TITLE: Digestibility of (Poly)phenols and Antioxidant Activity in Raw and Cooked Cactus Cladodes (Opuntia ficus-indica)

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ABSTRACT

This study aims to investigate whether heat treatment applied to cactus cladodes influences on the bioaccessibility of their (poly)phenolic compounds after simulated gastric and intestinal digestion. A total of 45 (poly)phenols were identified and quantified in raw and cooked cactus cladodes by UHPLC-PDA-HR-MS. Both flavonoids (60-68% total), mainly isorhamnetin derivatives, and phenolic acids (32-40%) with eucomic acids as the predominant ones, significantly ($p<0.05$) increased with microwaving and griddling processes. After *in vitro* gastrointestinal digestion, 55-64% of the total (poly)phenols of cooked cactus cladodes remained bioaccessible *versus* 44% in raw samples. Furthermore, digestive conditions and enzymes higher degraded or retained flavonoids (37-63% bioaccessibility) than phenolic acids (56-87% bioaccessibility). Microwaved cactus cladodes contributed the highest amount of (poy)phenols (143.54 mg/g dm) after gastrointestinal process, followed by griddled samples (133.98 mg/g dm), showing the highest antioxidant capacity. Additionally, gastrointestinal digestion induced isomerizations among the three stereoisomeric forms of piscidic and eucomic acids.

KEYWORDS: Polyphenols, cactus, *Opuntia ficus-indica*, heat treatment, *in vitro* gastrointestinal digestion, bioaccessibility.

INTRODUCTION

Cactus (*Opuntia ficus-indica*) is a plant belonged to the family *Cactaceae* which produces edible seeds, fruits and stems (cladodes) with nutritional and bioactive compounds [1]. In America, especially in Mexico, cladodes, known as "nopales", are commonly eaten as a fresh or cooked vegetable.

Previous studies have reported that cactus cladodes are a rich source of bioactive compounds including (poly)phenols, mainly flavonoids such as isorhamnetin, quercetin and kaempferol glycosides, as well as a minor quantity of phenolic acids such as ferulic, hydroxy benzoic, salicylic, chlorogenic and eucomic acids providing antioxidant capacity [2,3].

Cooking methods such as boiling, microwaving [4], frying and griddling [5] can induce changes in vegetables composition, influencing the concentration of polyphenols. Recently, it has been shown that heat treatment impacts on total (poly)phenolic content of cactus cladodes depending on the cooking technique. For instance, microwaving and griddling processes could increase the total (poly)phenol content of cactus cladodes, while there is a decrease in its concentration when cactus were boiling because of leaching into the water [6].

Several studies have revealed a positive correlation between a diet rich in plant-based foods and reduced risk of chronic diseases associated with oxidative stress such as cancer and cardiovascular and neurodegenerative diseases [7,8]. Protective effects of fruits and vegetables are mainly attributed to the presence of antioxidant phenolic compounds [9], which are usually bound to other structures like cellulose, hemicellulose, and could be partially released in the gastrointestinal tract from the food matrix to be absorbed [10].

Bioaccessibility is defined as the amount or fraction of a food compound, which is released from the food matrix in the gastrointestinal tract, becoming available for

absorption. *In vitro* digestion studies have been developed to simulate the physiological conditions taking place in the human gastrointestinal tract, including the mouth, stomach and intestine. Results reported in literature are controversial. While some studies have shown that *in vitro* gastrointestinal digestion decreases the phenolic content of vegetables as artichoke [11], pepper [12] and cardoon [13], others have reported that compounds are not affected by digestion process [14]. Up to now, none studies in cactus cladodes have been found.

Therefore, the aim of this work was to evaluate the bioaccessibility of (poly)phenolic compounds of both raw and cooked cactus cladodes monitoring them by UHPLC-PDA-HR-MS after a simulated gastric and intestinal digestion. To our best knowledge, this is the first work that investigates whether heat treatment applied to cactus cladodes influences on the bioaccessibility of their (poly)phenolic compounds and antioxidant capacity.

MATERIAL AND METHODS

Chemical and reagents. Raw cactus cladodes (*Opuntia ficus-indica*) were obtained from BioArchen company located in Murcia, Spain. Olive oil and soybean oil were obtained from local stores. Methanol and acetone solvents were of analytical grade from Panreac (Barcelona, Spain). Acetonitrile and formic acid (HPLC grade) were purchased from Panreac (Barcelona, Spain). Potassium chloride and sodium chloride were obtained from Merk (Darmstadt, Germany). Human saliva α-amylase (852 U/mg protein), pepsin (674 U/mg), pancreatin (4xUPS), bile salts (for digestion), sodium hydrogen carbonate, potassium phosphate monobasic, magnesium sulfate monohydrate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH·), the pure phenolic standards used for high-performance liquid

chromatographic and mass spectrometry (HPLC-MS) (isorhamnetin, kaempferol, quercetin, rutin and ferulic acid) were purchased from Sigma-Aldrich (Steinheim, Germany).

Samples preparation. Cactus cladodes were washed and the thorns were removed manually. Then, they were cut into small pieces, mixed well and divided into six portions of approximately 300 g. One portion (raw sample) was lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa, Spain) and stored at −18 °C until analysis. The other five portions were cooked as described below. Cooking conditions were previously normalized by preliminary experiments.

Boiling: 300 g of chopped fresh cactus cladodes were added to 600 g of boiling water in a stainless steel pan and maintained for 10 min. The samples were drained off and immediately cooled.

Microwaving: 300 g of chopped fresh cactus cladodes were placed in a silicone case (Lékué, Barcelona, Spain) and cooked in a domestic microwave oven (Whirlpool, Michigan, USA) at 900W for 5 minutes. Samples were drained off and immediately cooled.

Griddling: 300 g of chopped fresh cactus cladodes were submitted to heating at 150 ºC for 5 minutes and then at 110 ºC for 5 minutes in a non-stick griddle (Jata Electro, Vizcaya, Spain) without oil addition.

Frying: 300 g of chopped fresh cactus cladodes was fried with 30 mL of olive oil and another 300 g with 30 mL of soybean oil at 100 ºC for 10 minutes in a non-stick frying pan. Then, temperature was decreased to 90 ºC for 5 minutes.

After each heat treatment, every sample was lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa, Spain) and stored at -18ºC until analysis.

Simulated gastrointestinal digestion. A three step *in vitro* digestion model was carried out in a bioreactor according to Minekus et al. [15] and Monente et al. [16] adapted to our laboratory. Briefly, 2 g of each sample was weighted in a 100 mL vessel placed and heated in a water bath at 37 °C. The vessel was magnetically stirred and connected to a pH sensor. The three steps were carried out in absence of light. Simulated salivary, gastric and intestinal fluids (SSF, SGF and SIF) (Table 1S Supporting Information) were employed for each step. First, oral digestion was performed by adding 14 mL of the stock SSF solution, 250 μL of α-amylase solution (1.3 mg mL^{-1}), 0.10 mL of 0.3M $CaCl_2$, and water up to 20 mL. The sample was shaken for 30 min at 37 °C. Second, the gastric digestion step was carried out at pH 3 by addition 1M HCl. It was started by adding 15 mL of SGF, 1.19 mL of a pepsin solution (1 g of pepsin in 10 mL of 0.1 M HCl), 0.01 mL of 0.3M $CaCl_2$ and water up to 20 mL. After 2 h incubation, the final intestinal step was carried out by adding 22 mL of SIF, 10 mL of a pancreatin solution (0.008 g mL^{-1}), 5 mL of bile salts (0.025 g mL^{-1}), 0.08 mL of 0.3M $CaCl_2$ and water up to 40 mL. The pH was then adjusted to 7 with 1M NaOH and the samples were incubated for 2 h. Samples were taken after gastric and intestinal digestion and then were frozen and lyophilized in a freeze dryer Cryodos-80 (Telstar), and stored at -18ºC until further analysis. Each cactus sample was digested in duplicate and then the two repetitions were mixed and homogenized.

Extraction of (poly)phenols. Extracts of raw and cooked cactus cladodes, both digested and undigested, were prepared using the method of Avila-Nava et al. [17] with some modifications. Briefly, 25 mL of a methanol/water solution (50/50, v/v) was added to 2 grams of lyophilized cactus cladodes samples, stirred for 2 hours and then vacuum filtered through Whatman 1 filter paper. The resulting filtrate was saved and refrigerated. The residue was subjected to a second extraction with 25 mL of

acetone/water (70/30, v/v) solution, agitated for 2 hours and vacuum filtered through Whatman 1 filter paper. The resulting filtrate was also saved and refrigerated. A third extraction of the residue was performed using 25 mL of demineralized water for 30 minutes and then vacuum filtered through Whatman 1 filter paper. The resulting filtrates were mixed together and stored at -18ºC until analysis in less than 24 hours.

