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Evaluation of polyphenol bioaccessibility and kinetic of starch digestion of spaghetti with persimmon (*Dyospyros kaki*) flours coproducts during *in vitro* gastrointestinal digestion

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Vanillin (PubChem CID1183)
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ABSTRACT

The aim was to study the *in vitro* starch digestibility, the free and bound polyphenol profile and their bioaccessibility and antioxidant activity during *in vitro* gastrointestinal digestion of durum wheat semolina spaghetti added with two types of persimmon flour concentrates ("Rojo Brillante" flour and "Triumph" flour) at two concentrations (3 and 6%). Results obtained showed that persimmon flour improves the polyphenol profile of spaghetti by addition gallic acid and coumaric acid-o-hexoside, and increasing 2-fold and around 3-fold its content in spaghetti with 3% and 6% persimmon flours, respectively. Cooked process and digestion affected more to free polyphenol content than bound. Furthermore, 3% persimmon flour enriched spaghetti reduce kinetic of starch digestion, while 6% enriched spaghetti increased it. In conclusion, persimmon flours (Rojo Brillante and Triumph) at low concentrations could be used to develop spaghetti with more polyphenol content and less starch digestibility than traditional spaghetti.

1. Introduction

Nowadays, the population and scientific community are more aware of the relationship between diet and health, as well as the environmental impact of food production. Since diets rich in easy digestive carbohydrates, saturated fats, ultraprocessed foods and poor in fiber and bioactive compounds are related with development many non-communicable diseases such as cardiovascular diseases, diabetes or cancer, which are currently a public health issue, (WHO, 2013). For that reason, consumers and World Health Organization (WHO) claim to agro-food industry healthier and sustainable foods, but also, cheap, tasty and useful.

In the search for novel and sustainable ingredients with healthy and technological properties to formulate potential functional foods, many scientists have focused their research in agro-food industry coproducts, since these are rich in bioactive compounds: vitamins, polyphenols, carotenoids, etc. and macronutrients, especially fiber, but also protein and oil; furthermore, it have technological potential as water and oil holding agents, emulsifiers, colorants and antioxidants (Gullon, Pintado, Fernández-López, Pérez-Álvarez & Viuda-Martos, 2015; Lucas-González, Viuda-Martos, Pérez-Álvarez, & Fernández-López, 2017; Simonato, Trevisan, Tolve, Favati & Pasini, 2019), and also, use them in the food formulation is a strategy to reduce waste and value them.

Persimmon (*Dyospyros Kaki*) is an orange-red fruit of Asian origin,

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whose composition has been related with the prevention of heart diseases and diabetes for different *in vitro* and *in vivo* studies (Gorinstein et al., 1998; Sindu et al., 2019). Being their mechanisms of action related with: i) their ability to bile retention, which activates bile acid pathway, (and that are associated with modulation of metabolism of carbohydrate, protein, and lipids), ii) antioxidant activity and iii) inhibition of alpha-amylase and amiloglucosidase by their polyphenols compounds (Gorinstein et al., 1998; Kawakami, Aketa, Nakanami, Izuka & Hirayama, 2010; Matsumoto, Yokoyama & Gato, 2010; Sindu et al., 2019). Persimmon is found widely distributed in Mediterranean area, especially Spain, which experimented in the last decade and important increase in its production, specifically of "Rojo Brillante" and "Triumph" cultivars. Furthermore, a recent industry has been created around persimmon, being juice manufactured one of them. The co-product generated (peel and pulp) by persimmon manufactured present potential intermediate ingredients, due to their high gallic acid, carotenoids and dietary fiber content (Lucas-González et al., 2017; Lucas-González, Fernández-López, Pérez-Álvarez & Viuda-Martos, 2018).

