LWT - Food Science and Technology 155 (2022) 112961

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

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

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ARTICLE INFO

Keywords:
Diospyros kaki
Dehydrated persimmon
Astringency
Polyphenols
Microstructure

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.

1. Introduction

Consumption of fruits and vegetables has grown recently as many chose them for a healthier diet (Brito, Ferreira, & Fai, 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 and high 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, Giordani, & Yonemori, 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, Díaz-Rubio, & Saura-Calixto, 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, Morozova, Scampicchio, & Ferrentino, 2020; Domínguez-Rodríguez, Marina, & Plaza, 2017). Soluble and insoluble tannins can contribute to the health benefits of polyphenols because of their antioxidant activity, antiadipogenic, antitumor, and antidiabetic effects (Shin, Shon, Kim, & Lee, 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, García-Perez, Sanjuán, & Mulet, 2010; Munera et al., 2017; Novillo, Gil, Besada, & Salvador, 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

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https://doi.org/10.1016/j.lwt.2021.112961
Received 4 October 2021; Received in revised form 18 November 2021; Accepted 10 December 2021
Available online 14 December 2021

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, Hernando, & Moraga, 2021) and a freeze-drying process creates a sweet crispy product with a high quantity of bioactive compounds (González, Llorca, Quiles, Hernando, & Moraga, 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} and 30% relative humidity 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 of fresh samples and the equivalent dried mass of dehydrated samples) were homogenized (1 min) in an Ultraturrax (IKA T18 digital, Staufen, 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 mix supernatant containing soluble tannins was brought to 100 mL with 96% ethanol (Hernández-Carrión, Vázquez-Gutiérrez, & Hernando, 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). After, the solution was centrifuged at 30,024×*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, Qiao, Ren, & Li, 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 in darkness at 725 nm to determine the total tannin content (Arnal & Del Río, 2004). Results were expressed as grams of gallic acid equivalents (GAE)/100 g of dry basis (d.b). 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 (d.b) of sample. The analysis was made in triplicate (Hernández-Carrión, Vázquez-Gutiérrez, & Hernando, 2014).

2.5. 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 1280 × 1024 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.

2.6. 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, Luu, Dragsted, and Arrigoni (2017); Gómez-Mascaraque, Perez-Masiá, González-Barrio, Periago, and López-Rubio (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 mol/L 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 mol/L 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×*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.

2.7. 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, Viuda-Martos, PérezÁlvarez, & Fernández-López, 2018). The recovery index was measured according to equation (1).

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

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.

2.8. 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

Fig. 1 shows the soluble and insoluble tannins content along with the antioxidant activity detected in both fresh and dehydrated samples. Due to the difference in water content between fresh and dehydrated samples, the results are expressed on a dry basis for a better comparison. Regarding the soluble tannin content (Fig. 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, Ono, & Matsumoto, 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 is more suitable to increase extraction efficiency, because ice crystals formed in the plant matrix break the

