major reduction in a_w was recorded until day 35 and day 28 for S1 and S2, respectively, when it slowed down. Nevertheless, in the internal flesh, a_w did not change until day 21 for S1 and until day 28 for S2. After these dates, a gradual decrease was observed in both stages and the a_w values were always higher for S2.

The formation of the secondary epidermis could influence the reduction in water activity in the internal flesh. In dried foods it has been reported that the crust formation may maintain high values of water activity in the flesh since it forms a hard layer containing the free water inside (Telis and Sobral, 2002).

3.3. Changes in Firmness and External Color

The mechanical properties of dry fruits, such as firmness, can be related to the effect of water sorption. Dry fruit generally become softer due to the plasticizing effect of water, which leads to a depression in viscosity and loss of the crunchy/crispy texture (Sosa et al., 2012). However, depending on the drying type or intensity, hardness tends to increase again due to material shrinkage, which significantly compacts the structure, or due to excessive water evaporation and consequently, a higher concentration of solids (Kang et al., 2004; Sosa et al., 2012).

The results showed that the lowest deformation value of flesh fruit was at harvest, when fruit were still completely firm (Figure 4A). From day 7, fruit underwent drastic softening until day 14 for S1 and until day 21 for S2, when firmness tended to stabilize, being the deformation values of S2 fruits always higher. This tendency to firmness stabilization was possibly due to moisture being maintained in the secondary epidermis during approximately the same period. From day 42, the deformation values of S1 slightly lowered, which indicates pulp hardening with an average deformation of 22.7 % on day 63, while the average for S2 was 35.6 %.

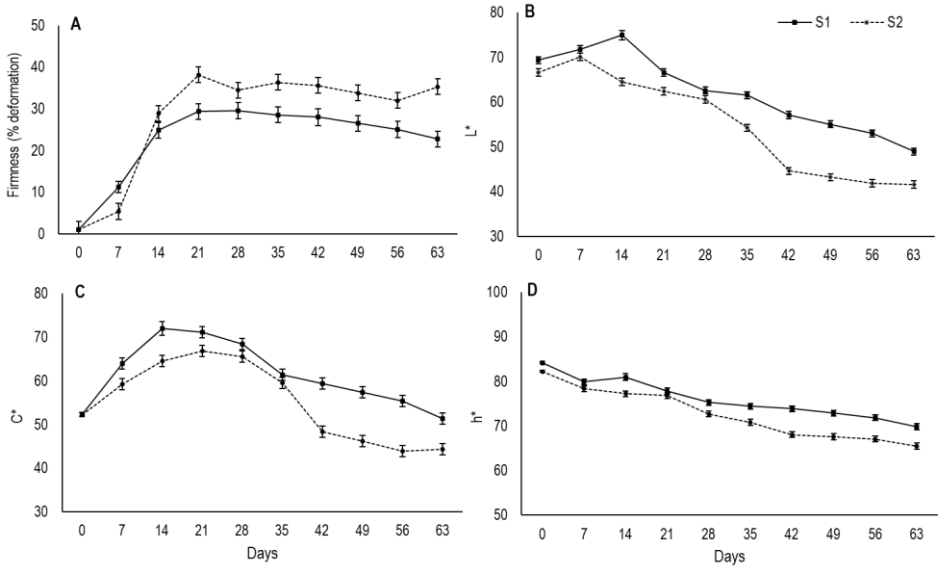

Figure 4. Changes in the (A) firmness, (B) lightness-L*, (C) chroma-C* and (D) hue angle-h* values during the drying process of persimmon cv. Rojo Brillante in two maturity stages (S1 and S2). Vertical bars represent Least Significant Differences (LSD) intervals ($p \leq 0.05$).

The lower deformation values in S1 compared to S2 could be due to the fruit in the first maturity stage developing a thicker secondary epidermis, which would imply greater resistance to deformation. Moreover, the smaller S1 fruit size would allow them to have a smaller gelatinous inner area volume, which would lead to less elasticity and more hardening.

Color is one of the most important sensory resources for determining consumer acceptability and product quality of dried food (Senadeera et al., 2020). During persimmon drying, browning occurs which leads to evident fruit color changes (Çelen, 2019; Yamada et al., 2009). The main factors reported to be involved in browning are the oxidative and nonoxidative degradation pathways of ascorbic acid (Yamada et al., 2009). Sodium metabisulfite solution, often used prior to drying to minimize microbial attacks, also prevents color deterioration during dehydration by retarding both enzymatic and nonenzymatic browning reactions (Hanif et al., 2015; Karakasova et al., 2013). In the present study, fruit were treated with sodium metabisulfite prior to drying, which proved effective in controlling pathogen attack. However, external fruit darkening took place.

To assess fruit external color, the values of the parameters lightness (L*), chroma (C*) and hue angle (h*) were recorded and are shown in Figure 4B–D. The maturity stage influences the evolution of these color parameters during the drying process. The L* values at harvest were 69.3 for S1 and 66.6 for S2, with a slight increase on the first 14 drying days for S1 and on the first 7 days for S2. Then in both cases, the L* values gradually lowered throughout what was left of the drying period, and the values in S1 were higher than S2. After 63 days, fruit had values of 48.9 and 41.6 for S1 and S2, respectively. In relation to C*, which measures color saturation, the fruit from both stages started with similar values, which came close to 52 and displayed increased saturation during the first few weeks, before lowering from day 21 in S1 and from day 28 in S2. By day 63, their average values were lower than their initial values, with 51.3 in S1 and 44.4 in S2.

Browning in semidried persimmon has been related to fruit water content. In ‘Atago’ persimmon, browning has been reported to occur when fruit goes from semidried to dried, with water content close to 50 % or less (Yamada et al., 2009). Accordingly, in the present study, the marked reduction in C* after 21 days in S1 and 28 days in S2 coincided with the time at which whole fruit moisture was about 50 %, or 0.5 gH_2O/g. This occurred when secondary peel moisture stabilized after a marked reduction.

Hue angle (h*) is related to color perception and indicates the primary color associated with angles of 0° (red), 90° (yellow), 180° (green) and 270° (blue) (Pathare et al., 2012). At harvest fruit had h* values of 84.2 and 82.2 for S1 and S2, respectively (Figure 4D). The Hue angle showed a gradual decrease in both maturity stages throughout drying and S1 fruits obtained higher values during the entire period. By day 63, h* values were 69.8 and 65.5 for S1 and S2, respectively.

3.4. Changes in Total Soluble Solids and Soluble Tannins

During persimmon drying, an increase in total soluble solids content occurred, mainly due to drop in moisture content as TSS are a major component of dry matter (Ashebir et al., 2009). The TSS content at harvest was 15.9 °Brix for S1 and 14.7 °Brix for S2 for whole fruit. Throughout drying, TSS content gradually increased and its evolution was similar in both stages with values close to 47 (Figure 5A).

When the TSS content was measured separately in the outermost area and internal flesh, major differences appeared (Figure 5B). In the external area, a major increase continued until day 28 in both stages, from which time S1 continued to gradually increase and S2 tended to stabilize before slightly increasing again from day 56. After 63 days, TSS content reached mean values of 48.4 °Brix and 49.1 °Brix for S1 and S2, respectively. In flesh, TSS content gradually rose, but it was slower than in the external area, albeit with similar values close to 45 by the end of the experiment. The differences in the TSS content concentration between the two fruit parts are related to different moisture losses during drying. Thus, the higher values in the secondary epidermis throughout the experiment would indicate a higher proportion of TSS due to the more marked reduction in water in this fruit area.

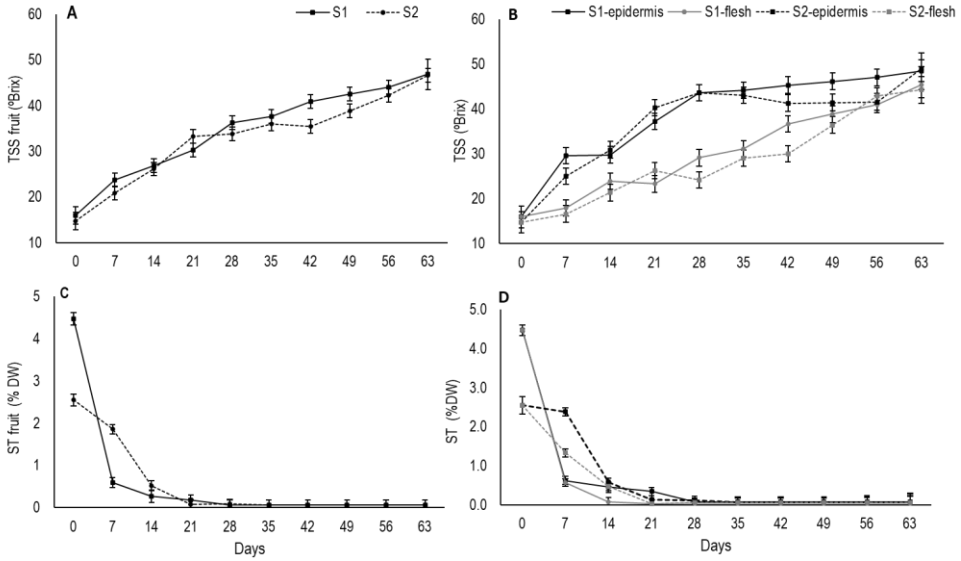

Figure 5. Changes in (A,B) total soluble solids (TSS) and (C,D) soluble tannins (ST) of whole fruit and by parts (secondary epidermis and internal flesh) during the drying process of persimmon cv. Rojo Brillante in two maturity stages (S1 and S2). Vertical bars represent Least Significant Differences (LSD) intervals ($p \leq 0.05$).

Drying also decreases astringency in fruit due to insolubilization of tannins. This process is generally used to remove astringency from persimmon fruit in Asian countries. Changes in soluble tannin contents in astringent persimmon during drying have been established, which indicate a constant reduction in the level of tannins throughout the water loss process (Wu and Hwang, 2002). In this study, the astringency of the variety 'Rojo Brillante' was evidenced by a high soluble tannin content at harvest, with 4.5 % in fruit at S1 and 2.6 % (Figure 5C). These values agree with previous studies about 'Rojo Brillante' (Salvador et al., 2007; Tessmer et al., 2016).

S1 fruits showed a marked decrease in the first week, which was similar in both zones, while this decline was slower in S2 fruit (Figure 5D). When assessing internal and external zones separately, internal flesh on day 21 already had ST non-astringency values, which were close to 0.03 % for both stages as shown

by Tessmer et al. (2016). During the same period, astringency was still residual in the secondary epidermis, with values of 0.23 % for S1 and 0.08 % for S2. Fruit completely lost astringency only from day 28 for both stages and fruit parts.

Previous studies reported the factors that may lead to insolubilization of tannins in persimmon fruit. This process was mainly studied during fruit ripening. The softening that occurs during fruit maturation was related to the degradation of cell wall polysaccharides, which induce an osmotic pressure that causes the concentration of soluble tannins in the tannin cells, leading to its insolubilization (Fukushima et al., 1991). Taira and Ono (1996) suggested that the flesh cells collapsing during the ripening process leads to the production of cell wall fragments that adhere to the tannins, thus reducing astringency. Taira et al. (1997) also reported that the softening and flesh cells collapse that occur during the fruit ripening lead to a solubilization of pectins which form a complex with tannins and causes their insolubilization. So, the tannins insolubilization during the drying process may also be associated to the structure changes in the fruit flesh. In this way, Asgar et al. (2004) related the flesh softening that occurs during sun-drying of japanese persimmon to the solubilization and depolymerization of pectic polysaccharides.

