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DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS

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TRATAMIENTOS DE SECADO PARA LA OBTENCIÓN DE INGREDIENTES DE ALTO VALOR NUTRITIVO A PARTIR DEL DESTRÍO POSTCOSECHA DE CAQUI

TESIS DOCTORAL

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MI QU ALI
Grupo de Investigación Microestructura y Química de Alimentos

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HACEN CONSTAR QUE:

El trabajo de investigación **"Tratamientos de secado para la obtención de ingredientes de alto valor nutritivo a partir del destrío postcosecha de caqui"**, que presenta Dña. Cristina Martínez González por la Universitat Politècnica de València, y que ha sido realizado bajo nuestra dirección en el Grupo de Investigación de Microestructura y Química de Alimentos de la Universitat Politècnica de València, reúne las condiciones para optar al grado de Doctor.

Valencia, septiembre 2021

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Resumen

La presente Tesis tiene como objetivo ofrecer estrategias que permitan incrementar la rentabilidad del cultivo de caqui "Rojo Brillante" mediante la valorización de los excedentes y destríos generados en los almacenes. La tesis aborda como objetivos principales la obtención de productos e ingredientes deshidratados con alto contenido en compuestos bioactivos y la evaluación de la digestibilidad *in vitro* de los mismos.

La primera estrategia consistió en obtener caquis semisecos mediante secado "natural" evaluando la necesidad de aplicar un tratamiento previo de desastringencia. El tratamiento de secado en los caquis astringentes disminuyó significativamente el contenido en taninos solubles responsables de la astringencia de la fruta, alcanzando valores similares a los presentes en el caqui no astringente. Además, se observó que, el color de la pulpa permaneció con un tono anaranjado y poco pardeado cuando se partió de caqui astringente, por lo que no sería recomendable la aplicación de un tratamiento previo de desastringencia.

Una segunda estrategia se centró en la liofilización para obtener snacks de caqui de alta calidad, al ser éste un tratamiento que permite obtener productos o ingredientes con alto valor añadido. Se establecieron las condiciones óptimas de procesado y almacenamiento mediante la obtención de los valores de humedad y actividad del agua críticos que garantizasen el estado vítreo de la matriz, evitando un aumento en la tasa de reacciones de deterioro, cambios de textura y color, y la pérdida de los compuestos bioactivos de la fruta.

Como última estrategia se utilizó el tratamiento de secado por aire caliente en caqui astringente y no astringente, en distintos estados de madurez, para obtener snacks de caqui. Los snacks desarrollados presentaron un tono más anaranjado, en comparación con la fruta fresca, y menor contenido en taninos solubles. Se observó una alta correlación entre el nivel de astringencia percibido por los consumidores y la disminución del contenido en taninos solubles. Los snacks obtenidos

a partir de caqui astringente en etapas de madurez avanzadas fueron igualmente aceptados por los consumidores que los obtenidos a partir de caqui no astringente.

Seguidamente se estudió el efecto del secado con aire caliente y del estado de madurez sobre la fracción de carotenoides mediante diferentes técnicas cuantitativas y cualitativas como la fotoluminiscencia. El secado no afectó al contenido en carotenoides, pero disminuyó la capacidad antioxidante de los snacks. La fotoluminiscencia evidenció la isomerización de carotenoides y la formación de productos de degradación térmica, por lo que ambos hechos podrían explicar la pérdida de capacidad antioxidante en el caqui sometido a tratamientos de secado por aire caliente.

Por último, se llevaron a cabo estudios de digestión *in vitro*. La recuperación de taninos solubles en la fase del intestino delgado fue mayor en los snacks obtenidos mediante secado por aire caliente que en la fruta fresca y en los snacks liofilizados. Los snacks obtenidos a partir de caqui no astringente presentaron un índice de recuperación de taninos solubles mayor que los obtenidos a partir de caqui astringente. Los taninos insolubles llegaron intactos a la fracción no absorbida, por lo que podrían llegar al colon y ejercer su potencial antioxidante.

Resum

La present Tesi té com a objectiu oferir estratègies que permeten incrementar la rendibilitat del cultiu de caqui "Roig Brillant" mitjançant la valorització dels excedents i destriaments generats en els magatzems. La tesi aborda com a objectius principals l'obtenció de productes i ingredients deshidratats amb alt contingut en compostos bioactius i l'avaluació de la digestibilitat *in vitro* d'aquests.

La primera estratègia va consistir a obtindre caquis semisecs mitjançant assecat "natural" avaluant la necessitat d'aplicar un tractament previ de desastringència. El tractament d'assecat en els caquis astringents va disminuir significativament el contingut en tanins solubles responsables de la astringència de la fruita, aconseguint valors similars als presents en el caqui no astringent. A més, es va observar que, el color de la polpa va romandre amb un to ataronjat i poc marró quan es va partir de caqui astringent, per la qual cosa no seria recomanable l'aplicació d'un tractament previ de desastringència.

Una segona estratègia es va centrar en la liofilització per a obtindre snacks de caqui d'alta qualitat, en ser aquest un tractament que permet obtindre productes o ingredients amb alt valor afegit. Es van establir les condicions òptimes de processament i emmagatzematge mitjançant l'obtenció dels valors d'humitat i activitat de l'aigua crítics que garantiren l'estat vitri de la matriu, evitant un augment en la taxa de reaccions de deterioració, canvis de textura i color, i la pèrdua dels compostos bioactius de la fruita.

Com a última estratègia es va utilitzar el tractament d'assecat per aire calent en caqui astringent i no astringent, en diferents estats de maduresa, per a obtindre snacks de caqui. Els snacks desenvolupats van presentar un to més ataronjat, en comparació amb la fruita fresca, i menor contingut en tanins solubles. Es va observar una alta correlació entre el nivell de astringència percebut pels consumidors i la disminució del contingut en tanins solubles. Els snacks obtinguts a partir de caqui astringent en etapes de maduresa avançades van ser igualment acceptats pels consumidors que els obtinguts a partir de caqui no astringent.

Seguidament es va estudiar l'efecte de l'assecat amb aire calent i de l'estat de maduresa sobre la fracció de carotenoids mitjançant diferents tècniques quantitatives i qualitatives com la fotoluminiscència. L'assecat no va afectar el contingut en carotenoids, però va disminuir la capacitat antioxidant dels snacks. La fotoluminiscència va evidenciar la isomerització de carotenoids i la formació de productes de degradació tèrmica, per la qual cosa tots dos fets podrien explicar la pèrdua de capacitat antioxidant en el caqui sotmés a tractaments d'assecat per aire calent.

Finalment, es van dur a terme estudis de digestió in vitro. La recuperació de tanins solubles en la fase de l'intestí prim va ser major en els snacks obtinguts mitjançant assecat per aire calent que en la fruita fresca i en els snacks liofilitzats. Els snacks obtinguts a partir de caqui no astringent van presentar un índex de recuperació de tanins solubles major que els obtinguts a partir de caqui astringent. Els tanins insolubles van arribar intactes a la fracció no absorbida, per la qual cosa podrien arribar al còlon i exercir el seu potencial antioxidant.

Abstract

This thesis proposes different strategies to increase the profitability of "Rojo Brillante" persimmon by valuing the surpluses and discarded fruit generated in the warehouses. This thesis addresses the development of dehydrated products and ingredients with a high content of bioactive compounds and the evaluation of their *in vitro* digestibility.

The first strategy was to obtain semidry persimmons by "natural" drying, evaluating the need to apply a previous desastringency treatment. The drying treatment in the astringent persimmons significantly decreased the soluble tannin content responsible for the astringency of the fruit, reaching values similar to those present in the non-astringent persimmon. In addition, the color of the pulp remained with an orange hue angle and moderate browning, when astringent persimmon was used, thus the application of a previous desastringency treatment would not be recommended.

A second strategy focused on freeze-drying to obtain high-quality persimmon snacks, as a treatment that allows obtaining products or ingredients with high added value. Optimum processing and storage conditions were established by obtaining the critical water content and water activity values that would guarantee the glassy state, avoiding an increase in the rate of deterioration reactions, changes in texture and color, and the loss of bioactive compounds in the fruit.

As a last strategy, the hot air-drying treatment was used in astringent and non-astringent persimmon, in different ripening stages, to obtain persimmon snacks. The snacks had a more orange hue angle, compared to fresh fruit, and lower soluble tannin content. A high correlation was observed between the level of astringency perceived by consumers and the decrease in soluble tannin content. Snacks obtained from astringent persimmon in advanced ripening stages were equally accepted by consumers as non-astringent ones.

Then, the effect of hot air drying and the ripening stage on the carotenoid fraction was studied using different quantitative and

qualitative techniques such as photoluminescence. Hot air-drying did not affect the carotenoid content but decreased the antioxidant capacity of the snacks. The photoluminescence evidenced the isomerization of carotenoids and the formation of thermal degradation products. Both facts could explain the loss of antioxidant capacity in persimmon subjected to hot air-drying treatments.

Lastly, *in vitro* digestion studies were carried out. The recovery of soluble tannins in the small intestine phase was higher in the snacks obtained by hot air drying than in the fresh fruit and the freeze-dried snacks. The snacks obtained from non-astringent persimmon had a higher recovery index of soluble tannin than those obtained from astringent persimmon. The insoluble tannins reached the unabsorbed fraction intact, so that they could reach the colon and exert their potential antioxidant capacity.

Introducción

EVOLUCIÓN HISTÓRICA

El caqui pertenece a la familia Ebenaceae y al género Diospyros. Este género contiene más de 2000 especies, es originario de Asia y existen registros de que fue cultivado siglos antes de Cristo. La mayor parte de la producción comercial de esta fruta se deriva de *Diospyros kaki* L., que se originó en China con registros de producción de hace 3000 años (Novillo et al., 2015).

El caqui se introdujo en Japón y Corea durante los siglos VII y XIV, respectivamente. Posteriormente, se importó a Europa y se extendió a la costa mediterránea (Kluge & Tessmer, 2018). El caqui se introdujo en América del Norte a mediados del siglo XIX y más tarde en Australia y Nueva Zelanda. En América del Sur, el cultivo del caqui comenzó a finales del siglo XIX (Matheus et al., 2020). Hoy en día, las bases de datos estadísticas de FAOSTAT (2019) indican una producción mundial de 4.270.074 toneladas y 992.425 ha de superficie cultivada en 2019, así como un aumento continuo de la producción desde el año 2000 (Figura 1).

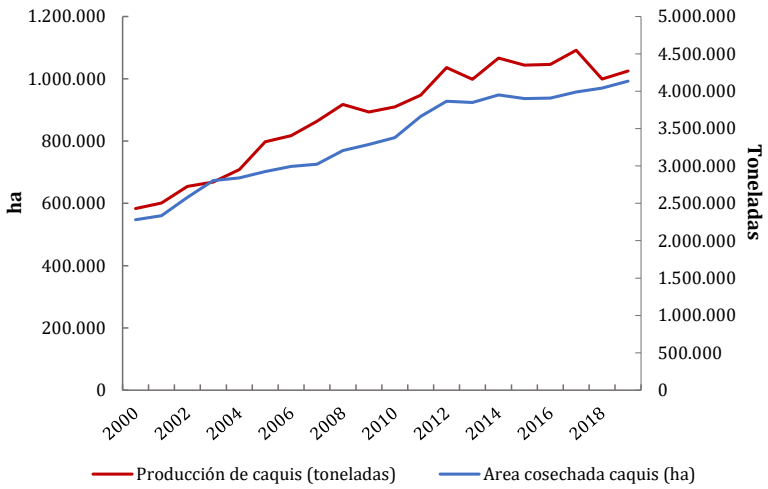

Figura 1. Producción/Rendimiento de caquis a nivel mundial (2000-2019). Fuente: FAOSTAT 2019.

La introducción del caqui en España data de finales del siglo XIX (Llácer & Badenes, 2002), como árbol ornamental y por la calidad de su madera. Los pequeños huertos comerciales de caqui comenzaron a establecerse a mediados del siglo XX con variedades nativas astringentes que reflejan la influencia de la selección natural y humana (Naval et al., 2010). En los últimos años, España es el país del área mediterránea que ha sido protagonista de la mayor y más rápida expansión de este cultivo. La producción de caqui ha aumentado exponencialmente en los últimos 20 años, pasando la superficie de cultivo de 3.518 ha en 2007 a más de 18.000 ha en 2017 (Figura 2). La primera expansión importante tuvo lugar en Andalucía y casi simultáneamente tuvo lugar otra gran expansión en la provincia de Valencia, sobre todo en la Ribera del Xúquer, comarca de gran tradición en el cultivo de cítricos y frutales de hueso (Llácer & Badenes, 2002).

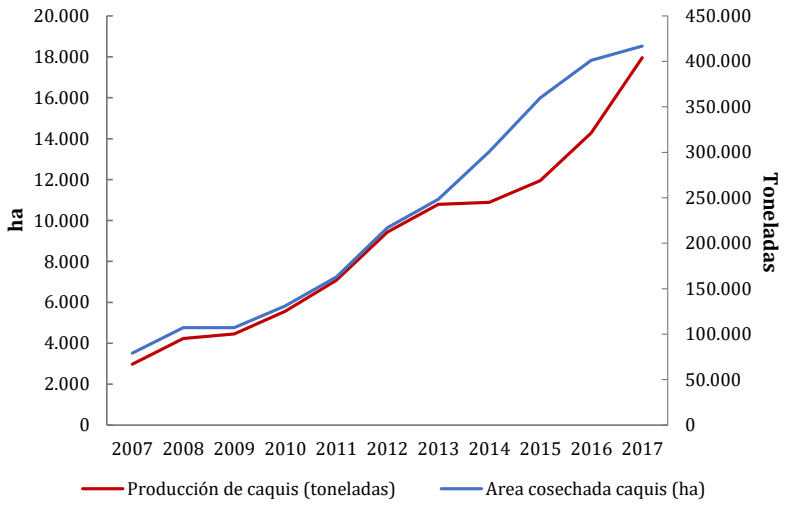

Figura 2. Producción/Rendimiento de caquis en España (2007 – 2017). Fuente: FAOSTAT 2017.

El caqui "Rojo Brillante"

La gran expansión producida en España, actualmente situada como segundo país productor después de China (FAOSTAT 2018), se basa en el cultivar "Rojo Brillante", producido principalmente en la Comunidad Valenciana (Llácer & Badenes, 2002). La producción española se destina principalmente a mercados de exportación donde existe demanda de caqui con textura firme. Tradicionalmente el cultivo de caqui en la Ribera del Xúquer (Valencia) estaba basado en pequeñas plantaciones que se utilizaban para el consumo propio o para venta en mercados locales. El cultivar "Rojo Brillante" surgió debido a una mutación espontánea y la primera plantación homogénea se injertó en el término municipal de L´Alcudia (Besada Ferreiro, 2008). La variedad "Rojo Brillante" es astringente, por lo que no es comestible en estados de madurez tempranos cuando tienen una textura firme, y para que los frutos puedan ser consumidos es necesaria la aplicación de técnicas de reducción de la astringencia en postcosecha. Una de las causas de expansión del "Rojo Brillante" en la Ribera del Xúquer fue la introducción de técnicas de reducción de astringencia que permiten eliminar la astringencia sin detrimento de la firmeza (Arnal, 2003). La introducción de la tecnología basada en la aplicación de altas concentraciones de CO_2 ha permitido obtener un producto novedoso para el consumidor, que ha tenido gran aceptación, ofreciendo ventajas para la manipulación y transporte a largas distancias.

Todo esto llevó a la creación del Consejo Regulador de la Denominación de Origen (CRDO) para garantizar la calidad y procedencia del caqui "Rojo Brillante" así como la Norma desarrollada por las Naciones Unidas, facilitando el comercio internacional y nacional de los caquis (Besada et al., 2008; *UNECE*, 2016).

Valorización de excedentes y destríos

El rápido crecimiento que la producción del cultivo de caqui "Rojo Brillante" ha experimentado en los últimos años se ha visto acompañado de un aumento importante del volumen de pérdidas postcosecha; se estima un desperdicio entre el 5-20% del producto recolectado (Méndez et al., 2021). En la actualidad, la estacionalidad y la sobreproducción dan lugar a excedentes que incluso pueden llegar a ser no recolectados. Por otro lado, los problemas asociados con el almacenamiento a bajas temperaturas, los procesos de maduración, la ineficiencia del tratamiento de eliminación de la astringencia en estados de maduración avanzados, las enfermedades de la fruta y las estrictas exigencias estándar en cuanto a la apariencia de la fruta dan lugar a la producción de destríos en diferentes etapas de madurez (Munera et al., 2017; Novillo et al., 2014; Salvador et al., 2007). Además, no existe un sistema de gestión que aproveche estos excedentes y destríos de caqui generados en las áreas de manipulación. Estas pérdidas generan un gasto económico adicional causado por la necesidad de retirar el producto desechado de los almacenes. En este contexto, el sector se enfrenta al reto de introducir la valorización de los excedentes y destríos. Es importante ofrecer estrategias a los productores para aumentar la rentabilidad de los cultivos de caqui reduciendo las pérdidas postcosecha y aumentando el valor de la fruta que se ha desechado.

Una de las opciones para valorizar los excedentes y destríos generados en la industria agroalimentaria se basa en obtener productos e ingredientes naturales, ricos en compuestos bioactivos, para su utilización en el desarrollo de nuevos alimentos o su consumo directo. Siendo el caqui un fruto con alto contenido en compuestos bioactivos (carotenoides, compuestos fenólicos, vitaminas, etc.) (Yaqub et al., 2016), esta estrategia se presenta como una oportunidad de valorización de la gran cantidad de excedentes y destríos postcosecha generados en este cultivo. La aplicación de tratamientos de secado

parece ser una alternativa para dar salida a la fruta descartada (Akyildiz et al., 2004). Dentro de los métodos de secado, el secado "natural" y por aire caliente son los más empleados. La conservación de los alimentos mediante el secado es el método tradicional y más común utilizado por la industria de procesamiento de alimentos. En épocas anteriores, el secado dependía del sol, pero hoy en día se utilizan muchos tipos de equipos y métodos sofisticados para deshidratar los alimentos. El secado reduce la actividad del agua, preservando así los alimentos al evitar el crecimiento microbiano y las reacciones químicas de deterioro (Shafiur, 2007). Otros tratamientos de secado no térmicos como la liofilización, a pesar de ser costosos, permiten minimizar las pérdidas de calidad en el producto deshidratado, dando lugar a productos e ingredientes estables con alto contenido en compuestos bioactivos (Krokida et al., 1998; Moraga et al., 2012). La liofilización se basa en la deshidratación por sublimación de un producto congelado. Debido a la ausencia de agua líquida y a las bajas temperaturas requeridas para el proceso, se detienen la mayoría de las reacciones de deterioro y microbiológicas, lo que da lugar a un producto final de excelente calidad (Ratti, 2001).

Los consumidores actuales son cada vez más conscientes de la relación entre alimentación y salud, y es por ello que, en la última década, ha aumentado el consumo de ciertos alimentos que aportan beneficios a la salud humana (Yaqub et al., 2016). Esto ha producido que las investigaciones estén enfocándose en el desarrollo de alimentos funcionales a partir de la valorización de subproductos industriales ricos en compuestos bioactivos. Este tipo de compuestos tienen un efecto beneficioso para la salud, más allá del efecto nutritivo de sus ingredientes (Ferguson, 2009). Los compuestos bioactivos más importantes hoy en día son los polifenoles junto con los carotenoides, ya que presentan una alta capacidad antioxidante, y se encuentran en gran abundancia en frutas y hortalizas (George & Redpath, 2008). Algunos de los beneficios que se les atribuyen son la mejora de la salud cardiovascular, la reducción de inflamación y la modificación de la microbiota intestinal, entre otros. Estos beneficios derivan de

sus propiedades antioxidantes, anticancerígenas, antimutagénicas, antimicrobianas, antiinflamatorias y neuroprotectoras (Del Rio et al., 2013; Shahidi & Ambigaipalan, 2015).

Aunque el contenido en polifenoles sea alto en el caqui "Rojo Brillante", para corroborar sus efectos beneficiosos es necesario estudiar otros aspectos, como la eficiencia en la extractabilidad de estos compuestos desde la matriz cuando se consumen (Balasundram et al., 2006). En este sentido es interesante estudiar el efecto que tiene el proceso de digestión *in vitro* y el comportamiento de estos compuestos bioactivos en la matriz alimentaria durante dicha digestión. Para ello se utilizan modelos gastrointestinales *in vitro* con el objetivo de simular el tránsito de los alimentos por el sistema digestivo (Brodkorb et al., 2019).

Según estos antecedentes expuestos, el desarrollo de nuevos productos e ingredientes con elevado contenido en compuestos bioactivos puede ser de gran interés por los efectos beneficiosos sobre la salud. La introducción de nuevos productos e ingredientes a partir de caqui "Rojo Brillante" en el mercado permitiría reducir las pérdidas postcosecha, y, dado que es una fruta estacional, ayudaría a su consumo durante todo el año.

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In vitro and *in vivo* digestion of persimmon and derived products: A review

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Foods (Enviado)

Abstract

The link between nutrition and health has focused on the strategy of diet-based programs to deal with various physiological threats, such as cardiovascular disease, oxidative stress, and diabetes. Therefore, the consumption of fruits and vegetables as a safeguard for human health is increasingly important. Among fruits, the intake of persimmon is of great interest because several studies have associated its consumption with health benefits because of its high content of bioactive compounds, fiber, minerals, and vitamins. However, during digestion, some changes take place in persimmon nutritional compounds that condition their subsequent use by the human body. *In vitro* studies indicate different rates of recovery and bioaccessibility depending on the bioactive compound and the matrix in which they are found. *In vivo* studies show that the pharmacological application of persimmon or its functional components, such as proanthocyanidins, can help to prevent hyperlipidemia and hyperglycemia. Thus, persimmon and persimmon derived products have the potential to be a fruit recommended for diet therapy. This review aims to compile an updated review of the benefits and digestibility *in vitro* and *in vivo* of some important nutrients and bioactive compounds of persimmon and their derived products.

Keywords: *Diospyros kaki*, bioaccessibility, phenolic compounds, carotenoids, fiber.

1. Introduction

Persimmon (*Diospyros kaki L. f*) is a fruit belonging to the Ebenaceae family originated in China around 450 BC, before spreading to Korea and Japan, where it is considered a traditional crop (Luo & Wang, 2008). Persimmon arrived in Europe at the beginning of the XVII century, being Spain, its main producer. Over the past 19 years, there has been an exponential growth in persimmon production and around

80% is exported to the European market (Perucho, 2018). According to FAO 2019 data, world persimmon production rose to 4,270,074 tons, with a cultivated area of 992,425 ha. Currently, the largest persimmon producer worldwide is China, followed by the Republic of Korea, Japan, Brazil, Spain, and Azerbaijan. These countries make up 87% of world production (FAO, 2019). Persimmons are usually classified as astringent and non-astringent varieties. In astringent varieties, persimmons are edible when overripe, but they are astringent at harvest time (when the fruits are still firm). The astringency at the time of harvest is related to the amount of soluble tannins accumulated in the fruit, rendering them not edible. They are only edible after artificial removal of astringency or when the fruit overripen—as soluble tannins polymerize during the ripening progress. In the non-astringent varieties, the accumulation of soluble tannins ends in the early stages of fruit development, and they are edible at harvest time (Giordani, Doumett, Nin, & Del Bubba, 2011). Varieties such as *Giombo, Niuxin, Wolha, GongChengYueShi, Yongding*, *Hohrenbo, Ichida-gaki, Gongcheng Yueshi, Tone Wase, Hachiya, Hiratanenashi, Jiangsu, Atago, Aodanshi, Triumph, Rojo Brillante, Mopan,* and *Sangju* are astringent whereas *Fuyu*, *Hana-Fuyu*, *Cal-Fuyu*, *O'Gosho*, *Hana-Gosho*, *Eshi*, *Jiro*, and *Kaki tipo* are non-astringent (Giordani et al., 2011; Luo & Wang, 2008; Naval et al., 2010). *Tone Wase, Hachiya, Saijo* varieties are widely cultivated in Japan; *Fuyu*, *Hana-Fuju* or *Jiro*; *GongChengYueShi, Yongding*, *Hohrenbo, Ichida-gaki* and *Mopan* are cultivated in China; *Kaki Tipo* is typical in Italy; and *Rojo Brillante* along with *Triumph* are grown in Spain. The exponential growth of persimmon in Spain is mainly because of the appearance of the astringent variety *Rojo Brillante*, because of its excellent quality and adaptation to Spain's climate conditions (Khademi, Salvador, Zamani, & Besada, 2013).

The cultivation of persimmon has a limited shelf life compared to other fruits. It is a seasonal fruit and is perishable and difficult to store and transport; therefore, many persimmons are discarded (Zhou et al., 2019). Hence, it is necessary to search for alternatives for the use and valorization of the discarded persimmon cultivars. Dehydrated

persimmons are commonly commercialized in countries such as China, Korea, and Japan (Masahiko, Giordani, & Yonemori, 2012). Products derived from persimmon such as persimmon flour have also been obtained (Lucas-González, Fernández-López, Pérez-Álvarez, & Viuda-Martos, 2018) and used in pork liver pâté (Lucas-González, Pellegrini, Viuda-Martos, Pérez-Álvarez, & Fernández-López, 2019) or pasta formulation (Lucas-González, Pérez-Álvarez, et al., 2021). Ice cream prepared with persimmon puree (Karaman et al., 2014), dairy products accompanied by persimmon (Hernández-Carrión, Varela, Hernando, Fiszman, & Quiles, 2015), persimmon syrup (Yoo, Kim, & Jeong, 2011), vinegar (Moon & Cha, 2008), persimmon wine (M. Liu et al., 2018), and persimmon snacks obtained by different drying treatments (González, Hernando, & Moraga, 2021; González, Llorca, Quiles, Hernando, & Moraga, 2020), have also been prepared in different studies.

Both persimmon and the derived products have a high content of fiber, minerals, vitamins, and bioactive compounds, which gives different beneficial effects against diseases such as oxidative stress, hypertension, diabetes mellitus, and atherosclerosis (Butt et al., 2015). However, changes take place during digestion in the bioactive and nutritional compounds that condition the subsequent use of these substances by the human body. Determining food efficiency is conducted using concepts such as bioavailability, bioaccessibility, and bioactivity of food components. Bioavailability, the ingested fraction of a biocomponent that reaches the systemic circulation to be distributed to organs and tissues, is determined using *in vivo* studies. Bioaccessibility, the ingested fraction of a biocomponent that becomes accessible for absorption through the epithelial layer of the gastrointestinal tract, is determined using *in vitro* studies. The bioactivity represents the ability of a compound to manifest a biological effect (Dima, Assadpour, Dima, & Jafari, 2020).

This review aims to compile the current information on *in vitro* and *in vivo* digestion of persimmon and derived products, focusing on the main bioactive and nutritional compounds.

2. Bioactive and nutritional compounds of persimmon

The most prominent macro- and micronutrients present in persimmon are carbohydrates, fiber, organic acids, phenolic compounds, and carotenoids, which give antioxidant, cytotoxic, and antidiabetic properties (Matheus, Andrade, Miyahira, & Fai, 2020). According to scientific evidence, foods rich in bioactive compounds such as persimmon reduce the risk of cardiovascular disease, kidney disease, and colon and rectal cancer (Yaqub et al., 2016). Besides, components such as fiber help to regulate obesity and being overweight, reduce type II diabetes and regulate the glycemic index, as well as reducing the risk of cardiovascular disease (Chandalia et al., 2000; Slavin, 2005).

2.1. Antioxidant capacity

Antioxidant concept in food could be defined as the capacity of any substance that delays or inhibits the oxidation of substrates (Gülçin, 2012). Clinical and epidemiological studies have shown that certain micronutrients and secondary metabolites present in fruits and vegetables are beneficial for health because they are antioxidant, anti-inflammatory, and hypocholesterolemic. Therefore, there is a relationship between the consumption of foods with high antioxidant activity and their health benefits, the higher the consumption, the lower the incidence of diseases. The most prominent micronutrients and secondary metabolites with antioxidant capacity are phenolic compounds, carotenoids, water-soluble and fat-soluble vitamins, and phytochemicals (Yahia, García-Solís, & MaldonadoCelis, 2018). Persimmon is a fruit with higher antioxidant capacity because of its high content of phenolic compounds (especially tannins), carotenoids, and water-soluble vitamins, such as vitamin C (Kondo, Yoshikawa, & Katayama, 2004; Yaqub et al., 2016). Astringent varieties have higher antioxidant capacity because of the high content of soluble tannins

(Del Bubba et al., 2009; Novillo, Besada, Tian, Bermejo, & Salvador, 2015). Several studies have shown that the antioxidant potential of persimmon is much higher when compared to other fruits such as apples (Gorinstein et al., 2001), blueberries, or strawberries (García-Alonso, De Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2004).

2.2. Phenolic compounds

Phenolic compounds are secondary metabolites that contain at least a phenol group in their structure and can be classified as extractable (soluble) and non-extractable (insoluble). Extractable polyphenols (EP) are soluble in water-soluble organic solvents, and non-extractable polyphenols (NEP) are retained in the residue after extraction of EP therefore, in the food matrix. NEP have often been ignored, although they are also bioactive compounds with potential health properties (Arranz, Silván, & Saura-Calixto, 2010). EP and NEP are mostly flavonoids and non-flavonoids. Among flavonoids, monomeric flavan-3-ols (catechin, epicatechin, and epigallocatechin) and polymerized proanthocyanidins (condensed tannins) with various structural variations (dimers, oligomeric and polymeric), are vast in persimmon. Non-flavonoid polyphenols (phenolic acids) such as benzoic and cinnamic acid derivatives; ferulic, coumaric, and gallic acid have also been found (Giordani et al., 2011). Furthermore, EP are a complex mixture of low molecular monomeric compounds, including extractable proanthocyanidins along with other flavonoids and phenolic acids. NEP are polymeric polyphenols, including high molecular non-extractable proanthocyanidins and low molecular polyphenols bound to cell wall constituents (polysaccharides and proteins) or trapped within the food matrix (Arranz et al., 2010). A higher proportion of EP are found in astringent varieties, whereas higher NEP are found in non-astringent varieties.

The phenolic profile of 11 varieties of persimmon conducted by Veberic et al., (2010) concluded that the most important compounds

were gallic acid and catechin. The varieties with the highest concentration of gallic acid in fresh persimmon were *Triumph* and *Tone Wase* and those with the highest concentration of catechin were *Jiro, Triumph, Thiene, Fuji, Cal-Fuyu,* and *Tipo*.

Using HPLC, Sentandreu et al., (2015) detected 32 low molecular phenolic compounds in the variety of *Rojo Brillante* persimmon. Gallic acid and coumaric acid were found as the highest proportion as well as monomeric and polymeric flavonoids. Studies show that among the phenolic compounds of persimmon fruit, there are mainly simple and polymerized polyphenols (condensed tannins or proanthocyanidins) (Jiménez-Sánchez et al., 2015).

2.3. Carotenoids

Carotenoids are a group of fat-soluble compounds responsible for the yellow, orange, and red color of persimmon fruit. Carotenoids are divided into carotenes and xanthophylls and the structure comprises conjugated double bonds, which provide a certain color in the UV-visible spectrum (Giordani et al., 2011). The health benefits of carotenoids, especially α-carotene, β-carotene, and β-cryptoxanthin, come primarily from their provitamin A activity. Vitamin A is essential for skin, eyes, heart, and the immune system (Díaz et al., 2020). Furthermore, carotenoids have antioxidant properties associated with cell protection, regulation of cell growth, apoptosis (Pachisia, 2020), the prevention of certain types of cancer, and atherosclerosis (Hu et al., 2009; Krinsky & Johnson, 2005).

Persimmon contains a high content of carotenoids, such as α-carotene, β-carotene and β-cryptoxanthin, which contribute to the intake of provitamin A (Giordani et al., 2011). Veberic et al., (2010) studied the profile of carotenoids in 11 varieties of persimmon. β-carotene was the main carotenoid found in ripe persimmon; the highest concentration of this compound was found in the peel of the *Hana-Fuyu* variety. The concentration of β-carotene was higher in the peel than in the pulp,

and the most abundant carotenoids in the pulp were β-carotene, β-cryptoxanthin, α-carotene, and zeaxanthin.

Bordiga et al., (2019) determined the carotenoid content in both the peel and the pulp of *Kaki Tipo* variety in 13 ripening stages. As the ripening stage advanced, the carotenoid content increased both in the peel and in the pulp, being β-cryptoxanthin the most abundant carotenoid. The carotenoid content increases as the fruit ripens; the content of β-cryptoxanthin is usually the highest, followed by lycopene, β-carotene, zeaxanthin, and lutein. (Yaqub et al., 2016). The average value of β-carotene in the fresh persimmon pulp of *Rojo Brillante* according to Hernández-Carrión et al., (2014) is 0.581 mg/g. González, García, et al.,(2021) observed that the *Rojo Brillante* variety also presented β-cryptoxanthin, β-carotene, violaxanthin, zeaxanthin, and lutein; in addition, as the ripening stage advanced, the content of β-cryptoxanthin also increased. Multiple factors affect the carotenoid profile of different varieties of persimmon, such as environmental conditions (climate, cultivation techniques, and post-harvest conditions), the ripening process, and genetic factors (Qi et al., 2019).

2.4. Dietary fiber

According to the World Health Organization (WHO), dietary fiber is defined as carbohydrate polymers with 10 or more monomeric units not hydrolyzed by endogenous enzymes of the human small intestine, but it is partial or totally fermented in the large intestine by the action of the microorganisms. Total dietary fiber comprises two fractions, insoluble and soluble. Insoluble fiber is poorly fermented and has a marked laxative and intestinal regulating effect; soluble fiber is fermented in the colon, favoring the development of intestinal flora, intestinal transit speed, and decreasing blood cholesterol concentration and glucose (Tungland & Meyer, 2002).

Persimmon has both soluble and insoluble fiber in the peel and in the pulp, with a higher content in the peel (Gorinstein et al., 2001). The

average fiber content is around 3.9 g/100 g (fresh weight); this data was obtained from varieties such as *Fuyu* and *Hachiya* (Altuntas, Cangi, & Kaya, 2011; Celik & Ercisli, 2008). The *Triumph* variety showed average values of 1.5 g/100 g (fresh weight) where the content of insoluble fiber was found in a proportion higher than soluble fiber (Park et al., 2006). In the *Rojo Brillante* variety, the fiber content was 3.11 g/100 g, where around 1.97 g/100 g were insoluble fiber (Hernández-Carrión et al., 2014). The fiber content in persimmon is relatively high compared with fruits such as apple (2.4 g of fiber/100 g of fruit), orange (2.4 g of fiber/100 g of fruit), and grapefruit (1.6 g/100 of fruit) (Dreher, 2018). Moreover, some studies hypothesize that various bioactive benefits of dietary fiber are determined by the action of some bound compounds, such as phenolic compounds (Quirós-Sauceda et al., 2014). Persimmon fiber interacts with antioxidant phenolic compounds such as condensed tannins, which may play an important role in the human body (Mamet, Ge, Zhang, & Li, 2018).

3. Digestion methods

3.1. *In vitro* digestion

In vitro digestion reproduces the human physiological gastrointestinal process in the laboratory in a controlled and reproducible way (Bohn et al., 2018). *In vitro* digestion is widely used to study the gastrointestinal behavior of foods. It is relatively fast, less expensive than *in vivo* methods and require little labor without ethical restrictions. *In vitro* digestion methods can include an oral, gastric, and small intestine phase and even the large intestine phase (Minekus et al., 2014). According to these methods, the simulation of digestion considers the enzymes present in the human body, their concentrations, pH, digestion times, salt concentration, and temperature, among other factors. Besides, there are also computerized models such as TNO, Research2, and INRA3

that allow the simulation of the dynamic aspects and the variation of concentrations of the different substances involved in digestion. However, the most used models are the static ones (Brodkorb et al., 2019; Minekus et al., 2014). The INFOGEST commission, to improve the health properties of food, created a standard method of digestion, composed by the oral, gastric, and intestinal steps. The proposed static method is widely used (Brodkorb et al., 2019; Minekus et al., 2014), but it has limitations since structural changes in the food are not considered. Mulet-Cabero et al., (2020) proposed an intermediate model based on the method proposed by Minekus et al., (2014). This method also includes kinetic aspects related to the gastric phase, gradual acidification, and the secretion and emptying of enzymes and fluids.

The determinations conducted after the *in vitro* digestion process include the concepts of recovery index and bioaccessibility. The recovery index provide the food components present in the whole digested fractions (oral, gastric, and intestinal phases) of digestion compared with the amount in the undigested fraction (Pellegrini et al., 2017). The concept of bioaccessibility can be defined as the amount of a food component present in the small intestine, after the release from the food matrix, and can pass through intestinal cells (Saura-Calixto, Serrano, & Goñi, 2007). Both concepts are conducted by equations (1) and (2):

$$Recovery\ index\ (\%) = \frac{IN + OUT}{Total\ content\ of\ the\ compound\ in\ the\ food\ matrix} \times 100 \quad (1)$$

$$Bioaccessibility\ (\%) = \frac{IN}{IN + OUT} \times 100 \quad (2)$$

where, IN is the content of the component present in the soluble fraction and OUT is the content of component present in the pellet after centrifugation or dialysis of the digesta (Lucas-González, Viuda-Martos, Pérez Álvarez, & Fernández-López, 2018; Ortega, Macià, Romero,

Reguant, & Motilva, 2011). The recovery index can be applied in each of the *in vitro* digestion stages whereas the concept of bioaccessibility only applies in the small intestine stage, at the end of digestion.

3.2. *In vivo* digestion

Despite being very expensive and time-consuming, *in vivo* studies are often used to compare with *in vitro* studies and establish accuracy and precision. *In vivo* digestion studies the changes that foods undergo during digestion in a living organism, considering the complexity of organisms, to better understand the impact of these foods on human health. The digestion variability depends on different factors. Some are related to the nature of the food (composition, structure, and interactions), and others are related to human physiology (age, health, and type of diet) (Bornhorst & Singh, 2014). Currently there is no standardized design of an *in vivo* experiment on the digestibility of foods—the researcher oversees designing their own experiment. Even though, there are different European regulations such as the Council Directive 86/609/EEC of November 24, 1986, regarding the protection of animals used for experimentation and other scientific purposes. However, evidence shows that most publications do not include key information and there is significant scope to improve the reporting of studies involving animal research. The National Center for the Replacement, Refinement, and Reduction of Research Animals (NC3Rs) revealed that only 59% of the 271 random articles chosen specified the hypothesis, the aim of the study, and the number and characteristics of the animals used (species/strain, sex, age, and weight). Most of the studies neither report using randomization nor blinding to reduce bias in animal selection and outcome assessment. Kilkenny et al., (2010) emphasized that knowing the number of animals is essential to evaluate the biological and statistical importance of the experimental results. Details of the study information would avoid unnecessary use of animals in the future.