Pasta is widely consumed in Europe and greatly appreciated worldwide since their easy elaboration, versatility, carbohydrate value and protein content. Furthermore, in the market, a high diversity of pasta formulations are available, like pasta enriched with carrot, spinach, tomato, squid ink, spirulina, even chocolate. Among cereal-based foods, like bread, breakfast cereals, pizza, and others, pasta has the lowest glycemic index (GI) due to its complex matrix. However, based in the carbohydrate food classification (FAO/WHO, 1998) [GI: low (< 55), medium (> 55 to < 70) and high (GI > 70)], pasta made with durum wheat semolina has a GI between medium and high (Foster-Powell, Holt, & Brand-Miller, 2002). Furthermore, it can be accompanied by sauces and ingredients with high-fat, energy meals, since generating high postprandial oxidative stress (Miglio et al., 2014). So, many scientists have enriched pasta with other ingredients, such buckwheat, oats, barley, sorghum, fibers, mushroom or also agro-food co-products, with the purpose to modify their carbohydrate digestibility and or improve their polyphenolic content, showing in many case positive results in the modification of traditional spaghetti. (Chillo, Ranawana & Henry, 2011; Khan, Yousif, Johnson & Gamlath, 2013; Biney & Beta, 2014; Lu et al., 2018; Padalino, et al, 2017; Tackás et al., 2018; Simonato et al, 2019); However, pasta matrix stability can be modified with the inclusion of ingredients other than wheat semolina, which can cause porous in their structure (Padalino et al., 2017). So, although healthy ingredients are added to pasta, a functional study must be performed. In this sense, *in vitro* digestion models, are a useful screening tool to study the functional potential of various formulations, being able to compare between them their digestibility, bioaccessibility, interaction between nutrients, etc.

Taking into account the persimmon consumption benefits mentioned before, the hypothesis of the current study was that the use of persimmon juice coproducts to enrich durum wheat spaghetti could contribute to reduce postprandial insulin respond, to increase diversity of polyphenols and to reduce postprandial oxidation. However, for carrying out these benefits, polyphenol compounds must be released from the matrix.

For all exposed, the aim of this work was to study the *in vitro* starch digestibility and the free and bound polyphenol profile and their bioaccessibility and antioxidant activity after the simulated *in vitro* gastrointestinal digestion of durum wheat semolina spaghetti added with two types of persimmon flour concentrates ("Rojo Brillante" flour and "Triumph" flour) at two concentrations (3 and 6%). The effect of cooking treatment in the free and bound polyphenol profiles in spaghetti samples were also studied.

2. Materials and methods

2.1. Raw material

Durum wheat (cv. Marco Aurelio) was used to obtain durum wheat semolina with a particle size of 280 μm and with the following chemical composition: moisture 13.7 g/100 g, protein content 12.0 g/100 g d.w., gluten content 10.0 g/100 g d.w., and ashes 0.67 g/100 g d.w. Juice persimmon co-products from cultivars 'Rojo Brillante' and 'Triumph' were processed as described by Lucas-González et al. (2017) to obtain both persimmon flours with a particle size < 210 μm. White bread was purchased in a local bakery of Orihuela (Alicante, Spain).

2.2. Pasta formulation and processing

Five batches (2 Kg) corresponding with different spaghetti formulations [spaghetti with 100% durum wheat semolina (CS), spaghetti with 3% of Rojo Brillante flour (SR-3), spaghetti with 3% of Triumph flour (ST-3), spaghetti with 6% of Rojo Brillante flour (SR-6) and spaghetti with 6% of Triumph flour (ST-6)], were processed using a Namad (Rome, Italy) pilot extruder with two mixing vessels and one extruder.

In brief, durum wheat semolina and persimmon flours were mixed in a shaft mixer for 15 min, then different water (18 °C) amounts: 500 g (CS), 600 g (SR-3), 750 g (ST-3), 700 g (SR-6) and 850 g (ST-6), which were calculated taking into account the water holding ability to dry ingredients, were added and mixed for 12 min. The formed dough was mixed again for 10 min in the second vessel and extruded in a single screw extruder with a barrel length of 350 mm and a diameter of 40 mm, provided with a water-cooling system. The extruder was equipped with bronze round drawplate of 100 mm diameter provided with 18 bronze inserts of 12 mm having 7 dies of 1.2 mm diameter each. The input feed rate was of 15 Kg/h, the screw speed was set to 42 rpm and temperature of cooling water was of 5 °C. In these experimental conditions, the temperature reached a maximum of 30 °C and pressure behind the die reached 10 MPa. The output rate was of 1.0 m/min and spaghetti strands were cut in 300 mm long spaghetti using a cutting bar. An AFREM (Lyon, France) fan-assisted experimental dryer was used to dry spaghetti formulations for 24 h at low temperature (50 °C). Dry spaghetti formulations were then packed in sealed plastic pouches and stored at room temperature for 10 days in order to stabilize the pasta and prevent it from absorbing water. Formulations were kept at room temperature and dark conditions prior to analysis.

