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Evaluation studies of persimmon plant (*Diospyros kaki*) for physiological benefits and bioaccessibility of antioxidants by *in vitro* simulated gastrointestinal digestion

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ABSTRACT

This study aims to analyze the antioxidant benefits from persimmon leaf tea, fruit and fibres taking into account their changes along gastrointestinal digestion. The evolution of polyphenols, flavonoids and antioxidant capacity was studied using the recent harmonized *in vitro* protocol published by Minekus et al. (2014). The digestion was performed with and without digestive enzymes. Results showed aqueous leaf extract was richer in antioxidants than the fruit or the extracted fibres. Nevertheless, persimmon-leaf antioxidants were more sensitive to the digestive environment. In general, the oral conditions greatly affected the antioxidants, while gastric digestion led to slight additional losses. The intestinal step enhanced polyphenols and flavonoids solubility coming from the fruit and fibres. Additionally, the presence of digestive enzymes positively contributed to antioxidant release throughout digestion. Finally, the bioaccessibility of polyphenols, flavonoids and antioxidant activity of persimmon fruit were 1.4, 1.0 and 3.8 times higher than in aqueous leaf extract.

1. Introduction

Since it is better to prevent chronic diseases than to treat them, reducing the risk of cardiovascular diseases or cancer has become one of the priorities of sanitary authorities, scientists and the food industry. The consumption of functional foods enriched in antioxidants as well as fruits and vegetables naturally rich in bioactive compounds would contribute to reducing the risk of suffering from diseases associated with oxidative stress. Although it is true that the presence of bioactive compounds in the diet is linked to the aforementioned benefit, their repercussion in human health mainly depends on biochemical state they are in when they reach the bloodstream and, consequently the tissues. The digestion process itself leads to a series of changes in the macro and micronutrients conditioning their final bioaccessibility and bioavailability (Parada & Aguilera, 2007). The bioavailability of dietary compounds in general, and phytochemicals in particular, is dependent on their digestive stability, their release from the food matrix and the efficiency of their transepithelial passage. The best way to determine the benefits arising from the intake of foods, and its bioavailability, consists of subjecting the product to *in vivo* gastrointestinal digestion. However, *in vivo* trials are expensive and require long periods of observation, especially in human samples. Besides, they involve medical and ethical implications. Due to these limitations, scientific evidences positively support the alternative to employing *in vitro* models to accurately reproduce the biochemical conditions of different phases involved in the gastrointestinal digestion (During & Harrison, 2005). *In vitro* simulated digestion presents additional advantages compared with *in vivo* trials such as the possibility of sampling at different times of digestion process and eluding the inter-human body variability. Therefore, the evaluation of the influence of matrix food structure on antioxidant changes along gastrointestinal digestion by means of an *in vitro* simulation could consider as a valid approach. In this sense, authors decided to explore the influence of structure of food matrices all coming from Persimmon crop on the changes experimented by antioxidants during gastrointestinal digestion. Concretely, food matrices selected for this study were the persimmon fruit, the fibre, a functional ingredient extracted from the fruit, and the aqueous extract of persimmon dried leaves. Persimmon crop is native to China where it has been cultivated for centuries before Christ. It spread to Japan (c. VII) and Korea (c. XIV) in Middle Ages, and posteriorly to other continents. Persimmon cultivars can be divided into two distinct groups: astringent (*Rojo brillante, Triumph, etc.*) and non-astringent varieties (*Fuyu, Hana-Fuyu, Jiro, etc.*). Astringency is linked to the chemical structure of the tannins in the fruit, being insolubilized

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in non-astringency varieties and soluble in astringent ones. Soluble tannins can interact with proteins of saliva that gives rise to a bitter taste in mouth. As ripening progresses, the tannin content decrease and the risk of bitter taste in astringent varieties as well (Hernándiz, 1999). Nevertheless, some astringent varieties such as *Rojo brillante* are harvested unripened because of its demanding texture. *Rojo brillante* is the most important commercial variety in Valencian Community (Spain). According to data from Agricultural Minister of Spain, the land dedicated to persimmon crop has significantly increased in the recent past years (only in the last year it has increased an additional 20%) (Alòs, 2014), and subsequently the fruit production. This fact is partially due to the implementation of new technologies capable to remove astringency in unripened fruits that allow offering persimmons with firm texture and without astringency to consumers (Arnal Navarro & Del Río Gimeno, 2005). Parallely, this rapid increase of production has led to an overproduction due to both the seasonality of the persimmon culture and fruits do not meet market standards. Due to this situation, alternatives to obtain higher economical return from the culture have being explored. In this sense, the use of dried persimmon leaves in infusions or the inclusion of aqueous leaf-extract in functional food formulations could be attractive because of the high antioxidant content in this food matrix (Martínez-Las Heras, Quifer-Rada, Andrés, & Lamuela-Raventós, 2016). The extraction of functional ingredients from persimmon fruit such as fibre could be also interesting (Landines, Martínez-Las Heras, Heredia, & Andrés, 2014). Currently, fibres are used in many foods such as yoghourts or bakery products in order to improve the texture and viscosity of the product, and because of their prebiotic potential (Guevara-Cruz et al., 2013; Thebaudin, Lefebvre, Harrington, & Bourgeois, 1997). Fibre consumption is associated with the prevention and treatment of some diseases such as colon cancer, coronary diseases, constipation and, diabetes as well as antioxidants (Sorensen et al., 2014). In this context, the aim of this study was to determine the changes undergone by total polyphenols, flavonoids and antioxidant capacity of persimmon aqueous leaf extract, fruit and fibre, extracted from the pulp and peel of persimmon fruit and stabilized by hot air drying or freeze-drying, during gastrointestinal digestion by means of an *in vitro* simulation. Besides, the bioaccessibility of these antioxidant families was determined.