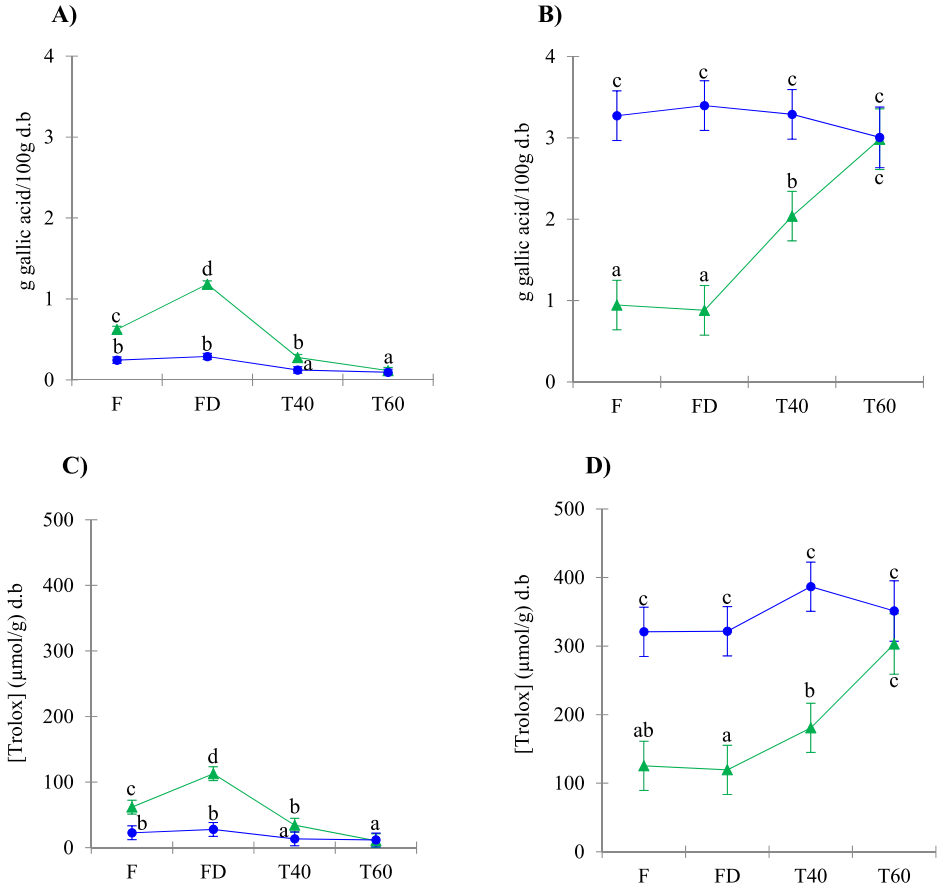

Fig. 1. Interactions plot with LSD intervals. Interaction between the sample (Astringent: green triangles, non-astringent: blue circles) and treatment (F: fresh, FD: freeze-dried, T40: dried at 40 °C, T60: dried at 60 °C) for total soluble tannin **(A)** and insoluble tannin content **(B)**. Antioxidant activity of soluble tannins **(C)** and insoluble tannins **(D)**. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cell structure, so the cell components flow out and enter the solvent, obtaining a better extraction effect (Seiber, Felsot, Rosen, Mitchell, & Barrett, 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, Oliveras, Quesada, Rufián-Henares, & Pastoriza, 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.

Fig. 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 (Fig. 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 and 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 (Fig. 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 (Fig. 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 (Fig. 1C and D). 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 (Fig. 1C and D). The antioxidant activity of the insoluble tannin extracts (Fig. 1D) was higher than the antioxidant activity of soluble tannin extracts (Fig. 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

Fig. 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 (Fig. 2A) showed both soluble and insoluble tannins, due to the natural ripening process when the soluble tannins become insoluble. However, in non-astringent fresh samples (Fig. 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 (Fig. 2B). These results correlate with the results in Fig. 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 (Fig. 2F and E). In these samples, most tannins appeared insolubilized inside the tannic cells, agreeing with the results in Fig. 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 (Fig. 2C and D). Zhao, Ameer, and 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 (Fig. 2G and H), as most of the tannins already appeared insolubilized and polymerized in fresh persimmon samples because of the deastringency treatment, which follows results shown in Fig. 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 Fig. 3. The chyme soluble fraction (IN) and the pellet fraction (OUT) were studied to comprehensively understand the release of tannins of both fractions from the persimmon matrix during the enzymatic and mechanic processes of *in vitro* digestion. The IN fraction (Fig. 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 (Fig. 3B). A60 had significantly ($p < 0.05$) higher values 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,