2.3. Extraction and identification of polyphenolic compounds (free and bound)

The extraction of phenolic compounds from crude and cooked spaghetti samples was divided in two fractions, free and bound. For the extraction of the polyphenolic compounds, 2 g of samples were mixed with 10 mL of acidified methanol (1% HCl), then the mix underwent sonication for 2 h at room temperature and centrifuged 10 min at 4 °C and 7100*g*. The supernatant, containing free phenolic compounds, was collected and evaporated under vacuum, and then the solids were re-suspended with 5 mL of methanol:water (50:50 v/v). For extraction of bounded polyphenolic compounds, the method described by Mpofu, Sapirstein and Beta (2006) was followed, using the pellet remaining after the free polyphenolic compounds extraction. Ethyl acetate extracts were evaporated under vacuum, and then re-suspended in 5 mL of methanol: water in the case of non-digested samples (crude and cooked) and 2 mL in the case of digested samples.

Detection of free and bound polyphenols in the different samples (crude, cooked and digested) was carried out in a Hewlett-Packard HPLC series 1200 instrument equipped with a C_{18} column Mediterranean Sea 18, 25 × 0.4 cm, 5 μm particle size (Teknokroma, Barcelona, Spain). In brief, 20 μL of the sample was eluted with a gradient of 1 mL/min. The mobile phases were composed of a mixture

of two solvents. Solvent A contained formic acid in water (1:99, v/v) and solvent B was composed of methanol (100%). The solvent gradient was at 0 min- 5% solvent B, at 20 min- 25% solvent B, at 40 min-50% solvent B; and at 45 min-5% solvent B. The detection of polyphenols was made by UV absorption at 270, 280, 325 and 360 nm. Identification was carried out comparing retention time and spectrum observed in the samples with the pure standards injected in the same conditions, while quantification was realized using calibrated curve of four point to following pure standards: gallic acid, protocatechuic acid, catechin, 4-hydroxybenzoic acid, epicatechin, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, vanillin, p-coumaric acid, ferulic acid, sinapic acid and L-tryptophan (aromatic aminoacid).

2.4. Antioxidant activity

Antioxidant activity of crude, cooked and digested spaghetti samples of both extracted fractions (free and bound) was evaluated by two spectrophotometric assays, using a spectrophotometer HP 8451 (Hewlett Packard, Cambridge, UK), as described below,

2.4.1. Ferric ion Reducing antioxidant power (FRAP)

Reducing power of samples to reduce Fe^{3+} to Fe^{2+} was evaluated following the methodology describe by Oyaizu (1986). In brief, 1 mL aliquots of methanolic extracts (crude, cooked and digested) were mixed with phosphate buffer (0.2 M, pH 6.6) and Potassium ferricyanide (1%), then the samples were incubated a 50 °C during 20 min. Time elapsed; 2.5 mL of trichloroacetic acid (10%) was added to the samples and vortex. Then 2.5 mL to the before sample were mixed with distillated water and iron trichloride (0.1%). After mixing the samples in a vortex, reaction was carried out 10 min, the absorbance was measured at 700 nm. The blank was made by replacing the sample with distilled water. A calibration curve was made with the Trolox reagent. Results were expressed as mg Trolox equivalent (TE)/g dry weight sample.