Identification and quantification of (poly)phenolic compounds by UHPLC-PDA-HR-MS. Qualitative and quantitative analysis of (poly)phenolic compounds in cactus samples were performed by UHPLC-PDA-HR-MS following the method described by Juániz et al. [5] with some modifications. The UHPLC equipment comprised with a PDA detector scanning from 200 to 600 nm, equipped with an autosampler operating at 4 ºC (Dionex Ultimate 3000 RS, Thermo Fisher Scientific, San José, USA) and an Exactive™ Orbitrap mass spectrometer fitted with a heated electrospray ionization probe (HESI) (Thermo Fisher Scientific, San José, USA). Separation was carried out using a column C18 5U Kinetex 100A (250 x 4.60 mm) (Phenomenex, Macclesfield, UK), and the volume of each sample injection was 20 µL. Chromatographic separation was performed at 40 ºC in 80 min using 5 to 30 % gradient of acid water with formic acid 0.1% (solvent A) and acetonitrile (solvent B) at a constant flow of 1 mL/min. After passing the PDA flow cell, the eluate was split and 0.2 mL/min was directed to the mass spectrometer with the HESI operating in negative ionization mode. Analysis was carried out in full-scan (100-800 m/z) and full-scan with In-Source Collision-induced dissociation (CID) (100-800 m/z; CID 25.0 eV). Capillary temperature was 300°C; sheath gas and auxiliary gas were 60 and 20 units/min, respectively; source voltage was 4.0 kV. Identification was achieved by comparing the exact mass and retention time with pure reference standards. In absence of standards, compounds were tentatively identified by comparing the theoretical exact mass of the molecular ion with the

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experimentally measured accurate mass of the molecular ion. In addition, identification was confirmed by the appearance of typical fragments produced from the molecular ion when the CID was applied. Quantification was performed at 280 nm for piscidic and eucomic acids and 325 nm for ferulic acid derivatives; and at 360 nm for flavonoids. Phenolic acids were expressed as ferulic acid equivalents, whereas isorhamnetin-, quercetin-, and kaempferol derivatives were quantified with their respective aglycones. Results were expressed as milligrams of each compound per gram of dry matter sample (mg /g dm).

Antioxidant Capacity by DPPH assay. The antioxidant capacity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) decolorization assay [18] with some modifications. A 6.1×10^{-5} M DPPH$^\bullet$ methanolic solution was prepared immediately before use. The DPPH$^\bullet$ solution was adjusted with methanol to an absorbance of 0.700 (±0.020) at 515 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV–VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). All the extracts were properly diluted in demineralized water prior to analysis. Samples (50 μL) were added to 1.95 mL of the DPPH$^\bullet$ solution. After mixing, the absorbance was measured at 515 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analog). The antioxidant capacity was expressed as micromoles of Trolox equivalent per gram of sample dry matter (μmol Trolox/g dm).

Statistical analysis. Each parameter was analyzed in triplicate. Results are shown as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied for each parameter. A Tukey test was applied as *a posteriori* test with a level of significance of 95%. All statistical analyses were performed using the STATA v.12.0 software package.

RESULTS AND DISCUSSION

Cactus cladodes are usually consumed fresh or cooked by boiling, griddling or frying. In a previous work, nutritional composition and antioxidant capacity changes in cactus cladodes after different heat-treatments have been reported [6]. However, the profile of the individual (poly)phenolic compounds, specifically in their glycoside form, of both raw and cooked cactus cladodes, as well as the effect of a gastrointestinal digestion on their bioaccessibility, remain unknown. This is of a great interest for further research in the claim of cactus cladodes health properties.

Influence of heat treatment on cactus cladodes (poly)phenolic compounds. Cactus cladodes were submitted to boiling, microwaving, griddling and both olive and soybean oil frying at domestic conditions, and the identification and quantification of individual (poly)phenolic compounds of raw and cooked cactus cladodes was carried out by UHPLC-PDA-HR-MS. A total of 45 (poly)phenolic compounds were identified and quantified. Flavonoids were the main compounds found in all samples, accounting for 60-68% of the total (poly)phenolic content, while phenolic acids accounted for 32-40% (Table 1). Details of the (poly)phenols identification are shown in the Supporting Information Table 2S.

Table 2 shows the content of individual identified and quantified flavonoids present in raw and cooked cactus cladodes. Before gastrointestinal digestion, isorhamnetin derivatives were the most abundant flavonoid compounds, showing more than 50% of total flavonoids content. Thirteen compounds were found, being isorhamnetin rutinoside II the most abundant, followed by isorhamnetin rutinoside rhamnoside and isorhamnetin hexose pentoside in all samples, except in microwaved cactus cladodes where isorhamnetin rutinoside rhamnoside was the highest. The rest of the isorhamnetin

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derivatives were minor. Five quercetin derivatives were also detected and quantified, being quercetin hexose dirhamnoside the most representative in all cactus cladodes samples. Finally, fourteen kaempferol derivatives were found, accounting for no more than 10% of the total flavonoids content, being kaempferol hexose pentose rhamnoside the main one in all samples. Similar profiles, with isorhamnetin glycosides as predominant flavonoids, have been reported for raw cactus cladodes of *Opuntia ficus-indica* and other *Opuntia* cultivars, even though the limited number of identified and quantified flavonoids in those studies [2,3,19,20].

Table 3 shows the content of each phenolic acid found in raw and cooked cactus cladodes. Two of the three eucomic acids identified were the most abundant, accounting for 50 to 60% of the total phenolic acids content; followed by three piscidic acids and seven ferulic acid derivatives. The 1-*O*-feruloylglucose compounds were the most representative ferulic acid derivatives in all samples. Piscidic and eucomic acids have been previously identified in *Opuntia ficus-indica* extracts [19] as unique compounds, but in the present study three stereoisomers of piscidic acid and other three of eucomic acid were identified and quantified. In the present study, eucomic acids were the most abundant, in contrast with Ginestra et al. [19] who reported piscidic acid as the major one. Piscidic acid is rarely found in nature and is restricted to those with crassulacean acid metabolism, being *Opuntia* species one of these succulent plants [21]. Other phenolic acids like 3,4-dihydroxybenzoic, 4-hydroxybenzoic, salicylic, chlorogenic and gallic acids, as well as iso-quercitrin in raw cactus cladodes of *Opuntia ficus-indica* have been identified [2,3,19]. However, they were not detected in the present study, most likely due to differences in cultivars, maturity stages, origin places, harvest seasons or environmental conditions.

The application of different cooking methods to cactus cladodes induces changes in their (poly)phenolic compounds profiles. The effect of heat treatment on the total (poly)phenolic compounds, as well as on individual flavonoids and phenolic acids, of cactus cladodes are shown in Tables 1, 2 and 3, respectively. All (poly)phenolic compounds found in raw and in boiled cactus cladodes are presented in their glycosidic forms; whereas isorhamnetin, quercetin and kaempferol aglycones were detected in traces or in low amount after microwaving, griddling and frying procedures.

Microwaving and griddling processes significantly ($p<0.05$) increased 1.4-fold and 1.2-fold the total amount of (poly)phenolic compounds, respectively. These increases were observed both in total flavonoids and in total phenolic acids content. Isorhamnetin derivatives showed a higher increment when cactus cladodes were submitted to microwaving, whilst quercetin and kaempferol derivatives increased higher after griddling (Table 2). Microwaved cactus cladodes also presented the highest amount of total phenolic acids, particularly in piscidic and ferulic acid derivatives (Table 3). These results are in agreement with those previously reported in cactus cladodes after microwaving and griddling [6]. Likewise, increases in (poly)phenolic compounds have been found in microwaved broccoli and cauliflower [22] as well as in griddled onion, pepper and cardoon [5]. However, the total content of (poly)phenolic compounds in cactus cladodes are substantially higher than in those observed in other vegetables.

In contrast, after frying with olive and soybean oils, the total amount of (poly)phenolic compounds decreased, with a 0.6-fold significantly ($p<0.05$) lower content than in raw cactus cladodes. Total flavonoid compounds showed a greater decrease when olive oil was used, whereas total phenolic acids decreased higher when frying with soybean oil. Likewise, the total (poly)phenolic compounds of cactus cladodes after boiling also decreased, but the reduction was much lower (0.9-fold) than during the frying

processes. These findings are in agreement with those results previously reported in fried green pepper, cardoon [5] and potatoes [23], as well as in boiled cauliflower [4] and red cabbage [24].

Heat treatment applied to vegetables induces several structural and chemical changes, which turn into (poly)phenolic compounds losses and gains depending on the cooking technique, technological parameters, as well as the food matrices. Increases after microwaving and griddling processes could be due to the release of (poly)phenolic compounds from the cell walls and sub-cellular compartments caused by thermal destruction as in other vegetables, but also due to their liberation from pectins, mucilages and other dietary fiber compounds [25]. Furthermore, both cooking techniques are applied without the addition of water avoiding leaching into the water, or at least minimized in the case of microwaved cactus cladodes due to a faster cooking time (5 min) than in boiling treatment (10 min). Additionally, high temperatures during griddling (110-150ºC) favor Maillard reactions and, consequently, the formation of melanoidins that could retain (poly)phenolic compounds into their structures. Besides, the inactivation of the enzyme systems (as polyphenoloxidases) lead to inhibit degradation of the (poly)phenolic compounds [26]. On the other hand, losses in boiled cactus cladodes mainly occur because of leaching of (poly)phenolic compounds into the water, as previously reported [6]. Similarly, frying process, in which oil acts as transfer medium for heat, induces a decrease probably due to a longer cooking time (15 min) than in the other heat treatments (5-10 min) making it a more deteriorative process [27].

Otherwise, total antioxidant capacity evaluates DPPH scavenging ability by phenolic and non-phenolic (ascorbic acid, carotenoids, melanoidins, etc.) compounds of raw and cooked cactus cladodes (Figure 1). DPPH antioxidant capacity increased after all cooking methods, except in the case of fried in soybean oil cactus cladodes, which

showed no significant differences (p<0.05) in comparison to raw samples. This is in agreement with antioxidant capacity results previously reported for heat-treated cactus cladodes [6], as well as for onion, green pepper [5] and eggplant [28].