3.5. Microstructural Changes

The microstructural study was carried out throughout persimmon drying by the Cryo-FESEM technique. For both maturity stages, from 7 days onward the structural evaluation was done for the two separate and di erentiated areas: (1) internal flesh; (2) the secondary epidermis formed during the drying process.

3.5.1. Internal Flesh

When Cryo-FESEM was used to observe a cross-section of persimmon internal flesh at harvest (day 0), the parenchyma was quite compact in both maturity stages (S1 and S2) and was formed by turgid and intact cells with intercellular

spaces full of air and cell walls and the plasma membrane was intact (Figure 6). During drying, cell structure deterioration became evident. In both stages, a drastic change in parenchyma structure was observed after 14 drying days onward. In S2, the parenchyma degradation process became slower while the drying process advanced in S1. On day 14 in S1, cell walls and cell membranes were hard to distinguish in the collapsed tissue. This cell structure deterioration continued as drying advanced. On day 28, the initial parenchyma structure had completely disappeared and had become a homogeneous compact mass in which no cells were visible. In S2, the cellular structure after 14 days had greatly deteriorated as most cells had lost their integrity and intercellular spaces were waterlogged, but unlike S1, it was still possible to see some structured cells. The total loss of the parenchyma structure with the appearance of a structure-less homogeneous mass occurred on day 42.

Parenchyma degradation is related to water removal, which occurs during drying. It is noteworthy that when the structure was completely lost and only a compact homogeneous mass was visible (on day 28 for S1, on day 42 for S2), fruit presented similar moisture content, which came close to 0.41 gH_2O/g, and similar water activity, close to 0.87, when the measurements were taken on the whole fruit. When we observed the internal flesh data obtained, similar values were recorded for moisture, close to 0.56 gH_2O/g, and water activity, close to 0.93, for S1 on day 28 and for S2 on day 42. After 63 days, the images of S1 showed more contracted tissue, but this was not found in S2.

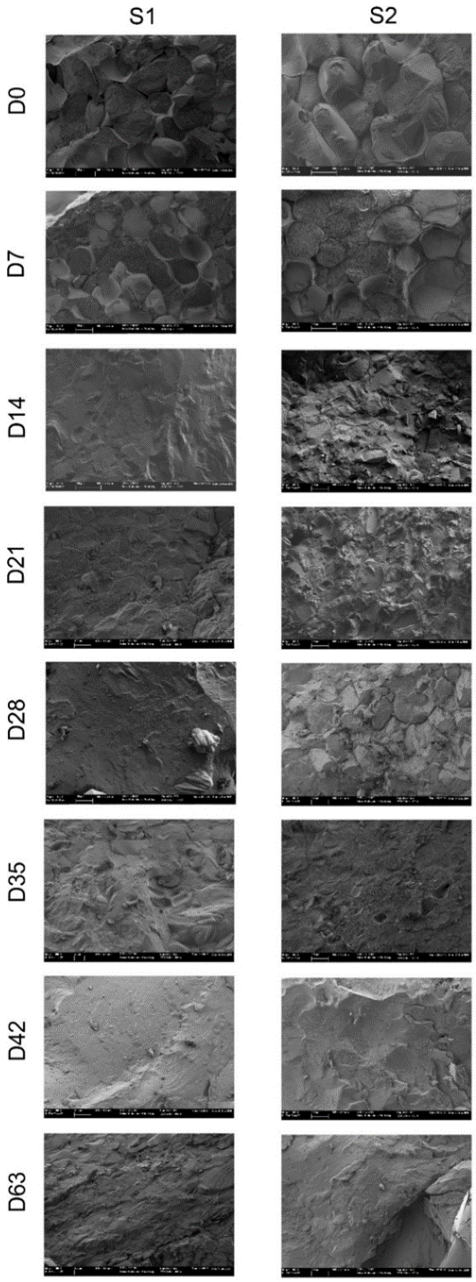

Figure 6. Internal flesh structure by the Cryo-field emission scanning electron microscopy technique of persimmon cv. Rojo Brillante in two maturity stages (S1 and S2) submitted to drying up to 63 days (D).

In persimmon, the soluble tannins responsible for astringency are located inside vacuoles of the so-called tannic cells. In previous studies, the insolubilization of tannins during the ripening process has been observed by the Cryo-FESEM technique (Salvador et al., 2007; Wu et al., 2002). When tannins are soluble, they can be observed as a network, which is the typical eutectic artefact generated during the water sublimation process when samples are prepared by Cryo-FESEM. On the contrary, when tannins are insolubilized, a compact mass inside the vacuole is shown and no eutectic artefact can be seen. In the present study, cells with soluble material inside them were observed in the samples upon harvest. After day 7 in S1 and until day 14 in S2, although tissue was already degraded, some cells with soluble material were seen. Nevertheless, in the subsequent samples, no soluble material was observed, which might indicate that tannin insolubilization had occurred during the drying process. This corroborates the drastic drop in the content of soluble tannins to non-astringency values, which took place after 14 days or 21 days of drying in S1 and S2, respectively. This process would be similar to ripening since in the maturity process, persimmon has been reported to be accompanied by a gradual insolubilization of tannins (Tessmer et al., 2016). Specifically, for the cultivar 'Rojo Brillante', complete astringency loss does not occur until fruit are overripe and their texture is very soft. In this state, pulp has a gel structure.

The gelling of flesh in the internal area was also observed under a light microscope by the fluorescence technique (Figure 7). Calcofluor was used as a staining agent, which is specific to cell walls (blue). The intact flesh upon harvest and the gelled tissue as a result of the drying process were compared. In the samples taken at harvest, cell walls were clearly visible. Cells are relatively spherical and uniformly distributed throughout the parenchyma. Nevertheless, in the samples of the gelled flesh, cell walls appeared torn and destroyed, which leads to the complete loss of structure tissue before collapse. The drastic degradation of cell walls would cause the leaching of cellular constituents into the tissue matrix. Tissue appears as tightly jumbled misshapen cells. At the end of the assay, after 63 days, tissue appears compacted as a result of the shrinkage process.

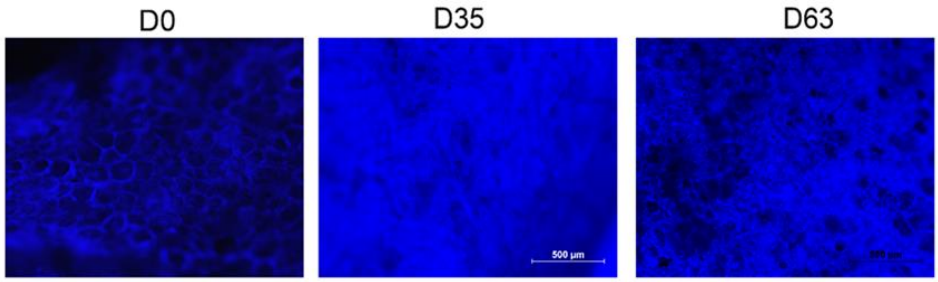

Figure 7. Tissue sections of persimmon cv. Rojo Brillante flesh submitted to drying observed by light microscopy (D: days of drying).

3.5.2. Secondary Epidermis

Figure 8 shows the microstructure of the secondary epidermis formed during drying process in both the maturity stages. The Cryo-FESEM evaluation was made from day 14 when the secondary epidermis was evident in both maturity stages.

After 14 days, the parenchyma of the fruit from both stages appeared to have collapsed and displayed a compact morphology. Cell walls were completely lost, and the cell and intercellular spaces were full of insoluble material. Samples presented hollows, which were not observed in internal flesh. While some cells remained rounded in S2, the S1 tissue seemed more contracted and displayed squashed cell layers.

With the drying process, parenchymatic tissue evolves to become a more compact and destroyed structure due to the shrinkage process and leads to cell deformation. When observing internal flesh, the collapse of the structure in the secondary epidermis was faster in S1 than in S2.

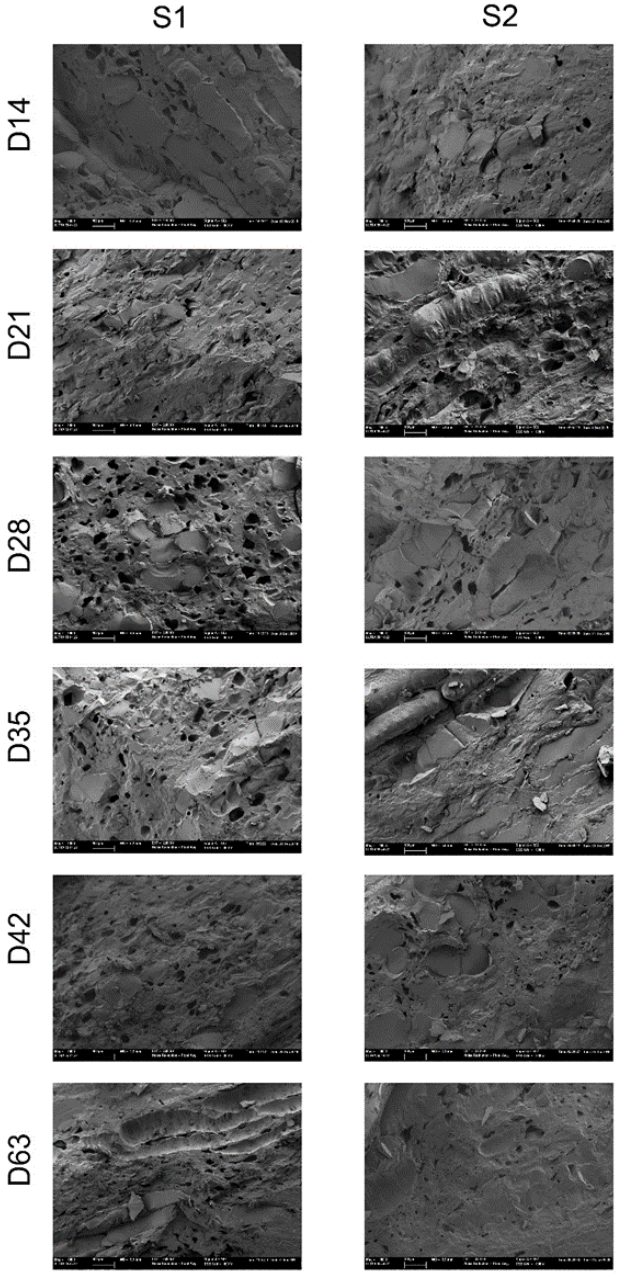

Figure 8. External flesh structure by the Cryo-field emission scanning electron microscopy technique of persimmon cv. Rojo Brillante in two maturity stages (S1 and S2) submitted to drying up to 63 days (D).

The hollows displayed in these samples would be generated by the water vapor outflow during the drying process. The pore size in the samples from S1 was bigger than that from S2. The presence of pores in this structure could explain that internal flesh continues to lose water, even though this second epidermis had formed. Furthermore, the larger pore size in S1 would also explain the faster internal dehydration in S1 than in S2.

4. Conclusions

The physico-chemical and microstructure changes that take place during the drying of persimmon cv. Rojo Brillante in two maturity stages were studied. A characterization of the internal area (flesh) and external area (secondary epidermis formed during drying) of fruit was made for the first time. The obtained results revealed that maturity stage influences fruit characteristics during the drying process. Moisture loss was faster in S1 than in S2. Fruit had moisture values of semidried persimmon, i.e., around 0.5 gH_2O/g, after 21 and 28 days for S1 and S2, respectively.