2.4.2. ABTS radical cation scavenging activity assay

ABTS assay was performed with the procedure carried out by Leite et al. (2011). In summary, ABTS radical active was prepared mixing the radical ABTS with Potassium persulfate ($K_2S_2O_8$) and distilled water. The ABTS radical active was diluted in distilled water until the absorbance (at a wavelength of 734 nm) was between 0.70 and 0.72. Then 10 μL of the methanolic extracts (crude, cooked and digested) was mixed with 990 μL of diluted ABTS radical. The reaction was led to 6 min. After that, absorbance was measured at 734 nm. The blank was made by replacing the sample with distilled water. A calibration curve was made with the Trolox reagent. Results were expressed as μg Trolox equivalent (TE) / g dry weight sample.

2.5. Simulated in vitro gastrointestinal digestion

Simulated *in vitro* gastrointestinal digestion was carried out following the consensus procedure describes by Minekus et al. (2014). In brief, three digestion phases were simulated: oral, gastric and intestinal. For each of them, simulated digestion fluids were prepared with its corresponding electrolyte stock solution, enzymes, $CaCl_2$ and water, in the quantities that are indicated in the protocol describe by Minekus et al. (2014). For adjust the pH, HCl 1 N and NaOH 1 N solutions were used. The digestion process was carried out in falcon 50 mL tubes, which were arranged horizontally in a reciprocal shaking bath (JP Selecta™ Unitronic Reciprocating Shaking Bat) and incubated with constant temperature (37 °C), agitation (30 rpm) and specific time of each digestion phase.

Before to start *in vitro* gastrointestinal digestion, crude spaghetti samples were cooked in boiling tap water (relation 1:10) during their optimum cooking time (disappearance of the white uncooked core in pasta) (CS: 8.5 min; SR-3: 6.0 min; SR-T: 7.5 min; SR-6: 5.5 min; ST-6: 5.5 min), which was previously determined by Lucas-Gonzalez et al. (2020). Then, the cooked spaghetti samples were cooled for 10 min while these were manually cut with a knife into small pieces, around 2.0–5.0 mm, and weighed.

Simulated gastrointestinal digestion start with oral phase, in which 5.00 ± 0.05 g of each cooked spaghetti samples were mixed with 5 mL of simulated salivary fluid (SSF) and incubated for 2 min. At the end of oral phase 10 mL of simulated gastric fluid was added to oral bolus, and when the pH was adjusted to 2.00 ± 0.02, gastric phase was left running for 2 h. For stop gastric phase 20 mL of simulated intestinal fluid was mixed with the gastric bolus and pH was adjusted to 7.00 ± 0.02. Another 2 h was needed of incubation for complete intestinal phase. Blanks of reagents were carried out of each studied simulated phase.

2.6. Polyphenol stability and their bioaccesibility after in vitro gastrointestinal digestion

The released polyphenols and their stability in the spaghetti matrix were study following the procedure describe by Gullon, et al. (2015). After each digestion phase, samples were centrifuged to 12 000*g* 10 min 4 °C, with the purpose to separate pellet fraction (PF) and chyme soluble fraction (CSF). Then, separated samples were collected, weighted and lyophilized. Two different methodologies were used for the extraction of phenolic acids from both digestion fractions; pellet fraction was extracted following the procedure for bound phenolic acid extraction described in the section 2.4, while soluble chime fraction was extracted with 2.5, 5.0 or 10.0 mL of 50% (v/v) aqueous methanol for 8 h at room temperature. After centrifugation at 127 *g* for 5.0 min, the supernatant was filtered through a 0.45 μm syringe filter prior to analysis by HPLC. Bioaccessibility index and colon available index (%) was calculated as following:

$$\%Bioaccessibilityindex = \frac{CSF_i}{TP_c} \times 100 \quad (1)$$

$$\%Colon\ available\ index = \frac{PF_i}{BP_c} \times 100 \quad (2)$$

where,

CSF_i: polyphenols sum in chyme soluble fraction after intestinal phase (μg /g)

TP_C: Total polyphenols after cooking treatment (μg/g)

PF_i: polyphenols sum in pellet fraction after intestinal phase (μg/g)

BP_C: Total bound polyphenols after cooking treatment (μg/g)

2.7. Total starch content

Total starch content of crude spaghetti samples was determined following the AOAC Official Method 996.11 (AOAC, 2000) using K-TSTA Megazyme assay (Megazyme Ltd, Ireland) for total starch determination. Results were expressed as percentage of dry weight.