To provide a solution, CONSORT (Consolidated Standards of Reporting Trials) proposed the guidelines called ARRIVE (Animal Research: Reporting of *In vivo* Experiments). The ARRIVE guidelines comprise a 21-point list that describes the minimum information that all scientific publications that use animals must include. Some of these points are the number and specific characteristics of the animals used (including species, strains, sex, and genetic background), details of care and breeding and statistical and analytical experimental methods. There is also a preparation stage of animals, followed by adaptation to the environment and the food ingested. After the feeding period or when the researcher considers, samples are taken with blood or plasma extraction. In other cases, the feces and urine of the animals or the arteries and organs are analyzed.

Reviewing this document when planning *in vivo* experiments will enable researchers to fully incorporate, implement research design recommendations, and prepare the information that needs to be collected during the experiment to study in accordance with the guidelines (du Sert et al., 2020).

4. *IN VITRO* DIGESTION STUDIES OF PERSIMMON AND DERIVED PRODUCTS

4.1. Phenolic compound digestibility

Phenolic compounds (EP and NEP) have an important antioxidant capacity. Therefore, it is necessary to know their digestibility and bioaccessibility to know if they provide health benefits. Most literature related to *in vitro* digestion of persimmon and derived products focus on the digestibility of phenolic compounds. Table 1 summarizes the studies related to the *in vitro* digestion of persimmon and derived products.

Table 1. Studies related to the in vitro digestion of persimmon and derived products.

Food matrix	Variety	*In vitro* method	Analytical method	Outcomes	Reference
Phenolic compounds					
Fiber, fresh fruit, and persimmon leaf	*Rojo Brillante*	Oral, gastric, and small intestine phases	EP and soluble flavonoids. Antioxidant capacity	The oral phase and α-amylase decreased the recovery index of EP. The intestinal phase increased the recovery index. The final bioaccessibility of the phenolic compounds was improved.	Martínez-Las Heras et al. (2017)
Persimmon tannins	*Niuxin*	Pancreatic lipase activity inhibition	Spectrophotometry analysis	Inhibitory effect of tannins on pancreatic lipase. Hypolipidemic effect.	Zhu et al. (2018)
Persimmon tannins	*GongChengYueShi*	In vitro starch digestibility	α-Amylase, α-Glucosidase Activity Assay Interaction of tannin with starch.	Tannins help prevent postprandial hyperglycemia.	Li et al. (2018)
Polymers and oligomers of proanthocyanidins from persimmon peel	-	-	α-Amylase, α-Glucosidase Activity Assay	Oligomers have inhibitory force on α-glucosidase and polymers have inhibitory force on α-amylase. Both have antidiabetic action.	Lee et al., (2007)
Persimmon fruit	*Mopan*	Oral, gastric, and small intestine phases	Total phenol content (EP and NEP) Antioxidant capacity α-Glucosidase inhibition activity	EP and NEP inhibit α-glucosidase. NEP were released and the gastric phase played a key role in their release.	Zhou et al. (2019)
Persimmon flours	*Rojo Brillante* and *Triumph*	Oral, gastric, and small intestine phases	EP and soluble flavonoids.	The fiber content, α-amylase interactions, pH differences, and polyphenols in the sample are the key factors that affect bioactive compounds during digestion.	Lucas-González et al. (2018)
Spaghetti with 3% and 6% of persimmon flours	*Rojo Brillante* and *Triumph*	Oral, gastric, and small intestine phases	Total phenol content (EP and NEP) Antioxidant capacity	Both persimmon flours added in 3% could develop spaghetti with higher polyphenol content. Bound polyphenols continue to the colon, being used by the intestinal microbiota.	Lucas-González et al. (2021)
Pork liver pâté with 3% and 6% of persimmon flours	*Rojo Brillante*	Oral, gastric, and small intestine phases	Total phenol content (EP and NEP)	NEP reach the colon intact and could be metabolized by the intestinal microbiota.	Lucas-González et al. (2021)
Persimmon peels	*Yongding*	Oral, gastric, and small intestine phases	Total phenol content (EP and NEP) Antioxidant capacity	EP decrease and NEP increase. 30% ethanol and 70% CO_2 improved the bioaccessibility of total polyphenols and antioxidant properties.	Liu, Qiao, Ren, & Li (2018)
Dehydrated persimmon	Local market (Turkey)	Oral, gastric, and small intestine phases	EP and soluble flavonoids. Antioxidant capacity	The higher temperature of drying, the higher increase of EP, soluble flavonoids, and antioxidant capacity bioaccessibility.	Kayacan et al. (2020)
Persimmon powders obtained using hot air drying (HAD) and freeze-drying (FD) treatments	*Rojo Brillante*	Oral, gastric, and small intestine phases *In vitro* colonic fermentation	Total phenol content (EP and NEP) Antioxidant capacity Characterization of Microbiota	Polyphenols and antioxidant capacity increased after the gastric phase and decreased after the intestinal phase. Positive correlations between polyphenols and bacteria genera after colonic fermentation.	Bas-Bellver et al. (2020)

Dehydrated persimmon	*Hohrenbo*	Oral, gastric, small intestine and large intestine phases	NEP determination Antioxidant capacity	NEP fermentative decomposition in the large intestine. The antioxidant properties increase.	Matsumura et al. (2016)
Dehydrated persimmon	*Ichida-gaki*	Gastric and small intestine phases	Total phenol content (EP and NEP) In vitro acid-binding capacity	Strong bile acid-binding activity of NEP. Dried persimmon as cholesterol-lowering agents.	Hamauzu & Suwannachot, (2019)
			Carotenoids		
Persimmon fruit	-	Oral, gastric, and small intestine phases	HPLC analysis of carotenoids	The bioaccessibility of carotenoids increases with the presence of a fat source.	Estévez-Santiago et al. (2016)
Persimmon fruit	*Rojo Brillante*	Oral, gastric, and small intestine phases	HPLC analysis of carotenoids	The pressurization and pasteurization processes increase the bioaccessibility of persimmon carotenoids.	Cano et al. (2019)
Persimmon-based dairy products	*Rojo Brillante*	Oral, gastric, and small intestine phases	HPLC analysis of carotenoids	Higher bioaccessibility of carotenoid in dairy products formulated with whole milk.	García-Cayuela et al. (2018)
Persimmon powders obtained by HAD and FD treatments	*Rojo Brillante*	Oral, gastric, and small intestine phases *In vitro* colonic fermentation	HPLC analysis of carotenoids Characterization of microbiota	The degradation of the carotenoids was evidenced in HAD and FD treatments. Greater positive bacteria genera in the colon.	Bas-Bellver et al. (2020)

4.1.1. Persimmon fruit

Martínez-Las Heras et al., (2017) analyzed the content of EP and the antioxidant capacity of the fruit, leaves, and fiber extracted from *Rojo Brillante* persimmon during its *in vitro* digestion. The results showed that the oral phase and the presence of α-amylase were the factors that most affected the reduction in the recovery index of the EP, especially in the leaves of persimmon. An increase in the recovery index of EP was observed in persimmon fruit and fibers during the small intestine phase. This could be because of the extent period of this phase (>2 h) and the effect of intestinal enzymes and bile salts, which could facilitate the release of polyphenols from the persimmon matrix. However, antioxidant capacity during digestion resulted in total losses at the end of digestion in leaves, persimmon fruit, and fibers. The bioaccessibility of the EP in the persimmon fiber was higher than in the fruit and persimmon leaves. Moreover, the bioaccessibility of the total

antioxidant capacity was lower than those of EP and never exceeded 40%. They concluded that the EP and total antioxidant capacity of the aqueous extract of persimmon leaf were more sensitive to the gastrointestinal environment than those derived from persimmon fruits or fibers. Although the bioaccessibility of the total antioxidant compounds in the persimmon fruit and the fiber was greater than in the aqueous extract of persimmon leaves, an infusion with persimmon leaf (1.5 g in 110 mL of water) and a fruit of persimmon (200 g) would provide similar bioaccessible antioxidants at the end of digestion.

Zhu, Jia, et al., (2018) analyzed the inhibitory effect of tannins extracted from persimmon *Niuxin* on pancreatic lipase. This critical enzyme is associated with hyperlipidemia and obesity. The results showed the tannins extracted from persimmon had a high affinity for pancreatic lipase and inhibited the activity of this enzyme; the interaction was spontaneous through non-covalent bonds. Therefore, the binding and inhibition capacity of persimmon tannins on lipid digestive enzymes may have effectiveness for the treatment and prevention of obesity.

Li et al., (2018) showed the effect of the tannins extracted from the fresh *GongChengYueShi* persimmon on the digestibility of corn starch and on the activity of α-amylase and α-glucosidase; two of the main digestive enzymes involved in the hydrolysis of the starch. The digestibility of starch decreased with the addition of tannins from persimmon, the higher the concentration of added persimmon tannins, the greater the inhibition of starch digestibility. Moreover, the results showed persimmon tannins interacted with the starch, interacting with amylose more than amylopectin. However, tannins exerted a great inhibitory effect on α-amylase and α-glucosidase. Generally, data suggest that tannins in persimmon may help reduce postprandial hyperglycemia, regulating glucose levels in the human body.

Y. A. Lee et al., (2007) evaluated the polymers and oligomers of proanthocyanidins from persimmon peel against diabetes. The oligomeric proanthocyanidins exerted a higher interaction with

α-glucosidase and the polymeric proanthocyanidins had greater interaction with α-amylase. This suggests that the inhibition of both enzymes depends on the degree of polymerization of the phenols. Therefore, persimmon peel could have an antidiabetic action.

In a study conducted by Zhou et al., (2019), the total content of EP and NEP present in *Mopan* persimmon was evaluated. The action against α-glucosidase of EP and NEP and their antioxidant capacity were also compared. In addition, the release of EP from NEP was studied throughout *in vitro* digestion, considering the oral, gastric, and small intestinal phases. The results showed the NEP content in fresh persimmon was higher than EP. The EP had a greater inhibition capacity of α-glucosidase than the NEP. This could be because EP comprised low molecular weight molecules, which can be more easily attached and have a greater ability to interact with the enzyme. Both had higher α-glucosidase inhibition capacity than acarbose—an oligosaccharide used as a drug reducing the speed of carbohydrate digestion. After *in vitro* digestion of NEP, the polyphenols content and the antioxidant capacity were lower in the oral phase and significantly increased in the gastric and intestinal phases. The acidic conditions of the stomach environment can enhance the release of NEP. Therefore, NEP were released after simulated gastrointestinal digestion, and the gastric phase played a key role in their release. Thus, NEP were the most effective antioxidant in persimmon fruit after digestion. Nevertheless, Zhou et al., (2019) suggested further investigations to explain the composition and structure of EP and NEP to clarify their differences in biological activities.

4.1.2. Persimmon derived products

Several authors have studied the *in vitro* digestibility of polyphenols in products such as persimmon flour, spaghetti, and pork liver pâté enriched with persimmon flour, persimmon peels, and dehydrated persimmon. Lucas-González, Viuda-Martos, et al., (2018) evaluated

the recovery index, bioaccessibility of EP, and total soluble flavonoids during the *in vitro* digestion of persimmon flour of *Rojo Brillante* and *Triumph* varieties. The recovery index of the EP in both varieties was similar, except in the oral phase, where *Triumph* showed lower values, and the recovery index of total soluble flavonoids was higher in the *Rojo Brillante* variety. These could be related to the different compositions of the persimmon varieties (total dietary fiber is higher in the *Triumph* variety) and the interaction of phenolic compounds with α-amylase. The highest recovery index was obtained after the gastric phase and the lowest after the small intestine phase in both varieties. This could be explained by the different pH in the digestion phases. At acidic pH present in the stomach, phenols are often found in a very stable chemical form—the Flavilium cation. Therefore, the bioaccessibility in the stomach could be higher. However, this bioaccessibility drastically decreases in the intestine because of interactions with dietary compounds such as fiber, chemical reactions of oxidation, and polymerization; or molecular changes because of enzyme action. Regarding the bioaccessibility of EP, the results indicated both flours presented similar values. *Triumph* presented greater bioaccessibility of soluble flavonoids than the *Rojo Brillante* variety, probably because of the different flavonoid profile of the persimmon flours. Therefore, the different fiber content, the interaction of α-amylase with polyphenols, the pH during the gastric and intestinal phase, and the total content of polyphenols and flavonoids in the persimmon samples are the main factors affecting the behavior of these bioactive compounds during digestion (Lucas-González, Viuda-Martos, et al., 2018).

In another study, Lucas-González, Pérez-Álvarez, et al., (2021) formulated spaghetti with 3% and 6% of the persimmon flours obtained from the *Rojo Brillante* and *Triumph* varieties. The profile of EP and NEP, and their bioaccessibility and antioxidant capacity after simulated *in vitro* digestion, were determined. Spaghetti enriched with persimmon flours modified the polyphenolic profile with the appearance of two new compounds, gallic acid and p-coumaric-o-hexoside, increasing the

antioxidant capacity. After *in vitro* digestion, numerous polyphenols remained bound to the cell wall or to indigestible polysaccharides. The EP bioaccessibility determined in the small intestine phase was poor and did not improve with the addition of persimmon flours; many EP could become part of the NEP. The authors concluded that although most NEP did not release from the food matrix during gastrointestinal digestion, they may still have a health-promoting effect as they could be available in the colon (Lucas-González, Pérez-Álvarez, et al., 2021).

Furthermore, Lucas-González, Pérez-álvarez, et al., (2021) enriched pork liver pâté with 3 and 6% of *Rojo Brillante* persimmon flour. In both samples of enriched pâté, 2 EP and 21 NEP were detected, provided by the persimmon flour. After *in vitro* digestion of the pâté samples, EP and NEP were evaluated. The *in vitro* digestion consisted in the oral, gastric and two small intestine phases; one with pancreatin high lipase activity (C1) and other with pan creatine low lipase activity (C2). More NEP than EP were detected in all digestion stages. In addition, some observed that the intestinal phase C1 was more suitable to recover NEP after digestion than the intestinal phase C2. This could be associated with a greater release of fatty acids in the digestive environment, which could have a protective effect on polyphenols by interacting with them. However, the observed polyphenols were NEP, which are not released. Therefore, they probably reached the colon intact, and some could be metabolized by the intestinal microbiome. Lucas-González, Pérez-álvarez, et al., (2021) concluded that high-fat foods such as pâté are excellent vehicles for preserving NEP, which could reach the colon intact and be metabolized by the intestinal microbiome. However, more studies are needed on lipid digestibility, colonic fermentation, and polyphenol transformations, to achieve the complete health implications of fortifying meat products with persimmon flours.

Liu et al.,(2018) selected optimal deastringency methods and evaluated the bioaccessibility of polyphenols in *Yongding* persimmon peels treated with 12 combinations of CO_2 and ethanol. EP and NEP content after the deastringency treatments as well as the antioxidant

capacity and bioaccessibility of the EP after *in vitro* digestion were determined. The results indicated that the EP content decreased, and the NEP content increased with increasing ethanol and CO_2 concentration in the non-digested samples. After *in vitro* digestion, there was also a significant decrease in the EP and antioxidant capacity of persimmon peels. This could be related to a higher NEP formation after the digestion process. The ethanol (30%) and CO_2 vapor (70%) method was the most effective with the highest bioaccessibility of EP. Therefore, it could be considered the best deastringency method.

Recent studies investigated the recovery index of EP, soluble flavonoids, and antioxidant capacity of persimmon affected using drying methods—ultrasound-assisted vacuum drying (USV), freeze-drying (FD), infrared drying (ID), and hot air drying (HAD) (Kayacan et al., 2020). The results showed that the USV, ID, and HAD led to a significant increase in the bioaccessibility of EP, soluble flavonoids, and antioxidant capacity compared to fresh persimmon. The samples obtained by FD did not show significant differences in the recovery index with respect to fresh persimmon. The recovery index increment was produced because of the heat treatment during the drying process. The heat facilitated the release of bioactive compounds from the food matrix. This would also explain the higher bioaccessibility obtained for ID and HAD because they are the drying processes with the highest thermal load. Therefore, the best dehydration processes to obtain higher bioaccessibility of EP, soluble flavonoids, and antioxidant capacity are HAD and ID.

Bas-Bellver et al., (2020) analyzed the effect of the gastric, small intestine phase, and colonic fermentation on the phenolic compounds and the antioxidant capacity of *Rojo Brillante* persimmon powders obtained using the HAD and FD methods. In other studies, EP and antioxidant capacity increased after the gastric phase and decreased after the intestinal phase. As they stated, most of the solubilized polyphenolic compounds remained in the precipitate. The bioaccessibility of the EP of both samples obtained by both drying treatments showed no differences between them, whereas the bioaccessibility of the

antioxidant capacity increased in the samples treated by freeze-drying. After the colonic fermentation of the predigested samples, a growth of beneficial bacteria was observed. Likewise, positive correlations were detected between polyphenols and different genera of bacteria related to beneficial effects on the immune system and health status. Thus, persimmon powders could be used in the food formulation to improve the content of bioactive compounds and could influence human health.

Matsumura et al., (2016) investigated the *in vitro* antioxidant potential of NEP from *Hohrenbo* dehydrated persimmon using HAD. They performed the *in vitro* digestion divided into four phases: oral, gastric, small intestine, and large intestine and determined the antioxidant capacity in each phase. The antioxidant capacity in the oral phase was low but increased in the gastric and small intestine phases. However, the highest values of antioxidant capacity were obtained in the large intestine phase. In the large intestine phase, the intestinal microflora produced the fermentative decomposition of the non-extractable fraction of the dried persimmon, enhancing its antioxidant capacity. Moreover, the authors concluded that more studies are required to confirm the health benefits of NEP and to distinguish between the dietary functions of EP and NEP.

Hamauzu & Suwannachot, (2019) analyzed the EP and NEP fractions in *Ichida-gaki* persimmon samples dehydrated using natural drying. After simulating the gastric and small intestine phase *in vitro*, the NEP fraction presented a strong bile acid-binding capacity. Therefore, dehydrated persimmon, with a large amount of NEP, could act as a cholesterol-lowering agent.

4.2. Carotenoids digestibility

To learn about the beneficial properties of persimmon carotenoids for human health, derived from their antioxidant capacity and provitamin A function, several studies have evaluated the bioaccessibility and stability of persimmon carotenoids during digestion.

4.2.1. Persimmon fruit

Estévez-Santiago et al., (2016) evaluated the bioaccessibility of provitamin A carotenoids from different fruits, including persimmon. The carotenoids evaluated were β-cryptoxanthin, β-carotene, and α-carotene in their trans/cis forms using HPLC quantification. The carotenoids bioaccessibility in persimmon was low, where β-carotene and α-carotene had the highest percentage of bioaccessibility. Estévez-Santiago et al., (2016) explains that the effect of the food matrix affects the bioaccessibility of carotenoids and bioaccessibility increases with the presence of a fat source. Fruit rarely is consumed with a fat source, as this mixture is not a common food offering. However, fruits are usually eaten as desserts after meals containing fat. This practice can have a positive effect on the bioaccessibility of carotenoids.

According to Cano et al., (2019), the bioaccessibility of carotenoids involves two processes: (i) release of carotenoids from the food matrix and (ii) subsequent micellization. This is limited by many factors, such as the presence of lipids, processing (milling, mechanical grinding), or the type of food matrix. Only the carotenoids present in the micellar phase are considered bioaccessible. Cano et al., (2019) evaluated the stability and bioaccessibility of carotenoids and carotenoid esters in the *Rojo Brillante* persimmon, in fresh fruit and after high hydrostatic pressure and pasteurization treatments. The number of carotenoids before and after digestion was quantified using the HPLC technique, and their recovery and bioaccessibility indexes were calculated after the oral, gastric, and small intestine phases. The results in fresh persimmon showed low bioaccessibility and no micellization of carotenoids; traces were found in the micellar phase of the small intestine phase. This was related to the fiber content of persimmon that traps bioactive compounds and reduces the micellization and bioaccessibility. Pressurized and thermally treated samples increased the overall carotenoid bioaccessibility to 54% and 25%, respectively. This increase in bioaccessibility could be because of structural modification (pressurized samples) or degradation plant of polysaccharides

(pasteurized samples), such as pectin—present in persimmon tissue—releasing the carotenoids and favor the subsequent micellization.

4.2.2. Persimmon derived products

García-Cayuela et al., (2018) assessed the *in vitro* carotenoids recovery index and bioaccessibility in *Rojo Brillante* persimmon-based dairy products. Dairy products were formulated with whole milk (3.6% fat) or skimmed milk (0.25% fat) and with whole freeze-dried persimmon, pulp, or peel. On average, the total carotenoids recovery was approximately between 25% - 39%. This means that the total carotenoids content decreased between 66-75% after *in vitro* digestion in all the formulated samples. The carotenoids bioaccessibility was significantly higher in dairy products formulated with the whole milk. Within the whole milk formulations, the highest amount of bioaccessible carotenoids was given by dairy products including peel, followed by those including whole persimmon, and those with the pulp. Furthermore, these formulations significantly improved the bioaccessibility of provitamin A total carotenoids (β-cryptoxanthin, α-carotene, β-carotene and lycopene). García-Cayuela et al., (2018) suggested, as Estévez-Santiago et al., (2016) did, that a higher fat content in the product exerts a significant improvement in carotenoids bioaccessibility. The highest amounts of bioaccessible carotenoids were found in whole milk + whole persimmon and whole milk + peel. Therefore, these formulations would be the most suitable for developing functional foods for people with low vitamin A consumption.

Bas-Bellver et al., (2020) determined the changes in persimmon carotenoids after FD and HAD treatments and during *in vitro* digestion. The total carotenoid content was slightly higher in the FD powders than in the HAD, because of the absence of oxygen and the low temperature during the FD treatment. α-Cryptoxanthin was the most abundant carotenoid in both persimmon powders. Nevertheless, the degradation of the carotenoids analyzed was evidenced during the

in vitro digestion in both drying treatments. This degradation during digestion depended on the content and characteristics of fiber and lipids present in the food matrix. After the colonic fermentation, the number of certain beneficial bacteria genera were slightly greater with the presence of the persimmon powder samples. There was a positive correlation with beneficial bacteria genera and a negative correlation with harmful bacteria, thus indicating that the presence of antioxidant compounds (polyphenols and carotenoids) is associated with high and low abundance of these genera. Therefore, persimmon waste powders could be included in the food formulation to improve the content of carotenoids and could have a positive effect on human health.

5. *IN VIVO* DIGESTION STUDIES ON PERSIMMON AND THEIR DERIVED PRODUCTS.

In vitro studies are often complemented by *in vivo* studies since they are more representative considering the complexity of organisms. The studies found about *in vivo* digestion on persimmon and derived products are focused on evaluating the beneficial effects on lipid metabolism, the regulation of glucose levels, and carcinogenic and anti-inflammatory effects. Table 2 summarizes the studies related to the *in vivo* digestion of persimmon and their derived products.

5.1. Effect on lipid metabolism

To determine the effect of persimmons, derived products, and phenolic compounds, such as tannins—on lipid metabolism—several markers have been measured. Based on literature, the most common markers are cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides (TG).

Gorinstein, Bartnikowska, et al.,(1998), Gorinstein, Kulasek, et al., (1998) and Gorinstein et al., (2011) observed a hypocholesterolemic

Table 2. Studies related to the *in vivo* digestion of persimmon and derived products.

Food matrix	Variety	*In vivo* method	Health benefits	Reference
Effect on lipid metabolism				
Dehydrated persimmon		Rats fed with 7% persimmon	Lower increase in TC, HDL, LDL, and TG levels. Hypolipidemic effect and antioxidant properties.	Gorinstein, Bartnikowska, et al., (1998)
Dehydrated persimmon		Rats fed with 7% persimmon peel and pulp	Persimmon peel has a greater hypocholesterolemic and antioxidant effect than persimmon pulp.	Gorinstein et al., (1998)
Persimmon freeze-dried	*Fuyu* and *Jiro*	Rats supplemented with 5% freeze-dried persimmon	*Fuyu* and *Jiro* varieties can be considered as hypocholesterolemic as they helped lower blood TG levels.	Gorinstein et al., (2011)
Mature and young persimmon fruit	*Fuyu-kaki* and *Hachiya-kaki*	Mice fed persimmon *ad libitum*	The young persimmon fruits exert beneficial effects on hepatic steatosis, plasma cholesterol, and dyslipidemia.	Matsumoto et al., (2006)
Young persimmon fruits	*Hachiya*	Mice fed 2% and 5% persimmon	Lower increase of TC, TG, HDL, and LDL levels. Young persimmon contributes to hypolipidemic effect.	Matsumoto et al., (2009)
Persimmon tannins	*Hachiya*	Mice fed 1% (w / w) of tannins	Tannins can help in the prevention and improvement of metabolic syndrome.	Matsumoto et al., (2011)
Three young persimmon fruits	*Fuyu Hiratanenashi* and *Hachiya*	Mice supplemented with 2% of each persimmon	*Hachiya* was considered the best of the three cultivars to maintain good health.	Matsumoto & Takekawa, (2015)
Tannins and freeze-dried whole persimmon	*Gongcheng Yueshi*	Rats fed 0.5% tannins extracted from persimmon and with 4.2% freeze-dried persimmon	Tannins were mainly responsible for the antihyperlipidemic effect of persimmon.	Zou et al., (2014)
Persimmon and satsuma mandarin peel extract	*Sangju*	Mice fed 50 and 200 mg/kg/day fruit extract	Extract with persimmon fruit and satsuma mandarin peel could attenuate some of the physiological changes that occur in obesity and be an anti-obesity agent.	Kim et al., (2016)
Persimmon tannin	*Niuxin*	Mice fed different dose of tannins (0, 50, 100 and 200 mg/kg weight)	Tannins altered the composition of the intestinal microbiota by increasing the bacteria inversely related to obesity.	Lin, et al., (2018)
Alcohol-free persimmon white wine		Hamsters supplemented with 7 mL/kg/day wine	Persimmon wine produce antiatherogenic and antioxidant effects.	Suh et al., (2011)
Persimmon vinegar	-	Mice with chronic alcoholism supplemented with 1 mL and 2 mL/ kg of body weight vinegar	Persimmon vinegar prevents alcohol-induced metabolic disorders.	Moon & Cha, (2008)
Persimmon peel powder		Rabbits supplemented with 0, 10 and 20% persimmon	The persimmon peel powder reduces the levels of TC, TG, and LDL cholesterol.	Yaqub et al., (2013)
Persimmon fiber rich in tannins	*Hiratanenashi*	Humans fed cookies supplemented with persimmon fiber (0, 3, 5 g of fiber)	The tannin-rich fiber of persimmon is a useful dietary component to treat hypercholesterolemia.	Gato et al., (2013)

Persimmon peels powders	*Sangju*	Rats supplemented with 5% persimmon	Supplementation of powdered persimmon leaf suppress body-weight gain, reduced plasma and liver lipid concentrations and increase the fecal lipids.	Lee et al., (2006)
Soluble and insoluble tannins extracted from persimmon	-	Key cells inhibition in adipogenesis	Tannin extracts could inhibit or alter the expression of specific genes involved in the adipogenesis.	Shin, Shon, Kim, & Lee, (2014)
Antidiabetic effects				
Persimmon peels	Local persimmon production	Diabetic rats supplemented with 5% and 10% persimmon	Useful dietary supplement for the synthesis of antidiabetic drugs.	S.-O. Lee, Chung, & Lee, (2006)
Persimmon tannins	*Atago*	Rats supplemented by 100, 200 and 300 mg/kg body weight tannins	Reduction of postprandial hyperglycemia.	Tsujita, (2016)
Persimmon tannins	*GongChengYueShi*	Rats administrated with 0, 25, 50, and 75 mg/kg body weight tannins	Persimmon tannins can alleviate postprandial hyperglycemia.	Li et al., (2018)
Persimmon extract		Mice administrated with 50 and 100 mg/kg persimmon	Persimmon extract has the potential to be a natural functional food material for improved cognitive function.	E. J. Shin et al., (2021)
Anti-carcinogenic and anti-inflammatory effects				
Persimmon extract		Cell culture. Human Lymphoid leukemia DNA fragmentation assay	Polyphenols induce the death of carcinogenic cells.	Yumiko et al., (1996)
Persimmon peel	*Aodanshi*	Anti-H. pylori activity. Assay anti-HIV activity Cytotoxic activity	Persimmon peel is a possible antitumor agent.	Kawase et al., (2003)
Persimmon extract		Rats treated with 15 mg crude extract per kg per day	Reduction of arthritis symptoms was observed with the administration of the persimmon extract.	Direito et al., (2020)

and antioxidant effect in rats fed with dried persimmon (peel, pulp, and different varieties such as *Fuyu* and *Jiro*). The diets supplemented with persimmon presented a lower increase in TC, LDL, HDL, and TG markers compared to the control diet. Persimmon peel and pulp could help prepare new foods in industrial processes lowering hyperlipidemia parameters in the consumers. Kim et al., (2016) studied the anti-obesity effect of an extract made with persimmon fruit (*Sangju*) and satsuma mandarin peel incorporated into a high-fat diet in mice. They observed that the extract inhibited triglyceride absorption by inhibiting pancreatic lipase, and a preventive effect on the visceral fat accumulation was also observed.

Matsumoto et al., (2006), Matsumoto et al., (2009), Matsumoto et al., (2011) and Matsumoto & Takekawa, (2015) studied the effect of dried mature persimmon (MP) and dried young persimmon (YP) (non-

astringent *Fuyu* and astringent *Hachiya,* and *Hiratanenashi*) on lipid metabolism. The main conclusions from these researchers were that none of the MP fed mice improved lipid metabolism, however, YP exerted beneficial effects such as preventing hepatic steatosis and reducing plasma cholesterol. Besides, the *Hachiya* YP-fed groups showed lower levels of TC, TG, LDL, and HDL. Matsumoto et al., (2011) observed that tannins from *Hachiya* persimmon had a high affinity for bile acids and significantly promoted their fecal excretion. Therefore, tannins would be beneficial compounds in the prevention and improvement of metabolic syndrome. Matsumoto & Takekawa., (2015) confirmed that astringent persimmon with a high content of soluble tannins improve plasma cholesterol conditions. *Hachiya,* the cultivar that obtained the highest bile acid-binding capacity and the highest content of soluble tannins, can be considered the best to maintain good health. These studies demonstrate that the beneficial effects of persimmon on lipid metabolism are related to bile acid-binding capacity and soluble tannin content. Zou et al., (2014) observed that tannins were mainly responsible for the antihyperlipidemic effect of persimmon. Besides, diets supplemented with tannins decreased the levels of the enzyme fatty acid synthase (FAS) responsible for catalyzing fatty acids and stimulating the genes responsible for lipogenesis and suppressed tumor necrosis factor (TNFα) and C-reactive protein (CRP) responsible for regulating inflammation.

Zhu, Lin, et al., (2018) evaluated the effect of *Niuxin* persimmon tannins on the intestinal microbiota. The diet with tannin altered the composition of the intestinal microbiota by increasing the bacteroidetes/proteobacteria ratio (bacteria inversely related to obesity). Therefore, the hypolipidemic effect of polyphenols could be attributed partially to the significant improvement of the microbial composition of the intestine contributing to health.

Some persimmon derivatives like white wine, persimmon vinegar, and peel powder have also prevented an increase of TG and TC levels in rabbits, hamsters, and rats; demonstrating an antiatherogenic effect

and reducing the risk of cardiovascular diseases (Moon & Cha, 2008; Suh et al., 2011; Yaqub et al., 2013).

Regarding the fiber present in persimmon, Gato et al., (2013) showed plasma cholesterol levels decreased significantly in humans fed a tannin-rich fiber diet. Lee et al., (2006) showed that food intake and body-weight gain were reduced with a diet supplemented with persimmon leaf (*Sangju*). They related these results to the fiber content acting as a satiating agent. A reduction in TC, HDL, and hepatic TG was also observed, and feces had a higher TG content in the diet supplemented with persimmon leaf.

Regarding antiadipogenic effect, Y. J. Shin, Shon, Kim, & Lee, (2014) determined whether extractable and non-extractable tannin extracts suppressed adipogenesis or the conversion of preadipocytes into adipocytes. 3T3-L1 cells were treated with extractable and non-extractable tannins from five types of persimmons. Treatment with extractable and non-extractable tannins for 7 days resulted in a significant inhibition of adipogenesis. Therefore, tannin extracts could inhibit or alter the expression of specific genes involved in the adipogenesis, although it is not clear how extractable and non-extractable tannin extracts regulate the adipogenesis process.

5.2. Antidiabetic effects

Diets supplemented with persimmon peel (S.-O. Lee, Chung, & Lee, 2006), persimmon tannins (*Atago*) (Li et al., 2018; Tsujita, 2016), and persimmon extract (E. J. Shin et al., (2021), improved characteristic symptoms of type II diabetes such as insulin resistance, hyperphagia, and the decrease of glucose blood levels delaying glucose into the bloodstream. The antihyperglycemic properties observed could be because of the combined effect of dietary fiber and antioxidants such as polyphenols and carotenoids present in persimmon. E. J. Shin et al., (2021) also observed the inhibition capacity of the digestive enzymes α-amylase and α-glucosidase, as seen in section 4.1. Besides, persimmon

extract also enhanced the cholinergic system damaged by oxidative stress and the endogenous antioxidant system in brain and liver tissues induced by the high-fat diet. Therefore, these results indicated that persimmon, persimmon tannins, and persimmon extracts could be a good dietary supplement for the synthesis of antidiabetic drugs; and could have potential as a natural functional food material to improve cognitive functions.

5.3. Anti-carcinogenic and anti-inflammatory effects

The possible beneficial effect of persimmon on carcinogenic cells and anti-inflammatory effects in different diseases has also been studied. Yumiko et al., (1996) showed phenolic compounds from persimmon such as catechin, epicatechin, epigallocatechin, and epigallocatechin gallate inhibited the growth of lymphoid cells. Furthermore, DNA fragmentation of the treated cells was observed, suggesting these compounds induce the death of carcinogenic cells.

Kawase et al., (2003) evaluated cytotoxic activity, activity against the human immunodeficiency virus (HIV), and *Helicobacter pylori* of persimmon peel (*Aodanshi*). The results indicate the existence of useful therapeutic bioactive compounds and the therapeutic value of persimmon peel extract. However, these fractions should be further purified to identify the main compounds related to a possible antitumor agent.

Direito et al., (2020) recently determined the anti-inflammatory effects of persimmon extract on rheumatoid arthritis disease through *in vivo* digestion in mice. A reduction in both edema and alterations caused by arthritis was observed after the consumption of the persimmon extract. Therefore, the administration of persimmon extract attenuated inflammation and tissue damage. This could be because of the powerful antioxidant characteristics that persimmon presents. The consumption of persimmon extracts can also be a useful pharmacological tool in the management of chronic arthritic conditions associated with active inflammation.

6. Conclusions

In recent years, persimmon production has increased and its chemical composition and the bioaccessibility of its nutrients and bioactive compounds has gained a lot of interest. Phenolic compounds and carotenoids have shown the greatest promise in persimmon digestibility and human health. The digestibility of these compounds throughout the gastrointestinal tract (oral, gastric, and intestinal phases) is mainly affected by the food matrix, enzymes, pH, and digestive fluids. The gastric phase plays an important role by increasing the release of EP mainly due to the acidic conditions of the stomach environment. However, the small intestine phase produces a reduction of EP because of interactions with dietary fiber, chemical reactions such as polymerization, or molecular changes by the action of bile salts and intestinal enzymes. Drying treatments with high temperature increased the recovery index and the antioxidant properties of the EP, favoring their release from the persimmon matrix. NEP with high antioxidant potential can reach the colonic phase intact; thus, they can interact with fiber and perform their function on the intestinal microbiota.

The intake of persimmon with foods rich in fat, and the introduction of derivatives of persimmon in matrices with a high percentage of lipids are key factors for increasing carotenoids bioaccessibility. Beneficial health properties have been found in dietary fiber from persimmon since it is rich in polyphenols and can be an ingredient in the formulation of functional foods.

In vivo studies show that the bioactive compounds of persimmon and, specifically, tannins have beneficial effects because of their high antioxidant and inhibitory capacity against the enzymes responsible for the transport and absorption of glucose and fat during digestion. These effects produce benefits in human health such as helping to reduce blood cholesterol levels, induce tumor cell death, help prevent cardiovascular diseases, regulate diabetes and, adipogenesis.

Author Contributions:

Conceptualization: Gemma Moraga and Isabel Hernando; methodology: Cristina M. González, writing—original draft preparation: Cristina M. González; writing—review and editing: Isabel Hernando and Gemma Moraga; visualization: Isabel Hernando and Gemma Moraga; supervision: Isabel Hernando and Gemma Moraga; funding acquisition: Gemma Moraga.

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The authors declare no conflict of interest.

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Objetivos

OBJETIVO GENERAL

El objetivo general de la presente tesis doctoral es explorar el uso de diferentes tratamientos de secado para el desarrollo de productos o ingredientes con alto contenido en compuestos bioactivos, a partir del destrío postcosecha de caqui variedad "Rojo Brillante".

OBJETIVOS ESPECÍFICOS

- Estudiar la cinética de secado, así como los cambios fisicoquímicos que ocurren en el caqui cuando se aplica un método de secado "natural". Evaluar el efecto de la aplicación de un tratamiento previo de desastringencia.
- Estudiar el efecto de la liofilización sobre las propiedades físicas (mecánicas y ópticas) y el contenido en compuestos bioactivos en snacks de caqui liofilizado. Obtener los valores críticos de actividad del agua y humedad que provocan el deterioro del producto durante su almacenamiento.
- Explorar el uso del secado por aire caliente para el desarrollo de un snack a partir de caqui astringente y no astringente. Evaluar el efecto del estado de madurez y la temperatura de secado sobre las propiedades físicas (mecánicas y ópticas), el contenido en taninos solubles y la aceptabilidad sensorial de los snacks desarrollados. Valorar la necesidad de la aplicación de un tratamiento previo de desastringencia.
- Analizar el efecto del estado de madurez y del secado por aire caliente, sobre el contenido y la capacidad antioxidante de los carotenoides. Valorar el uso de la fotoluminiscencia para evaluar estos cambios.
- Analizar el efecto del secado por aire caliente y por liofilización sobre la capacidad antioxidante y el contenido en taninos (solubles e insolubles) en caqui astringente y no astringente. Determinar los índices de recuperación tras su digestión *in vitro*.

Estructura de la tesis

El trabajo de investigación realizado ha dado lugar a seis trabajos científicos, los cuales se han incluido en la Introducción y en cuatro capítulos del apartado de Resultados. La tesis se enmarca en el proyecto del Ministerio de Ciencia, Innovación y Universidades titulado "Diseño de alimentos de alto valor nutritivo con ingredientes obtenidos a partir del destrío postcosecha de caqui" (RTA2017-00045-C02-02). Se trata de un proyecto coordinado, que se lleva a cabo junto con el Instituto Valenciano de Investigaciones Agrarias (IVIA), con el objetivo común de ofrecer estrategias que permitan incrementar la rentabilidad del cultivo del caqui.