During the drying process, the formation of a secondary epidermis, concomitantly to internal flesh gelification, was related to the moisture loss that occurred in fruit in each maturity stage. This process was slower in S2 than in S1. The changes in the parequimatic structure of the outmost area observed by Cryo-FESEM were related to the hardening of this area as a result of increased moisture loss compared to internal flesh. In internal flesh, texture changed as a result of parenchyma degradation, which led to a structure that looked like a homogeneous mass, in which cell membranes were completely degraded. Internal flesh gelling was also clearly observed under alight microscope using calcofluor to stain the cell walls. The collapsed tissue presented hollows generated by water outflow, which would allow water to be removed from the internal flesh. S2 presented a thinner secondary epidermis and a bigger volume of gelled area inside, which would make them more elastic and softer compared to S1. Astringency loss took place due to the insolubilization of tannins during the water loss process.

The present study revealed that 'Rojo Brillante' persimmon is a suitable astringent variety to be submitted to a drying process after taking into account that the final product characteristics depend on the maturity state upon harvest.

Acknowledgements

This research was funded by Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and Fondo Europeo de Desarrollo Regional (FEDER), through Project RTA2017-00045-C02. Nariane Quaresma Vilhena thanks the INIA for grant FPI-INIA (PRE2018-085833).

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CHAPTER VII

Influence of temperature on 'Rojo Brillante' persimmon drying. Quality characteristics and drying kinetics

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Pending submission

Abstract

A physico-chemical characterization and drying kinetics study was performed for whole 'Rojo Brillante' persimmon subjected to hot-air drying at 35 ºC, 40 ºC and 45 ºC. Fruit was dried to 30 % moisture, which is required to be considered dry persimmon. This moisture value was obtained after 12, 8 and 7 days when the drying process was applied at 35 ºC, 40 ºC and 45 ºC, respectively. All the drying treatments caused flesh structure changes, shrinkage and secondary epidermis formation. The final product's quality characteristics especially the texture, strongly depended on drying temperature. The fruit dried at 35 ºC exhibited a gelatinous internal texture and a thin secondary epidermis, similarly to that obtained during a natural drying process. The drying treatment at 40 ºC resulted in fruit having more rubbery flesh. However, the fruit dried at 45 ºC had a corky texture, with a very thick secondary epidermis that resulted in excessively tough fruit for commercialization purposes. The differences in flesh structure were revealed in the microstructural study by Cryo-FESEM. In all cases, soluble tannins content lowered to non-astringency values. Hot-air drying technology proved to be a good alternative to valorize discarded 'Rojo Brillante' persimmon.

Keywords: Dried persimmon; hot-air drying; texture; secondary epidermis.

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) is a crop of Chinese origin that has become one of the most important cultures in the Mediterranean region of Spain, with an annual production of approximately 500,000 tons, which centers mainly on the astringent cultivar Rojo Brillante (Mañez, 2019; Morales et al., 2022). High persimmon production associated with a short harvesting season has led to an increase in postharvest losses, which can reach up to 30 % of total production (Fernandez-Zamudio et al., 2020). Therefore, the development of value-added by-products emerges as a strategy to reduce fruit waste and to valorize surplus persimmon production.

Drying is one of the oldest fruit preservation methods that prolongs shelf life and enables fruit consumption during all seasons (Doymaz, 2012). In Asian countries, whole fruit drying is a traditional technique used for persimmon (Hur et al., 2014; Jia et al., 2019). Although the whole persimmon drying technology has not yet been implemented in Spain and other production areas, recent studies have revealed that cv. Rojo Brillante is suitable for being submitted to natural-air drying processes (González et al., 2021; Vilhena et al., 2020). Drying is also reported to eliminate 'Rojo Brillante' astringency due to tannins insolubilization, with the water loss process being an interesting aspect to allow its commercialization without the need for postharvest deastringency treatment (Matsumoto et al., 2007).

Dried persimmon can be classified into dried and semi-dried based on its water content, and the moisture limit for this classification is variable among countries and varieties (Choi et al., 2017). In South Korea, which is one of the main dried persimmon producing countries, the final water content is 50 % for semi-dried and 30 % for dried fruit. The period to achieve this moisture by natural-air drying lasts between approximately 25 and 60 days (Kang et al., 2004; Kim et al., 2018; Lee et al., 2011). For cv. Rojo Brillante, Vilhena et al. (2020) achieved moisture of 50 % at 25 drying days and of 30 % at 50 drying days.

However, the long time required to reach the desired moisture content, and the fact that fruit can be contaminated by airborne spores during the process, are some drawbacks for implementing natural-air drying on an industrial scale

(Cárcel et al., 2007). In addition, it does not seem to be a suitable technique for regions with high relative humidity and warm temperatures like the Spanish Mediterranean region. Therefore, strategies are being sought to improve the drying process. Hot-air drying appears to be a good solution to shorten the procedure without losing final product quality, as shown for different fruit crops like grapes (Adiletta et al., 2016), plums (Hedayatizadeh and Chaji, 2016) and dates (Falade and Abbo, 2007), and for different persimmon cultivars produced in Asia (Demiray and Tulek, 2017; Jia et al., 2020; Sugiura and Taira, 2009). Nevertheless, there are still no published references about the hot-air drying of whole 'Rojo Brillante' persimmon. So the fruit characterization throughout this process, and the study of drying kinetics, are necessary to estimate the optimal conditions to obtain a high-quality product (Brasiello et al., 2013; Doymaz, 2012).

Thus, the objective of this work is the optimization of whole fruit drying technology for 'Rojo Brillante' persimmon by applying different drying temperatures by means of evaluating fruit physico-chemical and microstructural characteristics.

2. Materials and Methods

2.1. Fruit Samples and Experimental Procedure

Persimmon cv. Rojo Brillante in the commercial maturity stage was obtained from a fruit cooperative (Cooperativa Agrícola Sant Bernart Coop. V.) located in Carlet (Valencian Community, Spain). Fruit was transported to the Instituto Valenciano de Investigaciones Agrarias (IVIA), where they were selected according to homogenous size, color and lack of external damage. The initial flesh firmness was 36±5 N and the peel color index was 14±6.

Twenty fruits were taken for the initial characterization. The rest were separated into three batches of 80 fruit to be subjected to three drying temperatures. Fruit was sanitized with sodium hypochlorite solution (1/10 v.v., for 4 min) and placed on absorbent paper to dry in the open air. Subsequently, fruit were manually peeled, individually identified and hung by the pedicel in

convective drying chambers (FED 260 Standard model, Binder, Tuttlingen, Germany) (Figure 1).

Fruit was subjected to forced air drying conditions at an air velocity of 2 m/s at 35 °C, 40 °C or 45 °C. In all cases, fruit were dried until moisture reached 30±2 % on a wet basis (w.b.) according to Choi et al. (2017). The duration of the process depended on the time when fruit achieved the established moisture.

Figure 1. Peeled 'Rojo Brillante' persimmon hung inside drying chambers.

2.2. Determinations

The following physico-chemical determinations were made on eight fruit per treatment before drying (day 0) and periodically throughout the drying process until samples reached 30 % moisture: water content, water activity (a_w), diameters, epidermis thickness, color, texture, total soluble solids (TSS) and soluble tannins (ST). In addition, a microstructural study was carried out with fresh fruit and at the end of the drying process.

2.2.1. Moisture, water activity and drying kinetics

Three fruit per treatment were taken to determine the moisture and water activity (a_w). For this purpose, they were crushed individually by a crushing machine (Moulinex AD560120) and the water content of samples was analyzed by vacuum drying (Vaciotem, J.P. Selecta vacuum oven) at 60 °C to constant weight according to the standard method of AOAC (2005). The results were expressed as a percentage of dry basis (d.b.). Water activity (a_w) was determined using a dew point hygrometer device (Aqualab CX-2 Decagon). All these determinations were replicated three times.

Given the aim of characterize fruit drying behavior at the three drying temperatures, the experimental moisture data were fitted to six mathematical models previously employed for whole persimmons dried by natural air (González et al., 2021) (Table 1).

Table 1. Mathematical models applied to drying curves.

Empirical Model	Equation	References
Newton	$MR = exp\ (-kt)$	Liu and Bakker-Arkema (1997)
Page	$MR = exp\ (-kt^n)$	Page (1949)
Midilli et al.	$MR = a\ exp\ (-kt^n) + bt$	Midilli et al. (2002)
Logarithmic	$MR = a\ exp\ (-kt^n) + c$	Toğrul and Pehlivan (2002)
Henderson & Pabis	$MR = a\ exp\ (-kt)$	Wang et al. (2007)
Verma	$MR = a\ exp\ (-kt) + (1-a)\ exp\ (-gt)$	Verma et al. (1985)

MR: moisture ratio; k, n, a, g, c, b: constants of each applied model; t: time in days (source: González et al., 2021).

These models relate the moisture ratio (MR) to the drying time (in days). The MR is calculated from the water content of the samples by applying **Equation 1:**

$$MR = \left(\frac{M_i - M_e}{M_0 - M_e}\right) \quad (1)$$

where M_0 is the moisture content at the initial time, M_i is the moisture content at any drying time, and M_e is the equilibrium moisture content, which can be neglected for being relatively low. Therefore, the MR can be expressed as M_i/M_0.

The regression analyses were determined by the Solver statistical software (Excel 2016). The criteria for selecting the best model to define the drying curves were a high determination coefficient (R^2) and lower values of the chi-square (X^2), mean bias error (MBE), root-mean-square error (RMSE) and relative percent error (PE). These parameters were calculated by using **Equations 2, 3, 4 and 5:**

$$X^2 = \frac{\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2}{N - z} \quad (2)$$

$$MBE = \frac{1}{n}\sum_{i=1}^{N}\left(MR_{pre,i} - MR_{exp,i}\right) \quad (3)$$

$$RMSE = \left[\frac{1}{n}\sum_{i=1}^{N}\left(MR_{pre,i} - MR_{exp,i}\right)^2\right]^{1/2} \quad (4)$$

$$PE\ (\%) = \frac{100}{N}\sum_{i=1}^{N}\frac{\left|MR_{exp,i} - MR_{pre,i}\right|}{MR_{exp,i}} \quad (5)$$

where $MR_{exp,i}$ is the experimental MR, $MR_{pre,i}$ is the predicted MR, N is the number of observations and z is the number of constants.

2.2.2. Diameters, secondary epidermis thickness and shrinkage

Fruit longitudinal and equatorial diameters, as well as the thickness of the secondary epidermis that formed during the drying process, were assessed individually on each fruit by a pachymeter (Mitutoyo 500-267V-CDL20CP, Japan).

The fruit volume on day 0 (V_0) and at each evaluation time point (V_t) was calculated from the values of the longitudinal and equatorial diameters. The shrinkage that occurred during drying was expressed as the relative volume ratio (V_t/V_0) according to the relative MR (M_t/M_0).

2.2.3. Color and Texture

Fruit external color was measured individually on two opposite equatorial zones without peel. A Minolta Colorimeter (model CR-400, Ramsey, NY, USA) was used by applying a D65 illuminant and 10 degrees standard observer. The Lightness (L*), Chroma (C*), and hue angle (h*) parameters were determined. The total color difference (ΔE) was calculated according to Equation (6):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (6)$$

where ΔL*, Δa*and Δb* are the difference of each parameter before and after all the drying treatments (Pathare et al., 2013).

Texture was assessed by a Texturometer Instron Universal Machine (model 4301, Instron Corp., Canton, MA, USA) using a 35-mm flat plunger and applying a compression force of 10 N along the fruit equatorial axis. Produced deformation was expressed as millimeters of deformation.

2.2.4. Soluble Tannin Content and Total Soluble Solids

Three fruits per treatment were chopped into small pieces and mixed. Three samples of 5 g of material were taken to determine ST and TSS. ST were determined by the Folin-Denis method (Taira, 1995) and the results were expressed as a percentage of dry basis (d.b.).