2.8. Kinetic of starch digestion and predicted glycemic index

The Protocol described by Goñi, Garcia-Alonso and Saura-Calixto (1997) was used for determining the *in vitro* kinetic of starch digestion and the predicted glycemic index. However, many modifications were carried out. So, *in vitro* digestion simulation was performed as describe above, but incubation time of gastric phase was of one hour. After 2, 30, 60, 75, 90, 120, 150 and 180 min, aliquots (100 μL) were collected and after enzyme inhibition by thermal shock (5 min to boiling water bath) were diluted. Then, 1 mL of the diluted aliquot was mixed with 3 mL of acetate buffer (pH 4.50) and 100 μL of amiloglucosidase (3,300 U/mL). The mixture was vortex mixed and incubated for 30 min at 50 °C. Then, the content was made up to 10 mL with distilled water and an aliquot (100 μL) was mixed with 3 mL of GOPOD reagent and incubated for

20 min at 50 °C. Absorbance of blank, sugar pattern and samples were measured a 510 nm. Results were expressed as percentage of starch hydrolyzed calculated following the equation (3)

$$\%\,Starch = A \times F \times V_D \times \frac{D}{W_d} \times 0.9 \quad (3)$$

A = Absorbance sample

F = factor to convert absorbance values to μg of D-glucose (100 μg of D-glucose divided by the GOPOD absorbance value for 100 μg of D-glucose)

V_D = Digestion phase volume (mL)

D = Dilution factor

W_d = Sample dry weight (mg)

0.90 = factor to convert from free glucose, as determined, to anhydroglucose, as occurs in starch.

Predicted glycemic index (pGI) was calculated as the area under the curve (AUC) of each studied spaghetti formulation, with the help of first order equation of hydrolytic process (Eqs. (4) and (5)), using white bread as reference food. The Eqs. (6) and (7) proposed by Goñi et al. (1997) was used for calculating the estimated glycemic index. The concentrations obtained at 120 min were used as final reaction time.

$$C = C_{(\infty)(1-e^{(-kt)})} \quad (4)$$

$$AUC = C_\infty(t_\infty - t_0) - (C_\infty/k)\left[1 - e^{-k(t_\infty - t_0)}\right] \quad (5)$$

$$I = AUC_{Spaghetti}/AUC_{white\ bread} \times 100 \quad (6)$$

$$eGI = 39.71 + 0.549\,HI \quad (7)$$

where,

C = % hydrolyzed starch

C_∞ = % hydrolyzed starch at final time

k = kinetic reaction constant

t_∞ = final reaction time (120 min)

t_0 = start reaction time

2.9. Statistical

Values were expressed as mean ± standard deviation of three repetitions. To know the differences between, total starch content and pGI among the samples a simple ANOVA was carried out. The differences in kinetic of starch digestion and starch hydrolysis during *in vitro* gastrointestinal digestion were evaluated carried out a two-ANOVA assay. The differences among the free and bound polyphenols content of spaghetti formulations were studied through two-ANOVA assays: Firstly, the differences among crude and cooked samples for the different spaghetti formulations and then, the differences among cooked and digested phases (oral, gastric and intestinal) for the different formulations. Statistical significances were considered when p value was < 0.05 after Tukey's post hoc test. Pearson correlation analysis was used to investigate the relations between polyphenols and ABTS and FRAP values. Statistical analyses were performed using the STAT graphic program.

3. Results and discussion

3.1. Phenolic profile of crude and cooked spaghetti

Among ten quantified bound polyphenols in crude CS, which can be shown in Fig. 1, ferulic acid was found in the biggest amount, following by sinapic acid, ferulic acid derivatives (I and II), protocatechuic acid, catechin, 4-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid and vanillin. These results were in agreement with scientific literature, where bound ferulic acid is the major phenolic acid present in durum wheat spaghetti (Biney & Beta, 2014; Fares, Platani, Baiano & Menga, 2010; Ciccoritti et al., 2017).