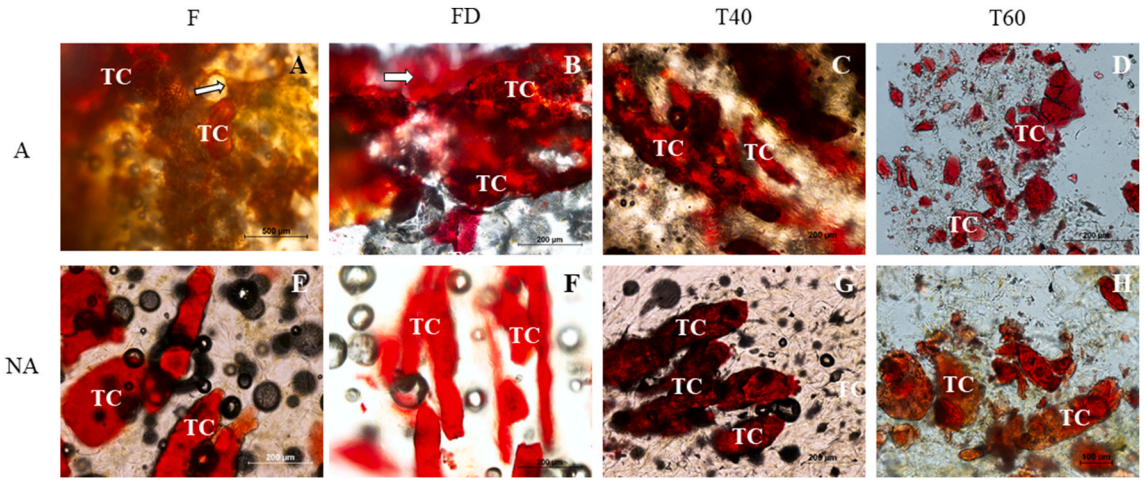

Fig. 2. Images of fresh and dehydrated persimmon samples. F: fresh, FD: freeze-dried, T40: samples dried at 40 °C, T60: samples dried at 60 °C; A: astringent; NA: non-astringent; TC: tannin cell, White arrow: soluble tannins.

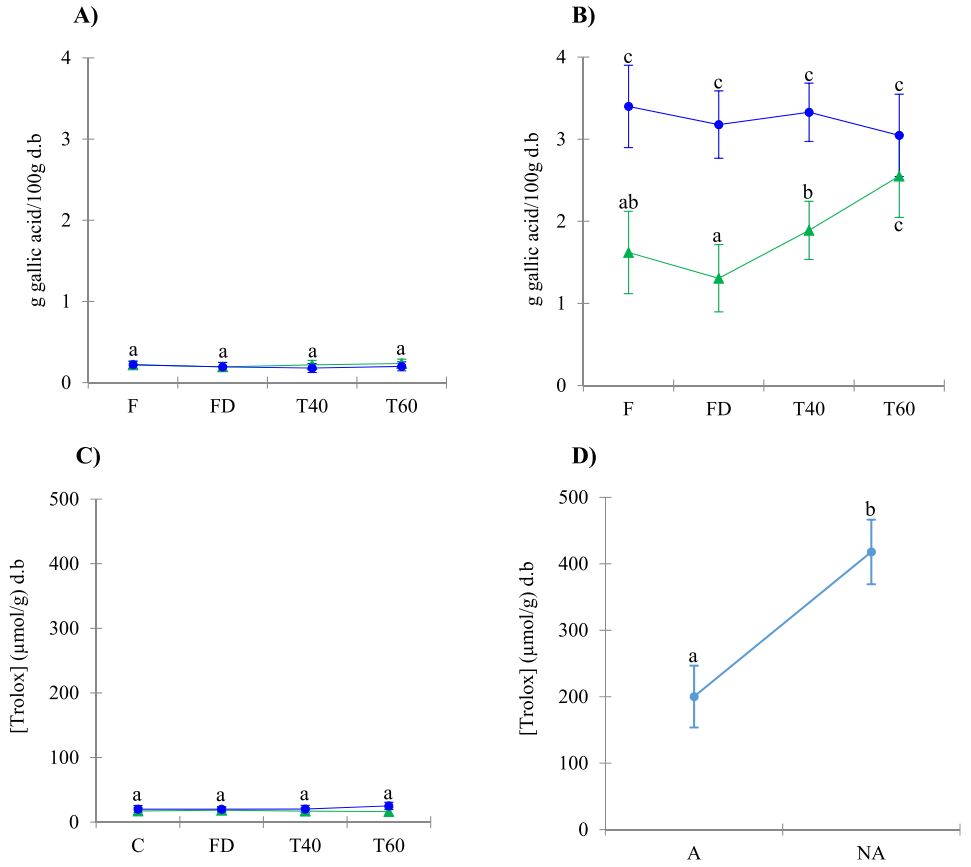

Fig. 3. Means and interactions plot with LSD intervals. Interaction between the sample (Astringent: green triangles, non-astringent: blue circles) and treatments (F: fresh, FD: freeze-dried, T40: dried at 40 °C, T60: dried at 60 °C) for total soluble tannins in the IN fraction **(A)** and insoluble tannins 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)**. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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, and 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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 Fig. 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 (Fig. 3B). In all the non-astringent samples (treated and fresh) (Fig. 4E, F, 4G, and 4H), a greater presence of insoluble tannins can be observed than in the astringent samples (Fig. 4A, B, 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).