Bioaccessibility of (poly)phenolic compounds of cactus cladodes after simulated gastrointestinal digestion. Up to our best knowledge, this is the first study where the effect of in vitro gastrointestinal digestion on the profile of individual (poly)phenolic compounds and antioxidant capacity of cactus cladodes has been evaluated. Simulated gastrointestinal digestion was developed in three steps: oral digestion with α-amylase, gastric digestion with pepsin at pH 3, and intestinal digestion with pancreatin and bile salts at pH 7. After gastric and intestinal digestion phases, individual (poly)phenolic compounds were identified and quantified.

After in vitro gastric digestion, the content of both flavonoids and phenolic acids significantly (p<0.05) decreased (Table 1). Flavonoids showed a higher decrease than phenolic acids after the gastric phase. Nevertheless, both reductions were lower when cactus cladodes were cooked. In raw cactus cladodes gastric digesta, 48% of total flavonoids and 73% of phenolic acids remain bioaccessible, whereas in cooked samples the bioaccessibility was 68-85% for flavonoids and higher than 90% for phenolic acids, except in boiling which was 74%. Therefore, the bioaccessibility of total phenolic compounds in raw samples accounted for 58%, while 76-83% remained bioaccessible in cooked ones.

Likewise, after the simulated intestinal phase, a significant (p<0.05) decrease in the (poly)phenolic compounds in raw and cooked cactus cladodes was also observed. Overall, 55-64% of the total (poly)phenolic compounds of cooked cactus cladodes remained bioaccessible after gastrointestinal digestion, while final bioaccessibility was

44% in raw samples. Furthermore, digestive enzymes and conditions higher retained or degraded flavonoids (37-63% bioaccessibility) than phenolic acids (56-87% bioaccessibility). In fact, the ratio between flavonoids and phenolic acids after the gastrointestinal digestion changed, accounting flavonoids for 45-60% (vs 60-68% before digestion) of the total (poly)phenolic content, while phenolic acids accounted for 40-54% (vs 32-40% before digestion).

In terms of total (poly)phenolic compounds content, microwaved cactus cladodes contributed the highest amount (143.54 mg/g dm) after the in vitro gastrointestinal process, followed by griddled samples (133.98 mg/ g dm). In contrast, digestion of cactus cladodes fried with soybean oil and olive oil had the lowest amount with 69.77 and 70.87 mg/ g dm, respectively.

Although (poly)phenolic compounds bioaccessibility after digestion might depend on food matrix, other authors also demonstrated a higher bioaccessibility of total (poly)phenolic compounds after heat treatment in boiled and steamed cauliflower (more than 100%) [26], as well as in griddled green pepper [12] and cardoon [13]. The high amount of pectins and mucilages which include bound (poly)phenolic compounds, along with those attached to the melanoidins formed by Maillard reactions after intensive heat treatment like griddling, might favor a protective effect against enzymatic action [12].

Individually, most (poly)phenolic compounds were partially, or even totally, degraded during gastrointestinal digestion (Table 2 and 3). Flavonoid aglycones were detected in traces or very low amount in cooked cactus cladodes after in vitro gastric digestion, but undetected after the intestinal phase. This confirms that the amylases added to simulate the salivary action and those present in pancreatin in the intestinal phase, which normally cleave α-linkages, are not able to break the β-glycosidic linkage between the

flavonoid aglycones and their glycosidic moieties [29]. Actually, the deglycosilation of flavonoids is due to membrane-bound and cytosolic β-glycosydases found in the brush border cells of the mammalian small intestine [30,31] or by the action of gut microbiota [12,13]. Hence, the loss of flavonoids glycosides during digestion could be mainly attributed to their affinity with the digestive enzymes [29].

Few (poly)phenolic compounds appeared after the gastric phase, like kaempferide 3,7-dirhamnoside in microwaved cactus cladodes (Table 2), eucomic acid I in raw, microwaved and griddled samples, and eucomic acid II in cactus cladodes fried with soybean oil an olive oil (Table 3). Other compounds as piscidic acid I, ferulic acid and dihydroferulic acid -O-glucuronide I increased after the gastric phase, but decreased during the intestinal phase. The stereoisomeric form of piscidic acid I could be favored by the pH acidic conditions, as well as the action of digestive enzymes, rather than piscidic acids II and III, which decreased. Similarly, eucomic acid I, as well as eucomic acid II in fried samples, enhanced by the isomerization of eucomic acids III and II due to gastrointestinal conditions. In addition, the acidic pH during the gastric phase might induce the hydrolysis of those ferulic acid moieties bound to the polysaccharides like pectins [32], and even partially from the glycosylated ones (feruloylglucoses), increasing free ferulic acid. Furthermore, gastric conditions favored the isomerization of dihydroferulic acid 4-O-glucuronide II into dihydroferulic acid -O-glucuronide I.

DPPH radical scavenging capacity of raw and cooked cactus cladodes after in vitro gastric digestion (Figure 1) was significantly ($p < 0.05$) reduced. The lowest decrease was observed in microwaved cactus cladodes with a 44%, followed by griddled ones, decreasing 50%. The rest of the cooked samples decreased more than 50%, showing frying with olive oil the highest decrease with a 90%. After the intestinal phase, the antioxidant capacity of raw and cooked cactus cladodes further decreased remained the

highest values in microwaved and griddled cactus cladodes, but being undetectable in both fried samples. This behavior is in agreement with the remained (poly)phenolic compounds found after the gastrointestinal digestion. The antioxidant capacity depends on the affected compounds, and even though there is a decrease, its health benefits still remain.

In summary, the current research confirms that heating processes may significantly influence the digestibility of dietary (poly)phenols of cactus cladodes from the food matrix. Thus, even (poly)phenols are retained by digestive enzymes or degraded by pH conditions during gastrointestinal digestion, most of them remain bioaccessible when cactus cladodes are cooked, especially by microwaving and griddling. Likewise, flavonoids and phenolic acids are unevenly affected, being the first more sensitive to gastrointestinal conditions than the latter. Additionally, isomerization reactions induce changes among the several stereoisomeric forms of piscidic and eucomic acids during gastrointestinal digestion. Nevertheless, because most (poly)phenolic compounds are not absorbed in the intestine and reach the colon, further investigations are needed to evaluate which are the main (poly)phenolic metabolites formed by the action of the human gut microbiota, as well as their bioavailability and biological activity in order to assess the health properties of cactus cladodes.

ABBREVIATIONS

DPPH, 2,2-diphenyl-1-picrylhydrazyl; SSF, simulated salivary fluid; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; UHPLC-PDA-HR-MS, ultra high performance liquid chromatography photodiode array detector high resolution mass

spectrometry; HESI, heated electrospray ionization; CID, collision-induced dissociation.

SUPPORTING INFORMATION

Two supplementary tables with the concentrations of electrolytes of simulated salivary, gastric and intestinal fluids (Table 1S) and the mass spectrometric characteristics of (poly)phenolic compounds identified in this study (Table 2S) have been included.

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Figure captions

Figure 1. Antioxidant capacity by DPPH of raw and cooked cactus cladodes before and after *in vitro* gastric and intestinal digestion. Different letters indicate significant differences ($p \leq 0.05$).

Table 1. Content (mg (poly)phenolic compound/g dry matter) and bioaccessibility (%) of total (poly)phenolic compounds in raw and cooked cactus cladodes before and after *in vitro* gastric and intestinal digestion. Results are expressed as mean ± standard deviation (n=3).

Compounds	Raw		Boiled		Microwaved		Griddled		Fried in olive oil		Fried in soybean oil	
	mg/g dm	(%)	mg/g dm	(%)	mg/g dm	(%)	mg/g dm	(%)	mg/g dm	(%)	mg/g dm	(%)
Total Flavonoids												
Before digestion	120.40 ± 0.49 d C	-	97.79 ± 0.52 c C	-	158.21 ± 0.11 f C	-	149.43 ± 1.05 e C	-	72.77 ± 0.19 a C	-	76.91 ± 0.48 b C	-
Gastric digestion	58.30 ± 2.28 b B	48	83.50 ± 1.06 c B	85	111.54 ± 0.59 d B	70	111.57 ± 0.54 d B	75	56.53 ± 0.02 ab B	78	52.62 ± 0.36 a B	68
Intestinal digestion	44.96 ± 0.89 c A	37	61.14 ± 0.54 d A	63	64.23 ± 0.68 e A	40	63.57 ± 0.82 de A	43	33.49 ± 0.07 a A	46	41.65 ± 0.52 b A	54
Total Phenolic Acids												
Before digestion	71.42 ± 0.37 d C	-	65.45 ± 0.22 c C	-	95.11 ± 0.30 f C	-	89.40 ± 0.98 e B	-	42.89 ± 0.31 b C	-	34.87 ± 0.20 a C	-
Gastric digestion	52.34 ± 0.10 d B	73	48.55 ± 0.64 c B	74	91.77 ± 0.50 f B	96	84.12 ± 1.30 e B	94	39.91 ± 0.05 b B	93	32.47 ± 0.38 a B	93
Intestinal digestion	39.64 ± 0.10 b A	56	44.05 ± 0.74 c A	67	76.98 ± 0.22 e A	81	70.41 ± 1.82 d A	79	37.38 ± 0.04 b A	87	28.11 ± 0.05 a A	80
Total Compounds												
Before digestion	191.87 ± 0.86 d C	-	163.27 ± 0.30 c C	-	260.08 ± 0.40 f C	-	238.82 ± 2.03 e C	-	115.66 ± 0.49 b C	-	111.79 ± 0.67 a C	-
Gastric digestion	110.64 ± 2.18 c B	58	132.07 ± 0.41 d B	81	208.84 ± 1.12 f B	80	195.70 ± 1.84 e B	82	96.44 ± 0.07 b B	83	85.09 ± 0.75 a B	76
Intestinal digestion	84.60 ± 0.78 b A	44	105.20 ± 0.20 c A	64	143.54 ± 0.51 e A	55	133.98 ± 1.64 d A	56	70.87 ± 0.11 a A	61	69.77± 0.57 a A	62

In each parameter, different capital letters in the same column denote significant differences (p ≤ 0.05) among digestions.
In each parameter, different small letters in the same row indicate significant differences (p ≤ 0.05) among cooking processes.