The enrichment of durum wheat spaghetti with both studied persimmon flours, modified its polyphenolic profile, because, two new compounds, gallic acid and p-coumaric-o-hexoside, have been detected in the enriched samples (SR-3, ST-3, SR-6 and ST-6). Furthermore, while in CS the biggest polyphenol was ferulic acid, in the enriched samples was gallic acid. However, persimmon flour in spaghetti did not reduce or increase the content of phenolic compounds derived from wheat ($p > 0.05$). In consequence, the content of total bound phenolic acid increased 2-fold and around 3-fold in spaghetti with 3% and 6% persimmon flours, respectively, in comparison with the content of CS ($p < 0.05$). These results were expected, because gallic acid represented around 90% among of the total of polyphenols detected and quantified (gallic acid, 4-hydroxybenzoic acid, coumaric acid-o-hexoside, catechin, epicatechin, quercetin, kaempferol, ellagic acid, and various glycosylated quercetin and kaempferol compounds) in both studied flours as can be observed in Lucas-González, Fernández-López et al. (2018); Lucas-González, Viuda-Martos et al. (2018). Thus, the presence of other polyphenols contributed by persimmon flours to spaghetti was limit by the low concentration in the persimmon flours and the possible loss during the making spaghetti process, although free phenol compounds are more susceptible to degradation during process than bound phenol compounds (Fares et al., 2010). However, as exception of this fact, it is the case of the coumaric acid o-hexoside, which although was found in fewer concentrations in Rojo Brillante and even in Triumph flour was not detected (Lucas-González, Viuda-Martos et al., 2018), it has been quantified in the enriched spaghetti. This fact could be due to the different extraction methods (in the referenced work about the polyphenol profile of persimmon flour, neither alkaline nor acid hydrolysis was carried out to determine bound phenol compounds), different harvest condition, postharvest treatment and fruit state maturation.

Significant differences between enriched crude spaghetti samples were observed, depending on persimmon flour type and dose; the amount of gallic acid and coumaric acid-o-hexoside was dose-dependent ($p < 0.05$) and SR-6 showed the biggest amount of gallic acid ($p < 0.05$), while ST-6 showed the highest amount ($p < 0.05$) of coumaric acid-o-hexoside. Other authors (Khan et al., 2013) have previously reported dose-dependent rise in polyphenolic content of durum wheat spaghetti by addition of novel ingredients.

As regard to the content of free polyphenols in crude spaghetti (Fig. 2), in the case of crude CS three phenolic acids (protocatechuic, vanillin and ferulic acid) were identified, and four (the same than for crude CS and the gallic acid) in the enriched spaghetti samples (SR-3, ST-3, SR-3 and ST-6). Other authors have detected another free phenolic acids, such as sinapic, caffeic, o-comuaric, hydroxybenzoic acid glucoside, 8-C-Glucosyl-6-C-arabinosyl-apigenin and vanillic acid, in whole wheat spaghetti (Takács, et al., 2018) or whole wheat noodles (Podio, Baroni, Pérez & Wunderlin, 2019). These differences could be due to the different extraction methods, the wheat varieties used treatment and environment (Mpofu et al., 2006). Furthermore, in all studied samples, L-tryptophan, an aromatic amino acid, was detected; this compound has been previously detected in free fractions of durum whole-wheat noodles (Podio et al., 2019).