For determining TSS, fruit samples were homogenized with distilled water at a 1/3 ratio (w/w) in a Polytron homogenizer (model PT 3100D, Kinematica, Switzerland). To avoid tannins interfering with the TSS measurements, their insolubilization was previously done according to Sugiura et al. (1983). Measurements were taken in triplicate with a refractometer (Atagomod. PR1). The results were expressed as ºBrix.

2.2.5. Microstructural Analysis

For the microstructural study, a Cryo-field emission scanning electron microscopy (Cryo-FESEM, ZEISS ULTRA 55, Oxford Instruments, Abingdon, UK) was used to observe flesh samples. Cubes (3 mm^3) were cut from the equatorial area perpendicularly to the main axis of the persimmon flesh with a stainless-steel cutter. These cubes were then immersed into slush nitrogen (-210 ºC) and transferred to a cryo-trans GeminiSEM 500 (ZEISS, Oberkochen, Germany) linked with a field emission scanning electron microscope, which operated at a temperature below -130 ºC. Samples were cryofractured at -180 ºC and etched at -90 ºC. Samples were observed at 3 kV at a working distance of 68 mm.

2.2.6. Statistical Analysis

The fruit physico-chemical data were subjected to analyses of variance (ANOVA) and multiple comparisons between means at $p \leq 0.05$, determined by the LSD test. The Statgraphics Centurion XVII.I software application was used for the statistical analysis (Manugistics Inc., Rockville, MD, USA).

3. Results and Discussion

3.1. Moisture and water activity. Drying kinetics

Fruit moisture at day 0 was close to 80.5 %. It continuously lowered during the drying process, which was faster the higher the drying temperature was (Figure 2a). Moisture at around 30 %, which is the required value for dried

persimmon, was reached at 12, 8 and 7 days when drying treatments were carried out at 35 ºC, 40 ºC and 45 ºC, respectively.

The effect of temperature on persimmon drying has been related to a higher evaporation rate from the fruit surface at higher temperatures (Demiray and Tulek, 2017). For the cv. Rojo Brillante submitted to natural-air drying, the time required to reach 30 % moisture was substantially longer, around 50 days (González et al., 2021; Vilhena et al., 2020).

The fresh fruit had a value of 0.98 for a_w (Figure 2b). Throughout the drying process, a decrease occurred that was temperature-dependent. The fruit dried at 35 ºC showed a steady decrease in a_w to values of 0.83 after 12 days when fruit reached 30 % moisture content. The fruit dried at 40 ºC showed a similar decrease as that recorded at 35 ºC until day 5, but this decrease became more marked from that time onward. At the end of drying, after 8 days it reached similar values to those of the fruit dried at 35 ºC. The a_w reduction in the fruit at 45 ºC was more pronounced, mainly from day 5, with values of 0.70 on day 7.

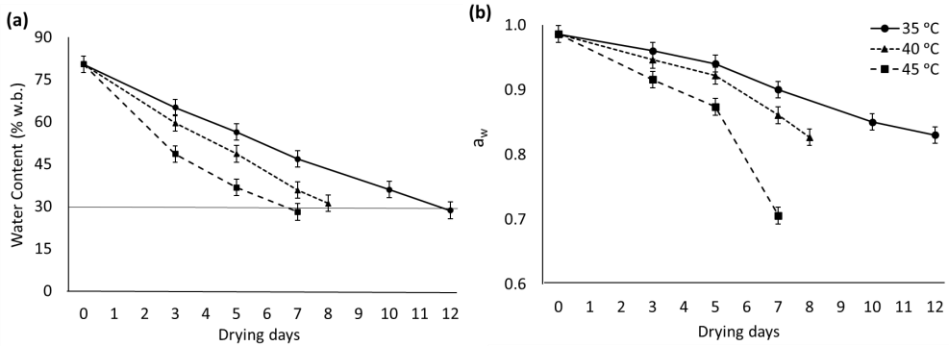

Figure 2. Water content (a) and water activity (a_w) (b) of 'Rojo Brillante' persimmon during the drying process at 35 ºC, 40 ºC and 45 ºC until 30 % moisture was reached. The vertical bars represent Least Significant Differences (LSD) intervals ($p \leq 0.05$).

The moisture ratios calculated from the experimental water content values were represented according to the drying time and fitted to six drying models (Table 1). Previous studies have employed these models for different products, such as apple slices (Sacilik and Elicin, 2006) and grape seeds (Roberts et al., 2008). For persimmon, they have been previously applied for slices (Sampaio et al., 2017; Senadeera et al., 2020) and for whole fruit undergoing natural-air drying (González et al., 2021).

The parameters from the six models were estimated using a nonlinear regression analysis with persimmon samples dried at the three temperatures (Table 2). The value of the k constant of all the models ranged from 0.121 to 0.704. This value was higher as the drying temperature increased. This effect of temperature on the k value has also been observed during the drying process of persimmon slices (Senadeera et al., 2020). For naturally dried persimmon, González et al. (2021) reported lower values for the k parameter, which were between 0.027 and 0.041.

The n parameter obtained values ranging from 0.789 to 0.636, and this value lowered with drying temperature increase. During natural drying, this parameter had higher values than in the present study, and ranged from 0.983 to 1.101 (González et al., 2021). Parameters a, g, c and b were similar between models and did not present any wide variations with drying temperatures changes.

In order to test goodness of fit, five statistical parameters were employed: the R^2, X^2, MSE, RMSE and PE values (Table 2). The model that best fitted the three drying processes was Verma's model because it had the highest R^2 value (> 0.999) and the lowest X^2 (< $1 \cdot 10^{-5}$), MBE (< $1 \cdot 10^{-3}$), and RMSE (< $1 \cdot 10^{-5}$) values. Page's and Midilli's models followed closely, and also obtained high R^2 (> 0.999) values and very low X^2, MBE and RMSE parameters. The PE values varied from 1 % to 12 % for all the models assessed for the three temperatures, and Page, Midilli and Verma's models were those with lowest values, which ranged between 1-2 %.

Table 2. Values of the model parameters and statistical parameters of six drying models employed for 'Rojo Brillante' persimmon dried at 35 °C, 40 °C and 45 °C.

	Model Parameters						Statistical Parameters				
Model	***k***	***n***	***a***	***g***	***c***	***b***	**R^2**	**X^2**	**MBE**	**RMSE**	**PE %**
					35 °C						
Newton	0. 218	-	-	-	-	-	0.99	$4.1 \cdot 10^{-4}$	$1.2 \cdot 10^{-2}$	$2.7 \cdot 10^{-4}$	8.45
Page	0.328	0.789	-	-	-	-	0.99	$9.2 \cdot 10^{-6}$	$1.7 \cdot 10^{-3}$	$6.1 \cdot 10^{-6}$	0.91
Midilli et al.	0.328	0.789	1	-	-	0	0.99	$9.2 \cdot 10^{-6}$	$1.7 \cdot 10^{-3}$	$6.1 \cdot 10^{-6}$	0.92
Logarithmic	0.261	-	0.938	-	0.061	-	0.99	$1.7 \cdot 10^{-5}$	$2.4 \cdot 10^{-3}$	$1.1 \cdot 10^{-5}$	1.83
Henderson & Pabis	0.217	-	0.995	-	-	-	0.99	$4.0 \cdot 10^{-4}$	$1.3 \cdot 10^{-2}$	$2.7 \cdot 10^{-4}$	8.43
Verma	0.121	-	0.391	0.363	-	-	1.00	$5.4 \cdot 10^{-6}$	$1.4 \cdot 10^{-3}$	$3.6 \cdot 10^{-6}$	1.01
					40 °C						
Newton	0.316	-	-	-	-	-	0.99	$7.9 \cdot 10^{-4}$	$1.8 \cdot 10^{-2}$	$5.3 \cdot 10^{-4}$	10.02
Page	0.457	0.76	-	-	-	-	0.99	$3.7 \cdot 10^{-5}$	$3.6 \cdot 10^{-3}$	$2.5 \cdot 10^{-5}$	1.99
Midilli et al.	0.456	0.761	1	-	-	0	0.99	$3.7 \cdot 10^{-5}$	$3.6 \cdot 10^{-3}$	$2.5 \cdot 10^{-5}$	1.99
Logarithmic	0.396	-	0.924	-	0.075	-	0.99	$1.5 \cdot 10^{-4}$	$7.0 \cdot 10^{-3}$	$9.8 \cdot 10^{-5}$	3.54
Henderson & Pabis	0.314	-	0.991	-	-	-	0.99	$7.7 \cdot 10^{-4}$	$1.9 \cdot 10^{-2}$	$5.1 \cdot 10^{-4}$	9.89
Verma	0.237	-	0.706	5.164	-	-	0.99	$1.4 \cdot 10^{-5}$	$2.1 \cdot 10^{-3}$	$9.4 \cdot 10^{-6}$	1.16
					45 °C						
Newton	0.429	-	-	-	-	-	0.99	$8.3 \cdot 10^{-4}$	$1.7 \cdot 10^{-2}$	$5.5 \cdot 10^{-4}$	12.61
Page	0.704	0.636	-	-	-	-	0.99	$1.8 \cdot 10^{-5}$	$2.3 \cdot 10^{-3}$	$1.2 \cdot 10^{-5}$	1.90
Midilli et al.	0.704	0.636	1	-	-	0	0.99	$1.8 \cdot 10^{-5}$	$2.3 \cdot 10^{-3}$	$1.2 \cdot 10^{-5}$	1.90
Logarithmic	0.561	-	0.924	-	0.076	-	0.99	$1.0 \cdot 10^{-4}$	$5.6 \cdot 10^{-3}$	$6.9 \cdot 10^{-5}$	4.36
Henderson & Pabis	0.427	-	0.995	-	-	-	0.99	$8.3 \cdot 10^{-4}$	$1.7 \cdot 10^{-2}$	$5.5 \cdot 10^{-4}$	12.6
Verma	0.258	-	0.524	5.164	-	-	1.00	$5.0 \cdot 10^{-6}$	$1.1 \cdot 10^{-3}$	$3.3 \cdot 10^{-6}$	1.01

k, n, a, g, c, b: constants of each applied model.

These results agree with those obtained for natural-air dried persimmon (González et al., 2021), where the models that best fitted were Midilli, Logarithmic, and Verma's models. Verma's model for the three drying temperatures is plotted in Figure 3 and shows a good fit to the experimental data at each drying temperature.

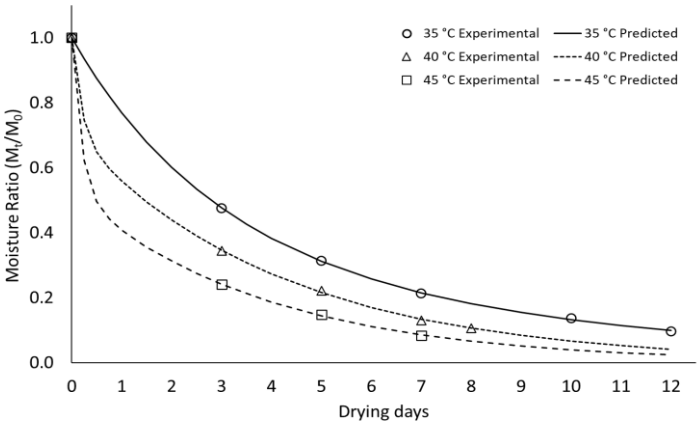

Figure 3. Experimental data and predicted drying curves using Verma's model for 'Rojo Brillante' persimmon dried at 35 °C, 40 °C and 45 °C.