Table 2. Content (mg (poly)phenolic compound/g dry matter) and bioaccessibility (%) of flavonoid compounds in raw and cooked cactus cladodes before and after *in vitro* gastric and intestinal digestion. Results are expressed as mean ± standard deviation (n=3).

Compounds	Raw mg/g dm	(%)	Boiled mg/g dm	(%)	Microwaved mg/g dm	(%)	Griddled mg/g dm	(%)	Fried in olive oil mg/g dm	(%)	Fried in soybean oil mg/g dm	(%)
Isorhamnetin derivates												
Isorhamnetin												
Before digestion	nd	-	nd	-	2.07 ± 0.03	-	tr	-	tr	-	tr	-
Gastric digestion	nd	-	nd	-	1.32 ± 0.00	64	tr	-	tr	-	tr	-
Intestinal digestion	nd	-	nd	-	nd	0	nd	-	nd	-	nd	-
Isorhamnetin hexose rhamnose hexoside												
Before digestion	1.88 ± 0.02	-	1.48 ± 0.02	-	1.79 ± 0.00	-	2.01 ± 0.13	-	1.21 ± 0.01	-	1.28 ± 0.02	-
Gastric digestion	1.90 ± 0.03	101	1.37 ± 0.03	93	1.28 ± 0.01	72	1.63 ± 0.00	81	1.05 ± 0.00	87	0.94 ± 0.01	73
Intestinal digestion	1.09 ± 0.00	58	1.16 ± 0.02	78	0.86 ± 0.02	48	1.27 ± 0.04	63	0.82 ± 0.00	68	1.08 ± 0.01	84
Isorhamnetin di-hexoside												
Before digestion	1.02 ± 0.02	-	0.89 ± 0.01	-	1.25 ± 0.04	-	1.11 ± 0.06	-	0.82 ± 0.01	-	0.86 ± 0.01	-
Gastric digestion	0.63 ± 0.04	62	0.80 ± 0.02	90	1.01 ± 0.02	81	0.83 ± 0.01	75	0.66 ± 0.01	80	0.73 ± 0.00	85
Intestinal digestion	0.70 ± 0.01	69	tr	0	0.96 ± 0.02	77	0.56 ± 0.00	50	tr	0	tr	0
Isorhamnetin rutinoside rhamnoside												
Before digestion	17.17 ± 0.11	-	13.67 ± 0.15	-	35.78 ± 0.07	-	20.91 ± 0.07	-	10.38 ± 0.03	-	10.21 ± 0.07	-
Gastric digestion	9.41 ± 0.04	55	11.65 ± 0.06	85	26.69 ± 0.18	75	16.26 ± 0.12	78	7.90 ± 0.01	76	7.17 ± 0.01	70
Intestinal digestion	6.33 ± 0.06	37	8.18 ± 0.19	60	14.11 ± 0.03	40	9.26 ± 0.11	44	4.53 ± 0.00	44	6.01 ± 0.08	59
Isorhamnetin hexose pentoside												
Before digestion	15.18 ± 0.11	-	12.39 ± 0.19	-	17.52 ± 0.03	-	19.31 ± 0.08	-	8.85 ± 0.01	-	9.69 ± 0.00	-
Gastric digestion	6.97 ± 0.36	46	10.52 ± 0.08	85	11.95 ± 0.09	68	15.44 ± 0.13	80	7.40 ± 0.04	84	6.66 ± 0.11	69
Intestinal digestion	5.76 ± 0.12	38	8.15 ± 0.14	65	8.30 ± 0.02	47	9.17 ± 0.30	47	4.54 ± 0.03	51	5.66 ± 0.04	58
Isorhamnetin rutinoside I												
Before digestion	0.76 ± 0.00	-	0.78 ± 0.01	-	1.27 ± 0.01	-	1.05 ± 0.05	-	0.71 ± 0.02	-	0.65 ± 0.00	-
Gastric digestion	0.64 ± 0.01	84	0.84 ± 0.01	108	0.89 ± 0.01	70	0.81 ± 0.02	77	0.54 ± 0.01	76	0.52 ± 0.00	80
Intestinal digestion	tr	0	0.78 ± 0.02	100	0.78 ± 0.03	61	0.53 ± 0.00	50	tr	-	tr	-
Isorhamnetin rutinoside II												
Before digestion	19.09 ± 0.03	-	15.33 ± 0.22	-	26.74 ± 0.04	-	24.74 ± 0.12	-	11.76 ± 0.01	-	13.54 ± 0.34	-
Gastric digestion	8.27 ± 0.20	43	13.26 ± 0.16	87	17.98 ± 0.05	67	17.35 ± 0.15	70	9.23 ± 0.03	78	8.17 ± 0.17	60
Intestinal digestion	6.57 ± 0.24	34	9.54 ± 0.30	62	10.31 ± 0.29	39	5.77 ± 0.26	23	5.55 ± 0.04	47	7.20 ± 0.37	53
Isorhamnetin 3-O-beta-(6-O-coumaroylglucoside)-7-O-beta-glucoside I												
Before digestion	0.45 ± 0.00	-	0.45 ± 0.00	-	0.57 ± 0.00	-	0.57 ± 0.01	-	0.51 ± 0.00	-	0.51 ± 0.00	-
Gastric digestion	0.37 ± 0.00	82	0.41 ± 0.00	91	0.38 ± 0.00	67	0.48 ± 0.00	84	0.40 ± 0.00	78	0.43 ± 0.00	84
Intestinal digestion	tr	0	tr	0	tr	0	tr	0	tr	0	tr	0
Isorhamnetin 3-O-beta-(6-O-coumaroylglucoside)-7-O-beta-glucoside II												
Before digestion	0.85 ± 0.01	-	0.83 ± 0.01	-	1.04 ± 0.01	-	1.03 ± 0.01	-	0.72 ± 0.00	-	0.83 ± 0.00	-
Gastric digestion	0.91 ± 0.02	107	0.80 ± 0.04	96	0.74 ± 0.00	71	0.74 ± 0.01	72	0.60 ± 0.01	83	0.57 ± 0.00	69
Intestinal digestion	0.59 ± 0.00	69	0.77 ± 0.02	93	0.53 ± 0.00	51	0.76 ± 0.02	74	tr	-	tr	-
Isorhamnetin 3-O-beta-(6-O-coumaroylglucoside)-7-O-beta-glucoside III												