Cooking treatment affected, indifferent ways, to bound and free phenolic acids (Figs. 1 and 2); On the one hand, it caused insignificant changes in the content of bound phenolic acids present in all spaghetti samples, given that, only was observed a significant lost in the vanillic acid content ($p < 0.05$). These results differed from those shown by other authors (Fares, Platani, Baiano, & Menga, 2010; Khan et al., 2013) who reported that cooked process increased the amount of bound phenolic acids in pasta, due to the links breakdown among phenolic acids and wall cells, and others that indicated losses after cooking process (Biney & Beta, 2014). These variations could be due to the different processing of spaghetti (Khan et al., 2013), the optimum cooking time, or the wheat variety. For example, the optimum cooking time used to cook the studied spaghetti was low (around 5.5–8.5 min),

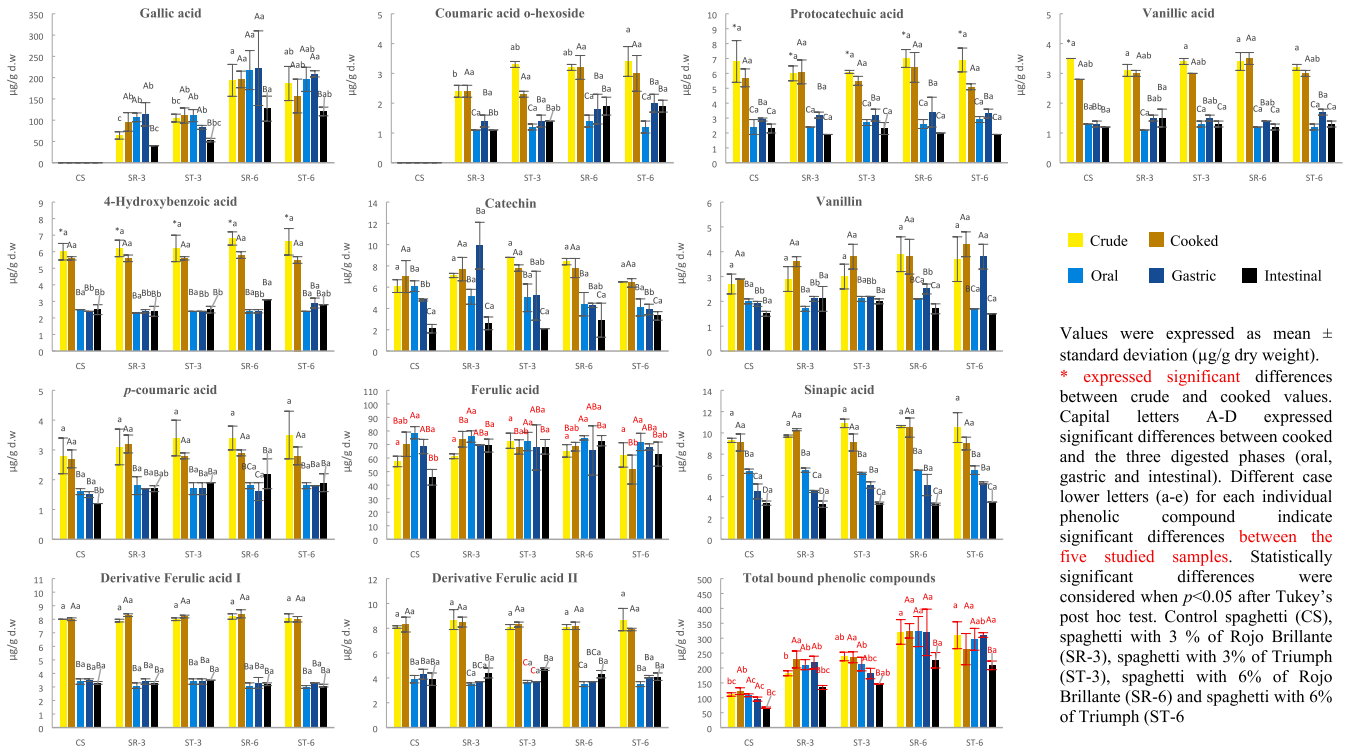

Fig. 1. Bound polyphenol profile of crude, cooked and digested spaghetti (after the oral, gastric and intestinal phase).

if it is compared with other works (around 10–15 min) and so they reported a higher bound polyphenol content (Fares et al., 2010; Khan et al., 2013). On the other hand, the cooked process significantly decreased the content of free polyphenols present in studied spaghetti ($p < 0.05$), probably due to the lixiviation of these compounds to cooking water. As in the case of bound polyphenols, conflicted results can be found in scientific literature, where cooking treatment can improve the free phenolic content in spaghetti (Ciccoritti, et al., 2017) or reduce it (De Paula, Rabalski, Messia, Abdel-Aal, & Maeconi, 2017)