Con el fin de aunar y discutir la información disponible sobre la composición y los estudios de digestión *in vitro* e *in vivo* realizados en caqui y productos derivados, se elaboró un trabajo de revisión bibliográfica, el cuál sitúa al caqui como una fruta con gran interés nutricional. Esta revisión forma parte de la Introducción, ya que contextualiza el resto de los trabajos desarrollados en la tesis.

En el trabajo incluido en el capítulo 1 de Resultados de la Tesis, la hipótesis de partida fue la aplicación de un tratamiento de secado "natural" tradicionalmente empleado en países asiáticos, para la obtención de un producto semiseco a partir de caqui "Rojo Brillante". Se estudió la cinética de secado y los cambios fisicoquímicos que ocurren en el caqui, evaluando el efecto de la aplicación de un tratamiento previo de desastringencia. Esta publicación ha permitido avanzar en el conocimiento sobre el impacto que puede tener esta tecnología de secado "natural", aún no aplicada en la industria del caqui "Rojo Brillante", que puede ser una buena estrategia para valorizar el excedente de esta fruta de temporada.

El capítulo 2, que incorpora un solo trabajo, se centra en la liofilización, por ser el proceso de referencia para la obtención de productos deshidratados de alta calidad. Este tratamiento de secado, a pesar de ser una tecnología más costosa, podría ser una alternativa adecuada para elaborar un snack saludable, o un ingrediente de alto

valor añadido, a partir del destrío postcosecha de caqui. En este sentido, en este trabajo se desarrolló un snack de caqui liofilizado y se estudió el efecto de la transición vítrea sobre las propiedades físicas y el contenido en compuestos bioactivos. Estudios preliminares demostraron que la liofilización no conlleva la eliminación de la astringencia típica de la fruta, por lo que se partió de caqui no astringente. El estudio permitió establecer las condiciones óptimas de procesado y almacenamiento para asegurar la calidad y estabilidad del producto desarrollado.

Con el objetivo de abordar tratamientos de secado económicamente más viables, en el capítulo 3 de Resultados de la Tesis, se plantea el uso del secado por aire caliente a dos temperaturas (40 y 60 ^{0}C) para el desarrollo de snacks con interés nutricional. Dentro de este capítulo se engloban dos trabajos:

En el primero de ellos, se evaluó el efecto del estado de madurez y la temperatura de secado sobre las propiedades mecánicas y ópticas, el contenido en taninos solubles y la aceptabilidad sensorial de los snacks desarrollados. En este trabajo se reflexiona sobre la necesidad de la aplicación de un tratamiento previo de desastringencia, el cual conlleva un coste económico asociado, en función de la materia prima de partida y el tratamiento de secado aplicado.

En el segundo trabajo, se evaluó el efecto del estado de madurez y la temperatura de secado sobre la fracción de carotenoides a través de diferentes técnicas experimentales como la fotoluminiscencia, en combinación con técnicas espectrofotométricas, cromatográficas y estructurales.

Finalmente, el capítulo 4, aborda la digestión *in vitro* del caqui. En este capítulo se engloba el último trabajo, en el cual se estudió el efecto del tratamiento de secado (liofilización y secado por aire caliente) y el tratamiento de desastringencia sobre la capacidad antioxidante y el contenido en taninos solubles e insolubles, y se analizó qué es lo que ocurre tras su digestión *in vitro*.

Las referencias de las publicaciones científicas derivadas de esta tesis se presentan a lo largo de los capítulos en el siguiente orden:

INTRODUCCIÓN:

González, C. M., Hernando, I., Moraga, G. *In vitro* and *in vivo* digestion of persimmon and derived products. A review. Enviado a Foods.

CAPÍTULO 1: SECADO NATURAL DE CAQUI "ROJO BRILLANTE" PARA LA OBTENCIÓN DE UN PRODUCTO SEMISECO. EFECTO DEL TRATAMIENTO DE DESASTRINGENCIA.

González, C. M., Gil, R., Moraga, G., & Salvador, A. (2021). Natural Drying of Astringent and Non-Astringent Persimmon "Rojo Brillante". Drying Kinetics and Physico-Chemical Properties. *Foods*, *10*(3), 647. MDPI AG. http://dx.doi.org/10.3390/foods10030647

CAPÍTULO 2. USO DE LA LIOFILIZACIÓN PARA EL DESARROLLO DE UN PRODUCTO DE ALTO VALOR NUTRITIVO A PARTIR DEL DESTRÍO POSTCOSECHA DE CAQUI.

González, C. M., Llorca, E., Quiles, A., Hernando, I., & Moraga, G. (2020). Water sorption and glass transition in freeze-dried persimmon slices. Effect on physical properties and bioactive compounds. *LWT*, *130*, 109633. https://doi.org/10.1016/j.lwt.2020.109633

Capítulo 3. Desarrollo de snacks saludables mediante el secado por aire caliente de caqui astringente y no astringente. Propiedades fisicoquímicas, estructurales y sensoriales.

González, C. M., Hernando, I., & Moraga, G. (2021). Influence of ripening stage and de-astringency treatment on the production of dehydrated persimmon snacks. *Journal of the Science of Food and Agriculture*, *101*(2), 603-612. https://doi.org/10.1002/jsfa.10672

González, C. M., García, A. L., Llorca, E., Hernando, I., Atienzar, P., Bermejo, A., ... & Quiles, A. (2021). Carotenoids in dehydrated persimmon: Antioxidant activity, structure, and photoluminescence. *LWT*, *142*, 111007. https://doi.org/10.1016/j.lwt.2021.111007

Capítulo 4. Efecto del tratamiento de secado y el tratamiento de desastringencia sobre la capacidad antioxidante y el contenido en taninos solubles e insolubles del caqui. Estudios de digestión in vitro.

González, C. M., Llorca, E., Quiles, A., Hernando, I., Moraga, G. An *in vitro* digestion study of tannins and antioxidant activity affected by drying "Rojo Brillante" persimmon. Enviado a LWT – Food Science and Technology.

Resultados y discusión

Capítulo 1:

Secado natural de caqui "Rojo Brillante" para la obtención de un producto semiseco.
Efecto del tratamiento de desastringencia.

Natural drying of astringent and non-astringent persimmon "Rojo Brillante". Drying kinetics and physico-chemical properties

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Foods, (2021), 10, 647

ABSTRACT

Persimmon *(Diospyros kaki* L.f.) crop has markedly increased in Spain, and "Rojo Brillante" persimmon is the main cultivated variety. This astringent cultivar requires de-astringency treatment before commercialization, which may involve an extra cost. Its short commercial season implies handling large volumes of fruits with consequent postharvest losses. Therefore, the development of derived added-value products is of much interest. In this study, astringent and non-astringent "Rojo Brillante" persimmons were dehydrated by following a natural drying method used in Asia. The drying kinetics and physico-chemical properties were analyzed for 81 days. The results indicated subsequent reductions in weight, water content, and water activity throughout the drying process, and the equatorial diameter decreased. All the employed thin-layer mathematical models were suitable for representing the drying characteristics of both products with similar behavior. The effective water diffusivity values were 5.07×10^{-11} m^2 s^{-1} and 6.07×10^{-11} m^2 s^{-1} for astringent and non-astringent persimmon samples, respectively. The drying treatment significantly decreased the soluble tannins content, and the astringent samples obtained similar values to those obtained for the non-astringent samples in 20 days. The external and internal flesh of the astringent fruit remained orange through the drying period, while brown coloration in the non-astringent fruit was observed after 57 drying days. Therefore, prior de-astringency treatment would not be necessary.

Keywords: *diospyros kaki*; post-harvest losses; dehydrated persimmon; thin-layer modeling; drying rate.

1. INTRODUCTION

In Spain, persimmon *(Diospyros kaki* L.f.) production has markedly increased over the last 20 years, and the cultivation area has expanded

almost 8-fold, from about 2253 ha in 2002 to over 18,000 ha in 2019. As a result, with close to 500,000 tons, Spain is now the second most important persimmon producing country worldwide after China (FAOSTAT, 2018). At present, cultivation is mainly based in "Rojo Brillante" cultivar, with a production of around 429,000 tons in Valencia Community (E Spain) and around 50 tons in Andalusia (S Spain) (Perucho, 2018).

"Rojo Brillante" is an astringent persimmon cultivar, which involves its presenting high soluble tannins at harvest (Tessmer et al., 2016) and, therefore, the postharvest de-astringency treatment is required before commercialization. The introduction of postharvest techniques based on exposing the fruit to high CO_2 concentrations to eliminate astringency has been one of the main causes of the expansion of persimmon production in Spain in recent years. With this de-astringency method, it is possible to obtain a fruit without astringency while preserving a firm texture (Novillo et al., 2014; Salvador et al., 2007). Currently, the Spanish production is mainly destined for exportation markets where there is a demand for persimmon as fresh fruit with a firm texture according to the current quality standards *(UNECE*, 2016). It is noteworthy that the short commercial season of this cultivar (between mid-October and the end of December) implies the postharvest handling of large fruit volumes with consequent product loss and without achieving the quality required by the fresh fruit market. Therefore, one of the current challenges for the persimmon industry is the search for strategies that increase the value of the discarded fruit.

Drying is a reliable preservation method for fruits in technical feasibility and nutritional quality terms. Unlike expensive energy-intensive artificial drying, natural drying can provide an alternative with adequate drying capacity (Mat Desa et al., 2019). Even though natural drying generates a significant loss of bioactive compounds, dried fruit can still be a valuable source of dietary fiber, minerals, and antioxidants. Based on scientific evidence, persimmon can be considered a functional food due to its high contents of bioactive

compounds that help reduce the risk of cardiovascular diseases, as well as kidney, colon, and rectal cancer, etc. Hence, dried fruit might be a potential snack that is healthier than most regular snacks (Sijtsema et al., 2012; Yaqub et al., 2016). In some Asian countries such as China, South Korea, and Japan, dehydrated persimmon is often consumed and commercially produced (Matsumoto et al., 2007; Oh et al., 2018; Sun-wun et al., 2011). The general procedure followed to make this dried product comprises removing the sepals of the calix and skin, and then hanging the fruit on strings. In China and Japan near the end of the drying period, the dried fruit is kneaded to distribute moisture uniformly in the fruit, and to produce the shape of the final product. However, in South Korea, they are left to hang in a well-ventilated place (Hur et al., 2014).

Presently, although this drying technology is not applied to "Rojo Brillante", it would be a good strategy to enhance the surplus fruit and to increase the value of the discarded fruit.

It is necessary to study drying kinetics to know the drying time required to attain a product of adequate quality. Semitheoretical models, based on a serial development of Fick's second law of diffusion, are the most widely used for food products (Akpinar, 2006; Roberts et al., 2008; Sampaio et al., 2017). Several studies have focused on drying kinetics in different persimmon formats and varieties, along with different treatments other than drying. García-Pérez et al., (2007) and Bozkir et al., (2019) studied the influence of ultrasound or osmo-convective pretreatments on drying cubes of and cylindrical-shaped persimmon. Sampaio et al., (2017) obtained the mathematical model of drying kinetics for the Fuyu persimmon variety in an osmo-convective drying procedure. Çelen, (2019) focused on the microwave effects on the drying characteristics of persimmon slices, whereas Doymaz, (2012) assessed the drying kinetics and activation energy of persimmon slices using hot air drying. Nevertheless, scarce information on drying kinetics applied in the whole persimmon fruit is reported. Demiray & Tulek, (2017) studied the effect of different

pretreatments and hot air-drying temperatures on the drying kinetics of whole persimmon. For the specific case of "Rojo Brillante", no studies have addressed the drying kinetics during the natural drying process of whole persimmon.

In this context, the aim of this research was to study the drying kinetics of persimmon"'Rojo Brillante" when the natural drying method (hanging in a well-ventilated place) is applied to astringent and non-astringent fruits (submitted previously to the de-astringency treatment). Moreover, the physico-chemical changes that occur during drying was also studied.

2. Materials and Methods

Persimmon fruits "Rojo Brillante" were harvested from commercial orchards in Valencia (E Spain) on 20 December 2018 at a commercial maturity stage (Color index (1000 a/Lb) between 15 and 17; firmness values between 33 and 35 N; initial water content between 77% and 78%). After harvest, the fruits were transported to the Instituto Valenciano de Investigaciones Agrarias (IVIA), where they were carefully selected for uniformity and separated into two groups. The first group was submitted to the astringency removal treatment in closed containers under standard conditions (95%–98% CO_2 for 24 h at 20 °C). The second group was not subjected to the de-astringency treatment. One hundred fruits from both the astringent and non-astringent groups (submitted to the de-astringency treatment) were manually peeled and immersed for 10 min in a 4.5% sodium metabisulfite ($Na_2S_2O_5$) solution used as a disinfectant. The fruits were individually hung by the pedicel for natural drying in the IVIA pilot plant. In order to study the drying kinetics and the evolution of the physico-chemical properties, one sample of 10 fruits was taken every 17–20 days up to a period of 57 days and at the end of the drying process, two more samples were taken at 67 and 81 days. The sampling dates were as follows: Day 0 (20

December 2018), Day 20 (9 January 2019), Day 40 (29 January 2019), Day 57 (15 February 2019), Day 67 (25 February 2019), Day 81 (11 March 2019).

The average temperature and relative humidity during the drying period were taken from the IVIA weather station, and ranged from 10.3 to 11.3 °C and from 68.6% to 75%, respectively.

2.1. Weight Loss and Equatorial, Longitudinal Diameters

The fruits were individually weighed with an Absolute Digimatic caliper (PB3002-S/FACT, Mettler Toledo, Switzerland). The equatorial and longitudinal diameters were measured with a pachymeter (Mitutoyo 500-171-20, Coventry, UK). Ten replicates were performed.

2.2. External Color

The external color was evaluated by a Minolta Colorimeter (model CR-300, Ramsey, NY, USA) on 10 fruits. "L", "a", "b" Hunter parameters were measured, and the results were expressed as a skin color index: (1000a)/(Lb) (Salvador et al., 2007).

2.3. Total Soluble Solids (TSS) and Soluble Tannins (ST)

Three samples of three individual fruits were used to determine TSS and ST. The fruits were cut into four longitudinal parts with the two opposite ends sliced and frozen at -20 °C to determine the ST content. The other opposite fruit parts were placed in an electric juice extractor (model 753, Moulinex, Barcelona, Spain) and filtered through a cheese cloth. The obtained juice was then used to determine the TSS content. ST were evaluated until Day 40 by the Folin–Denis method described by Arnal & Del Río, (2004), and the results were expressed as a percent of fresh weight. The TSS juice was measured in triplicate with a digital refractometer (model PR-1, Atago, Japan) and expressed as Brix.

2.4. Water Content and Water Activity

Three fruits were individually ground in a crushing machine. The water content and water activity (a_w) were measured using a vacuum oven (Vaciotem-T, J.P Selecta, Abrera, Barcelona, Spain) (60 ± 1 °C and pressure < 100 mm Hg) and an Aqualab CX-2 (Decagon Devices Inc, Pullman, WA, USA), respectively. Three replicates were measured per sample.

2.5. Mathematical Modeling of drying Curves

To investigate the drying characteristics of persimmon "Rojo Brillante", six commonly thin-layer drying semitheoretical models (Table 1) were used to fit the experimental drying data.

Table 1. Mathematical models given by several authors for drying curves.

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

k, n, a, g, c, b: Constants of each model applied; t: Time in days.

The non-linear least squares regression analysis was determined by the statistical software Solver (Excel 2016). In these models, *MR* is the dimensionless moisture ratio in Equation (1):

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

where M_i and M_0 are the moisture content (on a dry basis) at any drying time and at the initial time, respectively. M_e is the equilibrium moisture content and is relatively low (about 3%, wb) (Fang et al., 2009), so it can be neglected. Therefore, *MR* can be expressed as M_i/M_o.

The determination coefficient (R^2) is one of the primary criteria for selecting the best model to define the drying curves. Reduced chi-square (X^2), mean bias error (*MBE*), and root-mean-square error (*RMSE*) are used to determine the quality of fit. These parameters can be calculated using Equations (2)–(4):

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

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

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

The higher the R^2, and the lower X^2, *MBE,* and *RMSE,* the better the mathematical model fits the experimental data (Fang et al., 2009). $MR_{exp,I}$ is the experimental moisture ratio, $MR_{pre,I}$ is the predicted moisture ratio, N is the number of observations, and z is the number of constants.

Another criterion, the relative percent error (PE), is used to evaluate the predictive precision of models (Roberts et al., 2008). Lower relative PE values give better fitting models (Equation (5)):

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

The drying rate is represented as ΔM/Δt (the water content to time ratio to the product's average water content between two consecutive weight control times) vs. MR (dimensionless moisture ratio) (Contreras et al., 2008).

The experimental drying data for determining the effective water diffusivity were interpreted by Fick's second law of diffusion. To model the total amount of diffusing water entering the astringent and non-

astringent persimmon samples, the equation in spheres for "long times" was applied (Martinez-Navarrete & Chiralt, 1999). The effective water diffusivity coefficient (D_e) was obtained by fitting the corresponding linear equation (Equation (6)), where *r* is the radius obtained from the longitudinal diameter (m), t is the time in days, and *Y* is the reduced driving force defined by Equation (7), in the dry basis moisture content terms.

$$Ln\,Y = Ln\left(\frac{6}{\pi^2}\right) - \left(\frac{\pi^2 D_e t}{r^2}\right) \quad (6)$$

$$Y = \frac{M_i - M_0}{M_e - M_0} \quad (7)$$

M (g water/g dry solids) at each dehydration (M_i) time in the initial product (M_0) and at the equilibrium time (M_e).

2.6. Statistical Analysis

Data were subjected to an analysis of variance (ANOVA) using the least significant difference (LSD) test with a 95% confidence interval to compare the test averages (Statgraphics Centurion XVII Manugistics, Inc., Rockville, MA, USA).

3. Results and Discussion

3.1. Physico-Chemical Determinations

The drying process brought about a marked gradual weight loss for the first 57 days. Thereafter, the fruit weight decreased only slightly until the end of the assay (Figure 1a). Weight loss paralleled the reduced water content (Figure 1b). The water content dropped from 78% at harvest to 25% after 67 days, before lowering to 15% at 81 days. No significant differences in water loss were found between

the astringent and non-astringent fruits during the whole study period.

The water activity gradually decreased (Figure 1c) from values of 0.980 on Day 0 to values of 0.860 after 57 drying days, with no differences between the astringent and non-astringent fruits. Unlike the water content, the most marked drop in water activity was detected after 57 days. On Day 67, the a_w of the astringent fruit (0.830) was higher than that of the non-astringent fruit (0.760).

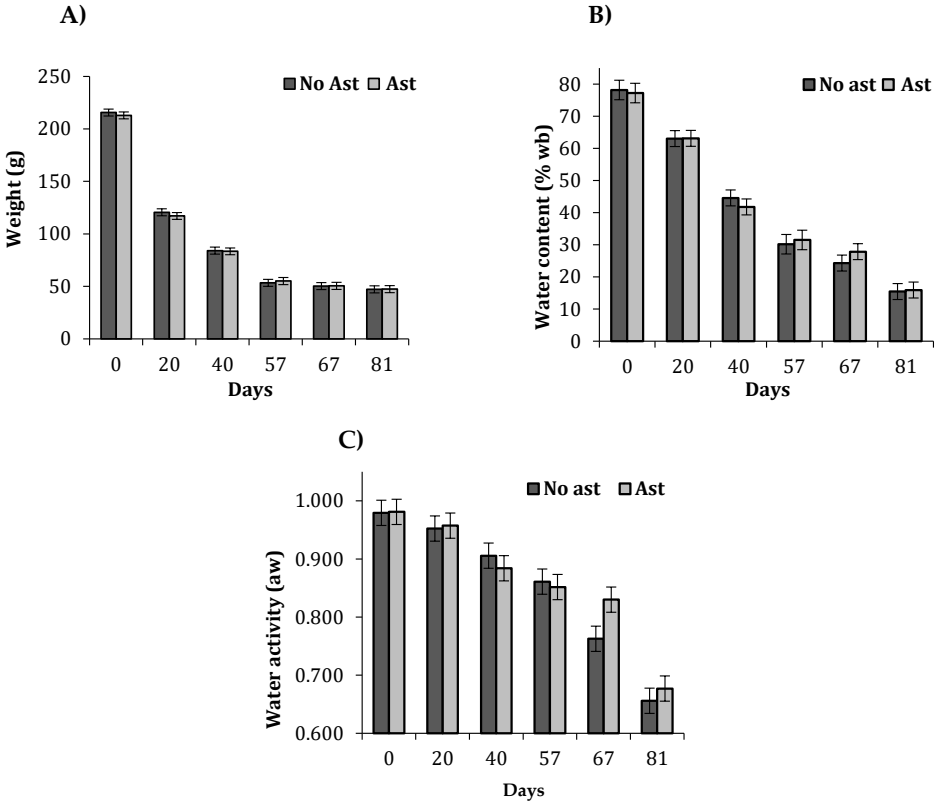

Figure 1. Weight loss (**a**), water content (g/100 g product on a wet basis) (**b**) and water activity (a_w) (**c**) of the non-astringent (No Ast) and astringent (Ast) persimmon samples during the drying treatment. Bars represent the least significant difference (LSD) intervals ($p \leq 0.05$).

According to previous authors, dried persimmon products are classified as semidried or dried depending on the water content (Choi et al., 2017). The final water content of the S Korean semidried and dried

persimmons are approximately 50% and 30%, respectively, with drying periods usually lasting 25 days to achieve 50% and approximately 60 days to accomplish 30% (Sun-wun et al., 2011). Similarly in our study, on Day 40, fruit samples showed 45% water content, which was 30% on Day 57. After 81 days, a drier product was obtained with 15% water content in both the astringent and non-astringent samples. The drying kinetics of this process could be the key to adjust the drying treatment.

Figure 2 illustrates the images of the astringent and non-astringent whole persimmon samples, which are cut longitudinally during the drying treatment from Day 0 to 81.

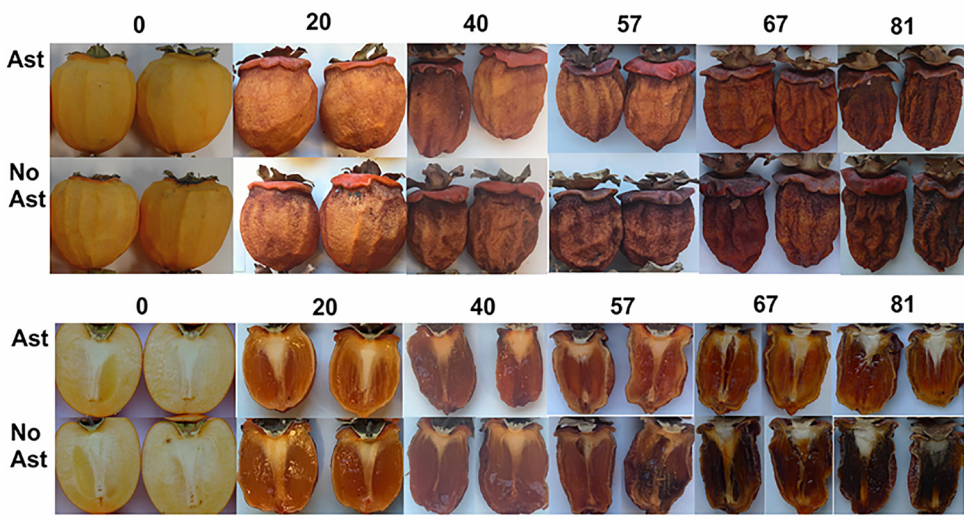

Figure 2. Images of the astringent and non-astringent persimmon cv. "Rojo Brillante" during the drying process.

The water content loss brought about a major reduction in the equatorial diameter up to 57 days (Figure 3a). The longitudinal diameter slightly lowered after 20 days to remain stable during the subsequent drying periods (Figure 3b). The minor changes in the longitudinal diameter that took place during the drying process were due to the position in which the fruits were hung. No significant differences were observed in the shape changes between the astringent and non-astringent fruits. These changes were accompanied by fruit shrinkage, warping, and

wrinkling, which became more evident with the drying time.

During the drying process, the external fruit color darkened (Figure 2). At the beginning of the process, the fruit color index (CI) came close to 18 and reached values near 30 after 40 days with no differences between the astringent and non-astringent fruits. Nevertheless, after 57 days the CI values were significantly higher in the astringent fruit (CI = 46) than in the non-astringent fruit (CI = 40). These differences were still found after 81 days (Figure 3c). The color changes that occurred during the drying period were the result of several biochemical reactions, such as degradation of carotenoids, decomposition of other color pigments, and enzymatic and non-enzymatic reactions (Jia et al., 2019). Yamada et al., (2009) suggested that the oxidative and non-oxidative degradation of ascorbic acid would contribute markedly to the browning of this product type, while enzymatic browning, by polyphenol oxidase, and the Maillard reaction, between amino acids and reducing sugars would not play a key role. From Day 67 to 81 of drying, no significant changes ($p > 0.05$) in the color index of the astringent samples were detected, while the non-astringent samples continued to change.

A marked change in the internal flesh structure was also observed after 20 days by showing gelling symptoms, which became much more evident while the drying process prolonged. Mamet et al., (2017) reported that persimmon tannins enhance the gel properties of pectin, even though mechanisms remain unclear. It is noteworthy that while the internal flesh color remained orange throughout the drying period in the astringent fruit, the flesh acquired a brown coloration from 57 drying days in the non-astringent fruit. This is consistent with the darker external coloration of non-astringent fruit and the significant difference in water activity at 67 days, which may be related to changes in both structure and water retention capacity.

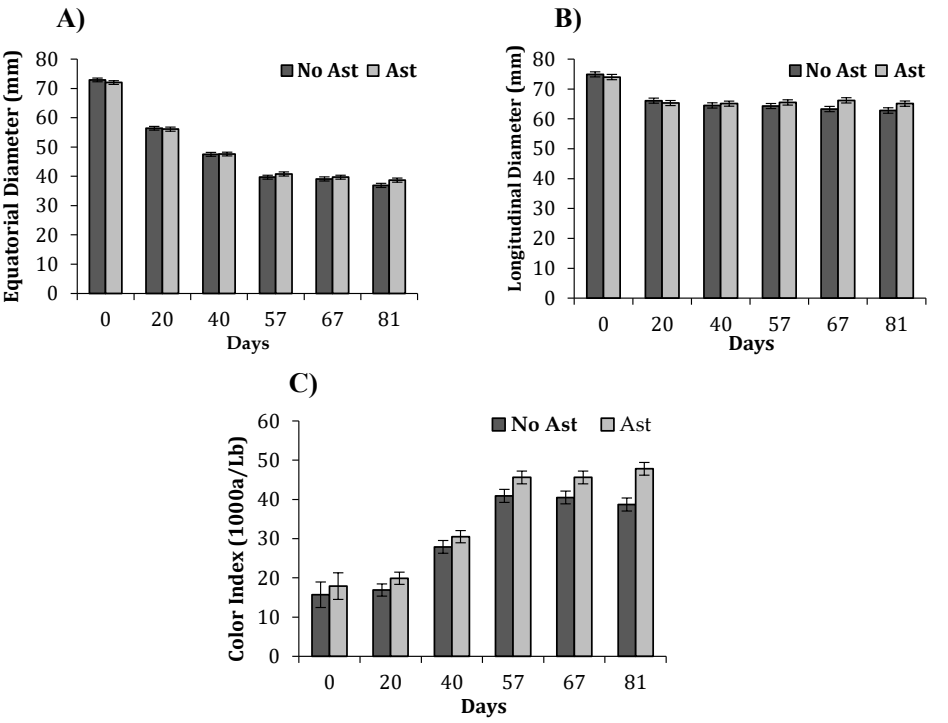

Figure 3. Equatorial diameter (**a**), longitudinal diameter (**b**), and external color index (**c**) of the non-astringent (No Ast) and astringent (Ast) persimmon samples during the drying treatment. Bars represent the least significant difference (LSD) intervals ($p \leq 0.05$).

For TSS (Figure 4a), a gradual increase was observed as the drying process advanced, with values going from close to 18 ºBrix on Day 0 to close to 55 ºBrix after 57 days, with no significant differences between the astringent and non-astringent fruits. After 81 days, the astringent fruit had higher TSS values (63 ºBrix) than the non-astringent fruit (57 ºBrix). As drying progressed, soluble solids became concentrated due to the fact that the water loss and new solids were also generated (Mat Desa et al., 2019). Similar TSS content have been reported in semidried and dried persimmon from South Korea (Im & Lee, 2007; Sun-wun et al., 2011).

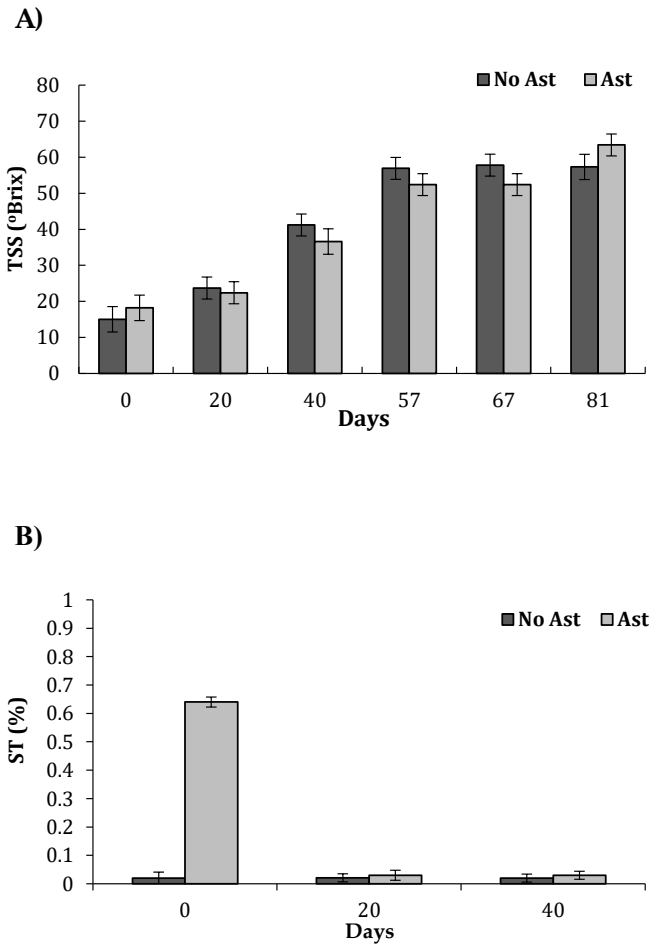

Figure 4. Total soluble solids (TSS) of the non-astringent (No Ast) and astringent (Ast) persimmon samples during the drying treatment (**a**). Total soluble tannin content (ST) of the non-astringent (No Ast) and astringent (Ast) persimmon samples up to the drying treatment at Day 40 (**b**). Bars represent the least significant difference (LSD) intervals ($p \leq 0.05$).

Initially on Day 0, the astringent persimmons, not previously submitted to the de-astringency treatment, had an ST content of 0.6% (Figure 4b). These values fall within the range found by most previous studies conducted on "Rojo Brillante", which have been related to high astringency levels in fruits (Arnal & Del Río, 2003; Salvador et al., 2007; Taira et al., 1997). In contrast, the non-astringency fruit, submitted to the de-astringency treatment with a high CO_2 concentration, gave ST content values of 0.02%, which are sensory non astringency values for "Rojo

Brillante" (Arnal & Del Río, 2003; Salvador et al., 2007). After 20 drying days, the ST content values in the astringency fruit notably dropped to 0.03%. The ST values were similar to those of the non-astringent fruit. Tannin insolubilization during the drying process could be associated with structural flesh changes (Figure 2), which happens during natural persimmon fruit ripening (Asgar et al., 2004; Jia et al., 2019; Tessmer et al., 2016). In astringent cultivars, the ripening process is accompanied by gradual tannin insolubilization, which leads to a progressive decline in ST with subsequent astringency reduction (Salvador et al., 2007). The softening that occurs during fruit ripening leads to pectin solubilization, which forms a complex with tannins and brings about their insolubilization (Taira et al., 1997). Asgar et al., (2004) have also related the flesh structural changes found during the sun-drying of Japanese persimmon to the solubilization and depolymerization of pectin polysaccharides.

3.2. Fitting of drying Curves and drying Rate Determinations

The water content data obtained at the different drying times were converted into a dimensionless moisture ratio (Equation (1)) and then fitted to six thin-layer drying models (Table 1). These models have been used for agricultural products (Akpinar, 2006; Sampaio et al., 2017). To estimate the parameters from those six models, a non-linear regression analysis was used with both the astringent and non-astringent persimmon samples. The statistical results of the models are summarized in Table 2 (astringent samples) and Table 3 (non-astringent samples). The best models describing the thin-layer drying characteristics of the persimmon samples were chosen with the highest R^2 values and the lowest X^2, *MSE*, *RMSE*, and *PE* values. In both cases, R^2 values were higher than 0.995, while X^2, *MBE*, and *RMSE* were ≤ 0.001, ≤ 0.018, and 0.000, respectively. PE values were between 2%–10% for all the models assessed in both samples. These results were in agreement with those found in fruits such as cape gooseberry (Vega-Gálvez et al., 2014), pomegranate (Mundada et al., 2010), apple or pumpkin (Akpinar, 2006).

Table 2. Values of the parameters of the models for astringent persimmon "Rojo Brillante".

	Models Parameters						Statistical Parameters				
Models	k	n	a	g	c	b	R^2	X^2	*MBE*	*RSME*	*PE%*
Newton	0.035	-	-	-	-	-	0.997	0.001	0.017	0.000	7.071
Page	0.030	1.043	-	-	-	-	0.997	0.001	0.017	0.000	9.021
Midilli et al.	0.027	1.101	1.028	-	-	0.000	0.998	0.000	0.011	0.000	10.243
Logarithmic	0.039	-	1.022	-	0.019	-	0.998	0.000	0.012	0.000	7.965
Henderson & Pabis	0.037	-	1.037	-	-	-	0.998	0.000	0.012	0.000	7.586
Verma model	0.036	-	0.007	0.036	-	-	0.998	0.001	0.018	0.000	7.663

k, n, a, g, c, b: Constants of each model applied. R^2: determination coefficient; X^2: reduced chi-square; MBE: mean bias error; RMSE: root-mean-square error; PE%: relative percent error.

Table 3. Values of the parameters of the models for non-astringent persimmon "Rojo Brillante".

	Models Parameters						Statistical Parameters				
Models	k	n	a	g	c	b	R^2	X^2	*MBE*	*RSME*	*PE%*
Newton	0.037	-	-	-	-	-	0.999	0.000	0.008	0.000	2.305
Page	0.032	1.040	-	-	-	-	0.999	0.000	0.010	0.000	4.632
Midilli et al.	0.041	0.983	1.042	-	-	0.000	0.999	0.000	0.002	0.000	1.943
Logarithmic	0.039	-	1.028	-	0.011	-	0.999	0.000	0.002	0.000	2.032
Henderson & Pabis	0.038	-	1.037	-	-	-	0.999	0.000	0.004	0.000	3.095
Verma model	0.037	-	0.017	0.037	-	-	0.999	0.000	0.008	0.000	2.305

k, n, a, g, c, b: Constants of each model applied. R^2: determination coefficient; X^2: reduced chi-square; MBE: mean bias error; RMSE: root-mean-square error; PE%: relative percent error.

Figure 5a,b shows the Midilli, Vermal, and Logarithmic models selected to represent the drying characteristics of the whole astringent and non-astringent persimmon samples. After fitting the experimental data, it took 34 days to reach a water content of 50% and 57 days to obtain 30%. Under these conditions, the desirable semidried and dried persimmon would be obtained. Figure 5c shows the effect of $\Delta M/\Delta t$ vs. MR on the drying rate of the astringent and non-astringent samples, where a good correlation was obtained (R^2 = 0.9855). In both the sample types, the drying rate was rapid during the initial period, but then slowed down in the later stages, and no constant rate of the drying period was observed. The entire drying process occurred during the falling-rate period. When the decrease in the drying rate was linear with the water content, water evaporation in the material continued

as during the constant rate period. This indicates that mass transfer is governed by intrinsic product properties and internal resistance to water diffusion to the surface (Mulet, 1994). This result was similar to those reported for the thin-layer drying of other biomaterials (Contreras et al., 2008; Doymaz, 2004, 2005).

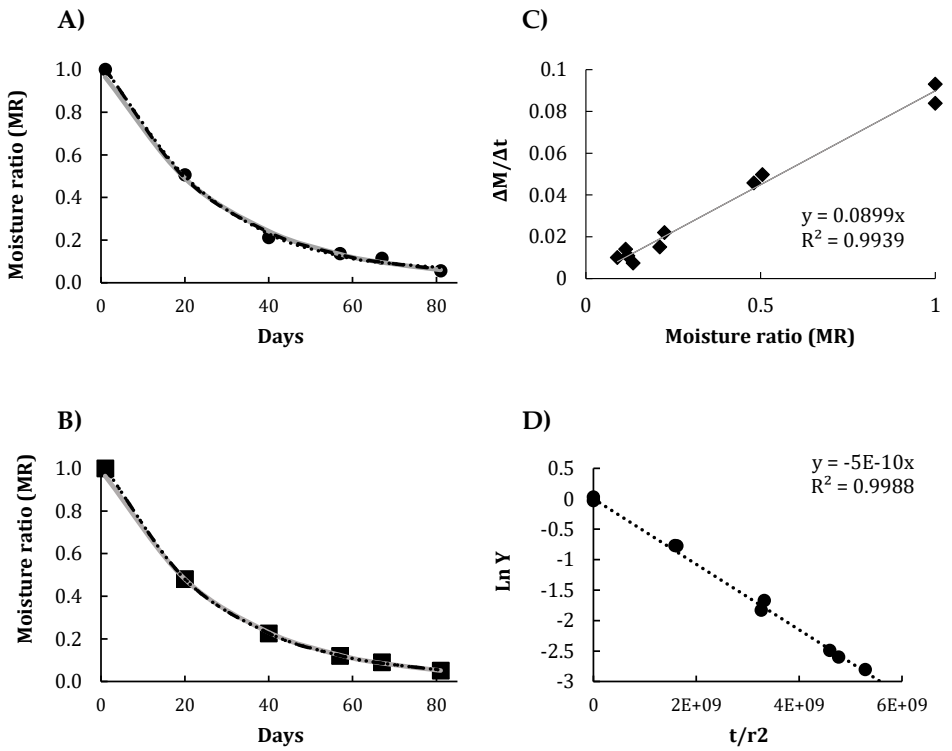

Figure 5. Modeling the drying curves of the astringent **(a)** and non-astringent **(b)** persimmon fruits with the Midilli (dotted line), Logarithmic (dashed line), and Verma models (gray line). Drying rate of the astringent and non-astringent persimmon samples **(c)**. Effective water diffusivity (D_e) determination by equation $Ln\,Y = Ln\left(\frac{6}{\pi^2}\right) - \left(\frac{\pi^2 D_e t}{r^2}\right)$ in the astringent and non-astringent persimmon samples **(d)**.