Before digestion	0.49 ± 0.01	-	1.73 ± 0.00	-	2.42 ± 0.04	-	2.72 ± 0.17	-	1.42 ± 0.00	-	1.49 ± 0.01	-
Gastric digestion	0.40 ± 0.00	82	1.43 ± 0.02	83	1.51 ± 0.01	62	1.84 ± 0.01	68	0.98 ± 0.01	69	1.01 ± 0.00	68
Intestinal digestion	0.46 ± 0.00	94	1.32 ± 0.04	76	0.86 ± 0.02	36	1.49 ± 0.01	55	0.87 ± 0.01	61	1.10 ± 0.00	74
Isorhamnetin 3-O-beta-(6-O-coumaroylglucoside)-7-O-beta-glucoside IV												
Before digestion	2.08 ± 0.01	-	0.46 ± 0.00	-	0.54 ± 0.00	-	0.46 ± 0.00	-	0.50 ± 0.00	-	0.50 ± 0.00	-
Gastric digestion	1.07 ± 0.02	51	0.45 ± 0.01	98	0.36 ± 0.00	67	0.41 ± 0.00	89	0.37 ± 0.00	74	0.41 ± 0.00	82
Intestinal digestion	1.04 ± 0.01	50	tr	0	tr	0	tr	0	tr	0	tr	0
Isorhhamnetin 3-O-beta-(6-O-coumaroylglucoside)-7-O-beta-glucoside V												
Before digestion	0.4 ± 0.00	-	0.37 ± 0.00	-	0.54 ± 0.00	-	0.38 ± 0.00	-	tr	-	tr	-
Gastric digestion	tr	0	0.36 ± 0.00	97	0.33 ± 0.00	61	tr	0	tr	-	tr	-
Intestinal digestion	tr	0	tr	0	tr	0	tr	0	tr	-	tr	-
Isorhamnetin 3-ferulylrobinobioside												
Before digestion	0.69 ± 0.03	-	0.57 ± 0.00	-	0.76 ± 0.00	-	0.73 ± 0.02	-	0.61 ± 0.00	-	0.59 ± 0.00	-
Gastric digestion	tr	0	0.66 ± 0.01	116	0.49 ± 0.00	64	0.56 ± 0.00	77	0.43 ± 0.00	70	0.48 ± 0.00	81
Intestinal digestion	tr	0	0.65 ± 0.00	114	0.41 ± 0.00	54	0.65 ± 0.00	89	tr	0	tr	0
TOTAL ISORHAMNETIN DERIVATES												
Before digestion	**60.06 ± 0.01**	**-**	**48.93 ± 0.17**	**-**	**92.29 ± 0.02**	**-**	**75.01 ± 0.33**	**-**	**37.50 ± 0.03**	**-**	**40.15 ± 0.43**	**-**
Gastric digestion	**30.58 ± 0.56**	**51**	**42.55 ± 0.17**	**87**	**64.92 ± 0.37**	**70**	**56.37 ± 0.16**	**75**	**29.57 ± 0.00**	**79**	**27.09 ± 0.29**	**67**
Intestinal digestion	**22.53 ± 0.42**	**38**	**30.55 ± 0.02**	**62**	**37.20 ± 0.32**	**40**	**29.48 ± 0.60**	**39**	**16.31 ± 0.05**	**44**	**21.04 ± 0.48**	**52**
Quercetin derivates												
Quercetin												
Before digestion	nd	-	nd	-	tr	-	tr	-	tr	-	tr	-
Gastric digestion	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
Intestinal digestion	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
Quercetin hexosyl pentosyl rhamnoside												
Before digestion	0.83 ± 0.04	-	0.88 ± 0.03	-	1.22 ± 0.05	-	2.19 ± 0.23	-	0.50 ± 0.03	-	0.47 ± 0.01	-
Gastric digestion	tr	0	0.66 ± 0.02	75	1.03 ± 0.01	84	0.62 ± 0.00	28	0.44 ± 0.00	88	0.24 ± 0.02	51
Intestinal digestion	tr	0	0.08 ± 0.01	9	0.03 ± 0.00	2	0.03 ± 0.01	1	tr	0	tr	0
Quercetin hexose pentoside												
Before digestion	0.29 ± 0.01	-	0.36 ± 0.01	-	0.42 ± 0.00	-	0.48 ± 0.04	-	0.04 ± 0.00	-	0.03 ± 0.00	-
Gastric digestion	tr	0	0.23 ± 0.01	64	0.22 ± 0.02	52	0.56 ± 0.00	117	0.02 ± 0.00	50	tr	0
Intestinal digestion	tr	0	tr	0	tr	0	tr	0	tr	0	tr	0
Quercetin 3-O-rutinoside (rutin)												
Before digestion	0.86 ± 0.05	-	0.83 ± 0.04	-	1.48 ± 0.08	-	0.93 ± 0.00	-	0.47 ± 0.01	-	tr	-
Gastric digestion	nd	0	0.37 ± 0.03	45	0.66 ± 0.00	45	0.91 ± 0.03	98	0.30 ± 0.01	64	tr	-
Intestinal digestion	nd	0	tr	0	nd	0	nd	0	tr	0	tr	-
Quercetin hexose dirhamnoside												
Before digestion	49.12 ± 0.51	-	37.72 ± 0.21	-	52.12 ± 0.11	-	58.89 ± 0.33	-	27.16 ± 0.10	-	28.95 ± 0.03	-
Gastric digestion	22.53 ± 1.67	46	32.17 ± 0.94	85	36.50 ± 0.25	70	43.47 ± 0.33	74	20.96 ± 0.06	77	20.02 ± 0.04	69
Intestinal digestion	17.75 ± 0.42	36	23.95 ± 0.40	63	20.75 ± 0.47	40	26.57 ± 0.29	45	13.17 ± 0.01	49	15.69 ± 0.03	54
TOTAL QUERCETIN DERIVATES												
Before digestion	**51.11 ± 0.52**	**-**	**39.78 ± 0.22**	**-**	**55.25 ± 0.14**	**-**	**62.5 ± 0.59**	**-**	**28.17 ± 0.13**	**-**	**29.46 ± 0.04**	**-**
Gastric digestion	**22.53 ± 1.67**	**44**	**33.43 ± 0.97**	**84**	**38.42 ± 0.22**	**70**	**45.56 ± 0.36**	**73**	**21.72 ± 0.05**	**77**	**20.26 ± 0.03**	**69**
Intestinal digestion	**17.75 ± 0.42**	**35**	**24.03 ± 0.41**	**60**	**20.78 ± 0.47**	**38**	**26.6 ± 0.28**	**45**	**13.17 ± 0.01**	**47**	**15.69 ± 0.03**	**53**
Kaempferol derivates												
Kaempferol												

Before digestion	nd	-	nd	-	tr	-	tr	-	nd	-	tr	-
Gastric digestion	nd	-	nd	-	tr	-	tr	-	nd	-	tr	-
Intestinal digestion	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
Kaempferol hexoside dirhamnoside I												
Before digestion	1.05 ± 0.00	-	1.01 ± 0.04	-	1.26 ± 0.01	-	1.49 ± 0.03	-	0.74 ± 0.01	-	0.81 ± 0.03	-
Gastric digestion	0.64 ± 0.02	61	0.86 ± 0.01	85	0.99 ± 0.01	79	1.11 ± 0.00	75	0.61 ± 0.00	82	0.58 ± 0.01	72
Intestinal digestion	0.56 ± 0.00	53	0.79 ± 0.01	78	0.80 ± 0.00	63	0.91 ± 0.01	61	0.52 ± 0.00	70	0.55 ± 0.00	68
Kaempferol hexoside dirhamnoside II												
Before digestion	1.26 ± 0.01	-	1.24 ± 0.02	-	1.43 ± 0.02	-	1.71 ± 0.01	-	0.88 ± 0.01	-	0.94 ± 0.01	-
Gastric digestion	0.65 ± 0.02	52	1.10 ± 0.07	89	1.10 ± 0.02	75	1.53 ± 0.00	89	0.74 ± 0.00	84	0.73 ± 0.01	78
Intestinal digestion	0.61 ± 0.00	48	1.07 ± 0.01	86	0.81 ± 0.02	55	1.02 ± 0.05	60	0.59 ± 0.00	67	0.72 ± 0.00	77
Kaempferol hexose pentose rhamnoside												
Before digestion	1.38 ± 0.02	-	1.35 ± 0.10	-	1.47 ± 0.03	-	1.82 ± 0.04	-	0.95 ± 0.01	-	1.06 ± 0.03	-
Gastric digestion	0.81 ± 0.02	59	1.05 ± 0.03	78	1.20 ± 0.00	84	1.67 ± 0.02	92	0.72 ± 0.01	76	0.74 ± 0.01	70
Intestinal digestion	0.62 ± 0.02	45	0.83 ± 0.10	61	1.06 ± 0.00	74	1.07 ± 0.01	59	0.53 ± 0.01	56	0.77 ± 0.00	73
Kaempferol hexose pentoside												
Before digestion	1.12 ± 0.02	-	1.14 ± 0.00	-	1.21 ± 0.03	-	1.57 ± 0.00	-	0.85 ± 0.00	-	0.88 ± 0.00	-
Gastric digestion	0.67 ± 0.01	60	0.97 ± 0.00	85	0.83 ± 0.01	69	1.38 ± 0.01	88	0.65 ± 0.01	76	0.65 ± 0.01	74
Intestinal digestion	0.67 ± 0.02	60	0.93 ± 0.00	82	0.65 ± 0.00	54	1.03 ± 0.00	66	0.55 ± 0.00	65	0.68 ± 0.00	77
Kaempferol rutinoside I												
Before digestion	1.02 ± 0.00	-	1.26 ± 0.10	-	1.32 ± 0.00	-	1.48 ± 0.05	-	0.82 ± 0.01	-	0.74 ± 0.02	-
Gastric digestion	0.64 ± 0.00	63	1.02 ± 0.00	81	0.84 ± 0.00	64	1.06 ± 0.00	72	0.60 ± 0.02	73	0.63 ± 0.01	85
Intestinal digestion	0.55 ± 0.01	54	0.80 ± 0.01	63	0.61 ± 0.00	46	0.74 ± 0.02	50	0.45 ± 0.00	55	0.61 ± 0.00	82
Kaempferol rutinoside II												
Before digestion	0.34 ± 0.02	-	0.25 ± 0.00	-	0.37 ± 0.00	-	0.37 ± 0.01	-	0.30 ± 0.00	-	0.33 ± 0.00	-
Gastric digestion	0.24 ± 0.00	71	0.23 ± 0.00	92	0.22 ± 0.00	59	0.29 ± 0.00	78	0.23 ± 0.00	77	0.26 ± 0.00	79
Intestinal digestion	0.26 ± 0.00	76	tr	0	tr	0	0.34 ± 0.00	92	tr	0	tr	0
Kaempferol rutinoside III												
Before digestion	0.31 ± 0.00	-	0.24 ± 0.00	-	0.28 ± 0.00	-	0.28 ± 0.00	-	0.28 ± 0.00	-	0.29 ± 0.00	-
Gastric digestion	tr	0	tr	0	tr	0	0.22 ± 0.00	79	tr	0	tr	0
Intestinal digestion	tr	0	tr	0	tr	0	tr	0	tr	0	tr	0
Kaempferol acetyl arabinopyranosyl hexoside												
Before digestion	0.42 ± 0.01	-	0.46 ± 0.02	-	0.54 ± 0.01	-	0.54 ± 0.01	-	0.44 ± 0.00	-	0.40 ± 0.00	-
Gastric digestion	0.29 ± 0.00	69	0.48 ± 0.03	104	0.97 ± 0.01	180	0.43 ± 0.00	80	0.34 ± 0.00	77	0.33 ± 0.00	83
Intestinal digestion	0.34 ± 0.00	81	0.45 ± 0.02	98	0.56 ± 0.01	104	0.50 ± 0.00	93	0.33 ± 0.01	75	0.38 ± 0.00	95
Methoxy kaempferol hexoside												
Before digestion	0.64 ± 0.02	-	0.72 ± 0.01	-	0.77 ± 0.01	-	0.55 ± 0.01	-	0.51 ± 0.00	-	0.41 ± 0.00	-
Gastric digestion	0.44 ± 0.01	69	0.43 ± 0.02	60	0.73 ± 0.00	95	0.58 ± 0.00	105	0.39 ± 0.00	76	0.33 ± 0.00	80
Intestinal digestion	0.44 ± 0.01	69	0.43 ± 0.02	60	0.62 ± 0.00	81	0.61 ± 0.01	111	0.34 ± 0.00	67	0.37 ± 0.00	90
Kaempferol acetyl hexoside												
Before digestion	0.57 ± 0.00	-	0.60 ± 0.01	-	0.68 ± 0.01	-	0.80 ± 0.01	-	0.45 ± 0.00	-	0.49 ± 0.00	-
Gastric digestion	0.27 ± 0.00	47	0.37 ± 0.00	62	0.40 ± 0.00	59	0.42 ± 0.00	53	0.33 ± 0.00	73	0.33 ± 0.00	67
Intestinal digestion	0.30 ± 0.00	53	0.35 ± 0.00	58	0.37 ± 0.00	54	0.47 ± 0.00	59	tr	0	tr	0
Kaempferide 3,7 – dirhamnoside												
Before digestion	tr	-	tr	-	tr	-	tr	-	tr	-	tr	-
Gastric digestion	tr	-	tr	-	0.31 ± 0.00	-	tr	-	tr	-	tr	-
Intestinal digestion	tr	-	tr	-	0.36 ± 0.00	116	tr	-	tr	-	tr	-
Kaempferol coumaryl glucoside glucoside I												