3.2. Physico-chemical Parameters

During the drying process, a reduction in the equatorial and longitudinal diameters due to water loss and a consequent reduction in the internal volume resulted in visible fruit wrinkling (Figure 4).

The reduction in the equatorial diameter was greater compared to the changes in the fruit longitudinal diameter (Table 3). Thus, when fruit reached 30 % moisture, while the equatorial diameter had lowered by about 45 % in all the drying treatments, the longitudinal diameter had decreased by 19 %. The slighter reduction in the longitudinal diameter compared to the equatorial diameter was due to the way fruit were hung because when they were suspended by the peduncle. So, the force of gravity caused fruit to stretch downwardly, with the consequent maintenance of longitudinal size.

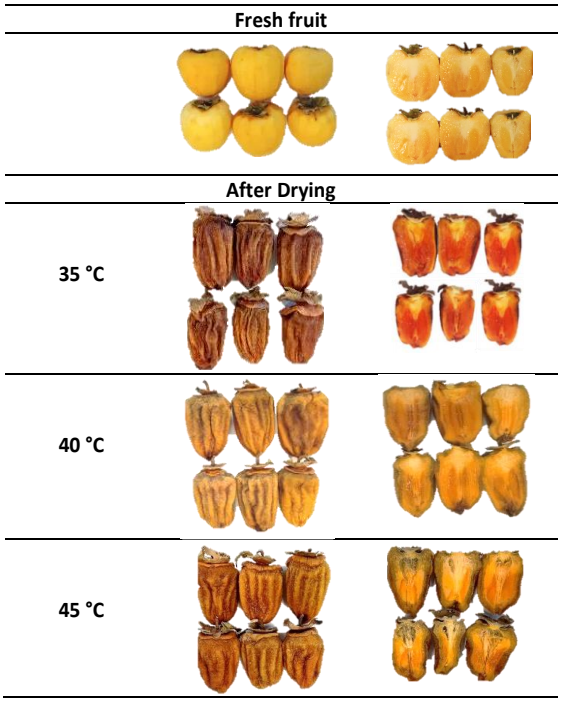

Figure 4. Appearance of 'Rojo Brillante' persimmon in the initial stage and at the end of each drying process at 35 °C (12 days), 40 °C (8 days) and 45 °C (7 days).

Table 3. Longitudinal and equatorial diameters of persimmon 'Rojo Brillante' before (fresh fruit) and after drying until reaching 30 % moisture at 35 °C, 40 °C or 45 °C.

		Longitudinal diameter (mm)	Equatorial diameter (mm)
Fresh fruit		77.76 ± 0.87 [a]	76.93 ± 0.97 [a]
After drying	**35 °C**	62.64 ± 1.5 [b]	38.80 ± 0.97 [b]
	40 °C	63.22 ± 1.25 [b]	42.81 ± 1.64 [b]
	45 °C	62.25 ± 1.30 [b]	41.47 ± 1.57 [b]

Different letters in the same column show significant differences according to the LSD test ($p \leq 0.05$).

The reduction in fruit overall volume, defined as shrinkage, is a physical phenomenon that occurs during the dehydration process affects textural quality (Sun et al., 2022). Shrinkage depends on moisture content, but also on other many factors, including material characteristics, microstructure, mechanical properties and process conditions (Mahiuddin et al., 2018). Some studies have reported the effect of temperature on material shrinkage during the drying process (Mahiuddin et al., 2018). Most fruit showed less tissue shrinkage at a higher temperature. At high temperature, the surface of samples dries very quickly, which means that the surface became rigid (secondary epidermis). This circumstance does not allow material to significantly shrink. In the present study, the drop in V_t/V_0 depended on the drying temperature, with the fruit dried at 35 °C obtaining the highest values on the first drying days (Figure 5). Nevertheless, when fruit reached 30 % moisture on days 12, 8 and 7 at 35 °C, 40 °C and 45 °C, respectively, no differences were shown. Indeed, only slight visual differences were observed in fruit wrinkling for the fruit subjected to the three drying temperatures, which was slightly more pronounced in the fruit dried at the lowest temperature (Figure 4).

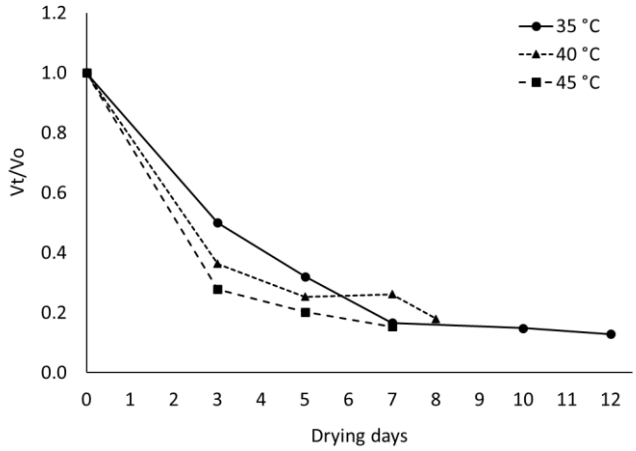

Figure 5. 'Rojo Brillante' persimmon shrinkage during the drying process at 35 °C, 40 °C and 45 °C until 30 % moisture was reached. V_t/V_0 represents the ratio of the fruit volume at any time (V_t) to the initial volume (V_0).

During the drying process, a rigid external layer formed as water loss was faster on the external fruit surface than in the internal zone. This process is called 'crust formation'. In persimmon, this layer has been referred to as secondary epidermis or secondary surface (Jia et al., 2020; Kang et al., 2004; Vilhena et al., 2020). In this study, the thickness of this secondary epidermis depended on the drying temperature (Figure 6). On day 3, the fruit of the three treatments presented similar epidermis thickness, which was close to 1.0 mm. Nevertheless, from that time onward, no increase in secondary epidermis thickness was observed in the fruit dried at 35 °C, but it continued to thicken up to 2.85 mm by the end of drying in the fruit dried at 40 °C. Epidermis thickness dramatically increased in the fruit dried at 45 °C, with values up to 4.12 mm on day 7.

An influence of drying conditions, such as temperature and drying rate, on crust formation has been reported. A slow drying rate could allow moisture migration from the inside to replenish the product surface by reducing or avoiding crust formation (Achanta and Okos, 1996; Rahman, 2008). This may explain the thinnest secondary epidermis in the fruit dried at the lowest temperature (35 °C).

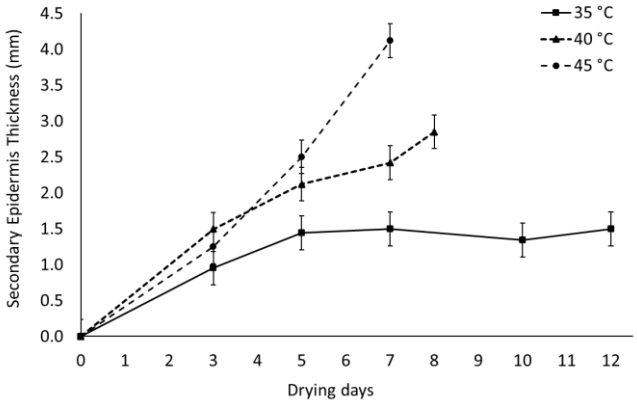

Figure 6. Secondary epidermis thickness of 'Rojo Brillante' persimmon during the drying process at 35 °C, 40 °C and 45 °C until 30 % moisture was reached. The vertical bars represent Least Significant Differences (LSD) intervals ($p \leq 0.05$).

The drying process in persimmon has also been reported to cause fruit darkening (Çelen, 2019; Yamada et al., 2009). Flesh browning is a result of several biochemical reactions, such as degradation of carotenoids and other pigments, enzymatic and non-enzymatic reactions, as well as the oxidative and non-oxidative breakdown of ascorbic acid (Yamada et al., 2009).

In the present study, secondary epidermis color was influenced by drying temperature. When fruit reached 30 % moisture, the fruit dried at 35 ºC had darker peel than that dried at 45 ºC, and the fruit dried at 40 ºC displayed the palest color (Figure 4). These differences among drying temperatures were reflected in the Lightness (L*), Chroma (C*), hue angle (h*) and total color difference (ΔE) values (Table 4). Thus, the fruit dried at 35 ºC had the lowest L*, C* and h* values, which indicated more fruit darkening. By considering the color changes previously reported for the natural drying of 'Rojo Brillante' persimmon, the fruit dried at 40 ºC was that with the most similar L*, C* and h* values when 30 % moisture was reached (Vilhena et al., 2020).

Total color difference is the deviation of the final product color from the color at the initial time (Milczarek et al., 2020). The lowest ΔE value was obtained in the fruit dried at 40 ºC, which indicates a less marked color change compared to the fresh fruit and, thus, depicts less fruit darkening (Table 4). However, for the fruit dried at 35 ºC, the ΔE value was 48, which implies the darkest coloration at the end of the process versus the initial values. Senadeera et al. (2020) evaluated the influence of different hot-air temperatures on the color of persimmon slices. They observed that the biggest color changes were for the fruit dried at a lower temperature, which was related to the longer time required to obtain the dried product, with consequent browning.

Table 4. Color parameters (lightness-L*, Chroma-C*, Hue angle-h* and total color difference-ΔE) of persimmon 'Rojo Brillante' before (fresh fruit) and after drying at 35 °C, 40 °C or 45 °C until 30 % moisture was reached.

		L*	C*	h*	ΔE*
Fresh fruit		71.7 ± 2.6 [a]	52.2 ± 0.8 [a]	83.9 ± 3.8 [a]	-
After drying	35 °C	32.9 ± 4.2 [d]	31.5 ± 1.6 [d]	53.6 ± 2.9 [c]	48.0 ± 4.7 [a]
	40 °C	43.5 ± 3.6 [b]	43.4 ± 1.6 [b]	61.4 ± 2.6 [b]	31.9 ± 2.4 [b]
	45 °C	36.3 ± 3.6 [c]	38.6 ± 1.6 [c]	59.7 ± 2.9 [b]	43.2 ± 4 [c]

Different letters in the same column show significant differences according to the LSD test ($p \leq 0.05$).

Regarding fruit texture, the drying process generally causes fruit softening and crisp texture loss. However, depending on drying intensity, hardness tends to increase again due to structure compaction (Kang et al., 2004; Sosa et al., 2012; Vilhena et al., 2020).

In our study, the deformation registered when applying a force of 10 N increased after drying. This indicated flesh fruit softening in relation to the initial fresh fruit (Figure 7). On drying day 3, similar fruit softening was observed at the three drying temperatures. From that point onward, the deformation values of the fruit dried at 35 °C, continued to increase until day 7, and then remained close to 11 mm until the end of the process. From day 5 onward, the fruit dried at 40 °C underwent less deformation with values up to 5.9 mm at the end of the drying process. This finding indicates flesh hardening. For the fruit dried at 45 °C, this hardening was observed from day 3, when the lowest deformation value (3.6 mm) was reached. This would be related to the marked secondary epidermis thickening on this fruit, which confers fruit rigidity, as well as tougher internal flesh.