In Figure 5d, Ln Y vs. t/r^2 is plotted to determine, from the slope ($\pi^2 D_e$), the effective water diffusivity of both the astringent and non-astringent persimmon samples. Fick's second diffusion law has been widely used to describe the drying process during the falling-rate period for biological material (Doymaz, 2008). The effective water diffusivity results were

similar in both the astringent and non-astringent persimmon samples, with values of 5.07×10^{-11} and 6.08×10^{-11} m^2 s^{-1}, respectively. The R^2 was 0.996. The D_e values fell within the general range of $10^{-12} - 10^{-8}$ m^2 s^{-1} in food materials (Zogzas & Maroulis, 1996). Similar results were reported by Doymaz, (2012) in persimmon slices (between 7.05×10^{-11} and 2.34×10^{-10} m^2 s^{-1}).

4. Conclusions

For the first time, the kinetics during the natural drying method followed in Asian countries (hanging the whole fruit in a well-ventilated place) was studied in "Rojo Brillante" persimmon. Since "Rojo Brillante" is an astringent cultivar, the behavior of the astringent and non-astringent fruits (submitted to the CO_2 treatment) was compared. The used thin-layer mathematical models were suitable for fitting the drying kinetics. No significant differences between the astringent and non-astringent fruits were found. Around 34 days were needed to reach a final water content of 50% and 57 days to reach one of 30%. This drying treatment was able to produce a natural decrease in ST contents. The astringent and non-astringent fruits obtained similar values in just 20 days. Different behaviors between the astringent and non-astringent samples were observed in a_w, external and internal color at 57 drying days. The astringent fruit remained orange, while brown coloration developed on the non-astringent fruit. Hence, the de-astringency treatment is not recommended. This natural drying technology, not yet applied to the "Rojo Brillante" persimmon industry, could be a good strategy to enhance the surplus of this seasonal fruit.

Author Contributions:

Investigation, validation, methodology, formal analysis, writing—original draft preparation, C.M.G.; investigation, validation, methodology,

formal analysis, R.G.; supervision, resources, conceptualization, funding acquisition, writing—review and editing, G.M.; supervision, resources, conceptualization, funding acquisition, writing—review and editing, A.S. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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Capítulo 2:

Uso de la liofilización para el desarrollo de un producto de alto valor nutritivo a partir de destríos postcosecha de caqui.

Water sorption and glass transition in freeze-dried persimmon slices. Effect on physical properties and bioactive compounds.

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ABSTRACT

The use of persimmon variety "Rojo Brillante", has seen a great expansion in recent years. Its production is associated with substantial amounts of post-harvest waste, therefore, development of products that allow its valorisation are of great interest. In this study, a freeze-drying technique was used to obtain a high quality product. Freeze-dried samples were conditioned in a range of water activities (0.113 - 0.680) at 20 °C at equilibrium, allowing for products of different water content. Water sorption isotherms were determined from persimmon slices, with BET (Brunauer, Emmett, and Teller) and GAB (Guggenheim, Anderson, and de Boer) models applied to the sorption data. The glass transition was analysed using differential scanning calorimetry (DSC); the Gordon & Taylor equation modelled the water plasticisation effect. Results confirmed a critical water activity (CWA) of 0.165 and a critical water content (CWC) of 0.0312 g water/g product. Below these critical values, the glassy state of the amorphous matrix and the crispness were guaranteed. This consequently avoids an increase in the rate of deterioration reactions, texture and colour changes, and the loss of the fruit bioactive compounds.

Keywords: Kaki, sorption isotherm, tannins, physicochemical properties, freeze-drying.

1. INTRODUCTION

Fruits are an essential part of the human food intake and a balanced diet which plays a crucial role in the prevention of chronic diseases and premature death (WHO, 2003). Persimmon is a consistent source of many bioactive compounds such as polyphenols and carotenoids besides vitamins, minerals, and dietary fibre (Jang et al., 2010). These components play an important role in the prevention of atherosclerosis, especially coronary atherosclerosis (Gorinstein et al., 2001). Many

studies related to persimmon's bioactive compounds and nutritional composition have been described (Hernández-Carrión et al., 2014).

Seasonal persimmon (*Diospyros kaki L.*) crops have been steadily increasing in Spain and have now reached a yearly production of 100,000 tonnes. This increase in production has been linked to a significant increase in volume losses, estimated as a post-harvest waste between 25 - 30% of the product collected (Munera et al., 2017). Since there is no management system that takes advantage of the persimmon waste, these losses generate an additional cost, caused by the need to remove the discarded product from the storage warehouses.

An interesting way of adding valuing to the waste from the agri-food industry is based on obtaining new products or alternative ingredients from this waste. There is also a constant interest for offering consumers new products that stimulate fruit consumption. Therefore, the development of dried fruits could be an interesting alternative. Freeze-drying appears to be the dehydration method which produces higher quality products and minimal changes in colour and nutrients (Krokida, Karathanos, & Maroulis, 1998). Thus, although freeze-drying is more expensive than other dehydration techniques, it is a good approach for obtaining new health products.

Because of their high hygroscopic and thermoplastic behaviour, freeze-dried fruits are especially sensitive to alteration over time. During the removal of water, the formation of an amorphous matrix, which may change from a glassy state to a liquid-like rubbery one, occurs at the glass transition temperature (T_g). In the rubbery state, the rate of diffusion-controlled reactions increases, thus crispy products undergo a loss of crispness, becoming texturally unacceptable (Roos, 1995; Mosquera, Moraga, & Martínez-Navarrete, 2012). Changes in the atmosphere's relative humidity (RH) in contact with the dried product will suggest an evolution in their water activity (a_w). According to the corresponding sorption isotherm, changes in the water content, that can induce phase transitions, affect food quality and stability. Therefore,

analysing their water sorption isotherms and phase transitions is key to obtaining the critical values of water activity and water content that define the quality loss of the product (Moraga, Martínez-Navarrete, & Chiralt, 2006; Telis & Martínez-Navarrete, 2010).

Here, we aimed to study the effect of glass transition on the mechanical and optical properties and bioactive compounds content during storage of freeze-dried persimmon slices. The state diagram of the fruit liquid phase and the water sorption isotherm were obtained to determine the critical water activity and water content, allowing maintenance of the crispness and stability of the final product.

2. Material and methods

2.1. Sample preparation

Persimmon "Rojo Brillante" fruit, treated by CO_2 95% during 24 h at 20 °C, was provided by "Instituto Valenciano de Investigaciones Agrarias" (IVIA, Valencia, Spain). These commercial persimmon fruits were harvested in early December corresponded to ripening IV (Tessmer et al., 2016). The fruits (pulp and skin) were washed and cut transversally into slices (10 mm thick), rapidly frozen in an ultra-freezer Infrico ULF700 (Equitec, Valencia, Spain), at -80 °C and freeze-dried in a Telstar Lioalfa-6 Lyophyliser (Telstar, Azbil Group, Terrassa, Spain) at 10^{-2} Pa and -40 °C for 68 h. Freeze-dried slices were placed at 20 °C in hermetic chambers containing saturated salt solutions (LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, and $NaNO_3$), allowing the slices to reach a constant relative humidity (RH) ranging between 11 and 68% (Greenspan, 1977). The sample weights were controlled until a constant value (μg < ± 0.005 g) was reached, where the equilibrium was assumed. Thus, assuring that the water activity (a_w) of each sample was equal to the corresponding RH/100. The samples with different a_w were analysed after 3 months of storage and recently freeze-dried samples

were used as the control. All analyses described were conducted in triplicate; except for the determination of the optical and mechanical properties, where 12 replications were performed.

2.2. Water content

The water content of the control and equilibrated samples was obtained by vacuum drying the samples in a vacuum oven (Vaciotem, JP Selecta SA, Spain) at 60 ± 1 °C and a pressure < 100 mm Hg until constant weight.

2.3. Water activity (a_w)

An Aqualab CX-2 (Decagon Devices, Inc., Pullman, USA) was used to measure the a_w of the control sample. With the samples equilibrated in the hermetic chambers, the a_w was assumed to be equal to the corresponding RH/100.

2.4. Sorption isotherms

In each equilibrated sample, the corresponding equilibrium water content was analysed as described in section 2.2. These values were used to construct the sorption isotherms. To predict the water sorption behaviour of the samples, the BET (Brunauer, Emmett, and Teller) and the GAB (Guggenheim, Anderson, & de Boer) models were used, Eq. 1 and 2, respectively.

$$w_e = \frac{w_o\, C\, a_w}{(1-a_w)\,(1+(C-1)\,a_w)} \qquad (1)$$

Where, w_e is the equilibrium water content (g water/g solids), a_w is water activity, w_o is the monolayer moisture content (g water/g solids), and C is the sorption energy constant.

$$w_e = \frac{w_o\, C\, K\, a_w}{(1-K\, a_w)\,(1+(C-1)\, K\, a_w)} \quad (2)$$

Where, w_e is water content (g water/g solids), a_w is water activity, w_0 is the monolayer moisture content (g water/g solids), and C is the sorption energy constant related to monolayer sorption heat, and K is the constant related to multilayer sorption heat. The nonlinear regression analysis was determined by the statistical software Solver (Excel 2016).

2.5. Calorimetric analyses

Calorimetric analyses were conducted in each equilibrated sample to analyse the glass transition temperature (T_g). Approximately 10 mg were placed into DSC pans (P/N SSC000C008, Seiko Instruments), sealed and analysed using a DSC Q2000 V24.11 (TA Instruments). The heating rate was 5 °C/min and the temperature ranged between -85 to 100 °C, depending on the sample's water content. The mid-point temperature was the glass transition. Experimental T_g-x_w (g water/g product) data was fitted to the Gordon & Taylor model (Eq. (3)).

$$T_g = \frac{(1-x_w)T_{g(as)} + kx_w T_{g(w)}}{(1-x_w) + kx_w} \quad (3)$$

Where, x_w is the mass fraction of water (g water/g product), T_g the glass transition temperature (°C), $T_{g(w)}$ the glass transition temperature of amorphous water (-135 °C), $T_{g(as)}$ the glass transition temperature of anhydrous solids predicted by the model (°C), and k is the model constant.

2.6. Mechanical properties and colour analyses

The mechanical behaviour of the control and equilibrated samples was registered with a puncture test using a Universal Texture Analyser (TA.XT2, Stable Micro Systems, Surrey, England). A cylindrical (4 mm

diameter) punch was used applying a relative deformation of 75% and a distance rate of 20 mm/s. The parameters analysed in the puncture test were the maximum force (Fmax), expressed in N and the area, up to a distance corresponding to the maximum force expressed in N/mm. The crispness was considered as the number of fracture peaks registered over 15 N, when produced in each plot, during the puncture test.

The colour was measured (12 replicates) with a Colorimeter Minolta (CM-3600d, Japan). A reflectance glass (CR-A51, Japan) was placed between the sample and the spectrophotometer. The measurement window was 6 mm diameter and a D65 illuminant/10° observer was selected to obtain CIE *L*a*b** colour co-ordinates, the chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($h_{ab}^* = \text{arctg}(b^*/a^*)$). The total colour difference ($\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$) was calculated with respect to the control sample.

2.7. Bioactive compounds

The total soluble tannin content, antioxidant capacity, and carotenoids content of fresh, control, and equilibrated samples were analysed according to Hernández-Carrión et al., (2014) with modifications.

2.7.1. Total soluble tannin content

Persimmon samples (5 g) were homogenised in an Ultraturrax (IKA T18 digital, Germany) with 25 mL of ethanol (96%). Homogenates were centrifuged (8000 rpm, 30 min, 4 °C) and filtered, while keeping the supernatant. More supernatant was extracted from the pellet with 25 mL of ethanol and added to the first supernatant. The total volume of supernatant was brought to 100 mL with ethanol. In a test tube, 1 mL of the extract and 6 mL distilled water were mixed and vortexed. Thereafter, 0.5 mL of Folin-Ciocalteu reagent was added. After 3 min, 1 mL saturated Na_2CO_3 was added, vortexed, and 1.5 mL distilled water was added. Absorbance was measured after 90 min at 725 nm

for determination of soluble tannin content. The calibration curve was performed at different concentrations of gallic acid in ethanol. Results were expressed as g gallic acid/100 g of dry matter.

2.7.2. Antioxidant capacity

Antioxidant activity was measured by ferric reducing antioxidant power assay (FRAP). Extracts were obtained in the same way as for total soluble tannin content determination. Distilled water (30 µL), sample (30 µL), and FRAP reagent (900 µL) were placed in each cuvette. Cuvettes were incubated over 30 min in a water bath covered with aluminium foil, at 37 °C, the absorbance was measured at 595 nm. The calibrated curve was performed using different concentrations of Trolox in 960 g/kg ethanol. Results were expressed as µmol Trolox/g (dry matter) of sample.

2.7.3. Carotenoid content

Homogenised persimmon samples (5 g) were extracted five times with 25 mL cool acetone using an Ultraturrax (IKA Ultraturrax T25 Basic) and vacuum filtered until no more colour was extracted. The extract was added gradually to 50 mL ethyl ether in a decanting funnel. With each addition of extract, enough NaCl solution (100 g/L) was added to separate the phases and to transfer the pigments to the ether, while the aqueous phase was removed. The process was carried out in several steps to ensure the greatest elimination of aqueous phase. The organic phase was treated several times with anhydrous Na_2SO_4 (20 g/L) to remove residual water, it was then evaporated until dry in a rotary evaporator (model RII; Buchi Labortechnik, Flawil, Switzerland) at a temperature ≤ 45 °C. The pigments were collected with acetone, a volume of 100 mL and the absorbance was measured at 450 nm using a spectrophotometer (CE 1021 1000 series, CECIL INSTRUMENTS Cambridge). The calibration curve was performed with different concentrations of β–carotene in acetone. Results were expressed as mg β–carotene/100 g of dry matter.

2.8. Field emission scanning electron microscopy (FESEM) and light microscopy (LM)

For FESEM observations, cubes (0.5 cm^3) of the samples were cut with a razor blade, vacuum coated with platinum, and observed in an Ultra 55 FESEM (Zeiss, Oberkochen, Germany).

For LM observations, thin sections (0.2 cm) were cut with a razor blade and placed on slides and stained with vanillin-HCl (1:1, v/v) to identify tannins. Images were captured under a light microscope (Nikon Eclipse E800 V-PS100E, Tokyo, Japan), andstored at 1,280 x 1,024 pixels using the microscope software (NIS-Elements F, Version 4.2, Nikon, Tokyo, Japan).

2.9. Statistical analysis

Data were subjected to variance analysis (ANOVA), using the least significant difference (LSD) test with a 95% confidence interval for the comparison of the test averages (Statgraphics Centurion XVII Manugistics, Inc., Rockville, MA, USA).

3. Results and discussion

3.1. Water sorption isotherms

After the freeze-drying process the control persimmon slices' a_w was 0.183 and the water mass fraction was 0.0378 g water/g sample. The conditioning of samples allowed plotting of the equilibrium water content (dry basis) with the respective water activity at 20 °C. To predict the water sorption behaviour, the BET and GAB equations (Eq. 1 and 2) were fitted with experimental points (Figure 1A).

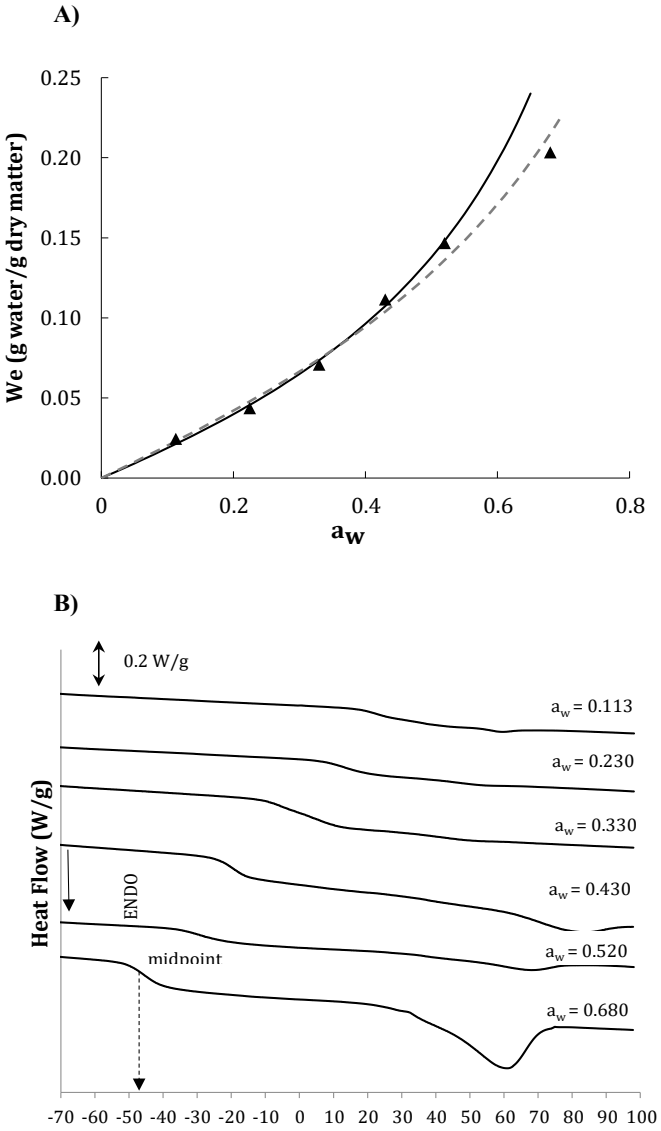

Figure 1. A) Water sorption isotherms of freeze-dried persimmon slices at 20 ºC. Experimental points (▲), fitted BET (■), GAB (———) models. **B)** DSC thermograms obtained in freeze-dried persimmon slices at different water activities.

Only experimental data up to $a_w \leq 0.5$ were fitted to the BET model. From that value, the model hypothesis fails, and the equation cannot predict sorption behaviour because of the prevailing effect of solute-solvent interactions. The parameters of w_0 (monolayer moisture content) and C (sorption energy constant) from both BET and GAB models, were similar despite the theoretical limitations of the BET adsorption analysis. The w_0 values ranged between 0.112 and 0.138 g water/g dry solids for BET and GAB, respectively. Comparable results were found by Sobral et al. (2001) and Telis et al. (2000) in persimmon fitted with the GAB model. The BET parameter indicates the amount of water adsorbed on the food surface and has been related to food stability in low-moisture products (Kubisiak et al., 1980). Therefore, in this study, it cannot overcome a water activity of 0.443, since the water content in equilibrium exceeds the value of w_0. This crucial tool is frequently used to control the stability of dried fruit during storage.

The constant C, relating to the sorption energy, allows the classification of sorption isotherms according to Brunauer's classification (Brunauer et al., 1940). Thus, persimmon isotherms can be classified as type III, since C values were < 2 (1.58 and 0.82 in BET and GAB, respectively), as has been found in other fruits (Yanniotis & Blahovec, 2009).

3.2. Glass transition (Tg) temperatures of freeze-dried persimmon.

All thermograms obtained from DSC (Figure 1B) showed the typical second-order transition with a clear endothermic shift in the heat flow curve related to the change in the heat capacity of the sample. No ice was formed during freezing since no endotherms associated with ice crystal melting were observed. For $a_w > 0.430$ a melting endotherm was observed, more pronounced as the water content increased, at temperatures between 60 - 85 °C (decreasing as the amount of water increased). This can be because of the fusion of solutes crystallised during equilibration. Figure 1B shows the T_g temperatures of each equilibrated sample. The sample equilibrated at a_w of 0.113 solely

presented a value of T_g > 20 °C (T_g mid-point = 23.01 °C), therefore, the only sample found in the glassy state. The increase in the water content drives the depression of T_g, which was modelled by the Gordon & Taylor equation (Eq. 3) obtaining the parameters T_{gs} = 43.10 °C and k = 4.628 (R^2 = 0.985). Similar results were determined in different fruits such as kiwi or borojó in the same domain of a_w, where T_g values were lower than 30 °C for a_w of 0.113 (Moraga et al., 2006; Mosquera et al., 2010).

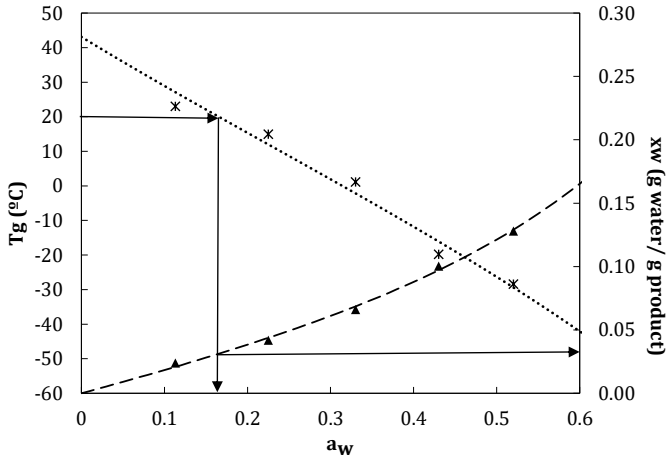

Figure 2. Relationships of freeze-dried persimmon slices considering the midpoint (×) of the glass transition. Experimental points (▲), BET, and Gordon & Taylor fitted models. Water content (Xw) water activity (a_w) (———); glass transition temperature (Tg) water activity (····).

To obtain the critical water content (CWC) and the critical water activity (CWA) to preserve the product in a glassy state, the combined T_g-x_w-a_w data and the BET and Gordon & Taylor fitted models were used (Figure 2). Despite the GAB model being the most common, the BET model fitted the experimental points better (Figure 1A) up to 0.520 a_w. Even though the last point at a_w = 0.680 fitted well with the GAB model; because of the high enzymatic browning of the sample at that point, it was rejected. At room temperature (20 °C), the CWA for the glass transition of freeze-dried persimmon was 0.165 and the corresponding CWC was 0.0312 g water/g product (Figure 2). Under these conditions,

it would ensure the glassy state throughout storage. Hence, the value of w_0 cannot be considered a critical water content to assure quality preservation during storage. Besides, the freeze-drying process applied in this study was not enough to ensure a complete glassy state of the persimmon slices at 20 °C. This can be for the high hygroscopicity the samples presented or the freeze-drying conditions used not being sufficient. Considering refrigeration storage conditions (4 °C), the expected CWA (a_w = 0.270) and CWC (0.0568 g water/g product) values would become higher, thus allowing the glassy state of the freeze-dried persimmon slices and increased stability range (data from Figure 2). The T_g-x_w-a_w relationship (Figure 2), considered as a state diagram, also allows setting 16.5 °C, as the maximum temperature at which the obtained freeze-dried control sample would be in a stable glassy state.

Therefore, this state diagram is a useful tool for the optimisation of processing requirements and storage conditions of the sample.

3.3. Mechanical properties and colour analysis

The puncture test performed on the samples allowed detection of changes in their mechanical properties relating to the different water activity levels (Table 1). Figure 3 and Table 1 show the sample at a_w = 0.113, in a glassy state, presented as crispy, shown by the many fracture peaks, without significant difference ($p > 0.05$) comparable to the control. In the glassy state, samples lose their ability to deform; the lower water content, the more brittle the sample (Boudhrioua et al., 2002). Once exceeding the critical values of CWC and CWA, the state change caused a significant decrease ($p < 0.05$) in the number of fracture peaks seen in the sample with a_w = 0.230. From a_w = 0.330 the curves were different, the crispiness of the samples disappeared, and no fracture peaks were detectable. Table 1 shows the average values of maximum force (N) and the area under the puncture curve (N/mm) at the different water activity levels. The sample at a_w = 0.113 and a_w = 0.230 compared to the control did not present a significant difference

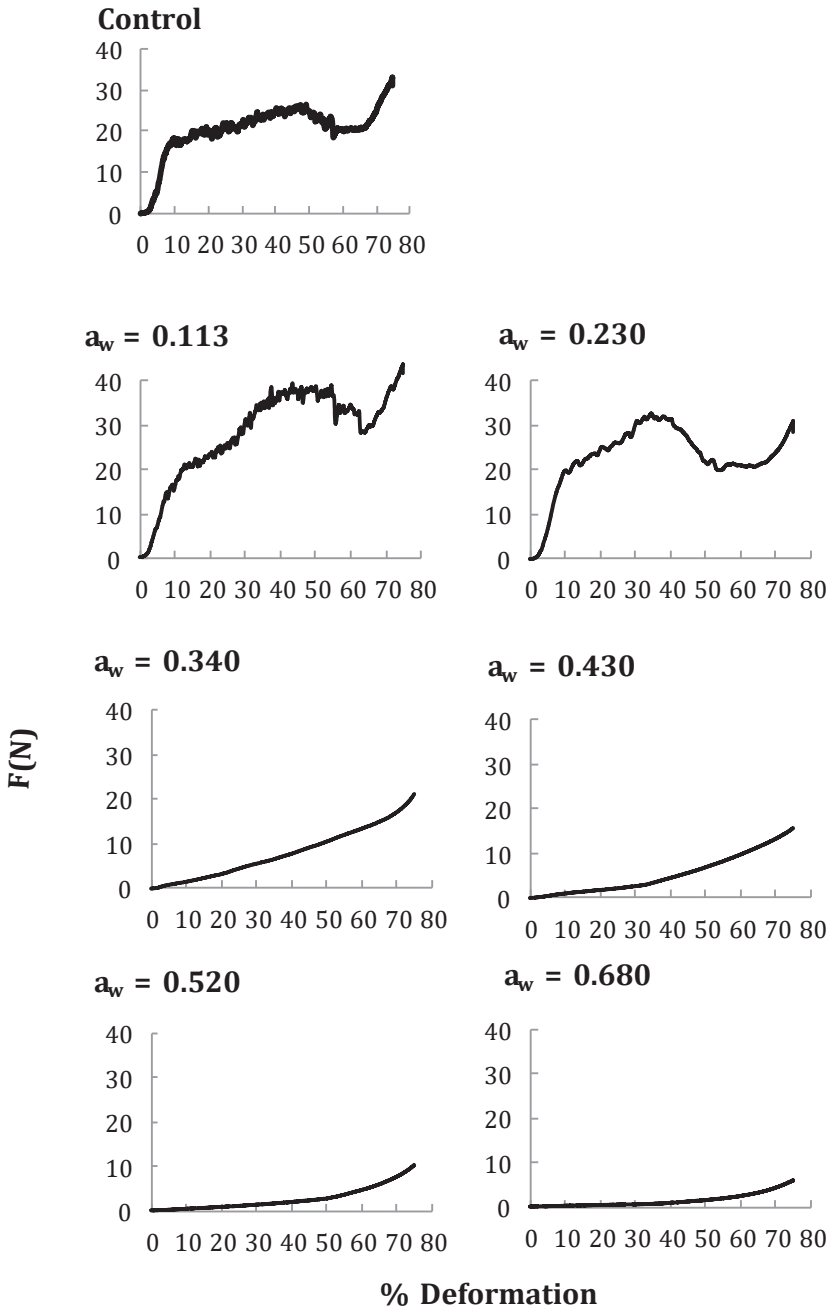

Figure 3. Force (N) vs % of deformation curves obtained from the puncture test of freeze-dried persimmon samples at different water activity levels.

Table 1. Maximum force (Fmax), area under puncture curve and number of fracture peaks of freeze-dried persimmon slices at different water activity levels.

Sample	Fmax (N)	Area (N.mm)	Number of Peaks
Control	27.7^{a} (4.9)	13.4^{a} (2.6)	66^{a} (13)
a_w = 0.113	30.3^{a} (6.1)	14.9^{ab} (2.8)	76^{a} (8)
a_w = 0.230	34.3^{a} (7.9)	17.1^{b} (3.4)	21^{b} (7)
a_w = 0.330	16.2^{b} (2.5)	7.5^{c} (2.2)	-
a_w = 0.430	14.0^{bc} (2.8)	4.7^{d} (2.3)	-
a_w = 0.520	11.0^{cd} (2.7)	2.5^{e} (0.7)	-
a_w = 0.680	8.9^{d} (1.9)	2.0^{e} (0.4)	-

Means values in a column with different superscript differ significantly ($p < 0.05$) according to ANOVA (LSD multiple range test).

($p > 0.05$) in the maximum force and area parameters. A significant decrease ($p < 0.05$) in all the mechanical parameters analysed was seen from a_w of 0.330 to 0.680, showing the collapse of the structure. This phenomenon occurs because of the continuous softening of the product when the water activity and the water content increased.

Therefore, an important change is observed in the mechanical properties relating to the glass transition. This is in agreement with the results seen in other samples, such as apple and banana chips or wafers in similar conditions (Martínez-Navarrete et al., 2004; Moraga et al., 2011).

Regarding the colour evolution, Table 2 shows the mean values and standard deviation of the luminosity (L^*), hue angle (h_{ab}^*), and chrome (C^*) of the control and freeze-dried persimmon samples at the different water activity levels. L^* values remained without significant difference ($p > 0.05$) from the control sample up to a_w of 0.430, showing a significant decrease ($p < 0.05$) after that point, because of the water

content uptake. The h_{ab}* value of the equilibrated samples significantly increased ($p < 0.05$) compared to the control, giving a greater yellow hue angle in all samples after 3 months storage. An exception was for the sample with $a_w = 0.680$, because of the enzymatic browning giving a more orange hue angle. Ling et al., (2005) described the enzymatic browning because of polyphenol oxidase (PPO) action, that can be inhibited at reduced water activity values. In samples with low a_w, colour changes can be related to the non-enzymatic browning reaction because of the concentration and interactions between sugars and amino acids in the fruit (Telis & Martínez-Navarrete, 2010).

Table 2. Colour attributes (L*, hab*, C*) and total colour differences (ΔE*) respect to the control sample of freeze-dried persimmon slices at different water activity levels.

Sample	L*	h_{ab}*	C*	ΔE*
Control	79^{a} (2)	68^{c} (1)	35^{a} (1)	0
$a_w = 0.113$	79^{a} (4)	79^{a} (2)	27^{b} (3)	10
$a_w = 0.230$	77^{a} (3)	74^{b} (3)	33^{a} (3)	4
$a_w = 0.330$	78^{a} (4)	75^{b} (4)	33^{a} (5)	4
$a_w = 0.430$	75^{a} (3)	73^{b} (3)	36^{a} (5)	6
$a_w = 0.520$	67^{b} (5)	73^{b} (3)	36^{a} (3)	13
$a_w = 0.680$	48^{c} (5)	62^{d} (5)	24^{c} (4)	33

Means values in a column with different superscript differ significantly ($p < 0.05$) according to ANOVA (LSD multiple range test).

The total colour difference values (ΔE*) (Table 2) were appreciable by the human eye (ΔE* > 3) for all the samples and increased as the water activity level increased. An exception was observed at a_w of 0.113, which produced a colour difference of 10 units, caused by the different physical state of the sample. The different physical state would also be responsible for the low value of C* in this sample. From the value

of a_w = 0.430, greater colour differences were seen. This agrees with several authors who considered this value a limit at which the rate of food enzymatic reactions can arise to (Venir et al., 2007). In addition, the water content at a_w of 0.430 was 11.2 g/100g dry basis coinciding with the value associated with the BET monolayer moisture content, much greater than the CWA and CWC. Therefore, the colour changes are associated with the glass transition and with the amount of water available, allowing movement of the reactants. Previous findings in banana fruit and grapefruit powder showed comparable results (Moraga et al., 2011; Telis & Martínez-Navarrete, 2010).

3.4. Total soluble tannin content

Soluble tannins are an important fraction of astringent persimmons. After a de-astringency treatment, astringency decreases and tannin compounds are transformed into their insoluble forms (Pérez-Burillo et al., 2018). There was a significant ($p < 0.05$) difference for soluble tannins found in the fresh fruit (0.154a ± 0.003 g/100 g dry basis) and the freeze-dried control (0.229b ± 0.016 g/100 g dry basis). Ice crystals formed during freezing can break the cellular structure, thus facilitating an entrance for a solvent; thus, the extraction of phenolic compounds is improved (Leong & Oey, 2012). Furthermore, (Wu et al., 2010) explained that the freeze-drying process increases the porosity of the food tissue, making it more efficient at the extraction of phenolic compounds.

Figure 4A shows the total soluble tannin content of the equilibrated samples. A significant ($p < 0.05$) decrease was seen in all the samples than the control. Previous studies have described that no significant changes for total phenols were observed after 3 months of storage (Moraga et al., 2012; Cheng et al., 2017). Hence, the lower soluble tannin content could be by the tannin insolubilisation. These results agree with previous studies using other techniques such as high hydrostatic pressure, where its application provoked the precipitation of soluble

tannins in "Rojo Brillante" persimmons (Hernández-Carrión et al., 2014). As expected, overcoming the BET monolayer moisture content when in the rubbery state, the decrease in the tannin content was more marked for the enzymatic and non-enzymatic reactions, besides the tannin precipitation; likewise results were found by Syamaladevi et al., (2011) and Fang & Bhandari, (2011).

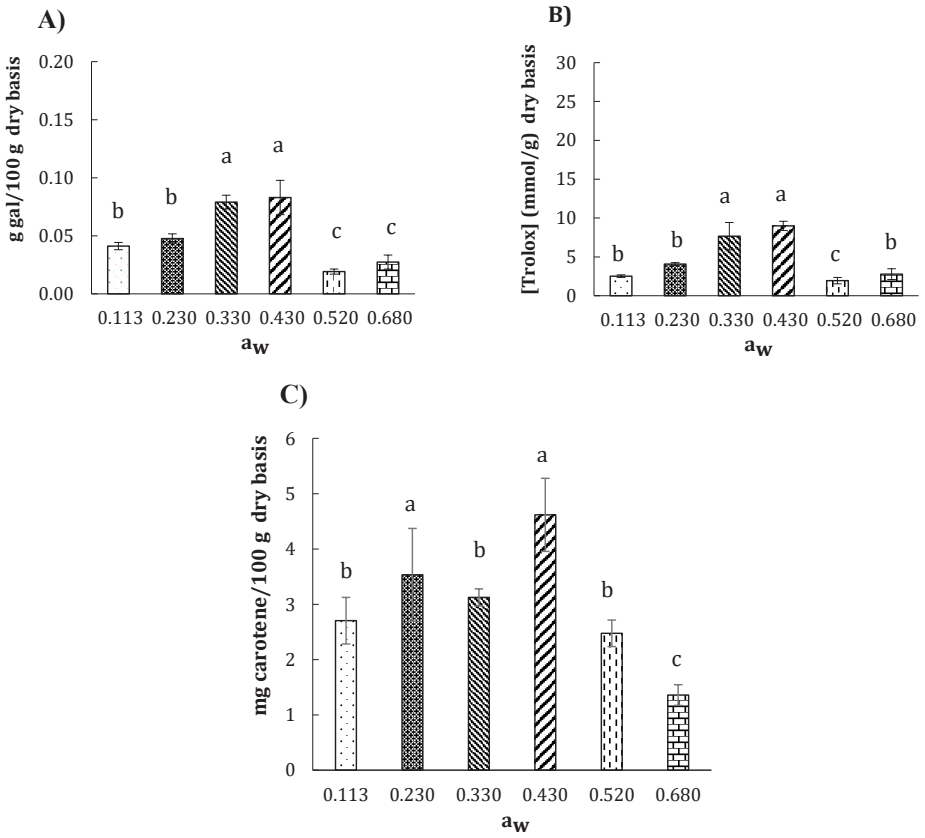

Figure 4. A) Total soluble tannin content; **B)** Antioxidant capacity; **C)** Carotenoid content of freeze-dried persimmon at different water activity levels after three months of storage. Different superscript in each bar differ significantly ($p < 0.05$) according to ANOVA (LSD multiple range test).

3.5. Antioxidant capacity

Regarding the antioxidant capacity, measured by the FRAP method, there was higher antioxidant capacity in the control (22^{b} ± 2 µmol/g Trolox dry basis) than in the fresh sample (10.8^{a} ± 1.2 µmol/g Trolox dry basis). Results had the same trend for total soluble tannin content.

Figure 4B shows the antioxidant capacity of equilibrated persimmons. A significant decrease ($p < 0.05$) was observed in all the samples when compared to the control. The trend was correlated with the soluble tannin content, since the method only detected the antioxidant capacity of soluble bioactive compounds (Karadag et al., 2009).

3.6. Carotenoids content

Like the soluble tannin content and antioxidant capacity, the content of carotenoids had significant difference between the fresh samples (3.168 mg/100 g ± 0.135 β-carotene dry basis) and the control (3.54 mg/100 g ± 0.042 β-carotene dry basis). As previously reported, the freeze-drying process permitted better extractability of carotenoids (Wu et al., 2010).

Figure 4C shows the carotenoid content of the equilibrated samples. All equilibrated samples presented similar values up to $a_w = 0.520$. For $a_w \geq 0.680$, the number of carotenoids decreased. In this range microbial growth, enzymatic, and non-enzymatic reactions speed up (Lavelli et al., 2007). The a_w range corresponding to the maximum carotenoid stability was next to the monolayer a_w. Arya et al., (2007) reported that in dehydrated carrots, stored in an a_w range of 0.113 - 0.730, total carotenoids were more stable in the a_w range of 0.320 - 0.570. Lavelli et al., (2007) corroborated these findings when studying the effect of water activity on the stability of carotenoids in freeze-dried carrots.