Before digestion	0.38 ± 0.01	-	0.39 ± 0.00	-	0.49 ± 0.00	-	0.47 ± 0.01	-	0.33 ± 0.00	-	0.39 ± 0.00	-
Gastric digestion	0.29 ± 0.00	76	0.33 ± 0.01	85	0.38 ± 0.00	45	0.36 ± 0.01	77	0.26 ± 0.00	79	0.30 ± 0.00	77
Intestinal digestion	0.33 ± 0.00	87	0.42 ± 0.01	108	0.44 ± 0.00	35	0.35 ± 0.00	74	0.34 ± 0.00	103	0.39 ± 0.00	100
Kaempferol coumaryl glucoside glucoside II											
Before digestion	0.74 ± 0.00	-	0.64 ± 0.03	-	0.85 ± 0.02	-	0.83 ± 0.02	-	0.54 ± 0.01	-	0.58 ± 0.00	-
Gastric digestion	0.24 ± 0.01	32	0.48 ± 0.01	75	0.55 ± 0.01	65	0.60 ± 0.02	72	0.38 ± 0.00	70	0.41 ± 0.01	71
Intestinal digestion	tr	0	0.47 ± 0.00	73	0.33 ± 0.00	39	0.46 ± 0.00	55	0.36 ± 0.00	67	0.45 ± 0.00	78
TOTAL KAEMPFEROL DERIVATES											
Before digestion	**9.23 ± 0.02**	**-**	**9.07 ± 0.14**	**-**	**10.68 ± 0.00**	**-**	**11.92 ± 0.12**	**-**	**7.09 ± 0.03**	**-**	**7.30 ± 0.09**	**-**
Gastric digestion	**5.19 ± 0.04**	**56**	**7.52 ± 0.08**	**83**	**8.21 ± 0.00**	**77**	**9.65 ± 0.02**	**81**	**5.25 ± 0.03**	**74**	**5.26 ± 0.05**	**72**
Intestinal digestion	**4.68 ± 0.05**	**51**	**6.57 ± 0.15**	**73**	**6.25 ± 0.03**	**59**	**7.49 ± 0.06**	**66**	**4.01 ± 0.01**	**57**	**4.93 ± 0.01**	**68**

Table 3. Content (mg (poly)phenolic compound/g dry matter) and bioaccessibility (%) of phenolic acids in raw and cooked cactus cladodes before and after *in vitro* gastric and intestinal digestion. Results are expressed as mean ± standard deviation (n=3).

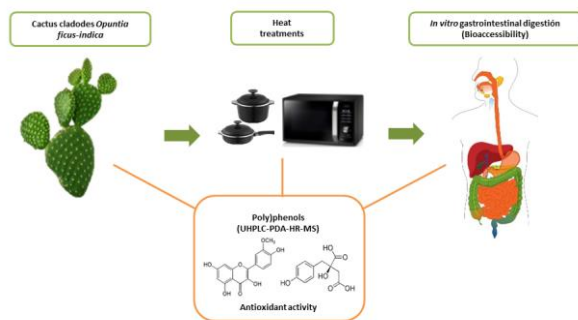| Compounds	Raw		Boiled		Microwaved		Griddled		Fried in olive oil		Fried in soybean oil	
	mg/g dm	(%)	mg/g dm	(%)	mg/g dm	(%)	mg/g dm	(%)	mg/g dm	(%)	mg/g dm	(%)
Piscidic acid derivatives												
Piscidic acid I												
Before digestion	13.15 ± 0.12	-	8.76 ± 0.17	-	21.44 ± 0.63	-	18.44 ± 0.51	-	10.76 ± 0.17	-	9.64 ± 0.02	-
Gastric digestion	17.83 ± 0.02	136	13.85 ± 0.21	158	25.19 ± 0.18	117	23.39 ± 0.35	127	12.11 ± 0.04	113	9.33 ± 0.08	97
Intestinal digestion	14.69 ± 0.05	112	13.43 ± 0.52	153	23.49 ± 0.17	110	21.84 ± 1.04	118	11.12 ± 0.02	103	10.26 ± 0.14	106
Piscidic acid II												
Before digestion	4.70 ± 0.17	-	7.76 ± 0.17	-	12.31 ± 0.09	-	9.33 ± 0.02	-	7.42 ± 0.08	-	6.62 ± 0.15	-
Gastric digestion	2.50 ± 0.14	53	3.92 ± 0.04	51	4.21 ± 0.12	34	3.89 ± 0.01	42	2.73 ± 0.02	37	3.08 ± 0.08	46
Intestinal digestion	2.29 ± 0.00	49	2.25 ± 0.10	29	4.00 ± 0.25	32	2.83 ± 0.11	30	2.54 ± 0.00	34	3.38 ± 0.03	51
Piscidic acid III												
Before digestion	0.06 ± 0.00	-	0.09 ± 0.01	-	0.21 ± 0.00	-	0.16 ± 0.01	-	0.12 ± 0.01	-	0.09 ± 0.01	-
Gastric digestion	tr	-	tr	-	tr	-	tr	-	tr	-	0.08 ± 0.00	89
Intestinal digestion	tr	-	tr	-	tr	-	tr	-	tr	-	0.02 ± 0.00	22
TOTAL PISCIDIC ACID DERIVATIVES												
Before digestion	**17.91 ± 0.05**	**-**	**16.61 ± 0.34**	**-**	**33.96 ± 0.72**	**-**	**27.94 ± 0.53**	**-**	**18.30 ± 0.23**	**-**	**16.36 ± 0.17**	**-**
Gastric digestion	**20.33 ± 0.17**	**114**	**17.77 ± 0.17**	**107**	**29.40 ± 0.06**	**87**	**27.28 ± 0.37**	**98**	**14.84 ± 0.03**	**81**	**12.48 ± 0.16**	**76**
Intestinal digestion	**16.98 ± 0.04**	**95**	**15.67 ± 0.62**	**94**	**27.49 ± 0.08**	**81**	**24.82 ± 1.10**	**89**	**13.66 ± 0.02**	**75**	**13.66 ± 0.17**	**84**
Eucomic acid derivatives												
Eucomic acid I												
Before digestion	nd	-	nd	-	nd	-	nd	-	nd	-	nd	-
Gastric digestion	2.29 ± 0.00	-	nd	-	16.65 ± 0.00	-	12.40 ± 0.33	-	nd	-	nd	-
Intestinal digestion	2.18 ± 0.06	95	nd	-	13.87 ± 0.08	83	11.42 ± 0.20	92	nd	-	nd	-
Eucomic acid II												
Before digestion	20.57 ± 0.14	-	21.66 ± 0.80	-	26.15 ± 0.68	-	26.35 ± 0.86	-	nd	-	nd	-
Gastric digestion	12.03 ± 0.91	58	19.63 ± 0.42	91	21.59 ± 0.27	83	22.32 ± 0.48	85	13.03 ± 0.02	-	7.71 ± 0.09	-
Intestinal digestion	7.79 ± 0.35	41	18.01 ± 0.08	83	19.29 ± 0.07	74	20.15 ± 0.52	76	11.72 ± 0.03	90	6.80 ± 0.08	88
Eucomic acid III												
Before digestion	27.80 ± 0.28	-	23.46 ± 0.21	-	28.23 ± 0.34	-	29.01 ± 0.63	-	20.85 ± 0.06	-	16.09 ± 0.02	-
Gastric digestion	15.07 ± 0.62	54	10.23 ± 0.37	44	18.59 ± 0.13	66	18.01 ± 0.11	62	11.20 ± 0.08	54	10.10 ± 0.03	63
Intestinal digestion	11.43 ± 0.20	41	7.49 ± 0.52	32	13.52 ± 0.07	48	12.02 ± 0.01	41	9.37 ± 0.08	45	7.04 ± 0.05	44
TOTAL EUCOMIC ACID DERIVATIVES												
Before digestion	**48.37 ± 0.43**	**-**	**45.12 ± 0.60**	**-**	**54.39 ± 1.03**	**-**	**55.35 ± 1.50**	**-**	**20.85 ± 0.06**	**-**	**16.09 ± 0.02**	**-**
Gastric digestion	**27.10 ± 0.28**	**56**	**28.24 ± 0.46**	**63**	**40.18 ± 0.41**	**74**	**40.33 ± 0.59**	**73**	**22.92 ± 0.06**	**110**	**17.81 ± 0.14**	**111**
Intestinal digestion	**19.22 ± 0.16**	**40**	**27.12 ± 0.10**	**60**	**33.28 ± 0.05**	**61**	**32.18 ± 0.53**	**58**	**22.41 ± 0.06**	**107**	**13.83 ± 0.13**	**86**
Ferulic acid derivates												
Ferulic acid												
Before digestion	0.11 ± 0.00	-	0.22 ± 0.00	-	0.46 ± 0.00	-	0.21 ± 0.00	-	0.14 ± 0.00	-	0.10 ± 0.00	-