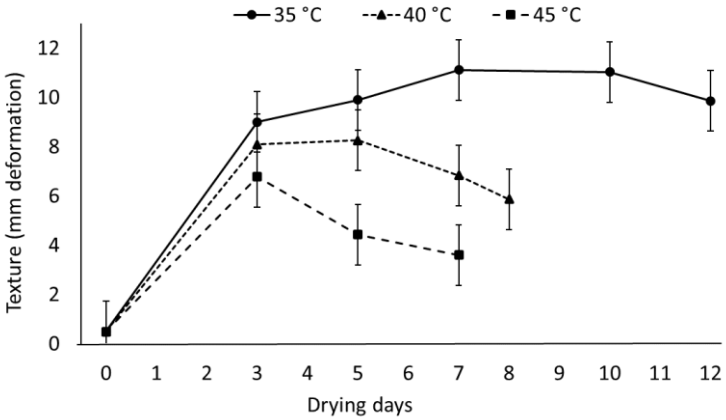

Figure 7. 'Rojo Brillante' persimmon texture during the drying process at 35 ºC, 40 ºC and 45 ºC until 30 % moisture was reached. The vertical bars represent Least Significant Differences (LSD) intervals ($p \leq 0.05$).

Thus, the observed internal structure was quite different depending on the drying temperature (Figure 4). The fruit flesh dried at 35 ºC showed a gel-like structure, while that of the fruit dried at 40 ºC was slightly more rubbery and drier. However, the process at 45 ºC resulted in a tough internal corky structure that conferred lower fruit deformation values. This structure would explain why, under these conditions, a_w was the lowest when fruit reached 30 % moisture.

The TSS in the fresh fruit were 16.6 ºBrix during persimmon drying as a result of water evaporation, and the TSS content increased (Figure 8a). This increase in TSS at all three temperatures was concomitant with the drop in moisture that took place throughout the drying process (Figure 2a). Drying at 40 ºC and 45 ºC resulted in a TSS of 60 ºBrix by the time that fruit reached 30 % moisture, while slightly lower values of 55 ºBrix were obtained for the fruit dried at 35 ºC. These values are similar to those reported for the natural drying of 'Rojo Brillante' (Vilhena et al., 2020).

'Rojo Brillante' persimmon is an astringent cultivar with a high tannin content at harvest (Tessmer et al., 2016; Vilhena et al., 2020). Thus, to be marketed fresh, it must undergo postharvest deastringency treatment, such as the application of ethanol or high CO_2 concentrations (Novillo et al., 2014). In some Asian countries, drying is used as a common treatment to eliminate astringency and to allow a market for astringent persimmon varieties. The time required to eliminate soluble tannins (ST) to non-astringent levels depends on both variety and drying conditions. Under natural drying conditions, 21 days are needed to completely remove 'Rojo Brillante' astringency (González et al., 2021; Vilhena et al., 2020). However, for different Japanese cultivars, fruit present no sensory astringency after 7-9 days under sun-drying conditions (Jia et al., 2020).

In this study, the fresh fruit had a ST content of approximately 3 % (d.b.) and this concentration dropped as drying progressed (Figure 8b). This decline was quicker at higher drying temperatures. After 5 days, the fruit dried at 40 °C and 45 °C had ST values below 0.3 %, which correspond to undetectable astringency values for 'Rojo Brillante'. However, for the fruit dried at 35 °C, these values were reached after 10 days. These results coincide with those of other authors, who have reported that astringency removal speed during drying increases with drying temperature (Manabe et al., 1980).

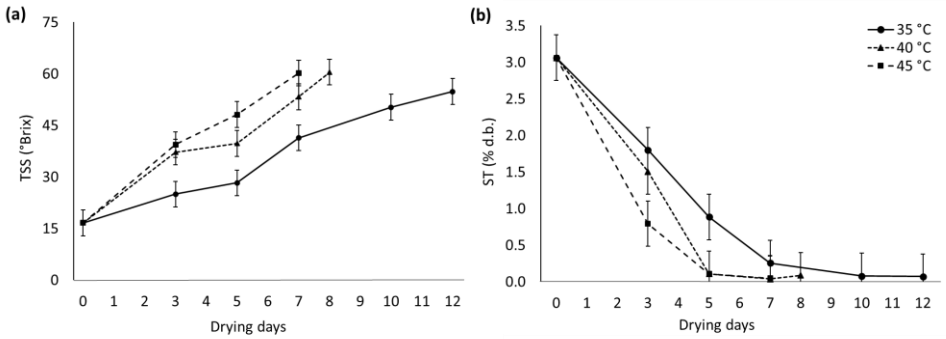

Figure 8. Total Soluble Solids (TSS) (a) and Soluble Tannins (ST) contents (b) of 'Rojo Brillante' persimmon during the drying process at 35 °C, 40 °C and 45 °C until 30 % moisture was reached. The vertical bars represent Least Significant Differences (LSD) intervals ($p \leq 0.05$).

3.3. Microstructural Characterization

The microstructural study was carried out on fresh persimmon fruit and on the final product after drying at 30 °C, 40 °C and 45 °C. In all cases, a cross-section of internal persimmon flesh was observed.

The fresh persimmon images show the typical flesh structure, where cells appear intact and turgid, and form a compact parenchyma (Figure 9). Cells are rounded with small air-filled intercellular spaces. The cell membrane appears intact and surrounds each cell. The cell interior looks like a network. Previous studies have described the appearance of the ST responsible for astringency inside vacuoles of the so-called tannic cells and their insolubilization during ripening (Salvador et al., 2007; Tessmer et al., 2016). Due to moisture content, ST together with other solutes, such as sugars, are observed to form a network, which is the typical eutectic artifact caused by the water sublimation process and the immobilization of these solutes when preparing samples for Cryo-SEM observations (Hernández-Carrión et al., 2014). It has been reported that, when tannins are insolubilized by ripening, the eutectic artifact disappears and the interior of vacuoles looks like a compact and continuous mass (Tessmer et al., 2016).

The fresh fruit had a tannins content of approximately 3 % (d.b.), which indicates high fruit astringency (Tessmer et al., 2016). Thus, in the Cryo-SEM images of these fruit, the cells with both ST and insoluble tannins are seen. In the images of the samples dried at different temperatures, the eutectic artifact does not form due to water loss during the drying process. Hence, it is not possible to differentiate between the cells with ST or those with insoluble tannins. Nonetheless, the results indicate complete tannin insolubilization because tannin content values dramatically dropped after the drying process in all cases and reached non-astringent values, lower than 0.3 % (d.b.) (Salvador et al., 2007; Tessmer et al., 2016; Vilhena et al., 2020).

The changes in fruit flesh noted after drying at 35 °C, 40 °C, and 45 °C are remarkable with evident cell structure deterioration. During drying, intracellular water transport causes cell shrinkage, pore formation and cell collapse. Finally, the overall flesh tissue is deformed due to water migration from the cell's interior (Mahiuddin et al., 2018). The images of the persimmon

dried at 35 ºC reveal that cells have slightly lost their rounded shape and become somewhat elongated. This may be due to the fact that fruit have remained suspended by their peduncle and the effect of gravity can be noted. Intercellular spaces are small due to the compaction that occurs. However, the areas with pores or empty spaces are still visible. The cell membrane is no longer continuous and is much thinner than the fresh fruit. The observed structure could be related to the higher deformation values of the fruit dried at 35 ºC, and with a gel-like and more flexible appearance compared to the persimmons dried at 40 ºC and 45 ºC.

On the flesh of the fruit dried at 40 ºC, cellular compaction is greater, and these cells are no longer rounded. They have contracted and show significant elongation. Empty spaces are still observed. Cell size has also changed and become much smaller due to contraction. The cell membrane has lost its integrity and appears blurred in some areas. Even so, the cellular structure can still be distinguished, although it appears to have collapsed. This structure may justify the texture values obtained at the end of the drying process at this temperature, which are intermediate between 30 ºC and 45 ºC, and they look rubberier with a dry flesh appearance.

Finally, for the fruit dried at 45 ºC, the parenchymal structure has completely disappeared. Such parenchyma degradation is related to water removal, which occurs during drying, as observed by the Cryo-FESEM technique in previous studies (Vilhena et al., 2020). In this fruit, cells have completely lost their integrity and, therefore, no cellular organization can be distinguished. The cell membrane is practically imperceptible, and flesh has become a homogeneous, continuous and quite compact mass. This structure is consistent with the tough corky appearance of flesh (Figure 4) and with the lowest deformation values.

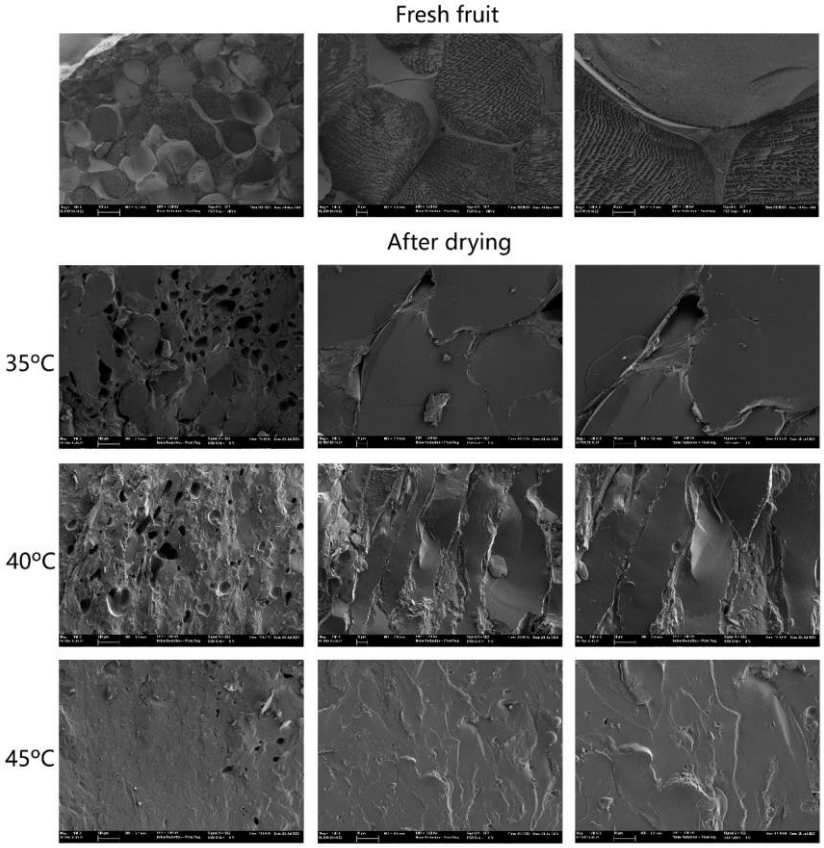

Figure 9. Internal flesh structure by the Cryo-field emission scanning electron microscopy technique of the 'Rojo Brillante' persimmon dried to 30 % moisture at 35 °C, 40 °C or 45 °C. Magnification: 100x, 500x and 1000x.

4. Conclusions

The present study addresses, for the first time, the drying behavior of whole 'Rojo Brillante' persimmon that have undergone hot-air drying at 35 °C, 40 °C and 45 °C. As expected, the drying temperature significantly influences the drying kinetics. The employed mathematical models are suitable for fitting the data from the three treatments, and Verma's model is that with the best fit.

The 30 % moisture required to consider dry persimmon fruit can be achieved at 12, 8, and 7 days when the drying treatment is carried out at 35 ºC, 40 ºC and 45 ºC, respectively. In all cases, the time is much shorter than when persimmons are naturally dried. The final product's quality characteristics differ depending on the drying temperature, especially in texture terms. The drying process causes shrinkage, flesh structure compaction and secondary epidermis formation.

The treatment run at 45 ºC is not recommended for presenting a thicker secondary epidermis and a hard texture. At 40 ºC however, the final product's color is more similar to that of the fresh fruit, and, applying a drying temperature of 35 °C results in a product with similar physico-chemical attributes and a gel-like structure to those achieved by natural drying methods. This approach significantly reduces processing times by enhancing commercial viability. The microstructural study reveals parenchyma degradation and cellular structure compaction of fruit flesh for the different drying treatments. Moreover, air-drying lower the ST content, which results in a non-astringent product and no prior deastringency treatment is necessary.