3.7. Field emission scanning electron microscopy and light microscopy.

FESEM images of the freeze-dried persimmon slices at the a_w values of 0.113, 0.330, and 0.520 are presented in Figure 5. The typical parenchyma cell structure is seen in all the samples and clusters of tannin cells (TC), with insoluble material being discerned in Figures 5A to 5C. These results are consistent with other literature (Salvador et al.,

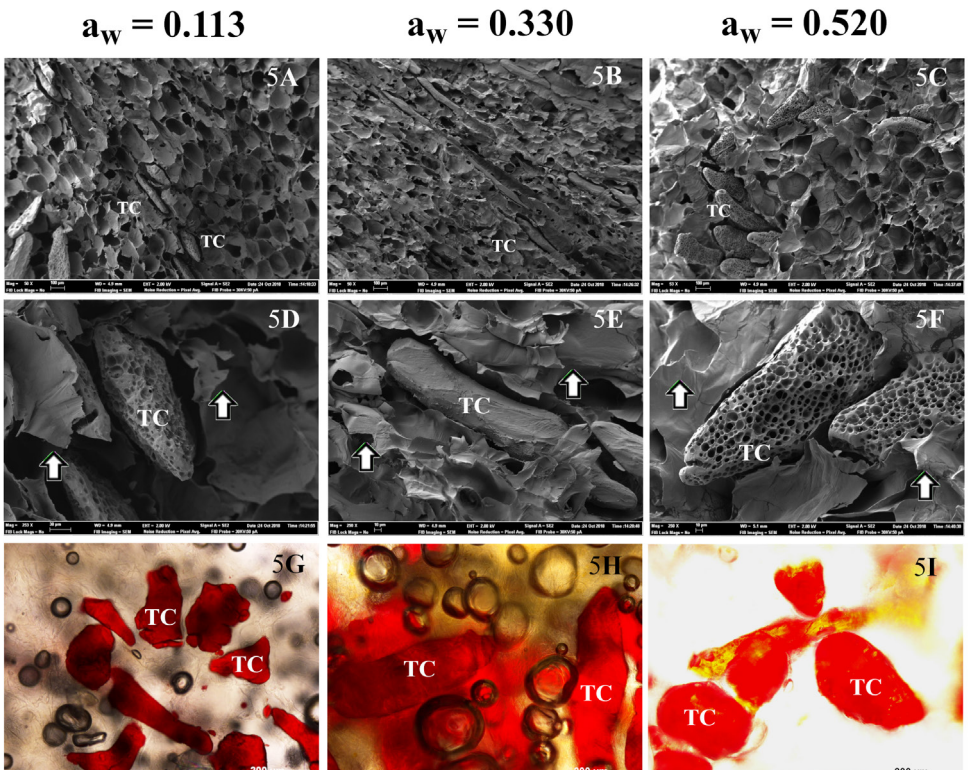

Figure 5. Field emission scanning electron microscopy (FESEM). (5A – 5C) - Images of freeze-dried persimmon slices a_w of 0.113, 0.330, and 0.520, magnification 50x, bar = 100 µm. (5D – 5F). Images of freeze-dried persimmon slices at a_w of 0.113, 0.330, and 0.520, magnification 250x, bar = 10 µm. Light Microscopy (LM). (5G – 5I) – Tissue sections from images of freeze-dried persimmon slices at a_w of 0.113, 0.330, and 0.520, magnification 10x, bar = 200 µm TC: tannin cell, White arrows: cell walls.

2008). The TC present distinct aspects depending on the sample water activity (Figures 5D to 5F). Samples with a_w of 0.113 and 0.520, present TC with a porous structure, whereas a clayey aspect of the TC was observed in the sample with a_w = 0.330 (Figure 5E). This structure can be related to soluble tannin content where the lower content results in the higher porosity in the TC.

For LM observation, samples were stained with HCL-vanillin, which reacts with tannins to give a red colour (Figures 5G to 5I). Cutting promotes the extravasate of tannins from TC, where they come in a soluble form. The microstructural study of samples seen by LM

revealed a greater amount of soluble tannins in sample a_w = 0.330 (Figure 5H), which is related with the results obtained for total soluble tannin content (Figure 4A). Tannins insolubility was more evident at a_w of 0.113 and 0.520 (Figures 5G and 5I). These images correlated with the soluble tannins content (Figure 4A) where the a_w of 0.520 had the lowest content.

In addition, as the adsorption of water in the sample's monolayer increased, a thickening of the cell walls was observed (Figures 5D to 5F). This is related to the water uptake of the samples, where water is captured by the cell walls, leading to the thickening. Further, these results corroborated the texture and the glass transition findings.

4. Conclusions

The freeze-dried treatment applied in this study is a practical alternative to develop dehydrated persimmon slices, ready for consumption. The application of the freeze-drying process creates a sweet crispy product with a high quantity of bioactive compounds. At 20 °C, the critical values for water activity and water content related to glass transition are CWA = 0.165 and CWC = 0.0312 g water/ g product, respectively. Above these values, a physical change to the rubbery state, provokes changes in the mechanical properties. Below these values, persimmon slices are stable in the glassy state and the insolubility of the tannins is favoured. To ensure the physical and functional quality to preserve freeze-dried persimmon slices during long-term storage, the glassy state must be a guarantee. This can be achieved by storing the freeze-dried persimmon slices at an RH < 16.5%, before packing, to be commercialised at 20 °C, or setting the temperature of commercialisation below 16.5 °C.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Cristina M. González: Investigation, Validation, Formal analysis, Writing - original draft.

Empar Llorca: Methodology, Investigation.

Amparo Quiles: Methodology, Investigation.

Isabel Hernando: Conceptualization, Supervision, Writing - review & editing.

Gemma Moraga: Supervision, Resources, Funding acquisition, Writing – review & editing.

DECLARATION OF COMPETING INTEREST

The authors declare that they do not have any conflict of interest.

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Capítulo 3:

Desarrollo de snacks saludables mediante el secado por aire caliente de caqui astringente y no astringente. Propiedades fisicoquímicas, estructurales y sensoriales.

Influence of ripening stage and de-astringency treatment on the production of dehydrated persimmon snacks.

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ABSTRACT

Background: Seasonal persimmon (*Diospyros kaki L.*) crops have steadily increased in Spain; this has been linked to a significant increase in the post-harvest production waste. Therefore, development of valorised products is of great interest. In this study, a hot air-drying technique was used to obtain persimmon snacks. Slices from astringent and non-astringent persimmons (submitted to de-astringency treatment) at three different ripening stages were dried at 40 and 60 ◦C to reach 15% ± 3 water content.

Results: After the drying treatment, dehydrated samples were harder, turned into a more orange hue angle, and had a reduced soluble tannin content. Dehydrated samples obtained from the astringent fruit at the most advanced ripening stage had similar soluble tannin content as the samples obtained from non-astringent fruit, especially at 60 ◦C. Besides, a high correlation was observed between the level of astringency perceived by consumers and the decrease of soluble tannin content. Although, in the first ripening stage, consumers preferred the snacks obtained from non-astringent fruits; in the last ripening stage, snacks produced from astringent fruits were equally accepted than the non-astringent ones.

Conclusion: Therefore, well-accepted persimmon snacks are obtained from both astringent and non-astringent fruits when advanced ripening stages of persimmon are used.

Keywords: Astringency**,** *Diospyros kaki*, physicochemical properties, tannins, sensory analysis, hot air drying.

1. Introduction

The featured role of food to improve health by decreasing the risk of diseases has highlighted a new class of foods, now known as functional foods (Yaqub et al., 2016). The beneficial value of functional foods depends on the presence of dietary fibres, total and major phenolics, essential minerals, and trace elements. Fruits and vegetables are essential parts of a human diet and a rich source of these nutritional compounds (Yaqub et al., 2013). Some of these compounds have beneficial effects on human health because of their ability to prevent or control various illnesses (Yaqub et al., 2016). Among the fruits, persimmon (*Diospyros kaki L.*) is a popular fruit with a relatively high dietary fibre content, bioactive and antioxidant compounds and minerals (Jung et al., 2005).

The persimmon is an edible fruit native to East Asia. Over recent years, diffusion of persimmons growth, outside of the traditional production countries, has made it a promising crop worldwide, including Mediterranean countries such as Spain (Bordiga et al., 2019). There are over 400 species of persimmon, varying in shape and colour, although they are generally classified into two main groups: astringent and non-astringent varieties. Among the persimmon varieties, the "Rojo Brillante" is valued as a fresh fruit owing to its high productivity and commercial quality (Cárcel et al., 2007). However, as the fruit's appearance has strict quality control, a significant proportion of persimmon has no commercial value as a fresh product, with 25 - 30% of the crop production wasted (Cárcel et al., 2010). Moreover, no management system can take advantage of the persimmon waste, thus generating economic and environmental problems.

An additional disadvantage of "Rojo Brillante" is its high degree of astringency; because of the high soluble tannin content of the fruit, precipitation with salivary proteins makes the product inedible at stages of maturity when the pulp is still firm (Nicoleti et al., 2005). Even though soluble tannin content decreases as the state of ripening

progresses, the removal of astringency is necessary to commercialize firm fruit (Arnal & Del Río, 2003). Therefore, different techniques are applied, such as anaerobic treatments (CO_2), precipitating tannins while maintaining firmness without damaging the product (Salvador et al., 2007).

Nevertheless, the ripening stage is a key factor influencing the effectiveness of the treatment. It has been reported that the de-astringency process occurs slower in fruits at advanced ripening stages, than in early and middle stages (Novillo et al., 2015). This may involve an extra cost when arriving to marketing, generating persimmon fruits out of the quality control if the de-astringency treatment is not applied properly, especially in advanced ripening stages.

An interesting way of valuing waste from the agro-food industry is based on obtaining products or ingredients with high added value. It could be beneficial for producers to develop dried fruits as an alternative. Dried fruits are increasingly valued and might be very convenient for consumers, which is an important motivation for their consumption (Sijtsema et al., 2012). Drying is one of the oldest and the most important food preservation methods practiced by humans. This process improves the food stability, as it reduces the water and microbiological activity of the material while minimising physical and chemical changes during its storage (Doymaz, 2012). There are several studies related to the drying kinetics of dehydrated persimmon slices applying different techniques and temperatures of drying (Demiray & Tulek, 2017; Doymaz, 2012; Giovagnoli-Vicuña et al., 2017). Although drying processes lead to a significant loss of bioactive compounds, dehydrated fruits can still be a valuable source not only of energy, but also of antioxidant activity (Sijtsema et al., 2012). Besides, methods such as hot air drying, produce a reduction in persimmon's total polyphenols without affecting dietary fibres, minerals, and trace elements content (Park et al., 2006). Hence, the dehydrated persimmon portions may contribute to the human health and could be also used as ingredients in products such as muesli, breakfast cereals, and snacks.

Therefore, the aim of this study was to explore using hot air drying, at two different temperatures (40 and 60 °C), with astringent and non-astringent (fruit submitted to de-astringency treatment) persimmon slices, to produce a healthy and well-accepted snack, which could increase persimmon production profitability. Three different ripening stages were used to obtain the final product, evaluating the effect of the ripening stage on the mechanical and optical properties, soluble tannin content, and sensory acceptability of consumers.

2. Material and methods

2.1. Sample preparation

Persimmon (*Diospyros kaki* Thunb. cv. Rojo Brillante) unseed fruits, astringent (A) and non-astringent (NA) samples (treated by 95% CO_2 over 24 h at 20 °C) at three different commercial ripening stages (Figure 1), were provided by the Instituto Valenciano de Investigaciones Agrarias (IVIA, Spain). These three commercial ripening stages were from fruits harvested from a local grove in L'Alcudia (Valencia, Spain) between mid-November and early December 2018. The criterion for harvesting each ripening stage was the visual evolution of skin colour corresponding to ripening IV (yellow-orange), V (orange), and VI (intense orange). (Tessmer et al., 2016) The fresh fruits were washed and transversally cut into slices (5 mm thick) with a mandolin (OXO good grips mandolin slicer 2.0, USA), without removing the peel; the stalk and the opposite end were discarded. Hot air drying was conducted in a cabinet dryer (Binder model FED 260 standard, Germany) using an air velocity of 2 m s^{-1} at 40 and 60 °C, until reaching 15% ± 3 water content (23 and 9 h were needed, respectively). The final point were set based on the literature (A. Akyildiz et al., 2004; Ben-arie & Sonego, 1993; Doymaz, 2012; Megías-Pérez et al., 2014) and previous experimental trials. These groups of fruits are named as shown in Figure 1A.

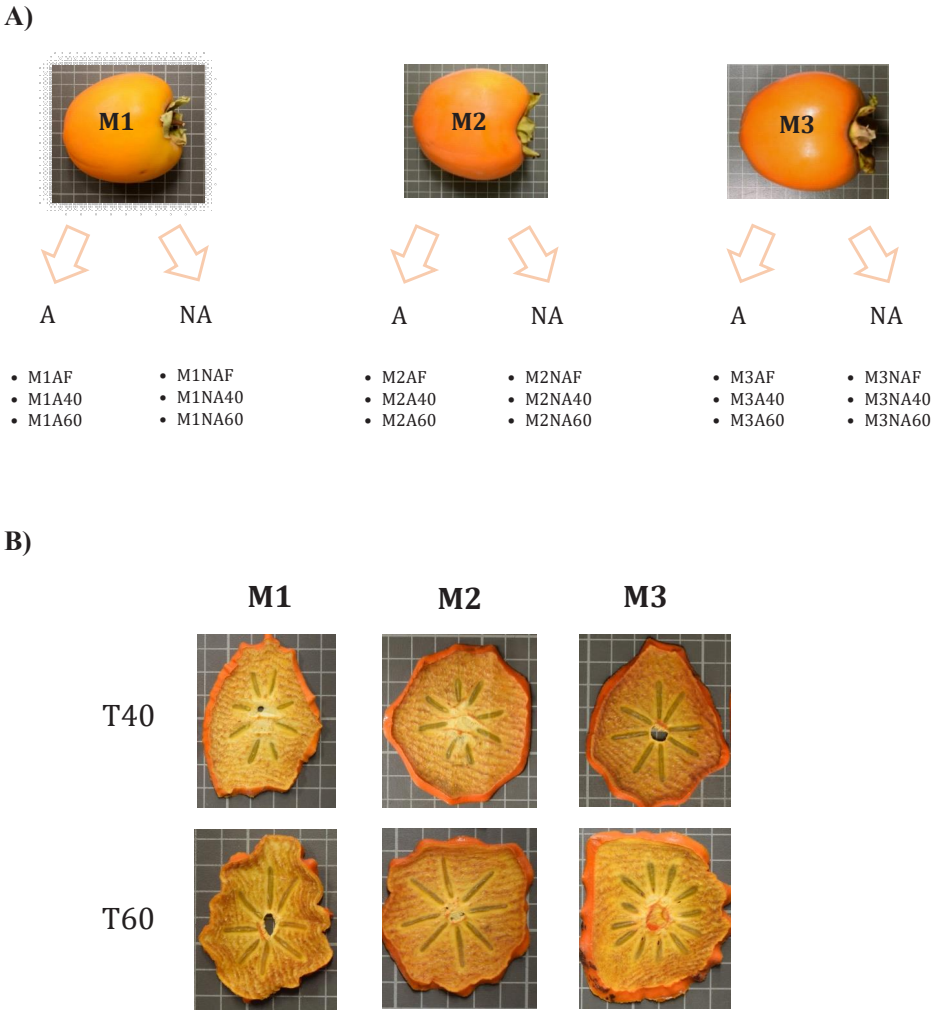


Figure 1. A - Schematic representation of persimmon samples analysed. A = astringent, NA = non-astringent. M1, M2, and M3 are referred to the three ripening stages. Letter F means fresh fruit; numbers 40 and 60 are related to the temperature of the air drying treatment. B - Captured images of persimmon snacks in the three ripening stages (M1, M2, M3) obtained by hot air drying at 40 (T40) and 60 ºC (T60).

2.2. Water content, Water activity, and ºBrix determinations.

The fresh samples and persimmon snacks were ground in a crushing machine; the water content and water activity were measured by a Vaciotem, J.P. Selecta vacuum oven (60 ± 1 ◦C; pressure < 100 mm Hg) and an Aqualab CX-2 Decagon Device, respectively. A refractometer (Hand-held refractometer, ATAGO ATC-1, Japan) was used to measure the ◦Brix of the fresh samples. Three replicates were measured per sample.

2.3. Mechanical properties

The mechanical behaviour of the fresh samples slices, and persimmon snacks was measured using a puncture test on a Universal Texture Analyser (Stable MicroSystems, TA.XT2, Ltd, Godalming, England). A cylindrical (2 mm diameter) punch was used, applying a relative deformation of 85% and a distance rate of 1 mm s^{-1}. The parameters analysed in the puncture test were the maximum force (F_{max}) (N) and the area under the curve (N mm^{-1}). Twelve replicates were performed per sample.

2.4. Optical properties

The optical properties (translucency and CIE*L* a* b** colour coordinates) of the fresh samples and persimmon snacks were obtained from the surface reflectance spectra (between 400 and 700 nm), measuring the pulp on black and white backgrounds using a Minolta CM-3600d spectrocolorimeter (Minolta Co., Tokyo, Japan), considering the standard light source D65 and the standard observer 10◦. Translucency (K/S) was determined by applying the Kubelka-Munk theory for multiple scattering of reflection spectra (Talens et al., 2002). This theory assumes that the flow of light that passes through the sample is related to the absorbed relationship to scattered light (equations (1) and (2)). The reflectance of an infinitely thick layer of

the material (R_∞) calculated using Eq. (2) was used to obtain colour coordinates CIE*L*a*b** (Moraga et al., 2011).

$$K/S = \frac{(1 - R_\infty)^2}{2R_\infty} \quad (1)$$

where: K: absorption coefficient; S: scattering coefficient; and R_∞: reflectance of an infinitely thick layer of the material, calculated with Eq. (2),

$$R_\infty = 0.5\left(R + \frac{R_0 - R + R_g}{R_0 R_g}\right) - \sqrt{\left(0.5\left(R + \frac{R_0 - R + R_g}{R_0 R_g}\right)\right)^2 - 1} \quad (2)$$

where: R: reflectance of the sample with an ideal white background; R_0: reflectance of the sample with an ideal black background; R_g: reflectance of the ideal white background.

Colour attributes of chrome (C_{ab}*), and hue angle (h_{ab}*) were obtained from the CIE*L*a*b** colour coordinates applying $C_{ab}^* = (a^{*2} + b^{*2})^{1/2}$ and $h_{ab}^* = \text{arctg}\ (b^*/a^*)$ equations, respectively.

2.5. Total soluble tannin content

The total soluble tannin content of fresh samples and persimmon snacks was determined with a spectrophotometer (CE 1021 1000 series, CECIL INSTRUMENTS Cambridge) using the Folin-Ciocalteu colorimetric method as described by Arnal & Del Río, (2004), with modifications. Persimmon samples (5 g) were homogenised in an Ultraturrax (IKA T18 digital, Germany) with 25 mL of ethanol (96%). Homogenates were centrifuged (30,024 g, 20 min, 4 °C) and filtered, while keeping the supernatant. More supernatant was extracted from the pellet, with 25 mL of ethanol (96%), and added to the first supernatant. The total

volume of supernatant was brought to 100 mL with ethanol (96%). One millilitre of the extract and six millilitres of distilled water were mixed and vortexed in a test tube. Thereafter, 0.5 mL of Folin-Ciocalteu reagent was added; after 3 min, 1 mL saturated Na_2CO_3 (20%) was added and vortexed, followed by 1.5 mL distilled water was added. Absorbance was measured after 90 min at 725 nm to determine soluble tannin content. The calibration curve was performed at different concentrations of gallic acid in ethanol (96%). Results were expressed as g gallic acid equivalents (GAE) kg^{-1} of dry matter. Total soluble extractions were made in triplicate.

2.6. Sensory analysis

Sensory analysis was conducted in persimmon snacks over three different sessions, one per each ripening stage. There were 255 consumers (153 women and 102 men) in total (85 consumers / session) recruited among the employees and students of the Universitat Politècnica de València. The persimmon snacks were analysed in a sensory testing room equipped with individual booths. A slice of each sample (A and NA dried at 40 or 60 ^{o}C) was presented in each session following a balanced complete block experimental design. The samples were served in small plastic dishes coded with random three-digit numbers. They were served at room temperature (20 ^{o}C) in random order. Water and bread were supplied to cleanse the consumers' palettes between each sample.

The consumer acceptance test was performed using a nine-point hedonic scale (1 = dislike extremely to 9 = like extremely) following the International Standard ISO / FDIS 11136:2014 (E). For each sample, the consumers scored their degrees of liking in the following order: 'appearance', 'flavour', 'texture', and 'overall acceptability'. Additionally, the consumers were asked to check the following characteristics: "astringent", "I would buy it", and "I wouldn't buy it".

2.7. Statistical analysis.

All the data were analysed using the Statgraphics Centurion XVII software package (Statistical graph Co., Rockville, MD).

A categorical multifactorial experimental design with two factors, ripening stage, and type of sample (A and NA), was used to characterise the fresh samples. A categorical multifactorial experimental design with three factors, ripening stage; type of sample; and drying treatment, was used to characterise the persimmon snacks. In the consumer acceptance tests, one-way analysis of variance was applied (ANOVA). The honest significant difference (Tukey´s HSD test) with a 95% confidence interval was used to compare the mean values obtained ($p < 0.05$).

3. Results and discussion

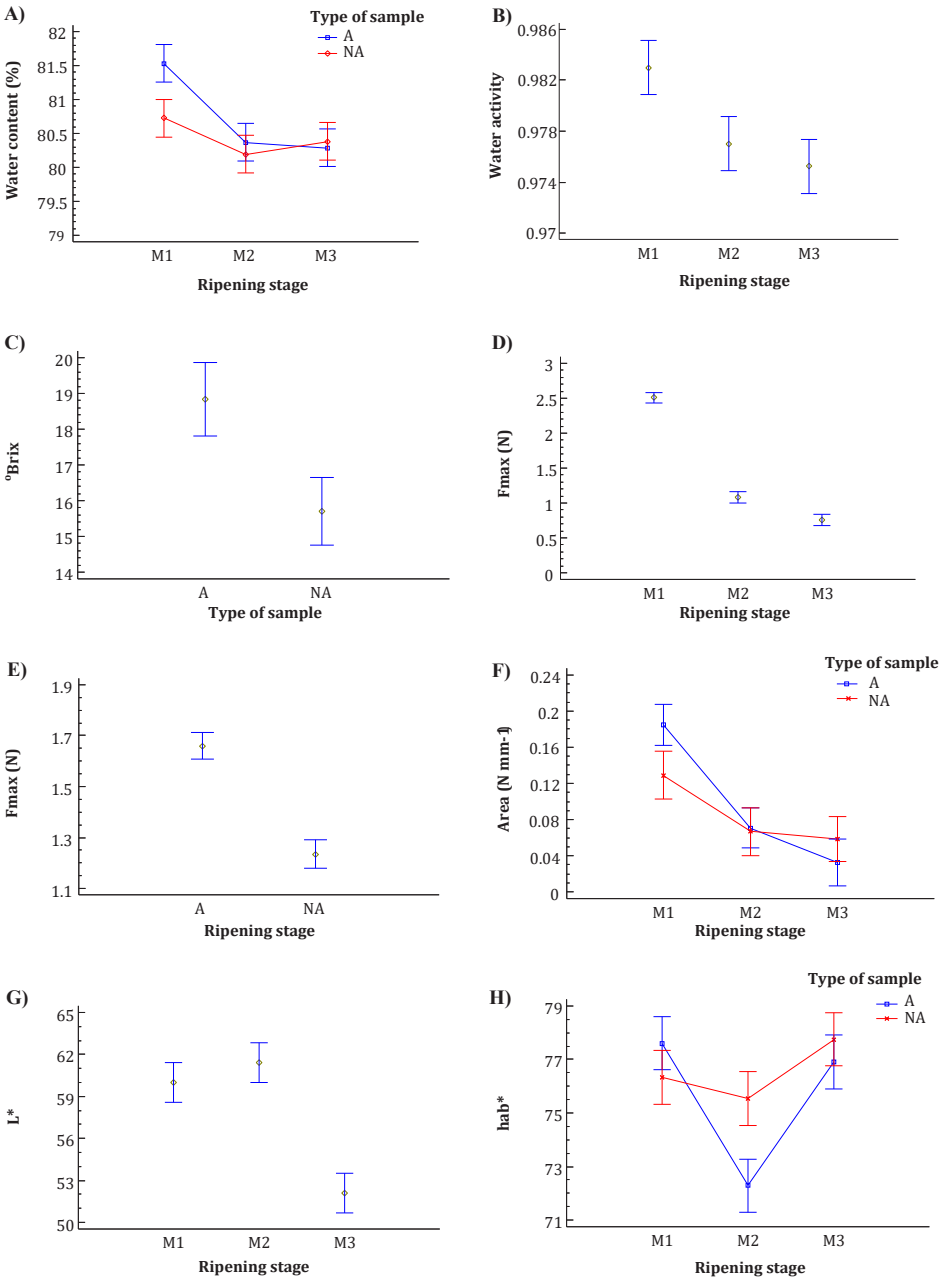
3.1. Fresh samples

The water content, water activity, and ºBrix are presented in Figure 2. Regarding to the water content (Figure 2A), significant interactions ($p < 0.05$) are observed between the ripening stage and type of sample factors. M1AF presents a greater moisture content than M1NAF, M2AF, M2NAF, M3AF, and M3NAF. The parameter of water activity (Figure 2B) does not show significant interactions between factors, only the ripening stage factor presented a significant effect ($p < 0.05$); the samples corresponding to the first ripening stage presented significantly higher water activity values. ºBrix (Figure 2C) shows no significant interactions ($p > 0.05$) observed between the ripening stage and type of sample factors, only the type of sample factor had a significant effect ($p < 0.05$). The de-astringent treatment led to a reduction in the ºBrix values, as the total soluble tannin content was reduced because of the treatment (Salvador et al., 2005).

Mechanical parameters show no significant interactions ($p > 0.05$) between the F_{max} and factors (Figure 2D and 2E) but both factors had a significant effect ($p < 0.05$) on the fruits. The samples show a significantly ($p < 0.05$) greater firmness of the pulp in the first ripening stage, decreasing in the second and third ripening stage (Figure 2D); moreover, the A samples present higher F_{max} ($p < 0.05$) than NA (Figure 2E). The effect of CO_2 on firmness loss in persimmon fruit was also observed by other authors (Harima et al., 2003). Regarding area parameter (Figure 2F), a significant interaction ($p < 0.05$) between the ripening stage and type of sample factors exists. This parameter represents the energy or work required to compress the sample and it is related with the sample toughness. The same as occurred in F_{max}, samples present a significantly higher area in the first ripening stage, decreasing in the second and third ripening stages. This effect of ripening on mechanical properties has also been reported in other studies (Mohammadi et al., 2015), because of the degradation of the primary cell wall (Salvador et al., 2007).

The colour parameters show no significant interactions between factors ($p > 0.05$) for the luminosity (L^*), only the ripening stage factor had a significant effect (Figure 2G). L^* values remain without significant differences ($p > 0.05$) up to the third ripening stage where a reduction occurs. The h_{ab}^* value and C_{ab}^* values show significant interactions between the ripening stage and type of sample factors ($p < 0.05$) (Figures 2H and 2I). The samples present an orange yellow hue angle (Figure 2H) with no significant differences ($p > 0.05$) except for the M2AF, turning into a more orange hue angle. A significantly lower value of C_{ab}^* (Figure 2I) is observed in the third ripening stage.

Figures 3A and 3B shows the spectral distribution of Kubelka-Munk's index (K/S ratio) of A and NA fresh samples, at the three ripening stages, respectively. The greater the increase of this ratio, the more translucent the samples become (Moraga et al., 2011). In the fresh samples, the advanced ripening stage shows a greater ratio of K/S, therefore, samples in the third ripening stage presented greater


A)
Type of sample
A
NA
Water content (%)
82
81.5
81
80.5
80
79.5
79
M1
M2
M3
Ripening stage
B)
Water activity
0.986
0.982
0.978
0.974
0.97
M1
M2
M3
Ripening stage
C)
ºBrix
20
19
18
17
16
15
14
A
NA
Type of sample
D)
Fmax (N)
3
2.5
2
1.5
1
0.5
0
M1
M2
M3
Ripening stage
E)
Fmax (N)
1.9
1.7
1.5
1.3
1.1
A
NA
Ripening stage
F)
Type of sample
A
NA
Area (N mm-1)
0.24
0.2
0.16
0.12
0.08
0.04
0
M1
M2
M3
Ripening stage
G)
L*
65
62
59
56
53
50
M1
M2
M3
Ripening stage
H)
Type of sample
A
NA
hab*
79
77
75
73
71
M1
M2
M3
Ripening stage

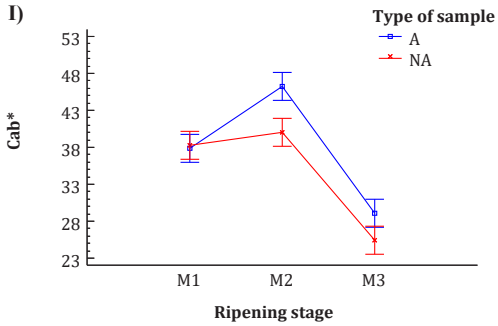
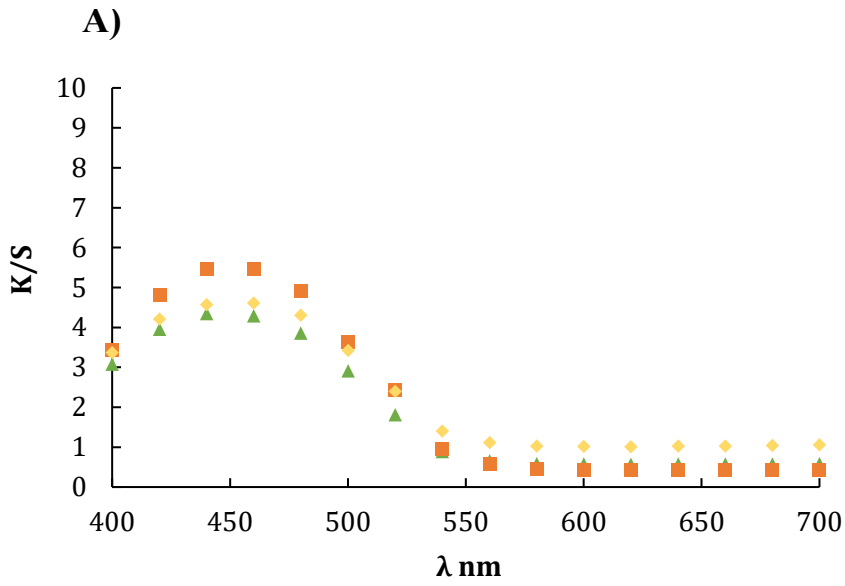
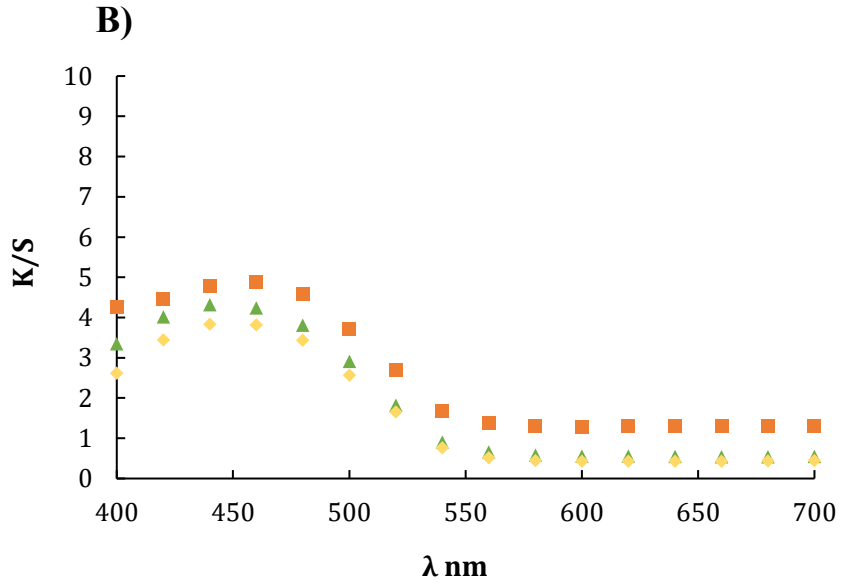


Figure 2. Means and interactions plots with Tukey HSD intervals. **A:** Interaction between the ripening stage and the type of sample for water content of fresh samples. **B:** mean values for the water activity according to the ripening stage for the fresh samples. **C:** mean values for the °Brix according to the type of sample for the fresh samples. **D** and **E:** mean values for the Fmax according to the ripening stage and the type of sample for the fresh samples, respectively. **F:** Interaction between the ripening stage and the type of sample for area of fresh samples. **G:** mean values for the L* according to the ripening stage of fresh samples. **H:** Interaction between the ripening stage and the type of sample for hab* of fresh samples. **I:** Interaction between the ripening stage and the type of sample for Cab* of fresh samples.

translucency, with no flesh changes because of the CO_2 treatment. This phenomena is because of the degradation of the primary cell wall and as a consequence production of water content was more exposed thereby increasing translucency (Salvador et al., 2007). Changes in sample translucency have a great impact on the colour of samples, since selective absorption occurs at differing degrees, changes in clarity, hue, and chrome will occur (Moraga et al., 2011). These effects explain the most notable colour changes of pulp in the third ripening stage of this study. However, since the Kubelka-Munk´s theory describes the ability of the materials to transmit light depends on their light scattering (S) and absorbing (K) properties, low K/S values imply that a more light is scattered by the samples indicating that the persimmon samples have closed structures, therefore, presented certain opacity (Agudelo et al., 2015).

The soluble tannin content of fresh samples is shown in Table 1. The soluble tannin content of the A fresh samples decreases significantly ($p < 0.05$) as ripening progresses. Loss of astringency takes place during


A)
K/S
10
9
8
7
6
5
4
3
2
1
0
400
450
500
550
600
650
700
λ nm


B)
K/S
10
9
8
7
6
5
4
3
2
1
0
400
450
500
550
600
650
700
λ nm

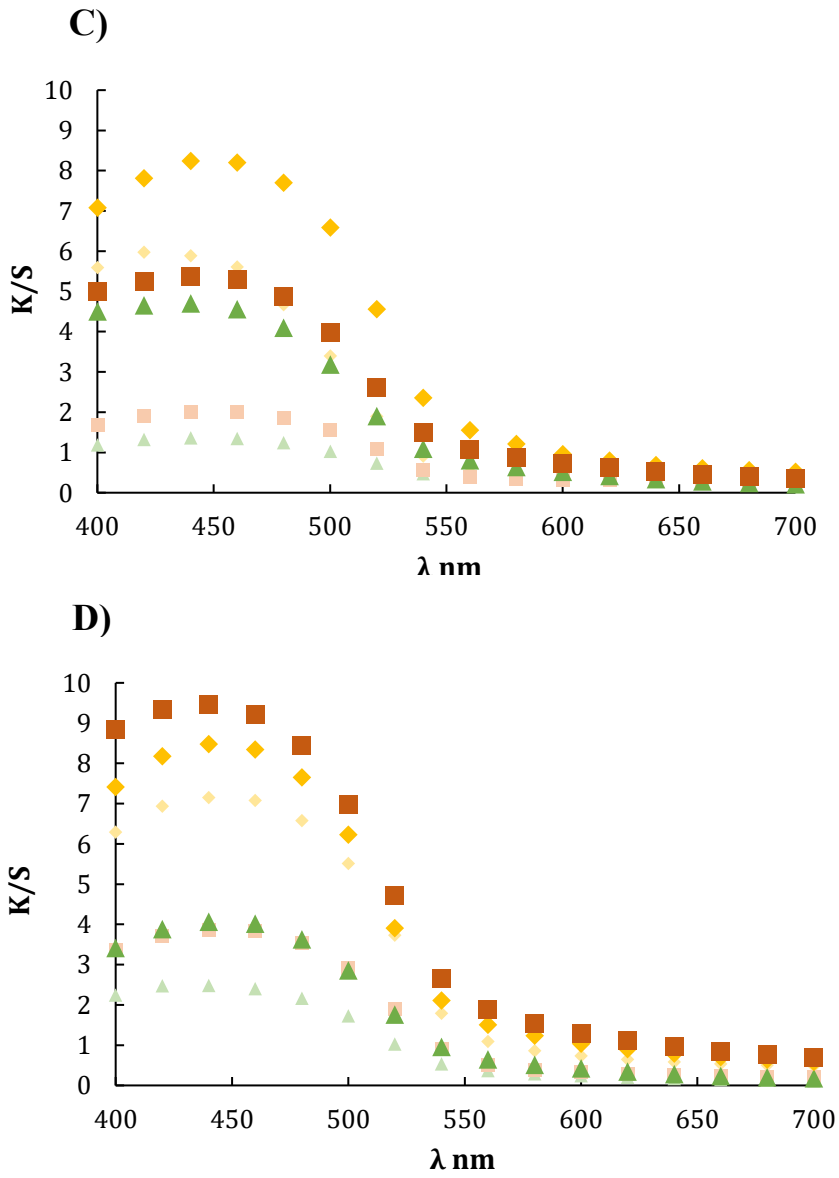


Figure 3. Spectral distribution of Kubelka-Munk's index (K/S ratio) for samples at the three ripening stages. **(A)** M1AF (▲), M2AF (♦), and M3AF (■). **(B)** M1NAF (▲), M2NAF (♦), and M3NAF (■). **(C)** M1A40 (▲), M1A60 (▲), M2A40 (♦), M2A60 (♦), M3A40 (■), and M3A60 (■). **(D)** M1NA40 (▲), M1NA60 (▲), M2NA40 (♦), M2NA60 (♦), M3NA40 (■), and M3NA60 (■).

Table 1. Soluble tannin content at the three ripening stages (M1, M2 and M3) in astringent (A) and non-astringent (NA) fresh samples and persimmon snacks obtained by hot air drying at 40 and 60 ºC.

Ripening stage	Persimmon samples	Soluble tannin content (g gallic acid equivalents (GAE) kg^{-1} dm)
M1	AF	9.10^{a} (0.16)
	A40	4.81^{d} (0.25)
	A60	4.04^{e} (0.21)
	NAF	1.17^{i} (0.22)
	NA40	0.99^{i} (0.10)
	NA60	0.93^{i} (0.12)
M2	AF	7.42^{b} (0.26)
	A40	3.75^{f} (0.15)
	A60	3.31^{f} (0.23)
	NAF	1.68^{h} (0.07)
	NA40	0.99^{i} (0.07)
	NA60	1.02^{i} (0.06)
M3	AF	5.64^{c} (0.04)
	A40	2.53^{g} (0.24)
	A60	1.05^{i} (0.06)
	NAF	2.26^{g} (0.45)
	NA40	1.11^{i} (0.21)
	NA60	0.86^{i} (0.08)

Means in the same column without a common letter are significantly different ($p < 0.05$) according to the Tukey´s multiple range test. Values in parentheses are the standard deviations.

the ripening of persimmon fruits and has been associated with the production of acetaldehyde, which must be involved in gradual tannins insolubilisation (Vázquez-Gutiérrez et al., 2011). The NA fresh samples show less soluble tannin content than the A samples, as a consequence of the de-astringency treatment; after the de-astringency treatment, soluble tannin compounds are transformed into their insoluble forms (Pérez-Burillo et al., 2018). A less marked decrease for soluble tannins was found in M3NAF than in M2NAF and M1NAF; therefore, as reported by other authors, ripening state is an important factor which compromises the effectiveness of the de-astringency treatment (Novillo et al., 2015).