Gastric digestion	0.46 ± 0.00	418	0.53 ± 0.00	241	1.71 ± 0.01	372	1.01 ± 0.00	481	0.65 ± 0.00	464	0.28 ± 0.00	280
Intestinal digestion	0.19 ± 0.01	173	0.33 ± 0.00	150	0.85 ± 0.00	185	0.52 ± 0.01	248	0.44 ± 0.00	314	0.21 ± 0.01	210
1-*O*-feruloylglucose I											
Before digestion	0.05 ± 0.00	-	0.03 ± 0.00	-	0.16 ± 0.01	-	0.22 ± 0.00	-	0.13 ± 0.01	-	tr	-
Gastric digestion	tr	0	0.02 ± 0.01	67	0.13 ± 0.01	81	0.18 ± 0.00	82	0.09 ± 0.00	69	tr	-
Intestinal digestion	tr	0	0.01 ± 0.00	33	0.09 ± 0.00	56	0.15 ± 0.00	68	0.07 ± 0.00	54	tr	-
1-*O*-feruloylglucose II											
Before digestion	0.13 ± 0.00	-	0.11 ± 0.00	-	0.23 ± 0.00	-	0.24 ± 0.00	-	0.15 ± 0.00	-	0.16 ± 0.00	-
Gastric digestion	0.09 ± 0.00	69	0.07 ± 0.00	64	0.11 ± 0.00	48	0.12 ± 0.00	50	0.08 ± 0.00	53	0.09 ± 0.00	56
Intestinal digestion	0.07 ± 0.01	54	0.04 ± 0.00	36	0.07 ± 0.00	4	0.07 ± 0.00	29	0.03 ± 0.00	20	tr	0
1-*O*-feruloylglucose III											
Before digestion	3.92 ± 0.00	-	2.76 ± 0.00	-	4.87 ± 0.01	-	4.68 ± 0.01	-	3.02 ± 0.01	-	1.81 ± 0.00	-
Gastric digestion	1.27 ± 0.00	32	1.39 ± 0.01	50	2.63 ± 0.01	54	1.64 ± 0.00	35	1.16 ± 0.01	38	1.53 ± 0.08	85
Intestinal digestion	0.32 ± 0.01	8	0.55 ± 0.01	20	0.78 ± 0.01	16	0.56 ± 0.00	12	0.53 ± 0.00	18	0.26 ± 0.00	14
1-*O*-feruloylglucose IV											
Before digestion	0.40 ± 0.00	-	0.29 ± 0.00	-	0.46 ± 0.00	-	0.30 ± 0.01	-	0.18 ± 0.00	-	0.14 ± 0.00	-
Gastric digestion	0.14 ± 0.00	35	0.22 ± 0.01	76	0.36 ± 0.01	78	0.16 ± 0.00	53	0.10 ± 0.00	56	0.12 ± 0.00	86
Intestinal digestion	0.11 ± 0.00	27	0.05 ± 0.00	17	0.15 ± 0.00	33	0.07 ± 0.00	23	0.01 ± 0.00	6	0.02 ± 0.00	14
Dihydroferulic acid -*O*-glucuronide I											
Before digestion	0.23 ± 0.00	-	0.12 ± 0.02	-	0.13 ± 0.00	-	0.21 ± 0.00	-	tr	-	0.06 ± 0.00	-
Gastric digestion	0.53 ± 0.00	227	0.26 ± 0.01	217	0.46 ± 0.01	354	0.25 ± 0.00	119	0.05 ± 0.00	-	0.12 ± 0.01	200
Intestinal digestion	0.46 ± 0.00	197	0.27 ± 0.00	225	0.30 ± 0.00	231	0.25 ± 0.01	119	0.21 ± 0.00	420	0.11 ± 0.01	183
Dihydroferulic acid 4-O-glucuronide II											
Before digestion	0.34 ± 0.00	-	0.22 ± 0.01	-	0.45 ± 0.01	-	0.24 ± 0.01	-	0.05 ± 0.00	-	0.16 ± 0.00	-
Gastric digestion	0.13 ± 0.00	38	0.08 ± 0.01	36	0.14 ± 0.01	31	0.05 ± 0.00	21	0.03 ± 0.00	60	0.03 ± 0.00	19
Intestinal digestion	0.10 ± 0.01	29	0.02 ± 0.01	9	0.09 ± 0.01	20	0.03 ± 0.00	13	0.02 ± 0.00	40	0.03 ± 0.00	19
TOTAL FERULIC ACID DERIVATIVES											
Before digestion	**5.18 ± 0.01**	**-**	**3.76 ± 0.03**	**-**	**6.76 ± 0.01**	**-**	**6.11 ± 0.02**	**-**	**3.74 ± 0.01**	**-**	**2.43 ± 0.01**	**-**
Gastric digestion	**2.62 ± 0.01**	**51**	**2.57 ± 0.02**	**68**	**5.53 ± 0.03**	**82**	**4.11 ± 0.01**	**67**	**2.16 ± 0.02**	**58**	**2.18 ± 0.08**	**90**
Intestinal digestion	**1.26 ± 0.03**	**24**	**1.37 ± 0.01**	**36**	**2.33 ± 0.02**	**34**	**1.99 ± 0.02**	**33**	**1.31 ± 0.00**	**35**	**0.62 ± 0.01**	**25**

Figure 1

DPPH

y-axis: µmol Trolox/ g sample dm

x-axis categories: Raw, Boiled, Microwaved, Griddled, Fried in olive oil, Fried in soybean oil

Legend: ☐ Before Digestion ☐ Gastric Digestion ■ Intestinal Digestion
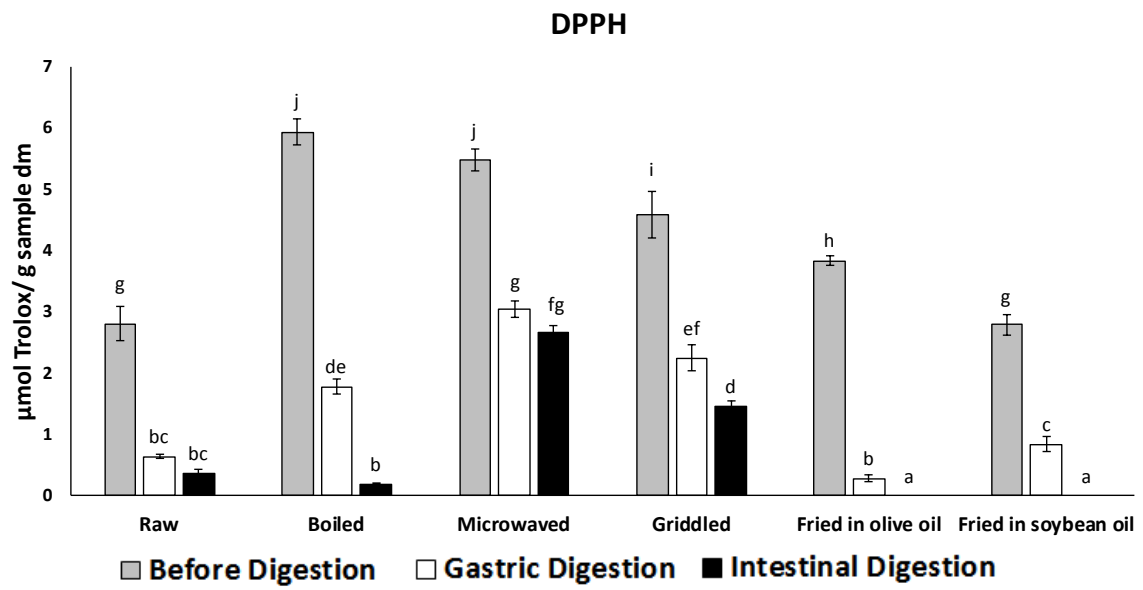
For Table of Contents Only