This technology, which has not yet been implemented in the 'Rojo Brillante' persimmon industry, may be a good strategy to increase the value of discarded fruit and to enhance the surplus of this seasonal fruit. Further research is needed to determine the appropriate packaging and storage conditions to prolong the product's shelf life.

Acknowledgements

This research was funded by Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and Fondo Europeo de Desarrollo Regional (FEDER), through Project RTA2017-00045-C02. The authors thank the Cooperativa Agrícola Sant Bernart Coop. V for supplying the fruit herein used. Nariane Q. Vilhena thanks the INIA for grant FPI-INIA (PRE2018-085833).

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IV. GENERAL DISCUSSION

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The major increase in 'Rojo Brillante' persimmon production in the Valencian Community has positioned Spain as the world's second largest producer and the leading exporter of fresh fruit. Nevertheless, in recent years, Spain has witnessed overproduction, which has resulted in falling prices. During recent seasons, close to 11 % of total production has not been harvested, which implies important economic losses and a consequent increase in food waste (Fernandez-Zamudio et al., 2020). Besides, close to 20 % of production is lost during the postharvest period (Fernández-Zamudio & Barco, 2021). These losses are due not only to inadequate postharvest managements that result in quality loss, but also to different preharvest factors that affect overall fruit quality at harvest.

It is noteworthy that the economic losses which result from unmarketed fruit include not only potential profit loss, but also the funds invested in all the pre- and postharvest operations before fruit are discarded (Xue et al., 2017). Therefore, one of the current challenges for the persimmon sector is to search for strategies to reduce the waste that originates throughout the supply chain.

Moreover, there are currently no cost-effective measures for the valorization of the persimmon waste generated in packing houses. Indeed, in most cases, the removal of non-commercial fruit involves an additional cost. This renders the development of strategies to reduce fruit losses during both pre- and postharvest, and to valorize the discarded product. In this context, the present Thesis approaches different pre- and postharvest aspects that influence final persimmon fruit quality and, thus, impact its marketability. In addition, drying technology is evaluated as a strategy to valorize surplus fruit and to increase the persimmon crop profitability.

PRE- AND POSTHARVEST ASPECTS RELATED TO FRUIT QUALITY TO REDUCE FOOD LOSS

Of the preharvest aspects related to fruit quality parameters, the plant nutritional status is a major factor that has been reported to affect plant material composition and fruit quality parameters (Ben-Arie et al., 2008; George et al., 2005). The relation between the leaf and flesh mineral concentrations and fruit quality has been well documented in some crops, such as cherry, orange and apple (El-Gioushy, 2016; Jivan and Sala, 2014; Milošević et al., 2015). However, there is very little information on the optimal nutritional requirements of persimmon to obtain high fruit quality at harvest (Ben-Arie et al., 2008; Choi et al., 2008; Xu et al., 2020). In this regard, it is not only important to know nutritional requirements but also how different management practices affect their absorption.

It is well-known that applying different fertilizer types in organic or conventional management affects ionomic plant material concentration (Bourn and Prescott, 2002). In conventional crops, a higher fertilization rate is usually applied. In organic crops, ecological management increase the organic matter in soil, which can modify the pH of the rhizosphere and, thus, alter plants' absorption capacity, which can improve their nutrient balance (Martínez-Alcántara et al., 2016). In Spain, most persimmon plots are cultivated following conventional farming practices, although production by organic farming is increasing (CAECV, 2021). In persimmon, few studies have been carried out to compare fruit nutritional concentrations from organic and conventional farms (Cardoso et al., 2015), and no studies are available for the cv. Rojo Brillante.

In this context, **Chapter I** of this Thesis focuses on evaluating the concentrations of the main macroelements in the leaf and flesh of 'Rojo Brillante' persimmon grown by organic and conventional practices and relating them to the physico-chemical parameters associated with fruit quality. In this study, the concentration of nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) was assessed throughout the commercial harvest period, from October to December. N mobilization from leaves to fruit

during this period was evidenced. However, P and K in leaves dropped, but this did not imply changes in fruit concentration, which indicates that these elements possibly move from leaves to other reserve organs before leaf senescence (Pomares et al., 2015). In leaves, Mg content gradually increased, and Ca remained stable during the studied period. The concentration of these two elements was much higher in leaves than in fruit due to their low mobility in the plant (Tagliavini and Scandellari, 2012; Zanotelli et al., 2014).

As a significant variation in the ripening of the apical and basal parts of flesh occurs in 'Rojo Brillante', in the present study the concentration of macroelements was separately evaluated in these flesh areas. Differences in N, P, K and Ca concentrations between fruit parts were found. The apical area accumulated higher N, P and K than the basal area. Ca translocation was observed from the basal to the apical flesh section throughout the study period. For 'Fuyu', Clark and Smith (1990) also reported the highest N and K concentration in the apical part of fruit. Concerning Mg, no differences between the two flesh areas were found and a drop occurred during the harvesting period, which has also been previously observed for 'Fuyu' (Clark and Smith, 1990). The correlation between the fruit macronutrient concentrations and quality parameters was dependent on the evaluated fruit area. In the basal flesh area, Ca and Mg correlated positively with firmness, soluble tannins and total soluble solids. In the apical area, positive correlations between Ca and color and total soluble solids were found, while Mg correlated with color, firmness, soluble tannins and total soluble solids.

Regarding the influence of crop management on nutrient concentration, it was dependent on the harvest moment. Nevertheless, in general, plants under organic management showed lower K and higher Mg in leaves, as well as lower Ca and higher N and P in fruit, when compared with conventional crops.

One of the drawbacks pointed out in organic crops is the lower yield compared to conventional crops. The higher productivity in conventional agriculture is associated with a greater uptake of mineral nutrients (De Ponti et al., 2012). In

the present study, although productivity was lower in the organic crop, its agronomic efficiency was much higher compared to conventional agriculture.

In a second study (**Chapter II**), in addition to macronutrients, microelements in leaf and fruit of organically and conventionally grown persimmon were evaluated at early commercial harvest (mid-October). The influence of crop system on biocomponents content and quality of fruit was also studied. This study was carried out in two consecutive seasons. The highest agronomic efficiency found in the organic crop was corroborated.

The greater macro and microelement supplied in the conventional compared with the organic system, did not imply in a higher concentration of these elements in leaf and fruit. The concentrations of P, K, Mg, Fe and B were similar in the two management systems, both in leaf and fruit. A higher concentration of Mn and Zn in leaves were found in organic management. In fruit, no influence of crop system was observed in Mn concentration, while the highest Zn concentration was observed in organic farming. For organic 'Rama forte' persimmon, Cardoso et al. (2015), also found higher Zn content compared to conventional fruit. In the present study, the nutrient concentrations detected in leaf from both crop systems are within the optimum range stablished for Morales et al. (2022) for this variety. This indicates the adequate fertilization performed in both managements and the important role of organic matter in favoring nutrient assimilation.

A more advance fruit color and lower firmness was found in organic fruit when compared to those conventionally grown, what was related to the lowest N input in organic crop (Agustí et al., 2004; Choi et al., 2008, 2011; Park, 2002). As reporter in the previous chapter, the Ca concentration in fruit was linked to the flesh firmness values. The influence of crop system on fruit biocomponents concentration was only observed for malic acid, β-Cryptoxanthin and ascorbic acid, that were higher in organic than conventional fruit. The highest ascorbic acid concentration in organic crop was related to the low N amount applied in this management, since, it has been reported that low N availability induces

the biosynthesis of non-nitrogen containing compounds (Lee and Kader, 2000; Mditshwa et al., 2017).

These results provide interesting information for a more efficient use of key nutrients for plant productivity and fruit quality.

Another aspect to consider in persimmon pre-harvest management is the application of phytoregulators as a common strategy to extend the harvest season (Besada and Salvador, 2018). For 'Rojo Brillante', the main usually employed phytoregulators are ethephon to advance harvest and gibberellic acid (GA3) to delay maturation (Agustí et al., 2015). The ethephon treatment induces fast fruit ripening, which allows earlier fruit harvesting, but it shortens the harvest window (Kim et al., 2004). Moreover, this fruit have a short shelf life and must be quickly marketed after harvest. Therefore, the application of 1-methylcyclopropene (1-MCP) after harvest is often needed to maintain fruit firmness during commercialization. On the other hand, the GA3 treatment delays fruit ripening, which prolongs the harvest period. So, the fruit intended for cold storage is usually preharvest-treated with GA3 and postharvest-treated with 1-MCP to avoid chilling Injury (CI) symptoms and to maintain fruit quality during conservation (Besada et al., 2008).

Considering that 1-MCP is widely employed in persimmon as a postharvest treatment throughout the harvest season, it would be interesting to study its effect when applied during the preharvest. Although current studies have shown remarkable benefits for different fruit crops with preharvest 1-MCP treatments (Li et al., 2021; Sakaldas and Gundogdu, 2015), there are very few studies about the effect of this treatment on persimmon.

So, in the present Thesis **(Chapter III)**, the effect of 1-MCP treatment (Harvista®) was studied in two scenarios for 'Rojo Brillante' persimmon: 1) to improve shelf life in the fruit treated with ethephon; 2) to enhance quality during cold conservation in the fruit treated with GA3. The results of this study showed that Harvista® applied 1, 7 and 10 days after ethephon treatment was effective in delaying the softening induced by ethephon during the harvest period. The application of Harvista® one day after ethephon treatment

maintained the highest flesh firmness after the commercialization period. Moreover, the pre- and postharvest 1-MCP treatment combination maintained the highest flesh firmness during the commercialization period than the single postharvest application and, thus, extended the persimmon shelf life. Similarly in other fruit, such as papaya and cherry, the preharvest 1-MCP applied after ethephon treatment has proven effective in maintaining fruit firmness during the postharvest life (Elfving et al., 2009; Sañudo-Barajas et al., 2008).

In the fruit treated with GA3, the application of Harvista® three days before harvest was shown to have the same effect on maintain fruit firmness as the usual postharvest 1-MCP treatment for up to 60 days of cold storage. Therefore, replacing the postharvest 1-MCP application with a preharvest 1-MCP treatment could be a useful tool to improve handling operations in packinghouses. These results reveal preharvest 1-MCP as a novel treatment to enhance persimmon postharvest quality.

As previously mentioned, the 'Rojo Brillante' persimmon destined for cold storage is usually preharvest-treated with GA3 to be harvested from November to December, with high firmness values. In addition, 1-MCP postharvest treatment is required to reduce softening, which is the main CI symptom in this variety (Besada and Salvador, 2018). Nevertheless, very different postharvest behavior has been commercially documented for the fruit harvested during this period. To explain these differences, the aim of **Chapter IV** was an in-depth study of the physico-chemical and microstructural changes that occurred in fruit (pretreated with GA3) during five commercial harvests, from November 11 to December 9. Moreover, the firmness changes during cold storage at 0 °C up to 90 days were evaluated periodically for the fruit from each harvest.