3.2. Persimmon snacks

Figure 1B shows the images of persimmon snacks obtained in the three ripening stages and dried at 40 and 60 °C. The A and NA persimmon snacks dried at 40 and 60 °C, over 23 and 9 h respectively, reached a final water content of 15% ± 3 with a water activity below 0.650. The low water activity inhibits the growth of most bacteria, yeasts, and moulds, reducing oxidative and enzymatic reactions and increasing product shelf-life (Bourdoux et al., 2016). The texture parameters show no significant differences ($p > 0.05$) in the maximum force (F_{max}) and the area values among all the persimmon snacks, ranging from 10.76 to 14.30 N and from 0.15 to 0.32 N mm^{-1}, respectively (data not shown). The values were higher than in the fresh samples because of the loss of water content caused by the drying treatment (Chung et al., 2017).

Figure 4A, 4B, and 4C show the *L** values measured in the persimmon snacks. The multifactor ANOVA shows interactions between ripening stage: type of sample, ripening stage: drying treatment, and type of sample: drying treatment. As the ripening stage progresses (Figure 4A and 4B), a reduction in luminosity is seen, as with the fresh samples. Snacks dried at 60 °C (Figure 4C) present lower luminosity than snacks

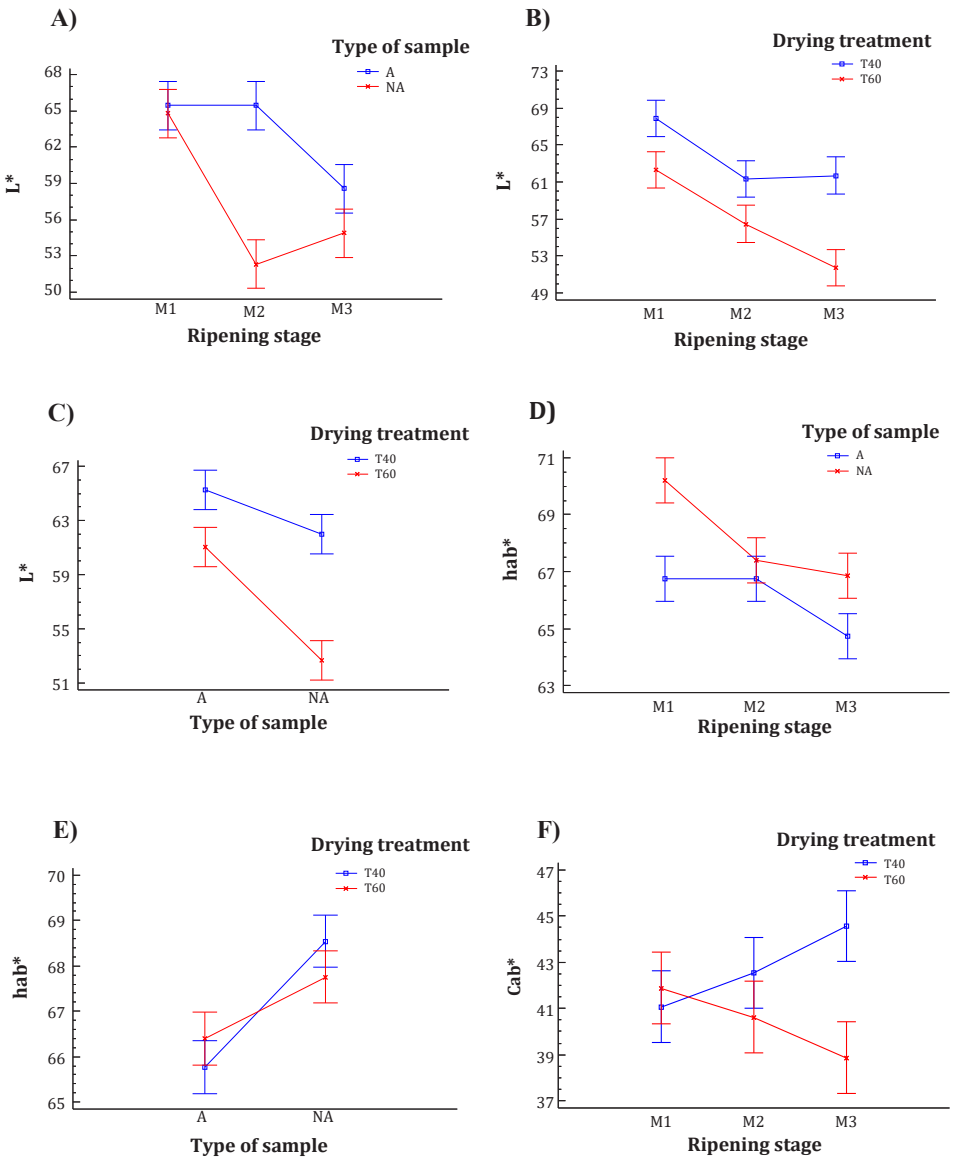


Figure 4. Means and interactions plots with Tukey HSD intervals. A, B, and C: Interactions between the ripening stage - the type of sample, the ripening stage - drying treatment, and the type of sample - drying treatment for L* of persimmon snacks obtained by hot air drying at 40 and 60 ^{o}C, respectively. C and E: Interaction between the ripening stage - the type of sample and the type of sample - drying treatment for hab* of persimmon snacks obtained by hot air drying at 40 and 60 ^{o}C. F: Interaction between the ripening stage - drying treatment for Cab* of persimmon snacks obtained by hot air drying at 40 and 60 ^{o}C.

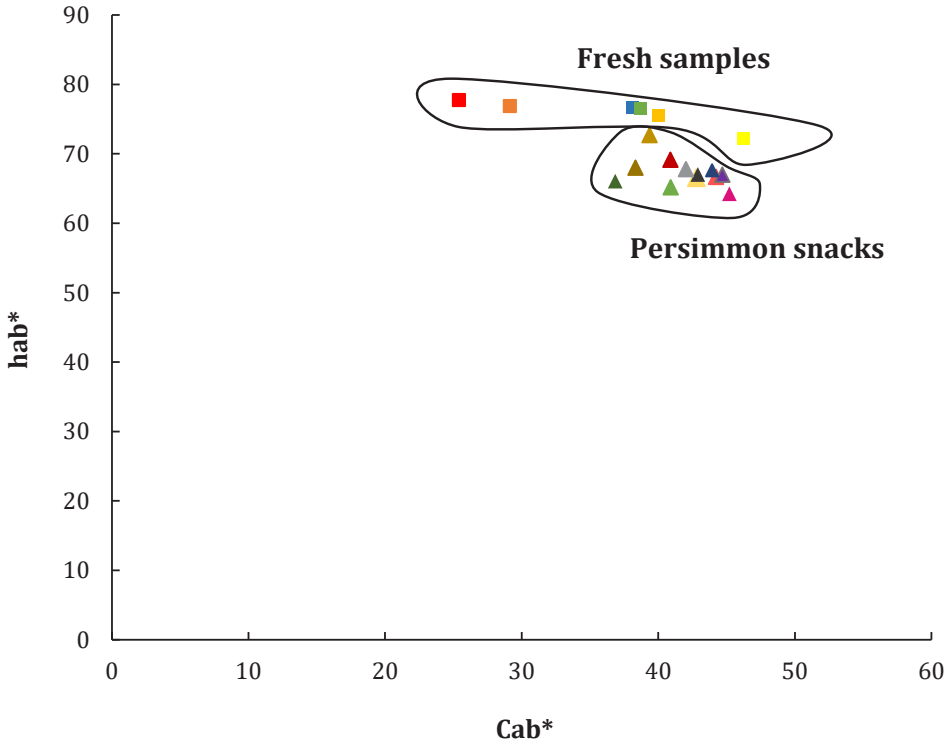


Figure 5. Comparison of hab* and Cab* colour values between the fresh samples and the persimmon snacks obtained by hot air drying at 40 and 60 oC. M1AF (■), M1NAF (■), M2AF (■), M2NAF (■), M3AF (■), M3NAF (■). M1A40 (▲), M1A60 (▲), M1NA40 (▲), M1NA60 (▲), M2A40 (▲), M2A60 (▲), M2NA40 (▲), M2NA60 (▲), M3A40 (▲), M3A60 (▲), M3NA40 (▲), and M3NA60 (▲).

dried at 40 °C while the NA samples dried at 60 °C show the lowest values. The *L** value slightly increases (Figure 4B) after drying at 40 °C and remains constant after drying at 60 °C compared to fresh samples (Figure 2G). The h_{ab}* values (Figure 4D and 4E) have interactions between ripening stage: type of sample and type of sample: drying treatment. A reduction of h_{ab}* values from the first ripening stage to the third ripening stage, turn to an orange hue angle (Figure 4D). Besides, A samples have a significantly ($p < 0.05$) lower hue angle than NA ones (Figure 4E). Comparing the snacks with fresh samples (Figure 2H), dehydration causes a decrease in the hue angle values. Chroma values, shown in Figure 4F, have an interaction between the ripening stage: drying treatment even though no significant differences ($p >$

0.05) are observed between the samples for M1 and M2. Only in the third ripening stage, snacks dried at 40 °C show higher chroma values than snacks dried at 60 °C (Figure 4F). In addition, the snacks at the third ripening stage (Figure 4F) show higher C_{ab}* values than fresh samples (Figure 2I). Several authors also observed the reduction of h_{ab}* values and the increase of C_{ab}* values during drying in different fruits, including persimmon (Asiye Akyildiz et al., 2008). The reduction in h_{ab}* values and the increase in C_{ab}* because of drying process can be observed in Figure 5. The development of these colour changes was likely to be related to non-enzymatic browning reaction (Krokida et al., 2001).

Figure 3C and 3D show the spectral distribution of Kubelka-Munk's index (K/S ratio) of A and NA persimmon snacks, respectively. Snacks dried at 60 °C present more translucency than snacks dried at 40 °C, therefore the higher drying temperature the greater translucency. This could be because of the high temperature effect on the cell wall degradation (Kunzek et al., 1999), which could generate intracellular fluid leakage and the increase of the refractive index homogeneity in the tissue, thus leading to a greater translucency (Talens et al., 2002). As reported by Talens et al., (2002) the greater the sample translucency, the darker the sample as can be seen in *L** and C_{ab}* values (Figure 4A, 4B and 4F). Despite drying treatments caused a slight increase in the K/S values of M2A40, M2A60, M3NA60, M2NA60, and M2NA40 and a slight decrease in M1A40, M3A40, and M1NA40, the values were low for all the snacks, as occurred in the fresh samples. Similar results were observed by Agudelo et al., (2015) in cocona chips.

Table 1 also shows the soluble tannin content of the persimmon snacks. After drying at 40 and 60 °C, a decrease in the soluble tannin content is observed over the fresh samples, specifically for snacks obtained from A persimmon. Previous researchers have indicated losses of bioactive compounds when samples are subjected to drying; the reductions or losses are attributed to the thermal degradation of phytochemicals (Çam et al., 2014) or the transformation of soluble forms of tannins into

their insoluble forms (Senica et al., 2016). Regarding the ripening stage, a significant decrease ($p < 0.05$) was observed for the snacks obtained from A persimmon from the first ripening stage to the third. M3A60 obtained the lowest values of all the A persimmon snacks.

Snacks obtained from NA persimmon have reduced the soluble tannin content than the fresh samples for the different drying treatments, both in M2 and M3 ripening stages (Table 1) with no significant differences ($p > 0.05$) observed among all the NA persimmon snacks. As seen in A persimmon snacks, the drying treatment favoured the formation of insoluble tannins. Moreover, M3A40 shows similar values to M3NAF, while M3A60 did not show significant differences ($p > 0.05$) with snacks obtained from NA persimmon at the different ripening stages.

Figure 6 (A, B, C and D) shows the results of the consumer acceptability test at the three different ripening stages. The attributes assessed in this test were appearance, flavour, texture, and overall acceptability.

In the first ripening stage (Figure 6A), the appearance of M1A40 and M1NA40 have higher acceptability ($p < 0.05$) than M1A60 and M1NA60. The acceptability of the flavour of snacks (Figure 6B) M1NA40 and M1NA60 are greater ($p < 0.05$) than snacks M1A40 and M1A60, regardless of the drying temperature. For the texture attribute (Figure 6C) only the M1A60 obtained low score ($p < 0.05$) and the overall acceptability (Figure 6D) follows the same tendency as the flavour attribute.

In the second ripening stage, the appearance (Figure 6A) of M2A60 and M2NA60 have the lowest values ($p < 0.05$). For the flavour attribute (Figure 6B), M2A60 presents the lowest score ($p < 0.05$) and the same tendency was observed in the overall acceptability (Figure 6D). The texture attribute (Figure 6C) does not show significant differences ($p > 0.05$). For the third ripening stage (Figure 6), no significant differences ($p > 0.05$) are observed between the snacks for the different attributes.

The appearance perception of the consumers could be related with the L^* values (Figure 4A, 4B, and 4C) and, with the translucency obtained

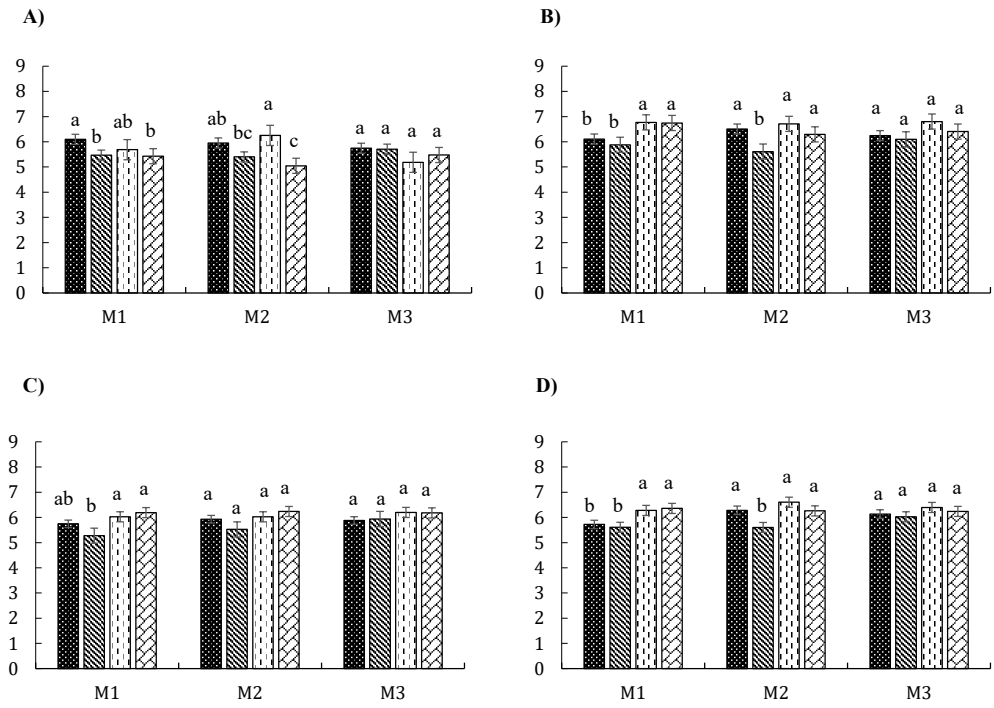


Figure 6. Liking scores for appearance (A), flavour (B), texture (C), and overall acceptability (D) of persimmon snacks obtained by hot air drying at 40 and 60 °C in the three ripening stages (M1, M2, M3). Different superscript in each bar differ significantly ($p < 0.05$) according to ANOVA (Tukey HSD multiple range test). - A40, - A60, - NA40, - NA60.

Table 2. Purchase intention and astringency perception of the persimmon snacks obtained by hot air drying at 40 and 60 °C in the three ripening stages (M1, M2, and M3). A: astringent persimmon; NA: non-astringent persimmon.

Ripening stage	Attributes	Frequency of mention			
		A40	A60	NA40	NA60
M1	Astringent	44	19	1	3
	buy it	30	38	50	29
	not buy it	54	46	34	55
M2	Astringent	23	19	6	6
	buy it	40	35	51	47
	not buy it	42	46	31	35
M3	Astringent	24	14	6	3
	buy it	46	45	52	48
	not buy it	38	39	32	36

in the persimmon snacks (Figure 3C and 3D), since both parameters were related. Snacks dried at 40 °C present higher luminosity than those dried at 60 °C and snacks dried at 60 °C present more translucency than snacks dried at 40 °C although in the third ripening stage the consumers did not detect the effect (Figure 6A). The evolution of flavour perception (Figure 6B) throughout the ripening stages could be related with the tannin insolubilisation and consequently a decrease in astringency caused by the drying temperature (Table 1). With texture no significant differences ($p > 0.05$) are detected in the acceptability by the consumers from the second ripening stage (Figure 6C) as occurred with the texture F_{max} and area parameters obtained in the characterization of persimmon snacks.

Regarding the overall acceptability (Figure 6D), although the most acceptable snacks in the first ripening stage are those obtained from the NA persimmon; as the ripening stage progressed, there are no significant differences ($p > 0.05$), with all snacks accepted at M3. Consequently, according to these findings, the flavour determines the overall acceptability of the persimmon snacks and relates to the transformation of soluble tannins to their insoluble forms leading to loss of astringency. Therefore, as the state of ripening progresses, an acceptable persimmon snack can be obtained without the need for a previous de-astringency treatment.

The frequency of mention, of the added questions presented to the consumers in the acceptability test, are shown in Table 2. The consumers detect less astringency in the snacks obtained from A persimmon as the ripening stage progressed and when the snacks are dried at 40 °C. The frequency of mention for the snacks dried at 60 °C is low, regardless the ripening stage. These results correlated to the content of soluble tannins (Table 1), with the correlation coefficient (R^2) being 0.8038 (Figure 7).

In addition, as the ripening stage progressed the frequency of mention of purchasing these products increases. In fact, 61% of

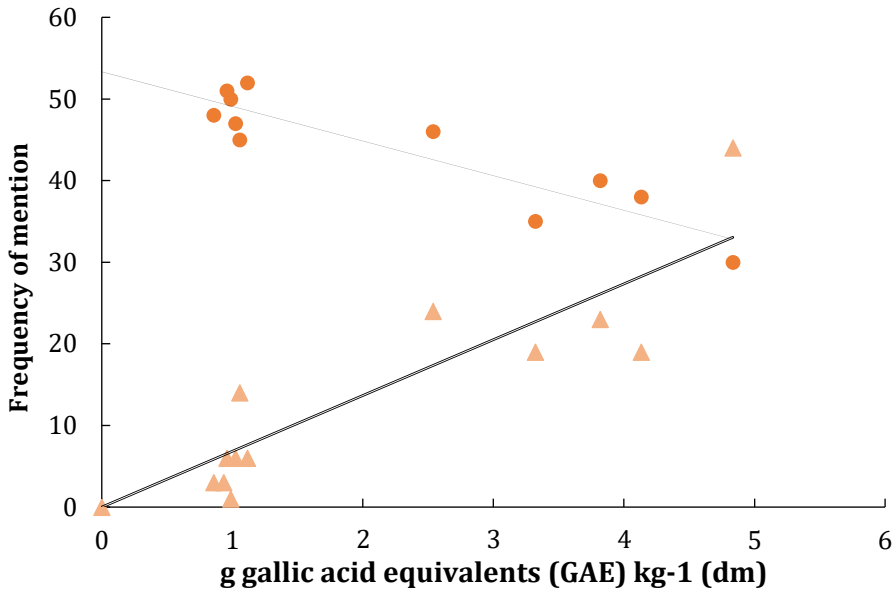


Figure 7. Correlation (R2) between the frequency of mention of astringent (triangles) and willing to buy (circles) terms and soluble tannin content of persimmon snacks obtained by hot air drying at 40 and 60 °C in the three ripening stages (M1, M2, and M3).

consumers declared they were willing to buy the M3NA40. Similar percentages of purchase intention were found in other products derived from persimmon (Castelló et al., 2011; Hernández-Carrión et al., 2015). Table 2 shows how the NA snacks present higher frequency of mention for the "buy it" term. However, in the third ripening stage, both M3A40 and M3A60 snacks present a high frequency of mention for "buy it", like M3NA40 and M3NA60. This could be related with the appearance, texture, flavour, and overall acceptability attributes in the third ripening stage as no significant differences were found between samples at this ripening stage. The declaration "willing to buy" could be also connected with the soluble tannin content, as they show an inverse correlation (R^2= 0.8273), showing that consumers are willing to buy the less astringent samples (Figure 7).

4. Conclusion

This study proves that hot air-drying treatment is a useful technique when developing a well-accepted dehydrated persimmon snack and could avoid the application of a de-astringency treatment when advanced ripening stages of persimmon are used.

Drying considerably decreases the content of soluble tannins, especially in astringent samples. As the ripening stage progresses, a snack can be made from astringent persimmon with a soluble tannin content like the snacks obtained from non-astringent fruits, especially when drying at 60 ºC. The astringency perceived by consumers is correlated to the soluble tannin content during ripening. Although at the beginning of the season they prefer the snacks obtained from non-astringent persimmon; snacks produced from astringent persimmon at the third ripening stage are also well accepted.

Therefore, the development of dehydrated persimmon snacks from astringent and non-astringent "Rojo Brillante" fruit can be an alternative, to increase persimmon production profitability, especially at the end of the harvest season, when a de-astringency treatment may be less efficient.

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Carotenoids in dehydrated persimmon: antioxidant activity, structure, and photoluminescence

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ABSTRACT

In this study, the effect of two hot air drying conditions (40 °C for 23 h and 60 °C for 9 h) on the content, antioxidant activity, microstructure, and luminescence properties of persimmon carotenoids in three ripening stages was studied. Based on the results from total carotenoids content and HPLC analysis, the carotenoid's content increased with the advance of ripening, highlighting the β-cryptoxanthin fraction. In addition, drying treatments did not affect the carotenoid content and profile but decreased the antioxidant activity. Microstructural studies showed that the ripening progress and/or drying treatments, led persimmon tissues to lose integrity, allowing the diffusion of carotenoids and their degradation. Photoluminescence measurements evidenced the synthesis of β-cryptoxanthin during the fruit ripening. After drying, a new emitting specie at 340 nm was attributed to the carotenoid's isomerization while the emission at 500 nm experienced a shift that was related to the formation of thermal degradation products. Both facts could explain the loss of antioxidant activity in persimmon submitted to drying treatments. In this sense, photoluminescence, in combination with spectrophotometric, chromatographic and structural techniques, helps to understand the phenomena caused by both, ripening and drying treatments, in the persimmon's carotenoids fraction.

Keywords: hot air drying, Rojo Brillante, light microscopy, fluorescence, β-carotene.

1. INTRODUCTION

Persimmon (*Diospyros kaki L.)* is a fruit crop originating from Asia, which has experienced a large growth along the Mediterranean over the last decades, because of its sensorial and nutritive properties. This fruit is a good source of bioactive compounds like polyphenols, ascorbic

acid, and carotenoids that have a beneficial effect on human health (Giordani et al., 2011). Thus, it has been used to treat mild pathologies, plus its potential against cardiovascular diseases and some cancer types has been proven (George & Redpath, 2008). Rojo Brillante persimmon is a much-appreciated variety because of its size and texture, and its crop has grown over years thanks to its high productivity (Cárcel et al., 2010). However, because of the compulsory quality control by the origin appellation there is a loss of ≤ 30% fruit, generating an economic and environmental problems. Therefore, valorisation techniques have been studied to avoid and reduce persimmon excess and to offer new products to the consumer throughout the year (Cárcel et al., 2007).

Drying techniques have been used for many years as a valorisation procedure for obtaining high quality and shelf-stable fruits (Hasan et al., 2019). Hot air drying (HAD) is one of the oldest and the most important food preservation methods practised; this improves the food stability and reduces the water and microbiological activity of the material, minimising physical and chemical changes during its storage (Doymaz, 2012). The effect of HAD on carotenoids is complex; several studies have shown how HAD affects carotenoids in different plant-based foods. Piyarach et al., (2020) found no differences in total carotenoid content in various vegetables when drying at 65 °C; whereas Zhang et al., (2018) showed the complex relationship between HAD and carotenoids because of differences in the food matrix.

Carotenoids are a group of hydrophobic molecules synthesised by plants and some microorganisms; the basic structure comprises a tetraterpenoid chain with possible terminal rings (Britton, 1995). In persimmon, most common carotenoids are β-cryptoxanthin, zeaxanthin, and β-carotene. However, the amount of each one is variable depending on the ripening stage; β-carotene appears in early ripening stages remaining constant throughout the shelf-life of the fruit whereas β-cryptoxanthin appears at advanced ripening stages (Bordiga et al., 2019).

The typical structure of trans-conjugated double bonds influences chemical and biochemical properties of carotenoids. They are responsible for vital physiological processes such as photosynthesis, structural stabilisation of protein-pigment photosynthetic complexes, provitamin A activity, and autoxidation to avoid cell damage. A small proportion of cis-isomers have been observed having lower provitamin A activity than their trans-equivalents (Nisar et al., 2015). The system of conjugated double bonds allows carotenoids to absorb UV-Vis light between 400-500 nm. This feature allows carotenoids in solution to obey the Lambert-Beer Law, thus its quantification from UV-Vis spectroscopic measurements can be obtained; therefore, several authors have reported the application of UV-Vis spectroscopy to determine carotenoid content (Hernández-Carrión et al., 2014; García-Cayuela et al., 2018). However, despite its simplicity, this system is not accurate enough to solve the complex nature of the excited states of carotenoids because of the low intensity of symmetry-forbidden, $\pi \rightarrow \pi^*$ transitions (Jørgensen et al., 1992). Therefore, the molecular and chemical changes that occur in the entire absorption spectrum cannot be detected. Although, luminescence spectroscopy may become an alternative in the analysis of carotenoids.

Luminescence is the light emission from atoms and/or molecules because of the energetic fall from excited states to the ground state. Luminescence is formally divided into two categories, fluorescence and phosphorescence, depending on the excited state. In fluorescence, deactivation occurs among the same multiplicity states (singlet-singlet and triplet-triplet) (Lakowicz, 2006). Fluorescence is the most common and useful type of luminescence in analytical chemistry. Excitation-emission spectrofluorimetry is an analytical technique of moderate selectivity and high sensitivity, which can be applied to the detection of a very wide range of analytes in environmental and biological samples. The capacity of detection is approximately one order of magnitude greater than molecular absorption spectroscopy, and its selectivity is greater than other spectroscopic methods (Andrade-

Eiroa et al., 2010). Fluorescence experiments are relatively easy to perform and can provide important information concerning molecular structure and chemical interactions (Strasburg & Ludescher, 1995). Many compounds in foods have been analysed by fluorometry, such as proteins, peptides, amino acids, vitamins, carbohydrates, enzymes, steroids, some inorganic compounds, and toxins (Jihad-René, 2000). To the best of our knowledge, this technique has not been used to study changes of carotenoids in foods.

Therefore, this study aimed to observe the changes in carotenoid fractions in persimmon because of air drying. Thus, their content, antioxidant activity, and microstructure were studied. Furthermore, the photoluminescence was investigated as a potential tool to easily show changes in carotenoid fractions.

2. Materials and methods

2.1. Sample preparation

Persimmon (Diospyros kaki Thunb cv Rojo Brillante) unseeded fruits in three ripening stages (M1, M2, and M3), treated using a de-astringency treatment (95% CO2 atmosphere over 24 h at 20 °C), were provided by "Instituto Valenciano de Investigaciones Agrarias" (IVIA, Valencia, Spain). These three commercial ripening stages were from fruits harvested from a local grove in L'Alcudia (Valencia, Spain) between mid-November and early December 2018. The criterion for harvesting each ripening stage was the visual evolution of skin colour corresponding to ripening IV (yellow orange), V (orange), and VI (intense orange) (Tessmer et al., 2016). The average water content of persimmon fruits was 80%. The fruits were washed and cut into slices (5 mm thick) with a mandolin (mandolin slicer 2.0, OXO good grips, Sheffield, UK). HAD was conducted in a cabinet dryer (FED 260

standard model, Binder, Tuttlingen, Germany) at 40 and 60 °C for 23 and 9 h respectively, using an air velocity of 2 m/s and 30% relative humidity to achieve ≈ 15 g water/100 g product (wet basis). The final point were set based on the literature and previous experimental trials (Doymaz, 2012; Megías-Pérez et al., 2014). These groups of fruits are named as shown in Figure 1. The analytical determinations were made in the pulp of the analysed samples.

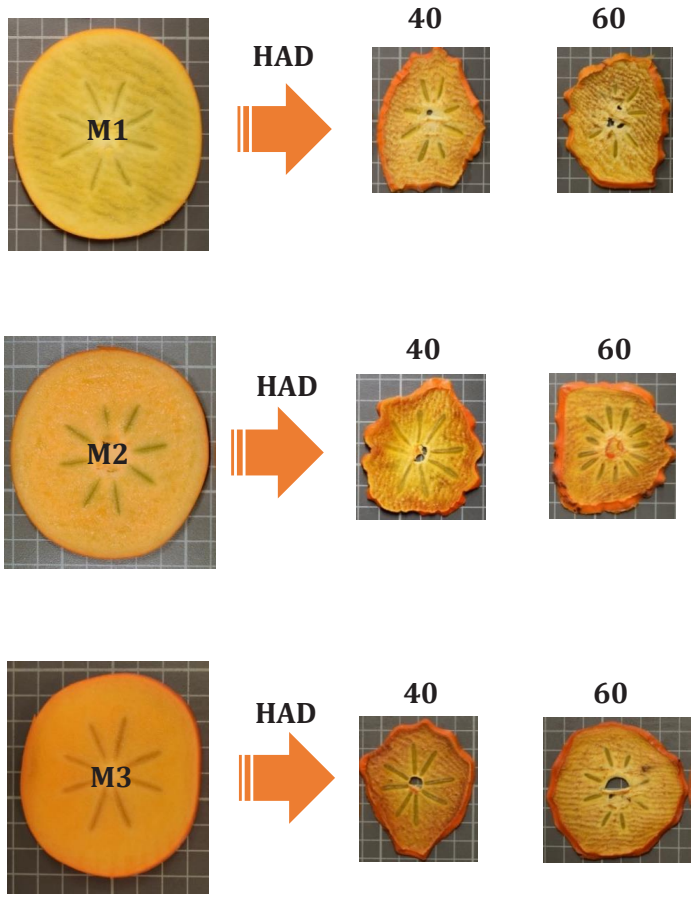

Figure 1. Images of persimmon samples analysed. M1, M2, and M3 are related with the three ripening stages. Numbers 40 and 60 correspond to the hot air drying treatments (HAD) applied at 40 and 60 °C, respectively.

2.2. Chemicals and standars

Acetone (99.5%): Panreac Quimica S.A. (Spain), diethyl ether (99.7% stabilised with ≈ 7 ppm BHT): Scharlab S. L. (Spain), food grade sodium sulphate anhydrous (E-514i, F.C.C.): Panreac Quimica S.A. (Spain), sodium acetate anhydrous (Reag. Ph. Eur.) Panreac Quimica S.A. (Spain), anhydrous sodium sulphate: Scharlab S. L. (Spain), basic magnesium carbonate (CO_3Mg) ≥ 40% : Riedel-de Haën (Germany), sodium chloride (NaCl) 99,0-100,5%: Panreac Quimica, S. A. (Spain), hydrochloric acid 37% (Reag. USP): Panreac Quimica S.A. (Spain), potassium hydroxide (KOH) ≥ 86%, Sigma-Aldrich (France), Triethylamine 99.5%: Panreac Quimica, S. A. (Spain), Ethyl acetate, acetonitrile, methanol and water were of LC-MS grade, purchased from Scharlab S. L. (Spain). β-carotene ≥ 97% (UV): Fluka Biochemika, Sigma-Aldrich (USA), β-cryptoxanthin 97% (UV): Extrasynthese (France), β-apo-8'-carotenal (trans) ≥ 96% (UV): Fluka Biochemika, Sigma-Aldrich (Switzerland), Violaxanthin 0.897 mg/L solution in ethanol: Carbosynth Ltd (UK), Lutein 88%: Carbosynth (UK), Zeaxanthin: Carbosynth (UK). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tris (2-pyridyn)-s-triazine (TPTZ), and β-carotene (provitamin A, ≥ 93% (UV), powder) were purchased from Sigma Aldrich (St. Louis, MO, USA). Trolox was obtained from Santa Cruz Biotechnology (Dallas, TX, USA). 2,6-Di-tert-butyl-4-methyl phenol (BHT) 99 % was obtained from Panreac Quimica, S. A. (Spain).

2.3. Total carotenoid content

Total carotenoid content of fresh and dehydrated samples were extracted according to Hernández-Carrión et al., (2014) with some modifications. Homogenised persimmon pulp samples (5 g of fresh samples and the equivalent dried mass from dehydrated samples) were extracted six times with 25 mL cool acetone using an Ultraturrax (T25 Basic, IKA, Staufen, Germany) and vacuum filtered until no more colour

was extracted. The extract was added gradually to 50 mL ethyl ether in a decanting funnel. With each addition of extract, enough NaCl solution (100 g/L) was added to separate the phases and to transfer the pigments to the ether, while the aqueous phase was removed. The process was conducted in several steps to ensure the greatest elimination of the aqueous phase. The organic phase was treated several times with anhydrous Na_2SO_4 (20 g/L) to remove residual water. Then, the sample was evaporated until dry in a rotary evaporator (model RII; Buchi Labortechnik, Flawil, Switzerland) at a temperature ≤ 45 °C. Finally, the pigments were collected with acetone to a volume of 30 mL, and the absorbance was measured at 450 nm using a spectrophotometer (CE 1021 1000 series, CECIL INSTRUMENTS, Cambridge, UK). The calibration curve was performed with different concentrations of β-carotene in acetone (R^2 = 0.9984). Results were expressed as mg β-carotene/100 g of dry matter. Carotenoid extractions were made in triplicate.

2.4. Individual carotenoids by HPLC-DAD analysis.

Five grams of fresh tissue was homogenized (Polytrom PT3100 homogenizer (Kinematica AG, Switzerland) with 0.2 g of basic magnesium carbonate and 15 mL of ethyl acetate (0.05% BHT). A volume of 100 mL of β-apo-8'-carotenal internal standard was added to sample. The homogenate was centrifuged (Centrifuge Eppendorf 5810R, Eppendorf Iberica, Madrid, Spain) at 12000 rpm at 4 °C for 30 min, the supernatant was collected in a 100 mL decantation funnel and the pellet was re-extracted with 5 mL of ethyl acetate (0.05% BHT) and the supernatant transferred to the decantation funnel. The organic phase was washed three times with water and NaCl-saturated water, and then collected and dried on a bed of anhydrous sodium sulphate. Ethyl acetate was removed in a rotary vacuum evaporator at 40 °C. The residue was dissolved in 5 mL of diethyl ether and saponified with 2.5 mL 20% KOH in methanol, and kept overnight in darkness. The

saponified extract was transferred to a 100 mL decantation funnel, mixed with 2 mL of diethyl ether and washed three times with water and NaCl-saturated water. Diethyl ether was collected, then dried with anhydrous sodium sulphate, and evaporated. Dried samples were dissolved in 0.4 mL ethyl acetate and analysed by HPLC-DAD Alliance liquid chromatographic system (Waters, Barcelona, Spain) equipped with a 2695 separations module coupled to a 2996 photodiode array detector, using a reverse-phase column ZORBAX Eclipse XDB-C_{18} (150 mm x 4.6 mm), 5 µm (Agilent). The flow rate was 1 mL/min and the injection volume was 5 µL. The mobile phase was of water (A): acetonitrile-water-triethylamine (900:99:1) and (B) ethyl acetate. 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; and 14 - 15 min, 100% A, with a total run time of 15 min. Compounds were identified on the basis of comparison of their retention times and absorption spectrum characteristics. Quantification of carotenoids was achieved using calibration curves with commercially available authentic standards: violaxanthin, lutein, zeaxanthin, β-cryptoxanthin and β-carotene. Their quantity was corrected for extraction efficiency based on the β-apo-8'-carotenal internal recovery standard. Empower 2 software was used for data acquisition. Results were expressed as percentage (mg individual carotenoid/total carotenoids) in dry basis.

2.5. Antioxidant activity

2.5.1. Determination of antioxidant activity using the FRAP method

The FRAP (ferric reducing antioxidant power) was performed in the carotenoid extracts obtained from fresh and dehydrated samples according to the method of Benzie & Strain, (1996) with some modifications. Sodium acetate buffer (300 mM; pH 3.6), 20 mM ferric chloride solution, and 10 mM TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine)

in 40 mM HCl solution were prepared. The FRAP reagent was made by mixing 2.5 mL of sodium acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of ferric chloride solution. Furthermore, 75 µL of distilled water, 75 µL of the sample extract, and 2.25 mL of the FRAP reagent were mixed and incubated at 37 °C for 30 min in darkness. After, the absorbance was measured at 595 nm using a spectrophotometer (CE 1021 1000 series, CECIL INSTRUMENTS Cambridge, UK). A standard curve was performed using Trolox as a standard and results were expressed as µmol Trolox eq/g.

2.5.2. Determination of antioxidant activity using the DPPH method

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method was performed on the carotenoid extracts obtained from fresh and dehydrated samples according to the method described by Matsumura et al., (2016) with some modifications. A stable DPPH radical was reduced because of the carotenoids resulting in a reduction of the absorbance; 40 µg/mL DPPH solution in acetone was prepared, 1 mL of sample extract was mixed with 4 mL of DPPH solution and stirred 30 sec in a Vortex. After 30 min of incubation at 37 °C in darkness, absorbance was measured at 517 nm using acetone as a blank. Results were expressed as a DPPH inhibition percent (Eq. (1)):

$$\%\ of\ DPPH\ inhibition\ = \frac{Abs\ control - Abs\ sample\ (extract) - Abs\ blank}{Abs\ control} \cdot 100$$

2.6. Microstructure

Microscopic analysis was performed in fresh and dehydrated samples with the aid of a Nikon Eclipse 80i® light microscope (Nikon Co. Ltd., Tokyo, Japan) which has incorporated a camera (Exwave HAD, nº DXC-19, Sony Electronics Inc., Park Ridge, New Jersey, USA). Twenty micrometre cryostat sections were obtained from persimmon slices and were transferred to a glass slide. Sections were displayed

using bright-field, with (LM-T) and without (LM), 1% toluidine blue as staining agent, and by fluorescence, using a mercury arc lamp with an FITC filter as excitation source (482/35 nm and 536/40 nm, excitation and emission wavelengths, respectively). The images were captured and stored at 1,280 x 1,024 pixels using the microscope software (NIS-Element M, version 4.0, Nikon, Tokyo, Japan).

2.7. Carotenoid determination using fluorescence

Fluorescence measurements for persimmon extracts from fresh and dehydrated samples were recorded using a Jasco FP-8500 Spectrofluorometer (Jasco Inc., Easton, MD). The emission spectra were measured between 360-650 nm using an excitation wavelength of 340 nm. The excitation spectra were recorded at 420 nm emission wavelength between 300-380 nm. The data interval was 0.5 nm, scan speed 500 nm/min, and the emission and excitation band were 2.5 nm. Absorbance of the extracts was adjusted, and the measurements were performed at ambient conditions. All the data were analysed using the OriginPro 9.0.0 software package (OriginLab Co., Northampton, MA, USA).