Cactus cladodes *Opuntia ficus-indica* → Heat treatments → *In vitro* gastrointestinal digestión (Bioaccessibility)

Poly)phenols (UHPLC-PDA-HR-MS)

Antioxidant activity

SUPPLEMENTARY INFORMATION

TITLE: Digestibility of (poly)phenols and antioxidant activity in raw and cooked cactus cladodes (*Opuntia ficus-indica*)

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Table S1. Concentrations of electrolytes Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF)

	Stock solution		SSF (250mL)	SGF (250mL)	SIF (250mL)
Constituent	g/50mL	mol/L	mL	mL	mL
KCl	1.87	0.5	9.44	4.31	4.25
KH_2PO_4	3.40	0.5	2.31	0.57	0.50
$NaHCO_3$	4.20	1	4.25	7.81	26.56
NaCl	5.90	2	-----	7.38	6.00
$MgCl_2(H_2O)_6$	1.53	0.15	0.31	0.25	0.69
$(NH_4)_2CO_3$	2.40	0.5	0.04	0.31	0.69

Table S2. Mass spectrometric characteristics of native (poly)phenolic compounds identified in this study.

Compound	Chemical formula	R$_t$ (min)	[M-H]$^-$ (m/z)	δppm	Fragment ions under low collision energy (m/z)
Piscidic acid I	C$_{11}$H$_{12}$O$_7$	5.4	255.0501	0.78	193.0499 / 179.0340 / 165.0546
Piscidic acid II	C$_{11}$H$_{12}$O$_7$	6.1	255.0501	0.78	193.0499 / 179.0340 / 165.0546
Piscidic acid III	C$_{11}$H$_{12}$O$_7$	6.6	255.0501	0.78	193.0499 / 179.0340 / 165.0546
Eucomic acid I	C$_{11}$H$_{12}$O$_6$	11.8	239.0550	-0.46	195.0658 / 179.0342 / 149.0599 / 107. 0490
Eucomic acid II	C$_{11}$H$_{12}$O$_6$	12.8	239.0550	-0.46	195.0658 / 179.0342 / 149.0599 / 107. 0490
Eucomic acid III	C$_{11}$H$_{12}$O$_6$	13.4	239.0550	-0.46	195.0658 / 179.0342 / 149.0599 / 107. 0490
Ferulic acid derivatives					
Ferulic acid	C$_{10}$H$_{10}$O$_4$	27.8	193.0497	1.04	
1-*O*-feruloylglucose I	C$_{16}$H$_{20}$O$_9$	15.1	355.1037	3.94	239.0558 / 193.0503 / 175.0391
1-*O*-feruloylglucose II	C$_{16}$H$_{20}$O$_9$	16.4	355.1037	3.94	239.0558 / 193.0503 / 175.0391
1-*O*-feruloylglucose III	C$_{16}$H$_{20}$O$_9$	17.5	355.1037	3.94	239.0558 / 193.0503 / 175.0391
1-*O*-feruloylglucose IV	C$_{16}$H$_{20}$O$_9$	18.6	355.1037	3.94	239.0558 / 193.0503 / 175.0391
Dihydroferulic acid -*O*-glucuronide I	C$_{16}$H$_{20}$O$_{10}$	9.4	371.0982	2.69	239.0558 / 179.0554 / 133.0135
Dihydroferulic acid -*O*-glucuronide II	C$_{16}$H$_{20}$O$_{10}$	9.9	371.0982	2.69	239.0558 / 179.0554 / 133.0135
Isorhamnetin derivatives					
Isorhamnetin	C$_{16}$H$_{12}$O$_7$	72.0	315.0504	1.59	151.0027
Isorhamnetin hexose rhamnose hexoside	C$_{34}$H$_{42}$O$_{21}$	32.4	785.2152	2.29	503.1777 / 371.0984 / 315.0503 / 151.0025
Isorhamnetin di-hexoside	C$_{28}$H$_{32}$O$_{17}$	34.6	639.1574	2.97	477.2342 / 361.1868 / 315.0503
Isorhamnetin rutinoside rhamnoside	C$_{34}$H$_{42}$O$_{20}$	36.4	769.2199	1.82	315.0503 / 145.0495
Isorhamnetin hexose pentoside	C$_{27}$H$_{30}$O$_{16}$	39.1	609.1462	1.97	477.1982 / 315.0508
Isorhamnetin rutinoside I	C$_{28}$H$_{32}$O$_{16}$	42.1	623.1627	1.76	477.2346 / 315.0501
Isorhamnetin rutinoside II	C$_{28}$H$_{32}$O$_{16}$	43.5	623.1627	1.76	477.2346 / 315.0501
Isorhamnetin 3-*O*-beta-(6-*O*-coumaroylglucoside)-7-*O*-beta-glucoside I	C$_{37}$H$_{38}$O$_{19}$	59.2	785.1940	2.16	315.0507 / 179.0554 / 145.0496
Isorhamnetin 3-*O*-beta-(6-*O*-coumaroylglucoside)-7-*O*-beta-glucoside II	C$_{37}$H$_{38}$O$_{19}$	61.8	785.1940	2.16	315.0507 / 179.0554 / 145.0496
Isorhamnetin 3-*O*-beta-(6-*O*-coumaroylglucoside)-7-*O*-beta-glucoside III	C$_{37}$H$_{38}$O$_{19}$	62.5	785.1940	2.16	315.0507 / 179.0554 / 145.0496
Isorhamnetin 3-*O*-beta-(6-*O*-coumaroylglucoside)-7-*O*-beta-glucoside IV	C$_{37}$H$_{38}$O$_{19}$	64.4	785.1940	2.16	315.0507 / 179.0554 / 145.0496
Isorhamnetin 3-*O*-beta-(6-*O*-coumaroylglucoside)-7-*O*-beta-glucoside V	C$_{37}$H$_{38}$O$_{19}$	66.1	785.1940	2.16	315.0507 / 179.0554 / 145.0496
Isorhamnetin 3-ferulylrobinobioside	C$_{38}$H$_{40}$O$_{19}$	63.9	799.2096	2.00	315.0509
Quercetin derivatives					
Quercetin	C$_{15}$H$_{10}$O$_7$	56.4	301.0354	3.98	178.9978 / 151.0027
Quercetin hexosyl pentosyl rhamnoside	C$_{32}$H$_{38}$O$_{20}$	30.4	741.1895	3.10	301.0351 / 151.0391
Quercetin hexose pentoside	C$_{26}$H$_{28}$O$_{16}$	31.5	595.1311	3.02	463.0881 / 433.2078 / 415.0884
Quercetin 3-*O*-rutinoside (rutin)	C$_{27}$H$_{30}$O$_{16}$	35.6	609.1470	3.27	301.0353 / 145.0496
Quercetin hexose dirhamnoside	C$_{33}$H$_{40}$O$_{20}$	36.8	755.2040	1.46	609.1467 / 301.0349
Kaempferol derivatives					
Kaempferol	C$_{15}$H$_{10}$O$_6$	68.9	285.0404	3.85	
Kaempferol hexoside dirhamnoside I	C$_{33}$H$_{40}$O$_{19}$	34.3	739.2102	2.98	431.2286 / 285.0402
Kaempferol hexoside dirhamnoside II	C$_{33}$H$_{40}$O$_{19}$	35.3	739.2102	2.98	431.2286 / 285.0402
Kaempferol hexose pentose rhamnoside	C$_{32}$H$_{38}$O$_{19}$	35.9	725.1945	3.03	285.0401
Kaempferol hexose pentoside	C$_{26}$H$_{28}$O$_{15}$	37.5	579.1362	3.11	496.2458 / 285.0402
Kaempferol rutinoside I	C$_{27}$H$_{30}$O$_{15}$	41.7	593.1520	3.22	496.2455 / 285.0403
Kaempferol rutinoside II	C$_{27}$H$_{30}$O$_{15}$	47.3	593.1520	3.22	496.2455 / 285.0403
Kaempferol rutinoside III	C$_{27}$H$_{30}$O$_{15}$	48.0	593.1520	3.22	496.2455 / 285.0403

Kaempferol acetyl arabinopyranosyl hexoside	$C_{28}H_{30}O_{16}$	42.5	621.1450	2.74	503.2504 / 285.0402
Methoxy kaempferol hexoside	$C_{22}H_{22}O_{12}$	44.6	477.1027	2.52	314.0434 / 285.0406
Kaempferol acetyl hexoside	$C_{23}H_{22}O_{12}$	45.8	489.1039	2.46	445.1141 / 285.0406
Kaempferide 3,7 – dirhamnoside	$C_{28}H_{32}O_{14}$	58.3	591.1708	2.37	285.0402
Kaempferol coumaryl glucoside glucoside I	$C_{36}H_{36}O_{18}$	60.5	755.1834	2.25	285.0404 / 179.0554 / 161.0446
Kaempferol coumaryl glucoside glucoside II	$C_{36}H_{36}O_{18}$	61.4	755.1834	2.25	285.0404 / 179.0554 / 161.0446

R_t, retention time; m/z, mass-to-charge ratio; [M-H]⁻, Negatively charged molecular ion