2. Materials and methods

2.1. Raw material

The raw materials used were persimmon tree (*Diospyros kaki, Rojo brillante*) leaves, fruit and fibre extracted from peel or pulp of the fruit.

Persimmon leaves were collected from an orchard in Alginet (Valencia, Spain). They were washed and blanched for 1 min at 100 °C and then dried by convection air current at 100 °C in an oven (30 min). Once they were dehydrated, they were grounded in a grinder (Severn) till they had a particle size of less than 1 mm. The resulting powder was finally used to prepare infusions. For this purpose, 1.5 g of leaf powder was mixed with 110 mL of boiling distilled water and were filtered with a Whatman paper (particle retention: 20–25 μm) after 5 min (Martínez-Las Heras, Heredia, Castelló, & Andrés, 2014).

The persimmon fruits were also collected in Alginet (Valencia, Spain). They were kept at environmental conditions for approximately 24 h. They were then subjected to a deastringency treatment in closed chambers with 95% of CO_2 at 20 °C and 90% of relative humidity for 24 h. Subsequently, the persimmon fruits were washed, peeled and the pulp cut into small cubes for digestion. Additionally, fibre was extracted from persimmon peel or pulp according to Escalada Pla et al. (2012). For this purpose, each fraction (peel and pulp) was separately homogenized with a high-performance dispersing instrument (T25 digital Ultraturrax IKA). Each fraction was mixed with boiling ethanol (96% v/v) in a ratio 1:2 (w/v) and the mixture was stirred at 600 rpm for 15 min. Finally, the ethanol was discarded by a sieve from the mixture and the semi-solid residue subjected to the Van Soest official method (Van Soest, Robertson, & Lewis, 1991) in order to confirm that it was fibre. Once the presence of fibre confirmed, it was dehydrated in a hot-air oven at 40 °C till it reached a constant mass (approximately 7 h), or frozen at −40 °C for 24 h and then freeze-dried (vacuum pressure of 10^{-1} mbar for 24 h). Therefore, four different fractions of fibre were obtained: PULP-A or PULP-F for hot-air dried or lyophilized pulp fibres; and PEEL-A or PEEL-F for hot air dried or freeze-dried/lyophilized peel fibres. The final moisture content of each fraction was: 6 ± 1, 7.8 ± 0.3, 6.9 ± 0.5 and 8 ± 1% for PULP-A, PULP-F, PEEL-A and PEEL-F, respectively.

2.2. In vitro simulated gastrointestinal digestion (GI) of persimmon food matrices

In vitro gastrointestinal digestion (oral, gastric and intestinal phases) was simulated following the harmonized INFOGEST protocol published by Minekus et al. (2014). According to this method, simulated salivary fluid (SSF), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared from stock solutions fresh daily and kept at 37 °C before their use. Stock solutions and simulated fluids were prepared with KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2(H_2O)_6$ and $(NH4)_2CO_3$ at the same molarity indicated in Minekus et al. (2014). Additionally, salivary α-amylase from human saliva was added to the SSF in a final concentration of 75 U/mL; pepsin from porcine gastric mucosa to the SGF in a final concentration of 2000 U/mL; and pancreatin from porcine pancreas and bile salts (bovine bile extract) to the SIF in a final concentration of 100 U/mL and 10 mM, respectively. All reagents and enzymes were obtained from Sigma-Aldrich Chemical Company (St Louis, MO, USA). Once simulated fluids prepared, *in vitro* digestion was carried out. For oral phase simulation, the different food matrixes (persimmon leaf aqueous extract, fruit and fibres) were mixed in a ratio food: SSF of 1:1 (w/v), and properly homogenized. The mixture was then placed in 50 mL falcon tubes and incubated without agitation for 2 min at 37 °C. Then, simulated gastric fluid (SGF) (pH 3) was added in a ratio of 1:1 (v/v) to each tube containing the oral mixture and the pH adjusted to 3 with HCl (1 N). The falcon tubes were placed in a head- over-heels stirrer (Intell-Mixer RM-2, Elmi Ltd, Riga, LV-1006, Latvia) at 55 rpm for 2 h at 37 °C in an incubator chamber (JP Selecta SA, Barcelona). Finally, simulated intestinal fluid (SIF) (pH 7), was added in a ratio of 1:1 (v/v) to each tube containing the gastric mixture, pH adjusted to 7 with NaOH (1 N) and the tubes placed in the head-over-heels stirrer at 55 rpm and in the incubator chamber to conduct the intestinal phase simulation for 2 h at 37 °C.

For analytical purposes, aliquots were taken at the end of each phase: oral (2 min), gastric (120 min) and intestinal one (120 min) as well as the following intermediate residence times: 10, 30, and 120 min for gastric and 30, 60, 90 and 120 min of intestinal digestions. Samples were placed in an ice bath for ten minutes and pH was adjusted to 9 to ensure enzyme inactivation.

The role of the pH of digestion on the antioxidants compounds changes along digestion was parallely evaluated by means of carrying out the entire simulation without digestive enzymes and bile salts. Simulation in the absence of enzymes served as control.

All simulations were performed twice and three aliquots were extracted at each of the specified sampling times in each one of the simulations.