2.8. Statistical analysis

A categorical multifactorial experimental design with two factors, ripening stage and drying treatment, was used to characterise the carotenoid content and the antioxidant activity of the samples.

The honest significant difference (Tukey's HSD test) with a 95% confidence compared the mean values obtained ($p < 0.05$). All the data were analysed using the XLSTAT statistical software 2019 4.2 (Addinsoft, Barcelona, Spain).

3. Results and discussion

3.1. Total carotenoid content (TCC)

The effect of both factors, ripening stage, and drying treatment conditions on the TCC of samples is presented in Figure 2A and 2B. There were no significant interactions ($p < 0.05$) between the ripening stage and the drying treatment conditions. The only factor with a significant effect ($p < 0.05$) on the TCC was the ripening stage. There was an increase in the TCC as the ripening stage progressed (Figure 2A), where M3 persimmon showed the highest TCC values; however, M1, M2, and M3 persimmon showed no significant differences ($p > 0.05$) TCC values. Figure 2B shows the effect of the drying treatment conditions on the TCC of samples. There were no significant differences ($p > 0.05$) among fresh and dehydrated samples; therefore, the TCC remained after both drying treatments.

3.2. Individual carotenoids by HPLC-DAD analysis.

The different carotenoids detected by HPLC-DAD are shown in Figure 3. β-cryptoxanthin and β-carotene were the main carotenoids in both fresh and dehydrated samples. A carotenoid increase was observed with the fruit ripening highlighting β-cryptoxanthin (Figure 3A); the content of violaxanthin, lutein and zeaxanthin was detected in a lower percentage. Therefore, the increase observed in the TCC (Figure 2A) could be related with the increase in β-cryptoxanthin. In agreement with De Ancos et al., (2000), the same carotenoids were also detected in the 'Rojo Brillante' persimmon. In Figures 3B, 3C, and 3D no differences were found in the percentage of individual carotenoids between fresh and dehydrated samples. This behaviour correlates with TCC determination (Figure 2B).

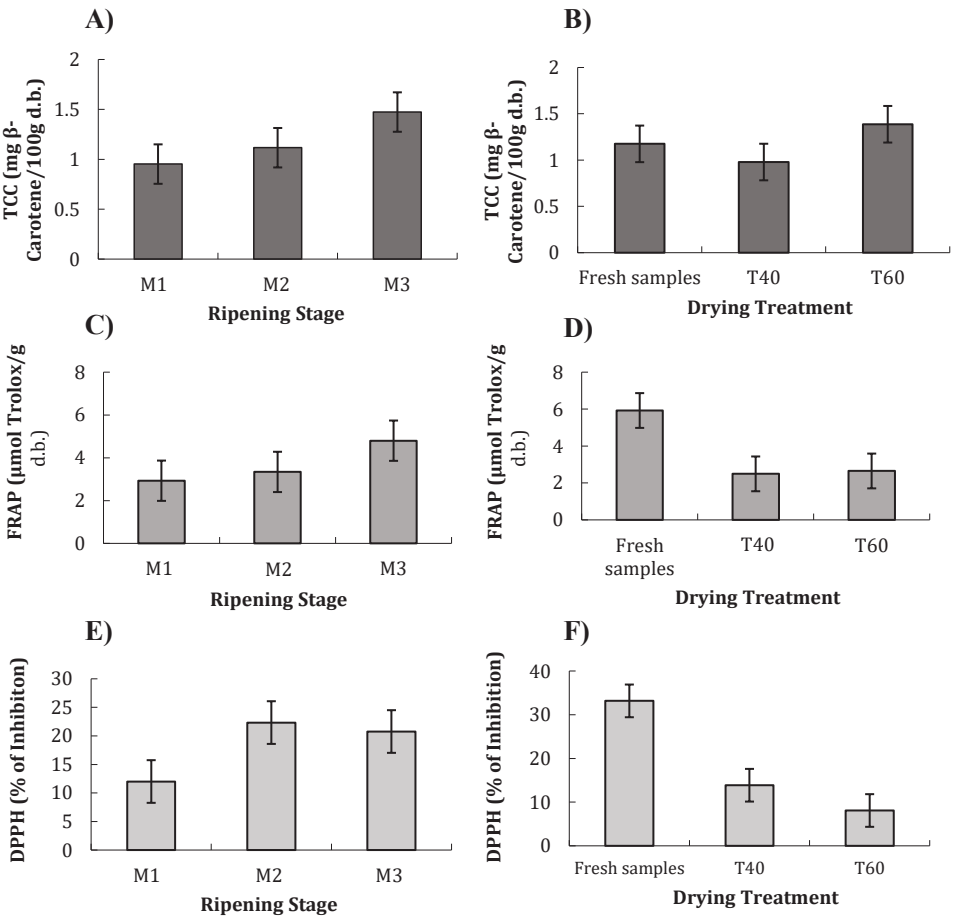

Figure 2. Mean plots with Tukey HSD intervals. A, B: mean values for the TCC according to the ripening stage and drying treatment, respectively for fresh and dehydrated samples. C, D: mean values for the FRAP according to the ripening stage and drying treatment, respectively for fresh and dehydrated samples. E, F: mean values for the DPPH (% inhibition) according to the ripening stage and drying treatment, respectively for fresh and dehydrated samples.

3.3. Antioxidant activity (AA)

The capacity to reduce the ferric-tripyridyl triazine (Fe^{III}-TPTZ) complex to the ferrous form (Fe^{II}) and the ability to inhibit DPPH free radical content of carotenoids in the fresh and dehydrated persimmon samples shows the AA. Results of the FRAP method showed there were no significant ($p > 0.05$) interactions between the factors; however, the

ripening stage and drying treatment conditions had a significant effect ($p < 0.05$). While the ripening stage progressed (Figure 2C), there was an increase in the AA. Significant differences ($p < 0.05$) were found between M1 and M3 as seen with the TCC. The FRAP method followed the same tendency as TCC along with the increase of β-cryptoxanthin fraction detected in the HPLC-DAD analysis, thus the higher content of carotenoids could be related with a higher AA; also determined by Yoo & Moon, (2016) in three citrus varieties. Figure 2D shows the effect of the drying treatment conditions on the AA, determined by the FRAP method with a significant decrease ($p < 0.05$) in the AA after the drying treatments at 40 and 60 °C was observed. This effect has also been reported previously by Martínez-Las Heras et al., (2017). This reduction in the AA could be related with the formation of cis-isomers when the samples were dehydrated (Marx et al., 2003). The conformation of carotenoids within the lipid membrane bilayer might have changed because of polarisation changes affecting its antioxidant properties (Grudzinski et al., 2017).

AA values obtained using the DPPH method show no significant interactions ($p > 0.05$) between the factors, but both factors had a significant effect ($p < 0.05$). Figure 2E shows the effect of the ripening stage factor in the DPPH values. A similar tendency to the FRAP method was obtained, where an increasing AA was seen when the ripening stage progressed. The samples M2 and M3 presented the highest ($p < 0.05$) DPPH values without significant differences. Considering drying treatment (Figure 2F), there was a significant decrease ($p < 0.05$) in the AA after both drying treatments without significant differences between them; also observed in the FRAP method.

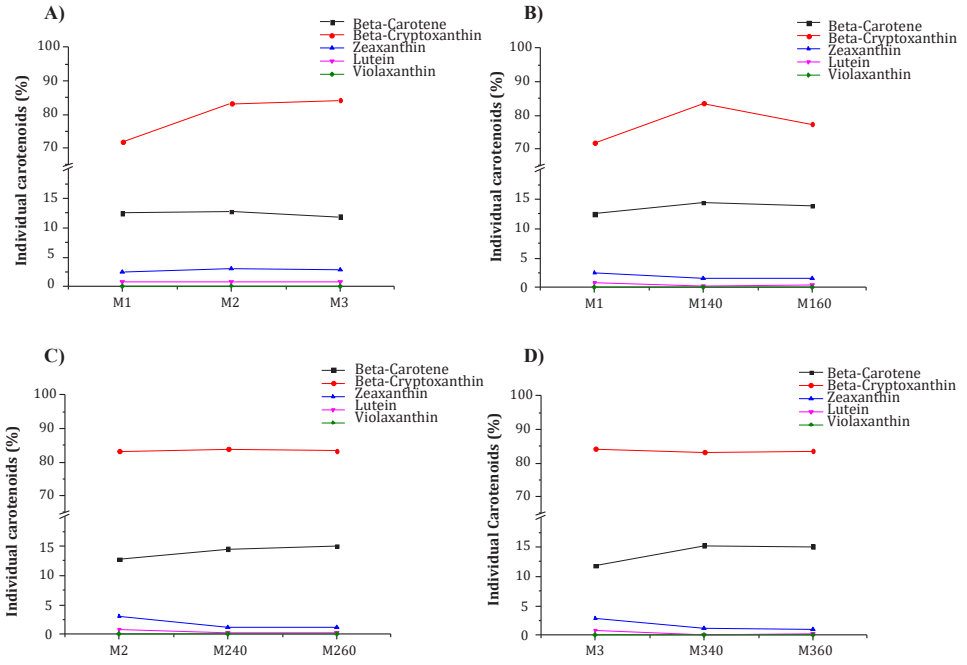

Figure 3. A, B, C, D: Individual carotenoids percentage detected by HPLC-DAD. A: effect of ripening stage (M1, M2, M3) in fresh samples. B, C and D: effect of dehydration at 40 and 60 °C in M1 (B), M2 (C), and M3 (D), respectively.

3.4. Microstructure

Figure 4 shows images of the fresh persimmon samples in the three ripening stages. In sections stained with toluidine blue, the cell walls appeared turgid, compact and with high physical integrity in the three ripening stages (Figure 4A, 4D, and 4G). In unstained sections of persimmon (Figure 4B, 4E, and 4H), the carotenoids could be seen with their characteristic yellow orange colour, distributed homogeneously throughout the cellular tissue. Carotenoid substances were observed grouped in two separate ways. They were inside the chromoplasts, close to the cellular wall, adopting a globular structure or spherical bodies. In addition, carotenoids appeared formed as crystalline clusters

inside the cell and cell wall. There was an increase in the carotenoid's accumulation in M3 samples (Figure 4H and 4I) compared to M1 and M2. These results agreed with the TCC, as M3 samples had more carotenoids than M1 samples.

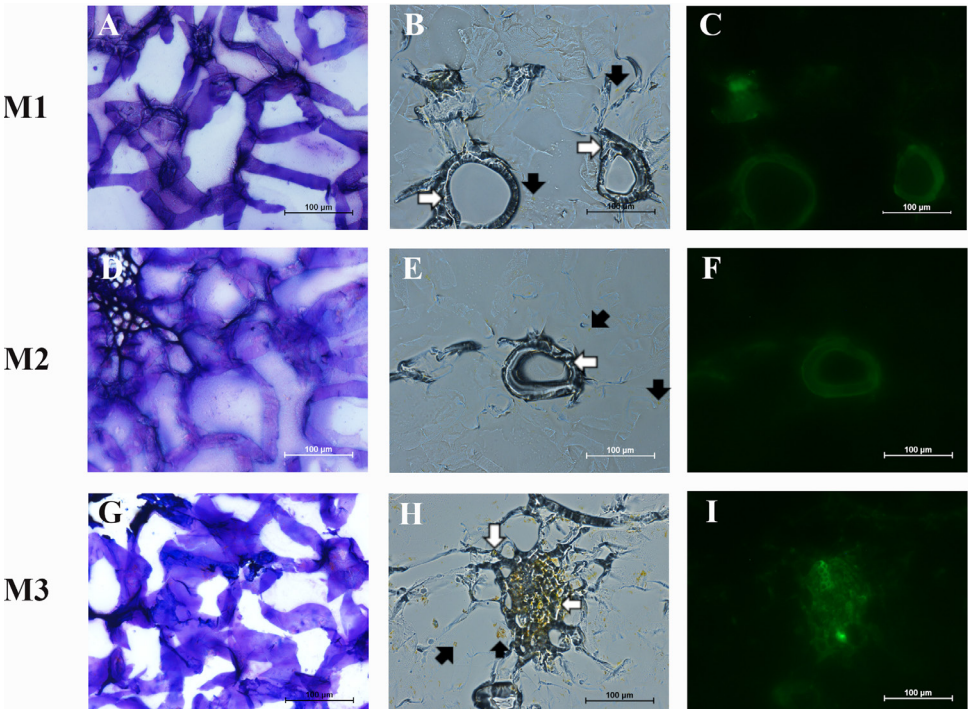

Figure 4. Microstructure of the fresh persimmon samples at the three ripening stages (M1, M2, M3). A, D, G: Blue toluidine stained images. B, E, H: unstained samples (White arrows: crystalline clusters of carotenoids; Black arrows: spherical bodies of carotenoids). C, F, I: autofluorescence images. Magnification 20x.

During ripening the transformation of chloroplasts into chromoplasts occurred, which is the most frequent modification of plastids in the ripening of fruits (Egea et al., 2010; Schweiggert et al., 2011; Vázquez-Gutiérrez et al., 2011). Autofluorescent emission was attributed to carotenoids because An, G.H., (2000) concluded that, using excitation with a 488 nm argon ion laser, the autofluorescence emitted at lengths greater than 515 nm was because of carotenoids. Autofluorescence images (Figure 4C, 4F, and 4I) showed that a substantial proportion of carotenoids were in the cell walls, in the three ripening stages.

Figure 5 shows images of dehydrated persimmon samples at the three ripening stages. The sections stained with toluidine blue (Figure 5A, 5D, 5G, 5J, 5M, and 5P) showed differences and changes in the cell wall structure of the samples after drying at 40 and 60 °C. In the unstained (Figure 5B, 5E, 5H, 5K, 5N, and 5Q) and autofluorescence (Figure 5C, 5F, 5I, 5L, 5O, and 5R) persimmon images the changes in the structure and location of the carotenoids can be observed.

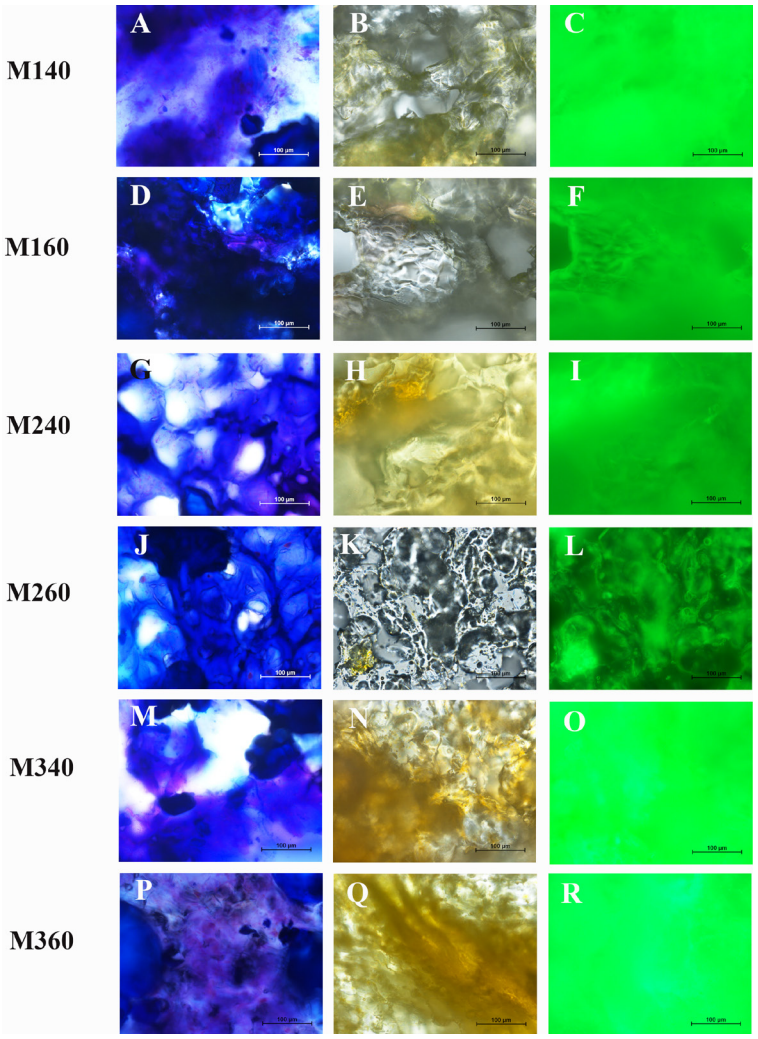

Figure 5. Microstructure of dehydrated persimmon samples dried at 40 and 60 °C at the three ripening stages (M1, M2, M3). A, D, G, J, M, P: Blue toluidine stained images. B, E, H, K, N, Q: unstained samples. C, F, I, L, O, R: autofluorescence images. Magnification 20x.

When comparing the fresh and dehydrated persimmon samples (Figures 4 and 5; blue toluidine stained images), both drying treatments produced the degradation of walls and membrane cells, thus loss of cellular structural integrity (Figure 5A, 5D, 5G, 5J, 5M, and 5P) in the three ripening stages. This degradation of the tissue could facilitate the diffusion of carotenoids. In all dehydrated persimmon samples, independent of the ripening stage, carotenoids lost a part of their crystalline appearance and were spread throughout the tissue (Figure 5B, 5E, 5H, 5K, 5N, and 5Q). In addition, the drying treatments enhanced the circulation and diffusion of carotenoids throughout the tissue, as seen in the autofluorescence images (Figure 5C, 5F, 5I, 5L, 5O, and 5R). The carotenoids diffusion could be related to the extractability of the carotenoids after the drying treatment. Therefore, it could improve the bioaccessibility of carotenoids because they would be more dispersed. However, this release from the cells could leave the carotenoids more exposed to environmental conditions affecting its antioxidant properties.

3.5. Fluorescence of carotenoids

Different excitation wavelengths were studied to determine the energy at which maximum emission occurs. Figure 6A shows the normalised excitation and emission spectra of the carotenoids extracted from the M1 fresh sample, which allowed selection of the 340 nm wavelength, used in the fluorescence analysis. Besides, emission spectra of pure β-carotene was conducted to compare with the extracted carotenoids, observing similar features, although with more definition, seen in Figure 6B. The emission spectra of β-carotene is characterised by a band from 400 to 500 nm with three characteristics peaks.

Figure 6 also shows the normalised fluorescence spectra for the fresh and dehydrated samples excited at 340 nm and recorded between 360 to 650 nm. Figure 6C shows the effect of the ripening stage, while ripening progresses on the spectra; Figures 6D, 6E, and 6F present the

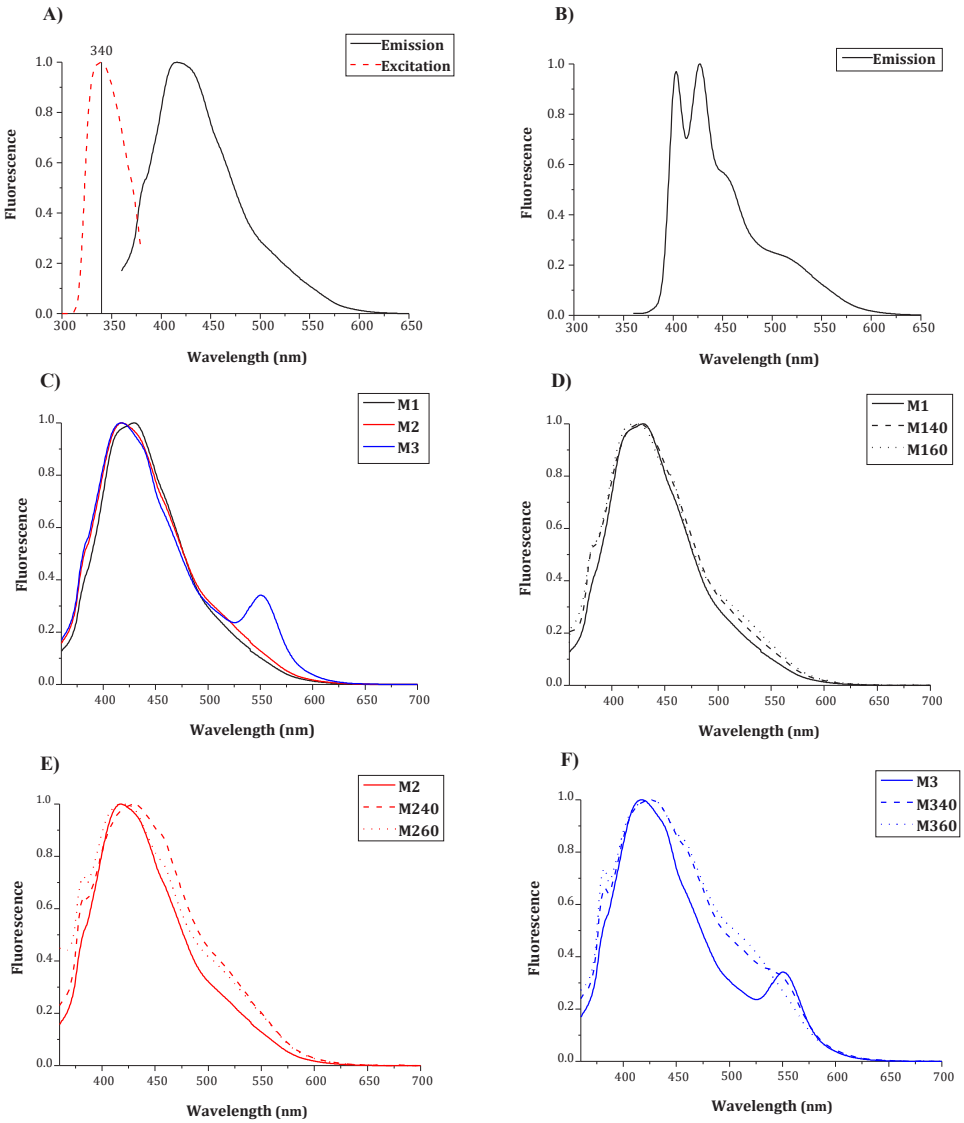

Figure 6. A: Normalised excitation (λexcitation = 340 nm; – – –) and emission (––) spectra of the carotenoids extract from fresh M1 sample. **B:** Normalised emission (––) spectra of the β-carotene pure extract excited at 340 nm. **C, D, E, F:** Normalised emission spectra of the carotenoids extract from fresh and dehydrated persimmon samples at λ excitation = 340 nm. **C:** the effect of the fresh samples in M1, M2, M3 ripening stages in the fluorescence. **D, E**, and **F:** the effect of the fresh and dehydrated samples dried at 40 and 60 °C in M1, M2, and M3 ripening stages on the fluorescence, respectively.

effect of the drying treatments in the earlier (M1), intermediate (M2), and advanced (M3) ripening stages, respectively.

All the samples were strong emitters when excited at 340 nm. A broad emitting band ranging from 375 nm to 500 nm was observed which corresponds to a hidden band in the UV/Vis spectra, associated to a symmetry-forbidden $\pi \rightarrow \pi^*$ transition (Jørgensen et al., 1992). This was demonstrated when the normalised spectra of the different samples were observed.

In Figure 6C a small difference of intensity among M1, M2, and M3 was noticed. An emitting peak at 380 nm can be observed in the M2 and M3 persimmon samples. This peak might be associated with different cis-isomers of β-carotene, as reported previously by Zaghdoudi et al., (2017). Although carotenoids are seen in an all samples, trans-conformation are the predominant proportion in nature and cis-isomers represent a minor fraction in fresh fruits (Aman et al., 2005). In Figures 6D, 6E, and 6F, this peak was remarkable with dehydrated samples, which showed a higher intensity at 380 nm if compared with the fresh persimmon samples, especially in the M2 and M3 ripening stages. Previous authors found some all-trans-β-carotene could be partially converted into cis-isomers by the heat action during the drying treatments (Schieber & Carle, 2005). Therefore, the formation of these cis-isomers could be related with the reduction of the AA after the drying treatments (Figure 2D and 2F) because the cis-isomers have lower AA (Pénicaud et al., 2011).

In Figure 6C a shift in the emission spectra, evidenced by a new band at 550 nm, was observed; especially in the most advanced ripening stage (M3). This could be related with the formation of new compounds such as β-cryptoxanthin. As seen in Figure 3A, β-cryptoxanthin was one of the main carotenoids that increased in advanced ripening stages. This also agrees with the study of Bordiga et al., (2019) where β-cryptoxanthin was the most abundant carotenoid as ripening progressed. Furthermore, the emission at longer wavelengths caused

by β-cryptoxanthin is strongly supported by Jørgensen et al., (1992), because of the nonradiative deactivation produced by n-states in xanthophylls, which reduces the energy gap between higher singlet state and the emissive state.

In Figures 6D, 6E, and 6F, there was a shift in the emission spectra of the dehydrated samples towards broader wavelengths. The evolution of the fluorescence spectra of the dehydrated samples towards longer wavelengths could be explained by the thermal degradation of carotenoids as described previously by Ehlers et al., (2007). In addition, several authors have studied the thermal degradation of carotenoids and proved that isomerisation and oxidation were the main degradative reactions of carotenoids (Kim et al., 2006; Colle et al., 2016). Pénicaud et al., (2011) investigated that isomerisation could be the first step of oxidation, leading to the formation of apocarotenones and apocarotenals from the epoxides.

4. Conclusion

As the ripening stage progresses, persimmon samples present higher TCC and AA values. The appearance of an emission peak at longer wavelengths, in the advanced ripening stage implies changes in the carotenoids fraction as the synthesis of β-cryptoxanthin confirmed by HPLC.

Hot air drying treatments do not affect the TCC or the carotenoids fraction but decrease the carotenoids AA values. At the microstructural level, both treatments lead to persimmon cellular integrity loss and favour the diffusion of the carotenoids throughout the vegetal tissue. This could expose them to external conditions favouring their degradation, which may explain the decrease in the persimmon antioxidant activity. The appearance of a new emission peak, at shorter wavelengths, and a shift in the spectra, both induced by drying treatments, could be related to isomerisation reactions and thermal

degradation, respectively. Therefore, the photoluminescence help to understand the phenomena caused by both, ripening stage and drying treatments, in the persimmon's carotenoid fraction.

The combination of spectrophotometric, chromatographic, structural, and photoluminescence techniques allows quantifying, detecting, and studying the changes in carotenoids of persimmon tissue produced by drying treatments.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

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Empar Llorca: Investigation, Validation, Methodology, Formal analysis.

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Gemma Moraga: Supervision, Resources, Conceptualization, Funding acquisition, Writing - review & editing.

Amparo Quiles: Investigation, Supervision, Resources, Conceptualization, Funding acquisition, Writing - review & editing.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Capítulo 4:

Efecto del tratamiento de secado y el tratamiento de desastringencia sobre la capacidad antioxidante y el contenido en taninos solubles e insolubles del caqui. Estudios de digestión in vitro.

An *in vitro* digestion study of tannins and antioxidant activity affected by drying "Rojo Brillante" persimmon.

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ABSTRACT

This study focuses on the evaluation of soluble and insoluble tannins and their antioxidant activity in fresh and dehydrated "Rojo Brillante" persimmon after *in vitro* digestion. Persimmon and its derived products contain a high amount of tannins with high antioxidant activity. An inversely proportional relationship between soluble and insoluble tannins was observed marked by the deastringency and hot air-drying treatments. Furthermore, the antioxidant activity after the hydrolysis of insoluble tannins was greater compared to soluble tannins. After small intestine *in vitro* digestion, the recovery of soluble tannins was higher in samples dehydrated at 40 and 60 °C, and insoluble tannins remained intact. Therefore, soluble tannins could be absorbed in the small intestine and insoluble tannins could reach the colon microbiota, both indicating potential health-promoting properties. Therefore, hot air drying and freeze-drying are alternative treatments to develop dehydrated persimmon snacks or powdery ingredients to improve nutritional properties of new foods.

Keywords: *Diospyros kaki*, dehydrated persimmon, astringency, polyphenols, microstructure

1. INTRODUCTION

Consumption of fruits and vegetables has grown recently as many chose them for a healthier diet (Brito et al., 2020). Following a diet with a high intake of fruits and vegetables entails a high nutritional value since they are excellent sources of vitamins, minerals, fiber, and bioactive compounds. Among the fruits, persimmon (*Diospyros kaki* L. f) is a popular fruit with high dietary fiber content, high bioactive, and antioxidant compounds such as tannins, vitamins, and minerals (Jung et al., 2005). Persimmon is considered a fruit with a large quantity of soluble and insoluble tannins, also called extractable and

non-extractable polyphenols (Matsumura et al., 2016). Soluble tannins are responsible for the astringency in persimmon fruit; however, they become insoluble as the ripening stage progresses, or after treatments with carbon dioxide, ethanol vapor, and other treatments (Arnal & Del Río, 2003; Masahiko et al., 2012; Salvador et al., 2007); thus, the astringency is no longer detected. Insoluble tannins—or bound polyphenols—are usually ignored and have long been neglected, although their content is usually more abundant than soluble tannins (Pérez-Jiménez et al., 2013). Soluble tannins are easily extracted with solvents (water or alcohol) whereas insoluble tannins remain in the extraction residue. The high molecular weight, complex structure, and binding to macromolecules makes insoluble tannins difficult to extract from a food matrix. However, acidic or basis hydrolysis can break insoluble tannins' structure, so they can be extracted and quantified (Ding et al., 2020; Domínguez-Rodríguez et al., 2017). Soluble and insoluble tannins can contribute to the health benefits of polyphenols because of their antioxidant activity, antiadipogenic, antitumor, and antidiabetic effects (Shin et al., 2014; Tian et al., 2012; Zhou et al., 2019).

According to the FAO, approximately 1.3 billion tons of food are wasted or lost every year worldwide. These losses mainly come from fruits and vegetables, accounting for ≤50%, highlighting that the food industry plays a role in waste production. Consumption of "Rojo Brillante" persimmon (astringent variety) has increased, especially in Spain. However, the high volumes of production, the strict quality control to which the fruit is subjected, and the inefficiency of deastringency treatment in advanced ripening stages have produced large surpluses that cannot be managed (Cárcel et al., 2010; Munera et al., 2017; Novillo et al., 2015). Therefore, one of the current challenges for the persimmon industry is to seek strategies to increase the value of discarded fruits and encourage a circular economy. Treatments such as hot air drying at low temperatures and freeze-drying have been demonstrated as good alternatives to address the surpluses of the "Rojo Brillante" persimmon industry. Hot air-drying treatments reduce the

soluble tannins, which become insoluble, and develop a well-accepted dehydrated persimmon snack (González et al., 2021) and a freeze-drying process creates a sweet crispy product with a high quantity of bioactive compounds (González et al., 2020). Hot air drying offers dehydrated products that can have a longer shelf life, whereas freeze-drying stops most deterioration and microbiological reactions, giving an excellent quality final product (Ratti, 2001). To guarantee the functionality of these dehydrated products, it is very important to know the impact of the digestion process on the main soluble and insoluble tannins, thus understanding the relationship between food composition, processing, and digestion steps.

This study aimed to evaluate the soluble and insoluble tannins content along with the antioxidant activity in astringent and non-astringent "Rojo Brillante" persimmon fruits and its dehydrated products. In addition, *in vitro* digestion determined the recovery index of soluble and insoluble tannins.

2. Material and methods

2.1. Sample preparation

Persimmon (*Diospyros kaki* Thunb. cv. Rojo Brillante) fruits—astringent and non-astringent samples (treated with 95% CO_2 over 24 h at 20 °C)—were provided by the Instituto Valenciano de Investigaciones Agrarias (IVIA, Spain). These persimmon fruits were harvested in a local grove in L'Alcudia (Valencia, Spain) in early December, and corresponded to ripening stage VI (intense orange) (Tessmer et al., 2016) and they had 80% initial water content. The fresh fruits were washed and transversally cut into slices (5 mm thick) with a mandolin (mandolin slicer 2.0, OXO good grips, Sheffield, UK) without removing the peel; the stalk and the opposite end were discarded. The samples

were dried using hot air drying and freeze-drying methods. Hot air drying was conducted in a cabinet dryer (model FED 260 standard, Binder, Tuttlingen, Germany) using an air velocity of 2 m s^{-1} at 40 and 60 °C, until reaching 15 ± 3% water content (23 and 9 h were needed, respectively). The freeze-dried samples were rapidly frozen in an ultra-freezer Infrico ULF700 (Equitec, Valencia, Spain) at -80 °C and freeze-dried in a Telstar Lioalfa-6 Lyophyliser (Telstar, Azbil Group, Terrassa, Spain) at 10^{-2} Pa and -40 °C over 48 h, until reaching 2 ± 0.5% water content. These groups of fruits were named as AF, NAF, AFD, NAFD, A40, NA40, A60, NA60, where A: astringent, NA: non-astringent, F: fresh, FD: freeze-dried, 40: hot air dried at 40 °C, 60: hot air dried at 60 °C.

2.2. Total soluble and insoluble tannins extraction

Samples (5 g) were homogenized in an Ultraturrax (IKA T18 digital, Staufen, Germany) with 25 mL of ethanol (96%). Homogenates were centrifuged (30,024 *x g*, 20 min, 4 °C) and filtered, while keeping the supernatant. More supernatant was extracted from the pellet with 25 mL of ethanol (96%) and added to the first supernatant. The mix supernatant containing soluble tannins was brought to 100 mL with 96% ethanol (Hernández-Carrión et al., 2014). The pellet, containing insoluble tannins, was soaked in 1% (v/v) hydrochloric acid (HCl) in 96% ethanol (25 mL) and stirred (orbital shaker Rotabit, J.P. SELECTA, Abrera, Barcelona, Spain) for 30 min at room temperature (≈25 °C) in. After, the solution was centrifuged at 30,024 *x g*, 20 min, 20 °C and the supernatant was kept. Then, the pellet was washed again with 1% HCl - 96% ethanol (25 mL) with the stirring and centrifuge steps repeated. Both combined supernatants, containing insoluble tannin fraction, were brought to 100 mL with 1% HCl-96% ethanol (Liu et al., 2018; Matsumura et al., 2016).

2.3. Tannin content determinations

Tannin content was measured using the Folin–Denis method. Briefly, 1 mL of the extract (1 mL of double-distilled water for the blank) and 6 mL of double-distilled water were mixed and vortexed. After, 0.5 mL of Folin-Ciocalteu reagent was added. After 3 min, 1 mL saturated Na_2CO_3 (20%) was added, vortexed, followed by 1.5 mL of double-distilled water. Absorbance was measured after 90 min at 725 nm to determine the total tannin content (Arnal & Del Río, 2004). Results were expressed as grams of gallic acid equivalents (GAE)/100 grams of dry basis. The analysis was conducted in triplicate.

2.4. Analysis of antioxidant activity

Antioxidant activity was measured using a ferric reducing antioxidant power assay (FRAP). Distilled water (30 µL), extract (30 µL), and FRAP reagent (900 µL) were placed in each cuvette. Distilled water was used as blank. Cuvettes were incubated for 30 min in a water bath covered with aluminum foil, at 37 °C; the absorbance was measured at 595 nm. The calibrated curve was performed using different concentrations of Trolox in 96% ethanol. Results were expressed as µmol Trolox/g (db.) of sample. The analysis was made in triplicate (Hernández-Carrión et al., 2014).

2.5. Simulated *in vitro* digestion process.

An *in vitro* gastrointestinal tract model was used to simulate the biological fate of ingested samples, following the methodology described by Eriksen et al. (2017); Gómez-Mascaraque et al. (2017); Minekus et al. (2014). Three phases were simulated: oral, gastric, and small intestine. All the enzymes used in the analysis were supplied by Sigma-Aldrich (Spain).

The digestion process was carried out in a Carousel 6 Plus reaction station (Radleys, UK). To mimic human physiological conditions, the

analysis was carried out at a controlled temperature (37 °C) agitation (150 rpm), and without light. Both the gastric and intestinal step were performed in an N_2 atmosphere to mimic human physiological reduction of oxygen levels during digestion. The digestions were carried out in duplicate and the results were expressed as a dry basis to facilitate comparison between the different treatments.

Solutions of simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) were prepared according to the compositions described by Minekus et al. (2014). First, for the oral stage, 5 g of sample was added in a beaker, then 4 mL of SSF + α-amylase (75 U/mL in the digestion mixture; pH 7), 19 µL of $CaCl_2$, and 0.981 mL of distilled water were added. The oral digesta was agitated for 2 min, then it was added to the digestion flask. Second, for the gastric phase, 16 mL of SGF + pepsin (2000 U/mL in the digestion mixture) and 8 µL of $CaCl_2$ were added. The pH was adjusted to 3 using 1 M HCl, and a volume of distilled water necessary for a total volume of 20 mL was added. The mixture was incubated at 37 °C for 1 h under agitation in anaerobic conditions. Third, for the intestinal stage, 12 mL of SIF + pancreatin (16.25 mg/mL), 45 µL of $CaCl_2$ and 12 mL of SIF + bile salts (37.80 mg/mL) were added. The pH was adjusted to 7 using 1 M NaOH. Once the pH was readjusted, a volume of distilled water necessary for a total volume of 30 mL was added. The mixture was incubated at 37 °C for 2 h and the final intestinal digesta was centrifuged (30,024 *x g*, , 20 min, 4 °C) and filtered (Whatman® Grade 4). The pellet was an unabsorbed material (OUT) and the filtered solution (supernatant) was the accessible fraction (IN). The samples were stored at -80 °C until further analysis.

2.6. Recovery index calculations

To analyze the effect of *in vitro* digestion on soluble and insoluble tannin content, the recovery index was used. The recovery index gives the tannin content recuperated after the intestinal digestion,

by comparison with the amount in the undigested fraction (Lucas-González et al., 2018). The recovery index was measured according to the equation 1.

$$Recovery\ index\ (\%) = \frac{DF}{UDF} \times 100 \quad (1)$$

Where DF (digested fraction) is the soluble and insoluble tannins content after the small intestine digestion; UDF (undigested fraction) is the soluble or insoluble tannin content quantified in the fresh matrix.

2.7. Microstructure analysis

Microscopic analysis was performed using a Nikon Eclipse 159 80i® light microscope (Nikon Co. Ltd., Tokyo, Japan) with a camera (Exwave HAD, nº DXC-19, Sony Electronics Inc., Park Ridge, NJ, USA). The images were stored at 1,280 × 1,024 pixels using the microscope software (NIS-Elements F, Version 4.2, Nikon, Tokyo, Japan). The samples were cut with a cryostat (Leica CM 1950, Barcelona, Spain), placed on slides and stained with vanillin-HCl (1:1, v/v) to identify tannins.