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.

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

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 °C, T60: samples dried at 60 °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

can occur because of the pH in the intestinal phase and/or enzymatic action, thus the solubility of the tannins increases (Diez-Sánchez, Quiles, & Hernando, 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 (Fig. 3B). AF and AFD samples obtained the highest percentage. These samples had higher insoluble tannin content compared to the undigested samples (Fig. 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, Espín, & Tomás-Barberán, 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

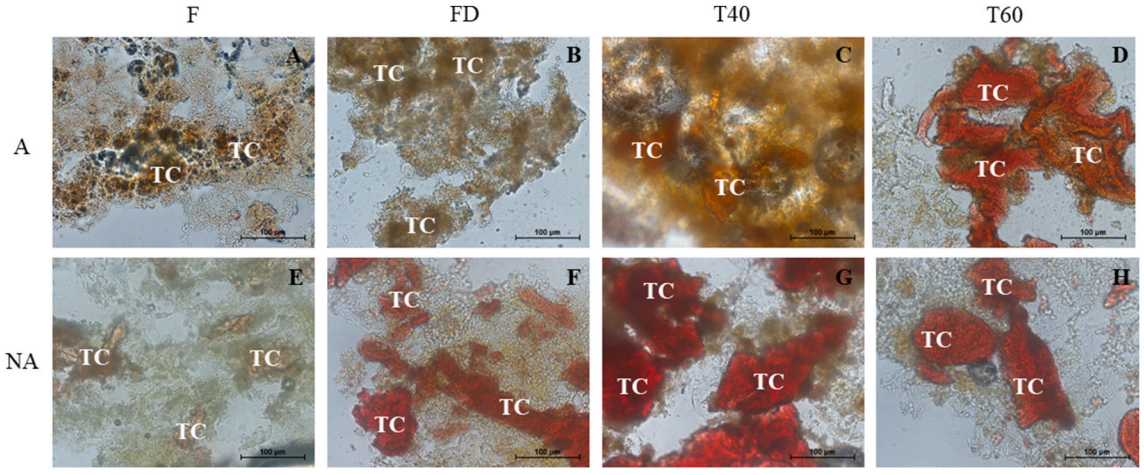

Fig. 4. Images of fresh and dehydrated persimmon samples in the OUT fraction after *in vitro* digestion. F: fresh, FD: freeze-dried, T40: samples dried at 40 °C, T60: samples dried at 60 °C; A: astringent; NA: non-astringent. TC: tannin cell.

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.

Funding

Grant RTA2017-00045-C02-02 funded by MCIN/AEI/ 10.13039/501100011033 and, by "ERDF A way of making Europe".

CRediT authorship contribution statement

Cristina M. González: Investigation, Validation, Methodology, Formal analysis, Writing – original draft. **Empar Llorca:** Investigation, Validation, Methodology, Formal analysis. **Amparo Quiles:** Investigation, Validation, Methodology, Formal analysis. **Isabel Hernando:** Supervision, Resources, Conceptualization, Funding acquisition, Writing – review & editing. **Gemma Moraga:** 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.

Acknowledgments

The authors thank Phillip Bentley for his assistance in correcting the manuscript's English.

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This key reference has helped to choose the dehydrated products by hot air-drying.

** 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. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 130*, 109633. https://doi.org/10.1016/j.lwt.2020.109633.

This key reference has helped to choose the dehydrated products by freeze-drying.

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This key reference has helped to know the results obtained after *in vitro* digestion and to compared the results.

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This key reference has helped to understand the function of insoluble tannins after the *in vitro* digestion and the possible health effects along the gastrointestinal tract.