During the harvest studied period, slight variations in firmness occurred and ranged from 48 to 40 N. As these values are above the minimum required to conserve this variety (40 N) (Besada et al., 2017), *a priori* no large differences would be expected in fruit behavior during cold storage. Nevertheless, the fruit softening observed during conservation very much depended on flesh firmness

values at harvest. Thus, the fruit collected in mid-November (November 11 and 18) with firmness around 48 N maintained high commercial values, over 30 N, after 90 days. The fruit harvested later (November 25) had lower firmness values than those of the earlier fruit after different cold storage periods. The firmness loss of the fruit harvested with around 40 N, on December 2 and 9 was faster, with values close to 30 N only after 30 storage days. The postharvest behavior of the fruit harvested during the studied period can be explained by means of the microstructural evaluation. Although some differences in parenchyma structure were observed by cryo-scanning electron microscopy (Cryo-FESEM) among the different harvests, all the fruit showed a structured parenchyma. However, the study by transmission electron microscopy (TEM) evidenced that the fruit harvested in mid-November, with the highest storage potential, displayed the greatest structural integrity of cell walls and membranes.

This is the first work to demonstrate that fruit with minor variations in flesh firmness at harvest can present major microstructural differences, which significantly influence fruit behavior during cold storage.

Of the usual postharvest handling processes to which persimmon fruit are subjected, the deastringency treatment is a critical step associated with postharvest losses because it directly influences the final fruit quality characteristics (Salvador et al., 2007). Of main deastringency treatments, high CO_2 concentrations are the most commercially applied (Besada et al., 2010). In Spain, the optimization of this process for 'Rojo Brillante' has been done by means of extensive studies conducted at the IVIA (Besada et al., 2010, 2015; Salvador et al., 2007).

Nevertheless, in some producing countries, deastringency treatment by exposing persimmon to ethanol vapor is the most widespread practice as it is a less costly alternative than high CO_2 concentrations (Tessmer et al., 2018; Vitti et al., 2009). Thus for 'Giombo' persimmon, one of the main varieties in Brazil, deastringency treatment consists in exposing fruit to ethanol vapor (Tessmer et al., 2018). However, in commercial terms, difficulty in completely losing

astringency has been reported for this cultivar. In addition, like many other cultivars, 'Giombo' is sensitive to low temperatures, with softening being the main CI symptom (Tessmer et al., 2019). This softening is accelerated when deastringency treatment is applied prior to conservation. (Besada et al., 2014; Salvador et al., 2007). To date, no studies have compared the effect of CO_2 and ethanol deastringency treatments on 'Giombo' quality during cold storage. In this context, **Chapter V** aims to evaluate the physico-chemical and microstructural changes during storage at 1 °C of 'Giombo' persimmons previously subjected to CO_2 or ethanol.

An interesting result of this study was that the deastringency of 'Giombo' with CO_2 resulted in faster tannins insolubilization than with ethanol. While the CO_2-treated fruit had undetectable astringency values one day after application, these values in the ethanol-treated fruit were achieved after 25 days of cold storage. The tannin insolubilization process during cold storage was observed by means of light microscopy (LM) and vanillin hydrochloric staining, which reacts with tannins to give a red color. Moreover, the CO_2-treated fruit still had high firmness after 25 storage days plus shelf life, while, with ethanol treatment, after 15 days the fruit showed firmness considered unmarketable for persimmon (Besada et al., 2014). This was closely related to the greatest parenchyma degradation caused by ethanol treatment, which was observed by (Cryo-FESEM).

These results suggest that, although ethanol is the usual deastringency treatment for 'Giombo', high CO_2 concentrations are recommended to maintain fruit quality, especially when fruit are to be cold stored.

DRYING AS A NEW STRATEGY TO VALORIZE DISCARDED PERSIMMON FRUIT AND PRODUCTION SURPLUS

In the last few years, the production Sector has faced the challenge of introducing new strategies to valorize losses associated with persimmon fruit management, seasonality and production surpluses. In this context, whole fruit drying appears as an interesting strategy to valorize fruit waste. Drying is a usual fruit preservation technique that is largely employed in Asian countries (Gardeli et al., 2010; Li, 2012). Nevertheless, this technology is not implemented in Mediterranean production areas.

This Thesis addresses the study of whole fruit drying as a novel strategy to valorize persimmon production in Spain. Thus, **Chapter VI** studied the physico-chemical and microstructural changes that occur during the natural-air drying of 'Rojo Brillante' persimmon fruit harvested in two maturity stages: early (S1) and late (S2). Maturity stages influenced moisture loss during the drying process. Moisture around the 50 %, limit to be considered semidried persimmon (Kang et al., 2004), was reached on days 21 and 28 for S1 and S2, respectively. In Asian countries, the semidried persimmon is more appreciated for its softer texture than fully dried fruit (Yamada et al., 2009).

The water loss that occurs during the drying process caused volume reduction, defined as shrinkage, which was concomitant to the formation of a rigid external layer, named the secondary epidermis. The formation of this secondary epidermis was in parallel to a drastic change in internal fruit structure. Flesh becomes gelatinous and soft as drying progresses due to the resistance near the fruit surface caused by the secondary epidermis, which makes the water loss from the innermost region difficult (Mayor and Sereno, 2004).

The formation of the secondary epidermis and internal flesh gelification were evidenced by a deep microstructural study performed by the Cryo-FESEM and LM techniques. The changes in the outmost and inner flesh areas were slower in S2 than in S1. The late-harvested fruits developed a thinner secondary

epidermis and a larger volume of gelled tissue inside, which would result in softer fruits compared to early-harvested ones.

As expected, drying led to an increase in total soluble solids and tannins insolubilization, which causes astringency loss. This fact has been associated with parenchyma degradation, which results in the solubilization and depolymerization of pectins, and in consequent tannins insolubilization (Asgar et al., 2004). Astringency loss was complete after 14 and 21 days in S1 and S2, respectively.

The results obtained in this study revealed that 'Rojo Brillante' persimmon is a suitable variety to be subjected to a natural drying process. However, the long time required to reach the desired moisture content by natural drying could be a drawback for its application on an industrial scale.

To improve the persimmon drying process, hot-air drying is an alternative that could be easily implemented on an industrial scale and would shorten the process. Indeed hot-air drying has been shown to be effective for different fruit crops (Demiray and Tulek, 2017; Falade and Abbo, 2007, Hedayatizadeh and Chaji, 2016; Jia et al., 2020).

Thus, **Chapter VII** of the present Thesis approached improvement of the drying technology by applying different drying temperatures to 'Rojo Brillante' persimmon. Fruit was dried at 35 ºC, 40 ºC and 45 ºC until 30 % moisture was reached, which is required to consider persimmon to be dried (Choi et al., 2017).

Six mathematical models were employed to characterize the drying kinetics. Verma's model was the best fitting one, which agrees with previous studies on the natural drying of 'Rojo Brillante' (González et al., 2021). As expected, the higher the drying temperature, the faster the drying process was. Moisture at around 30 % was reached on days 12, 8 and 7 when drying treatments were carried out at 35 ºC, 40 ºC and 45 ºC, respectively. In all cases, the process was substantially shorter than when fruit were submitted to natural-air drying (González et al., 2021; Vilhena et al., 2020). In all cases, when fruit reached 30

% moisture, the soluble tannin content displayed undetectable levels of astringency for 'Rojo Brillante' (Salvador et al., 2007).

Fruit shrinkage, flesh structure compaction and the formation of a secondary epidermis very much depended on drying temperature. The fruit dried at 35 °C exhibited a thin secondary epidermis and a gelatinous internal texture, similarly to that obtained after a natural drying process. The drying at 40 °C resulted in fruit having more rubbery flesh, but with a similar color to that of fresh fruit. However, the fruit dried at 45 °C had a very thick secondary epidermis and a corky internal texture, which resulted in fruit being excessively hard for marketing. The thinnest secondary epidermis of the fruit dried at the lowest temperature (35 °C) would be explained by the lowest drying rate, which would facilitate moisture migration from the inner to outmost flesh surface and would reduce crust formation (Rahman, 2008). Differences in fruit flesh observed in the fruit dried at the three temperatures were evidenced by microstructural studies.

According to the obtained results, for 'Rojo Brillante' persimmon the hot-air drying process is proposed as an alternative to natural drying. Nevertheless, further studies are necessary to optimize this process for it to be implemented into industry.

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V. GENERAL CONCLUSIONS

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- New information about macronutrients concentration (N, P, K, Ca, Mg) in leaves and in different flesh areas during the maturation period of 'Rojo Brillante' persimmon grown under conventional and organic management is reported. The influence of crop management on nutrient concentration, depended on the harvest moment. Nevertheless, in general, organic crops showed lower K and higher Mg in leaves, as well as lower Ca and higher N and P in fruit, when compared with conventional crops. The concentration of macroelements in fruit depended on the evaluated flesh part. Thus, during the harvest period, the flesh apical area accumulated higher N, P and K concentrations than the basal area, and Ca translocation from the basal to the apical area was evidenced. The correlation between macronutrients and fruit quality parameters revealed that the Ca and Mg and the N/Ca and Ca/(K+Mg) ratios were closely related to color, firmness, TSS and ST content.

- The greater macro- and microelements supplied in the conventional vs. the organic system did not imply a higher concentration of these elements in leaves and fruit. At early commercial harvest moment (mid-October), a similar concentration was observed for P, K, Fe and B in both management systems and the highest Mn and Zn concentrations were shown in the organic plots. In addition, the optimum range for the leaf concentrations detected in both crop systems indicates that adequate fertilization was performed for both managements, as well as the important role of organic matter in favoring nutrient assimilation. The greatest agronomic efficiency found in the organic crop indicates that the lower fertilization rate supplied during this management was adequate to obtain fruit with optimal nutrient concentrations. The results obtained in this study are of great relevance to reinforce the need for balanced fertilization during the growing season to achieve high fruit quality.

- The 1-MCP preharvest application was revealed as a novel and effective treatment to improve the postharvest fruit quality of 'Rojo Brillante' persimmon. At the beginning of the season, when applied to the ethephon-treated fruit, the preharvest 1-MCP extended the harvest window and prolonged the commercialization period. Moreover, when applied to the fruit intended for storage, the preharvest 1-MCP treatment can replace the 1-MCP postharvest application, which could be a useful tool for optimizing handling operations in packinghouses.

- Minor differences in persimmon firmness at harvest have been shown to highly influence fruit behavior during cold storage. From November to December, firmness slightly decreased. However, the fruit harvested in mid-November presented the highest storage potential, and high firmness was maintained for up to 90 days, which did not happen in the fruit from subsequent harvests. The differences in postharvest behavior were associated with structural parenchyma integrity at harvest.

- Although ethanol is the usual deastringency treatment for 'Giombo' persimmon, high CO_2 concentrations are recommended to maintain fruit quality during cold storage. CO_2 treatment resulted in faster tannins insolubilization and higher flesh firmness than ethanol application. The greater fruit softening caused by ethanol was closely linked with severer parenchyma degradation during storage.

- Natural drying is proposed as a new strategy to valorize persimmon production. An in-depth physico-chemical and microstructural study allowed to characterize the changes caused by the drying process in fruit in two maturity stages. 'Rojo Brillante' persimmon was revealed to be a suitable astringent variety to be subjected to the natural drying process after taking into account that the maturity stage can influence final product characteristics.

- To improve the drying of persimmon, hot-air drying is suggested as an alternative to shorten the process and can be more easily implemented on an industrial scale. After evaluating the drying at three temperatures (35 ºC, 40 ºC and 45 ºC), it was noted that the higher the drying temperature, the faster the drying process was. The final product characteristics differed depending on the drying temperature, especially in texture terms. The drying at 35 ºC resulted in a product with similar physico-chemical attributes to those achieved by the natural drying method, but in a much shorter time, which improves the commercial viability of this treatment.

This Thesis provides new information to implement pre- and postharvest strategies that improve fruit quality by resulting in reduced food loss. In addition, the drying technology is proposed as a potential solution to increase the value of discarded fruit and production surplus by increasing persimmon crop profitability.