2.8. Statistical analysis

All data were analyzed using the XLSTAT statistical software 2014 (Addinsoft, Barcelona, Spain). A categorical multifactorial experimental design with two factors, type of sample and treatment, was used to characterize the soluble and insoluble tannin content, and the antioxidant activity of the samples before and after *in vitro* digestion. The least significant difference (LSD test) with a 95% confidence compared the mean values obtained ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Total soluble and insoluble tannin content, and antioxidant activity in fresh and dehydrated samples

Figure 1 shows the soluble and insoluble tannins content along with the antioxidant activity detected in both fresh and dehydrated samples.

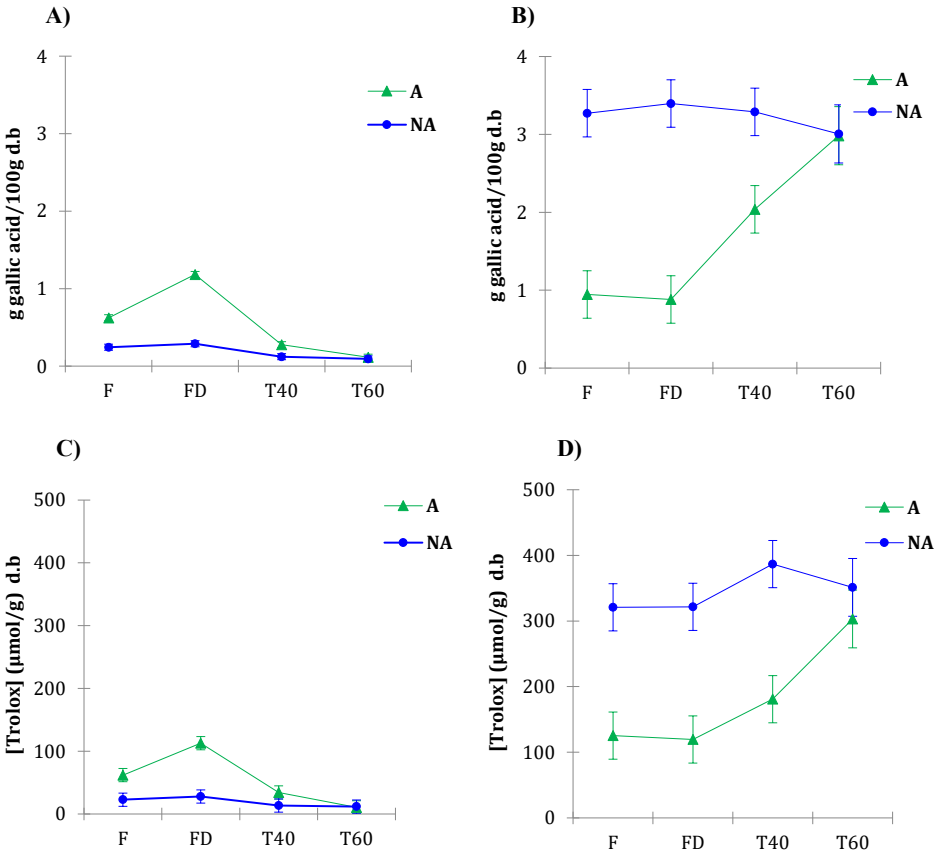

Figure 1. Interactions plot with LSD intervals. Interaction between the sample (A: astringent, NA: non-astringent) and treatment (F: fresh, FD: freeze-dried, T40: dried at 40 ^{0}C, T60: dried at 60 ^{0}C) for total soluble tannin **(A)** and insoluble tannin content **(B)**. Antioxidant activity of soluble tannins **(C)** and insoluble tannins **(D)**.

Regarding the soluble tannin content (Figure 1A), significant interactions were observed between the type of sample and treatment factors. Total soluble tannin content of the astringent (A) samples showed a wide range of change, depending on the drying method. The AF persimmon samples had a soluble tannin content of 0.624 ± 0.004 g/100 g gallic acid (db.), falling within the range found in most previous studies conducted on "Rojo Brillante", high soluble tannin content has been related to high astringency level (Arnal & Del Río, 2003; Taira et al., 1997). After the freeze-drying treatment, AFD samples' soluble tannin content increased significantly ($p < 0.05$) compared to the AF samples. Freeze-drying is carried out without the presence of oxygen at very low temperatures, which mostly avoids the degradation and enzymatic reactions. It can be considered the best drying method for preserving bioactive compounds or other nutrients in foods (Duan et al., 2016). Furthermore, freeze-drying has a higher extraction efficiency, because ice crystals formed in the plant matrix break the cell structure, so the cell components flow out and enter the solvent, obtaining a better extraction effect (Seiber et al., 2004). However, as the AFD samples have a high content of soluble tannins, they would not be edible because of their high astringency. Here, they could obtain easy-to-handle powder ingredients to develop nutraceuticals. A40 and A60 persimmon samples showed a significant ($p < 0.05$) reduction of soluble tannin content after drying at 40 and 60 °C. A60 samples presented the lowest soluble tannin content. Hot air-drying treatments usually reduce the soluble tannins content because of the transformation of soluble forms of tannins into their insoluble forms (González et al., 2021). Regarding the NAF samples, there was a significant reduction ($p < 0.05$) of the soluble tannin content compared to AF samples because of the deastringency treatment applied. After the deastringency treatment, soluble tannin compounds are transformed into their insoluble forms (Pérez-Burillo et al., 2018). NAFD samples did not show significant differences ($p > 0.05$) after the freeze-drying treatment compared to NAF samples, whereas both NA40 and NA60 showed a significant decrease ($p < 0.05$). Notably,

A40 samples showed similar values to NAF and NAFD samples but A60 gave values in the same range as NA40 and NA60. Therefore, a previous deastringency treatment would not be necessary for the production of dehydrated persimmon with both hot air-drying treatments.

Figure 1B shows the total insoluble tannin content, where significant interactions were observed between the sample and treatment factors ($p < 0.05$). The higher value of insoluble tannin content in AF samples compared to soluble tannin content (Figure 1A) suggest that several soluble tannins had been converted to insoluble tannins through the natural ripening process (Tessmer et al., 2016). There were no significant differences ($p > 0.05$) between AF and AFD samples; the freeze-drying treatment maintained the insoluble tannin content. After the hot air-drying treatments, an increase of insoluble tannin content was observed in the A40 and A60 samples. A60 samples presented the highest insoluble tannin content. This supported the hypothesis of the insolubilization of soluble tannins with the application of hot air drying, as previously explained. Hamauzu & Suwannachot (2019) also observed that "Ichida-gaki" astringent persimmon dried using natural drying were mostly composed of insoluble tannins.

NA samples showed a greater content of insoluble tannins without significant differences ($p > 0.05$) between them (Figure 1B). The significant differences ($p < 0.05$) found between AF and NAF samples are related to the deastringency treatment. Because of the tannins precipitation, a greater formation of insoluble tannins was generated in the NA samples (Salvador et al., 2007). Notably, A60 samples did not differ significantly with NA samples, as seen in the total soluble tannin content (Figure 1A). Hence, an inversely proportional relationship was observed between soluble and insoluble tannin content; the higher the soluble tannin content, the lower the insoluble tannin content and vice versa.

The antioxidant activity showed the same trend for soluble and insoluble tannin content (Figure 1C and 1D). These data reflect the

antioxidant activity present in the samples that come from the soluble and insoluble tannin content after hydrolysis. Several studies have reported the powerful antioxidant activity of persimmon derived from the tannins present in the matrix (Gu et al., 2008; Shin et al., 2014). Significant interactions were observed between type of sample and treatment factors in the antioxidant activity of soluble and insoluble tannins after hydrolysis (Figure 1C and 1D). The antioxidant activity of the insoluble tannin extracts (Figure 1D) was higher than the antioxidant activity of soluble tannin extracts (Figure 1C). In many scientific studies, insoluble tannins or non-extractable polyphenols have been underestimated. Non-extractable polyphenols have shown to possess interesting biological activities such as antioxidant, anti-inflammatory, chemo-preventive, among others; therefore, they play an important role in many foods (Domínguez-Rodríguez et al., 2017; Pérez-Jiménez et al., 2013; Saura-Calixto, 2012). Non-extractable polyphenols usually reach the colon almost intact, where they undergo most of the metabolic transformation. The mentioned health benefits may not come from the intact non-extractable polyphenols, but from their metabolites (Pérez-Jiménez et al., 2013). Zhou et al. (2019) and Matsumura et al. (2016) also observed a greater contribution of hydrolyzed non-extractable fractions in the antioxidant activity from "Mopan" fresh astringent persimmon and "Hohrenbo" astringent persimmon dried using natural drying.

3.2. Microstructural analysis

Figure 2 shows the different persimmon tissue samples; all samples appeared red due to the tannins being stained by vanillin. Insoluble tannins are observed enclosed in tannic cells, whereas soluble tannins appeared dispersed throughout the tissue. This distribution of insoluble and soluble tannins was also observed by Tessmer et al. (2016) during the ripening process of different astringent and non-astringent cultivars of persimmon. The tissue of fresh astringent persimmon

(Figure 2A) showed both soluble and insoluble tannins, due to the natural ripening process when the soluble tannins become insoluble.

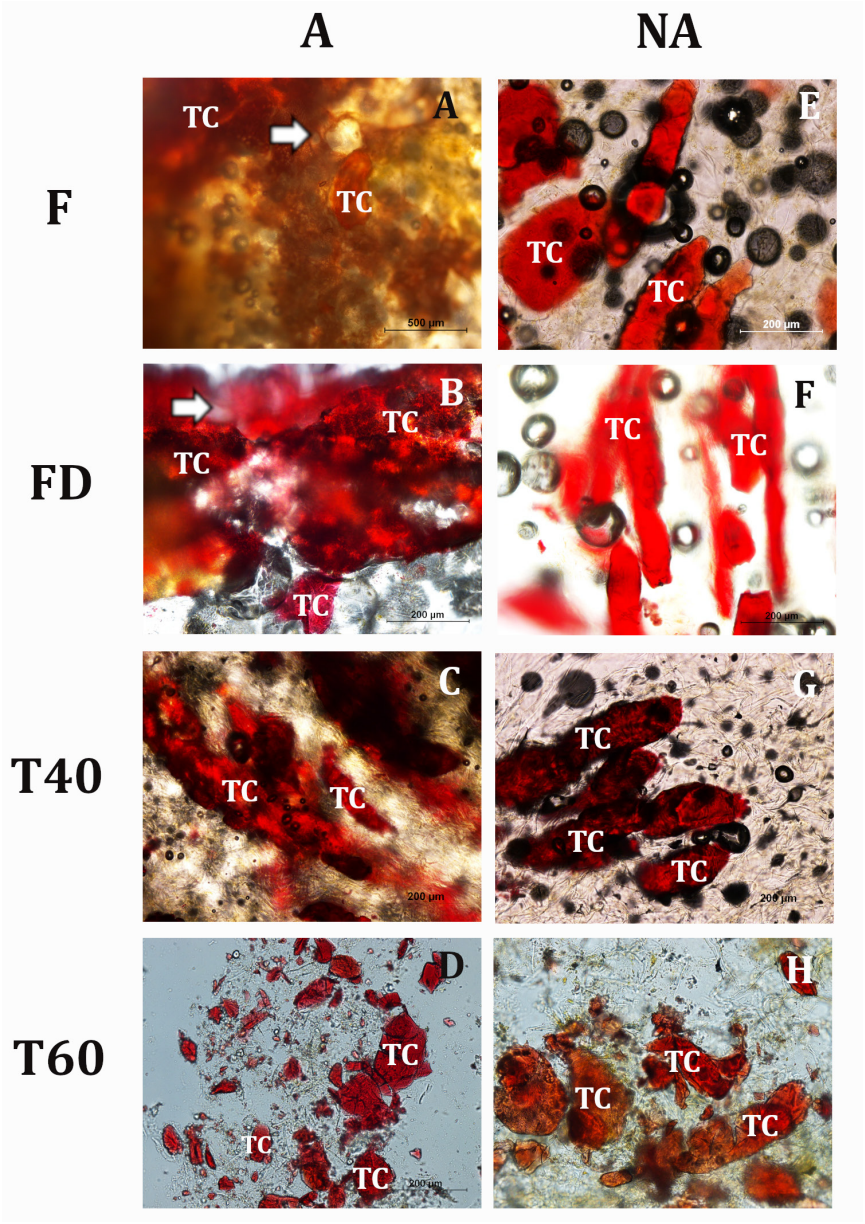

Figure 2. Images of fresh and dehydrated persimmon samples. **F:** fresh, **FD:** freeze-dried, **T40:** samples dried at 40 ^{0}C, **T60:** samples dried at 60 ^{0}C; **A:** astringent; **NA:** non-astringent; **TC:** tannin cell, White arrow: soluble tannins.

However, in non-astringent fresh samples (Figure 2E) most tannins were observed insolubilized, namely, inside of the tannic cells, because of the deastringency treatment, which produced the polymerization and insolubilization of tannins.

A higher content of soluble tannins is seen in the astringent freeze-dried samples than the fresh samples (Figure 2B). These results correlate with the results in Figure 1. The freeze-drying treatment favored the solubilization of the tannins and their migration throughout the persimmon tissue in astringent samples. However, freeze-drying did not affect the tannin structure in non-astringent persimmon (Figure 2F and 2E). In these samples, most tannins appeared insolubilized inside the tannic cells, agreeing with the results in Figure 1.

Regarding hot air-drying treatments, both treatments produced tannin insolubilization in astringent samples; a higher content of insoluble tannins was observed in T60 than in the fresh and freeze-dried samples, with tannins remaining mainly inside tannic cells (Figure 2C and 2D). Zhao, Ameer, & Eun, (2021) in their study on the influence of different drying techniques, observed that hot air-drying treatment increased the insolubilization of tannins. However, here, the hot air-drying treatments did not affect the solubilization or insolubilization of tannins in non-astringent persimmon (Figure 2G and 2H), as most of the tannins already appeared insolubilized and polymerized in fresh persimmon samples because of the deastringency treatment, which follows results shown in Figure 1.

3.3. Soluble and insoluble tannins content and antioxidant activity after *in vitro* digestion

The evolution of soluble and insoluble tannins in fresh and dehydrated samples after *in vitro* digestion is shown in Figure 3.

The chyme soluble fraction (IN) and the pellet fraction (OUT) were studied to comprehensively understand the release of tannins

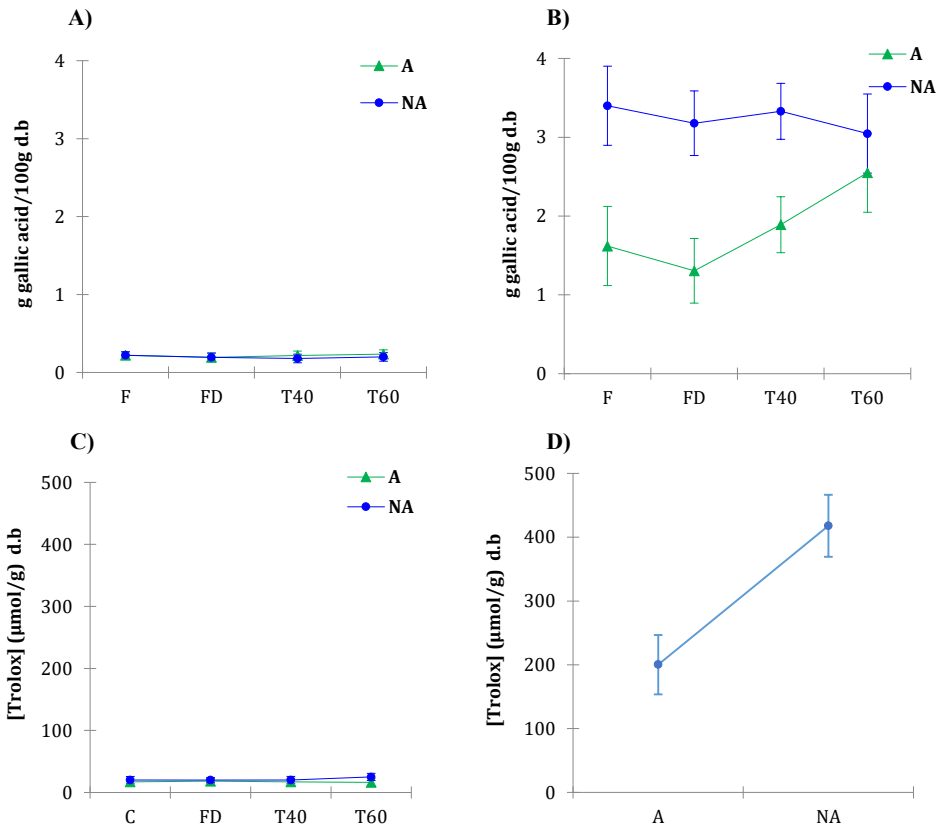

Figure 3. Means and interactions plot with LSD intervals. Interaction between the sample (A: astringent, NA: non-astringent) and treatments (F: fresh, FD: freeze-dried, T40: dried at 40 ^{0}C, T60: dried at 60 ^{0}C) for total soluble tannins in the IN fraction **(A)** and insoluble tannins in the OUT fraction of fresh and dehydrated samples **(B)**. Interaction between the sample and treatments for antioxidant activity from soluble tannins in the IN fraction **(C)**. Means values for antioxidant activity to the sample in the OUT fraction for fresh and dehydrated samples **(D)**.

of both fractions from the persimmon matrix during the enzymatic and mechanic processes of *in vitro* digestion. The IN fraction (Figure 3A) corresponds mainly to soluble tannin content because it is the accessible fraction and can therefore be absorbed in the intestinal phase. No significant interactions were observed between the sample and the treatment factors, and no factors had a significant effect (p > 0.05). All the samples presented similar soluble tannin content after the *in vitro* digestion (0.190–0.240 g gallic acid/100 g db.).

The OUT fraction only showed insoluble tannin content; soluble tannins were not detected. Significant interactions were observed between the sample and the treatment factors (Figure 3B). A60 had significantly ($p < 0.05$) values higher than AF, AFD, and A40. NA samples showed a greater content of insoluble tannins than A samples without significant differences ($p > 0.05$) between them. Furthermore, A60 samples had no significant differences with NA samples. Therefore, there was a correlation with the insoluble tannin content of the initial undigested samples, which showed that the insoluble tannins remained intact after *in vitro* digestion.

These results agree with other studies. Palafox-Carlos, Ayala-Zavala, & González-Aguilar, (2011) observed that because insoluble tannins are mostly composed of high-molecular-weight proanthocyanidins or interact with macromolecules such as the fiber in persimmons, they are not released from the food matrix when passing through the gastrointestinal tract. Therefore, a high amount of insoluble tannins could arrive to the colon and could be used by gut microbiota (Pérez-Jiménez et al., 2013).

Regarding the antioxidant activity in the IN fraction (Figure 3C), no significant interactions were observed between the sample and treatment factors, and no factors had a significant effect ($p > 0.05$). The antioxidant activity had the same trend as the soluble tannin content of the IN fraction (Figure 3A) where no differences were found between A and NA samples (16.3–20.1 μmol Trolox/g db.). No interactions were observed between the sample and the treatment factors in the antioxidant activity obtained in the OUT fraction after hydrolysis (Figure 3D), however, the sample factor had a significant effect ($p < 0.05$). NA samples had higher antioxidant activity values than A samples, which was probably related to the higher content of insoluble tannins in the OUT fraction (Figure 3B). Therefore, after *in vitro* digestion, a high antioxidant potential was observed. Liu et al. (2018) also observed a reduction of the antioxidant activity in

the IN fraction after *in vitro* digestion of "Yongding" persimmon peels. However, Matsumura et al. (2016) observed an increase in the antioxidant potential after *in vitro* digestion of non-extracted fractions, especially in the large-bowel phase. They suggested that fermentative decomposition of non-extractable fractions of dried persimmon by intestinal microflora could produce metabolites with antioxidant capacity. They also carried out *in vivo* assays with rats fed a non-extractable fraction of dried persimmon; and after collecting plasma from rats, they observed an increase in the antioxidant activity in the diets containing the non-extractable polyphenols.

3.4. Microstructural analysis after *in vitro* digestion

The microstructure of the OUT fraction is shown in Figure 4. All samples (astringent and non-astringent) presented insolubilized tannins, which were observed intact and inside the tannic cells in the *in vitro* digested samples. This agrees with the insoluble tannin content obtained in the OUT fraction (Figure 3B). In all the non-astringent samples (treated and fresh) (Figure 4E, 4F, 4G, and 4H), a greater presence of insoluble tannins can be observed than in the astringent samples (Figure 4A, 4B, 4C, and 4D). No tannic cells were detected in the IN fraction observed using light microscopy, even with the soluble tannins diluted with the digestion fluids (images not shown).

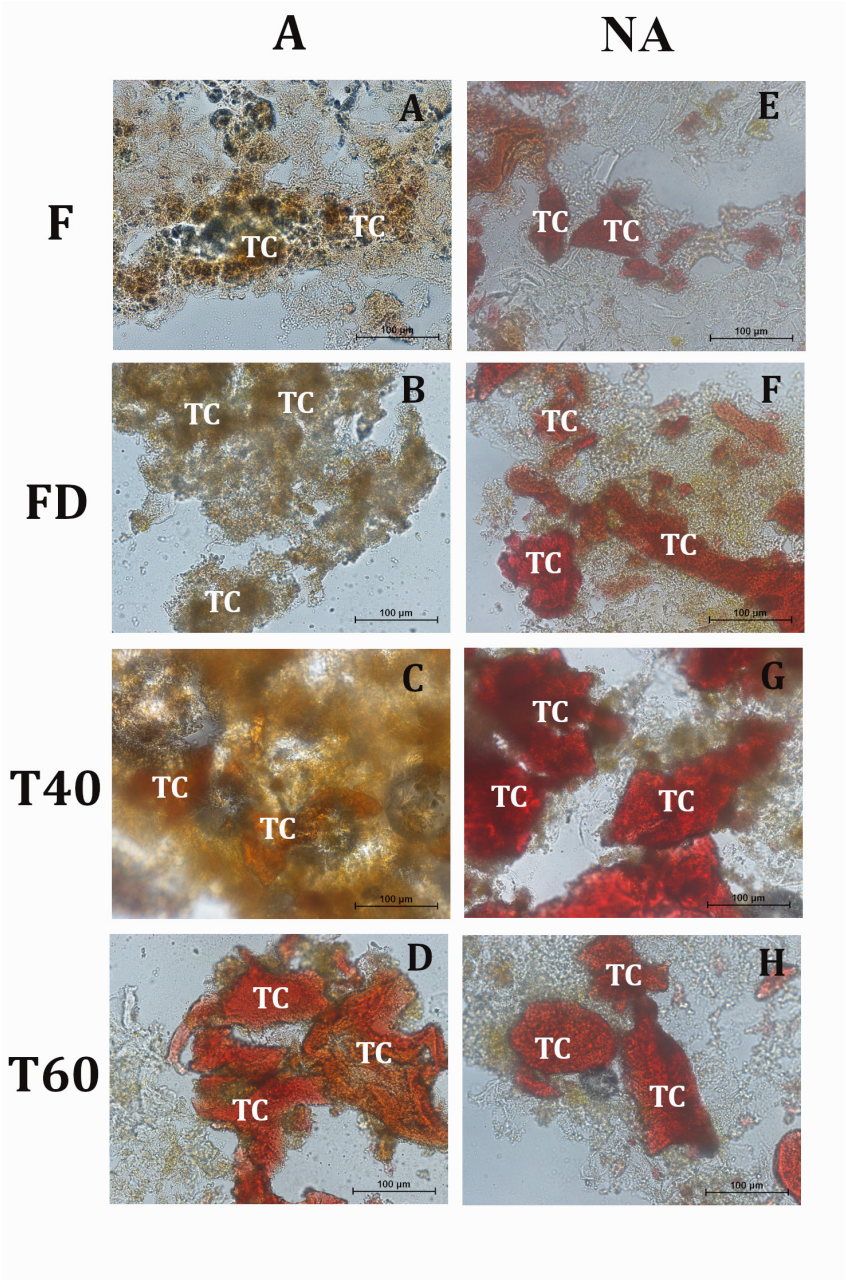

Figure 4. Images of fresh and dehydrated persimmon samples in the OUT fraction after in vitro digestion. **F:** fresh, FD: freeze-dried, **T40:** samples dried at 40 ^{0}C, **T60:** samples dried at 60 ^{0}C; **A:** astringent; **NA:** non-astringent. **TC:** tannin cell.

3.5. Recovery index

Once the content of soluble and insoluble tannins was obtained from the undigested and digested samples, the recovery index was calculated. Table 1 shows the recovery index of soluble and insoluble tannins. If the recovery of soluble tannins was low, the recovery of insoluble tannins was high and vice versa. This may be related to the insolubilization or solubilization of tannins as they pass through the gastrointestinal tract.

Table 1. Recovery index (RI%) of soluble and insoluble tannins after the in vitro digestion of fresh and dehydrated samples. **A:** astringent, **NA:** non-astringent. **F:** fresh, **FD:** freeze-drying, **T40:** samples dried at 40 ^{0}C, **T60:** samples dried at 60 ^{0}C.

Samples	RI (%) soluble tannins	RI (%) insoluble tannins
	A	
F	35	172
FD	16	151
T40	79	93
T60	205	86
	NA	
F	90	104
FD	67	94
T40	147	101
T60	213	101

Regarding the recovery index of soluble tannins, both in A and NA samples, those treated with hot air drying showed higher recovery index than fresh and freeze-dried samples. The A samples showed lower percentages of recovery than NA samples. These changes could be attributed to the interactions of soluble tannins with other dietary compounds, mainly fiber and proteins, and to the polymerization of soluble tannins to their insoluble forms in persimmon. During digestion, these interactions can be broken, and changes in the molecular structure can occur because of the pH in the intestinal phase and/or enzymatic

action, thus the solubility of the tannins increases (Diez-Sánchez et al., 2021; Lucas-González et al., 2018). Kayacan et al. (2020) also observed an increase in the recovery index of persimmon samples dried using hot air drying and infrared drying.

The recovery index of hydrolyzed insoluble tannins was high in all the samples. As seen previously, insoluble tannins reached intact to the OUT fraction after *in vitro* digestion (Figure 3B). AF and AFD samples obtained the highest percentage. These samples had higher insoluble tannin content compared to the undigested samples (Figure 1B), which could be related to the polymerization of soluble tannins into their insoluble forms, taking place during the *in vitro* digestion. Thus, insoluble tannins in the small intestine would become bioaccessible in the large intestine after the action of colonic microbiota (Hamauzu & Suwannachot, 2019).

Existing studies focusing on non-extractable polyphenols show they undergo extensive transformation in the large intestine (González-Sarrías et al., 2017; Pérez-Jiménez et al., 2013; Saura-Calixto, 2012). Once they reach the colon, several available routes can occur. These non-extractable polyphenols reach the colon intact or along with major indigestible macromolecules (fiber, carbohydrates, and proteins). The microbiota catabolizes these macromolecules, producing mainly short-chain fatty acids (acetic, propionic, butyric acids) (Saura-Calixto, 2012). Furthermore, low molecular weight polyphenols, along with some phenolic metabolites, are directly released from the non-extractable polyphenols (Pérez-Jiménez et al., 2013). Bacterial species that de-glycosylate dietary polyphenols in the gut include *Bacteroides*, *Enterococcus*, *Bifidobacterium*, and *Lactobacillus* (González-Sarrías et al., 2017). This catabolism by the bacteria present in the colon enhances the intestinal antioxidant status, which may protect against dietary pro-oxidants and free radicals, and also produces bioavailable metabolites with potential systemic effects (Saura-Calixto, 2012). Non-extractable polyphenols can exert potential health benefits through modulation of microbes locally in the gut, thus indirectly showing health effects as gut microbiota correlates with health status (González-Sarrías et al., 2017).

4. Conclusion

Drying techniques can remove the postharvest losses from "Rojo Brillante" persimmon, generating new products that may possess health effects for human. Hot air-drying treatments at 40 and 60 °C produce the insolubilization of soluble tannins, which removes the astringency of persimmon; however, in freeze-dried samples a deastringency treatment would be needed before consuming persimmon as a snack. Specifically, non-astringent dried products would be a good alternative to reduce food waste and increase persimmon consumption because of removing astringency and increasing insoluble tannins content. Drying astringent products at 60 °C is recommended as the tannin content almost equal to non-astringent products. Moreover, high soluble tannin recovery index values were obtained in persimmon dried at 40 and 60 °C. However, the recovery index of insoluble tannins was high in all samples; thus, they can reach the colon and potentially exert their antioxidant activity and health-promoting effects. Therefore, soluble, and insoluble tannins from fresh and dehydrated persimmon would show their beneficial effects in different parts of the gastrointestinal tract. This study helps understand the fate of soluble and insoluble tannins of dried persimmon after *in vitro* digestion and helps show how a product of food waste can be further processed to provide a healthy snack alternative.

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Discusión general de los resultados

La presente tesis abarca distintas estrategias para incrementar la rentabilidad del cultivo de caqui "Rojo Brillante" mediante la valorización de destríos o excedentes de esta fruta.

Se seleccionaron tratamientos de secado "natural", por aire caliente (40 y 60 ^{0}C) y liofilización para llevar a cabo el desarrollo de productos e ingredientes de alto valor nutritivo.

Durante la obtención de caqui semiseco por secado "natural" se determinó la cinética de secado, así como la evolución de algunos parámetros fisicoquímicos durante 81 días. Se registró la reducción de peso, el contenido en agua y la actividad del agua durante el proceso de secado. Los modelos matemáticos utilizados se ajustaron bien a los datos experimentales, por lo que todos fueron adecuados para representar las características de secado tanto de caqui astringente como no astringente. Los valores de la difusividad efectiva del agua fueron 5.07×10^{-11} m^2 s^{-1} y 6.07×10^{-11} m^2 s^{-1}, respectivamente. La cinética de secado en caqui "Rojo Brillante" siguió una tendencia similar a la de otras variedades asiáticas. El tratamiento de secado produjo una disminución en el contenido en taninos solubles, especialmente en el caqui astringente, dando lugar a un contenido similar a la fruta no astringente a partir de los 20 días de secado. La pulpa de la fruta astringente permaneció anaranjada durante el período de secado, mientras que se observó una coloración marrón en la fruta no astringente después de 57 días de secado. Por lo tanto, no sería recomendable aplicar un tratamiento previo de desastringencia, evitando así los costes del proceso.

El desarrollo de un producto de caqui semiseco, a partir de fruta astringente, podría ser una alternativa para aumentar la rentabilidad de la producción de caqui "Rojo Brillante" y con ello ayudar a reducir las pérdidas postcosecha.

El uso de la liofilización sobre caqui no astringente dio lugar a un producto crujiente y dulce con una mayor extractabilidad de compuestos bioactivos, y una capacidad antioxidante mayor que el caqui fresco. El almacenamiento de las rodajas liofilizadas a diferentes humedades

relativas permitió determinar las condiciones óptimas para mantener una textura crujiente y asegurar la calidad y estabilidad del producto obtenido. A 20^0C, los valores críticos de actividad del agua y humedad que provocaron la transición vítrea fueron 0.165 y 0.0312 g agua/ g producto, respectivamente. Por encima de estos valores, se produjo un cambio físico hacia un estado gomoso que dio lugar a importantes cambios en la textura del producto. Por debajo de estos valores, los snacks de caqui mantuvieron el estado vítreo y el carácter crujiente característico asociado a los snacks.

Para asegurar la calidad física y conservar los snacks de caqui liofilizados durante el almacenamiento a largo plazo, el estado vítreo debe mantenerse. Esto se puede lograr almacenando los snacks de caqui liofilizado a una humedad relativa por debajo de 16.5%, antes del envasado, para su comercialización a 20^0C, o estableciendo la temperatura de comercialización por debajo de 16.5 ^{0}C.

Los snacks obtenidos tras el secado por aire caliente (40 y 60 °C), mostraron una reducción de la luminosidad con una tonalidad más anaranjada en comparación con la fruta fresca. La aceptación por parte de los consumidores fue aumentando a medida que avanzó el estado de madurez del caqui utilizado como materia prima para la deshidratación. Los snacks obtenidos a partir de caqui no astringente fueron mejor valorados que los snacks obtenidos a partir de caqui astringente. Sin embargo, en el estado de madurez más avanzado, ambos fueron igualmente aceptados. Se observó una alta correlación entre la reducción del nivel de astringencia percibido por los consumidores y la disminución del contenido en taninos solubles. Así, los snacks obtenidos a partir de fruta astringente en la etapa de madurez más avanzada presentaron un contenido en taninos solubles similar a los obtenidos a partir de fruta no astringente, especialmente cuando se aplicó una temperatura de 60 °C.

El secado por aire caliente resultó una técnica útil para la valorización del destrío postcosecha de caqui, sin necesidad de aplicar

un tratamiento de desastringencia previo en estados de madurez avanzados, obteniendo un snack aceptado por los consumidores y pudiendo ser una alternativa para dar salida a la fruta descartada.

Tras la extracción de los carotenoides y su posterior detección y cuantificación por HPLC, se observó un aumento en su contenido a medida que avanzó el estado de madurez de la fruta, destacando la β-criptoxantina. Los tratamientos de secado por aire caliente no afectaron al contenido en carotenoides, sin embargo, se observó un descenso en la capacidad antioxidante de los snacks de caqui respecto a la fruta fresca. Los estudios microestructurales mostraron una mayor acumulación de carotenoides en las células a medida que aumentó el estado de madurez del caqui. Los tratamientos de secado provocaron la rotura de las paredes celulares permitiendo la difusión de los carotenoides por todo el tejido. Esto produjo una mayor exposición a las condiciones ambientales, afectando a su capacidad antioxidante. La técnica de fotoluminiscencia permitió ver bandas de emisión relacionadas con los carotenoides. La aparición de una nueva banda a mayor longitud de onda, en el estado de madurez más avanzado del caqui, se relacionó con la formación de nuevos compuestos como la β-criptoxantina. Por otro lado, una banda definida a menor longitud de onda se relacionó con la isomerización de los carotenoides pasando de su forma trans a su forma cis como consecuencia del proceso de secado. También se observó un desplazamiento de las bandas de emisión a mayor longitud de onda, que pudo estar relacionado con la degradación térmica que sufren los carotenoides tras el proceso de secado.

La combinación de técnicas espectrofotométricas, cromatográficas, estructurales y fotoluminiscentes permitió cuantificar, detectar y estudiar los cambios en los carotenoides producidos por la maduración y por los tratamientos de secado.

Finalmente, se decidió cuantificar los taninos solubles e insolubles de los caquis frescos utilizados como materia prima y de los productos obtenidos por liofilización y secado por aire caliente a 40 y 60°C, con el

objetivo de conocer su evolución tras la digestión *in vitro*. Se obtuvieron los índices de recuperación de ambas fracciones de taninos y se observó que cuando el índice de recuperación de los taninos solubles fue bajo, el índice de recuperación de los taninos insolubles fue elevado y viceversa. Esto se relacionó con la solubilización/insolubilización de los taninos a lo largo del tracto gastrointestinal. El índice de recuperación de los taninos solubles fue mayor en el caqui deshidratado a 40 y 60 °C que en la fruta fresca y que en los snacks liofilizados, mientras que el índice de recuperación de los taninos insolubles fue alto en todas las muestras. Además, el índice de recuperación de la fracción soluble en las muestras no astringentes fue mayor que en las astringentes.

Esto indicaría que los taninos solubles podrían ser absorbidos en el intestino delgado mientras que los taninos insolubles pueden llegar al colon donde potencialmente podrían ejercer su capacidad antioxidante. En este sentido, los taninos solubles e insolubles del caqui fresco y deshidratado podrían distribuirse y absorberse a lo largo del tracto gastrointestinal, produciendo beneficios en la salud. En conclusión, el uso de tratamientos como el secado "natural", secado por aire caliente y liofilización puede dar salida a excedentes y destríos del caqui "Rojo Brillante". Además, el desarrollo de estos productos o ingredientes puede tener un efecto beneficioso para la salud por el alto contenido en compuestos bioactivos y su alta capacidad antioxidante.

Conclusiones

Las principales conclusiones que se extraen de la presente tesis son:

- El método de secado "natural" seguido en los países asiáticos aplicado en el caqui "Rojo Brillante" produce una disminución natural del contenido en taninos solubles. Los productos semisecos obtenidos a partir de los frutos astringentes y no astringentes obtienen valores similares de taninos solubles en tan solo 20 días de secado. El caqui semiseco obtenido a partir de fruta astringente mantiene el color anaranjado y está menos pardeado que el caqui semiseco obtenido a partir de fruta no astringente. Por lo tanto, no sería recomendable aplicar un tratamiento previo de desastringencia. El tratamiento de secado "natural", aún no aplicado en la industria del caqui "Rojo Brillante", podría ser una buena estrategia para valorizar el excedente de esta fruta.

- La liofilización de caqui no astringente muestra ser una alternativa práctica para elaborar snacks de caqui listos para su consumo o ingredientes de interés nutricional. Su aplicación permite obtener un producto dulce y crujiente con una gran cantidad de compuestos bioactivos. El punto crítico de actividad del agua y humedad para mantener el estado vítreo del producto se encuentra en valores inferiores a 0.165 y 0.0312 g de agua /g producto, respectivamente. Por tanto, se puede asegurar el estado vítreo de los snacks de caqui liofilizado almacenándolos a una humedad relativa inferior a 16.5%, antes del envasado, y comercializándolos a 20 ^{0}C, o estableciendo la temperatura de comercialización por debajo de 16.5 ^{0}C.

- El secado por aire caliente es una técnica útil para desarrollar un snack de caqui bien aceptado. En estados avanzados de madurez, es posible elaborar un snack a partir de caqui astringente con un contenido en taninos solubles semejante a los snacks obtenidos a partir de caqui no astringente, especialmente cuando se secan a 60 ^{0}C; de este modo se puede evitar la aplicación de un tratamiento previo de desastringencia. El desarrollo de snacks de caqui a partir de frutos astringentes puede

ser una alternativa para incrementar la rentabilidad del cultivo de caqui, especialmente al final de la temporada de cosecha, cuando el tratamiento de desastringencia puede resultar menos eficaz.

- El secado por aire caliente no afecta al contenido en carotenoides, pero disminuye su capacidad antioxidante. El secado por aire caliente conduce a la pérdida de la integridad celular del caqui y favorece la difusión de los carotenoides por todo el tejido vegetal. La técnica de fotoluminiscencia permite observar cambios en los espectros obtenidos, que se relacionan con reacciones de isomerización y degradación térmica de los carotenoides .

- Los tratamientos de secado por aire caliente producen la insolubilización de los taninos solubles, lo que permite eliminar la astringencia en los derivados del caqui. Sin embargo, la liofilización necesita un tratamiento de desastringencia previo para su consumo. Tras la digestión *in vitro* del caqui y los snacks de caqui, los taninos solubles son mayoritariamente absorbidos en el intestino delgado mientras que los taninos insolubles pueden seguir su curso hacia el intestino grueso y ayudar a modular la microbiota intestinal.