3.2. *Polyphenol stability and their bioaccessibility after in vitro gastrointestinal digestion*

The evolution of polyphenols presents in spaghetti samples (CS, SR-3, ST-3, SR-6 and ST-6) during *in vitro* gastrointestinal digestion was studied in the three digestion steps (oral, gastric and intestinal), in both fractions, the chyme soluble fraction and in the pellet fraction (Fig. 2.A), with the propose to have a complete vision of their matrix release and bioaccessibility during enzymatic and mechanic process. The variations showed in their polyphenol content after digestion, were dependent on the own compound, the fraction and the digestive phase.

After oral phase in the chyme soluble fractions only protocatechuic and ferulic acids (traces amounts) were observed in low concentrations in comparison with that showed in cooked samples. Furthermore, spaghetti samples presented the same concentrations ($p < 0.05$). On the contrary, the highest content and diversity of free phenolic acids was found in gastric phase, since protocatechuic, vanillin, gallic acid (only in enriched spaghetti formulations), ferulic acid, catechin and epicatechin were detected. In the case of catechin and epicatechin, which have not been detected in the other studied extracts (crude, cooked and oral), their presence could be due to the acid degradation of proanthocyanidins (condensed tannins) (Zhu et al., 2002), a type of oligomeric flavonoids, whose common oligomers are (*epi*)gallocatechins, (*epi*)catechins, and (*epi*)afzelechins. However, after intestinal phase catechin, epicatechin, gallic acid and vanillin were not detected, which could be due to intestinal conditions, interaction with other dietary compounds or even biliary interactions (Kida, Suzuki, Matsumoto, Nanjo, & Hara, 2000; Zhu et al., 2002). On the other hand, a significant increase in the concentration of protocatechuic acid and ferulic acid, with respect to the other digestion phases (oral and gastric) were detected ($p < 0.05$). These increases could be due to the release of both polyphenols from their spaghetti matrix or to the degradation of flavonoids, like catechin, in the case of protocatechuic (Sánchez-Patán, Monagas, Moreno-Arribas & Bartolomé, 2011). Podio et al. (2019) also showed that ferulic acid increased after intestinal phase with respect to oral and gastric phases. As regard to L-tryptophan (Fig. 2.B), the content increased gradually after oral and gastric phases and strongly after intestinal phase, which was mediated by protease enzyme. Among studied spaghetti, CS showed the highest polyphenols bioaccessibility (18.7%, Fig. 3), whereas the bioaccessibility of polyphenols presents in enriched persimmon flours spaghetti (SR-3, ST-3, SR-6 and ST-6) was around 10%, showing SR-6 samples the lowest values. These differences were due to the higher total polyphenol content in enriched persimmon flours spaghetti than in the CS, since after intestinal phase, the 3% and 6% enriched spaghetti had 2 and 3 fold polyphenol content than CS, respectively ($p < 0.05$). The low bioaccessibility of wheat pasta determined *in vitro* have been previously reported by Tackás et al. (2018) and Podio et al. (2019).

As regard to bound fraction, in general, intestinal phase promoted a decrease in their concentration. However, if the behavior of each compound is detailed, substantial differences can be observed among individual compounds (Table 1). So, the amount of ferulic acid in studied spaghetti (CS, SR-3, ST-3, SR-6 and ST-6) increased discreetly in oral and gastric phases, while significantly decreased after intestinal phase ($p < 005$). In the case of *p*-coumaric acid, vanillic acid, vanillin, 4-hydroxybenzoic acid and both derivative ferulic acids (I and II), the oral digestion conditions caused significant loses in their concentration, in comparison with the amounts showed in cooked spaghetti ($p < 0.05$), which were remained along digestive process ($p > 0.05$). As regard to the content of sinapic acid and catechin it was also

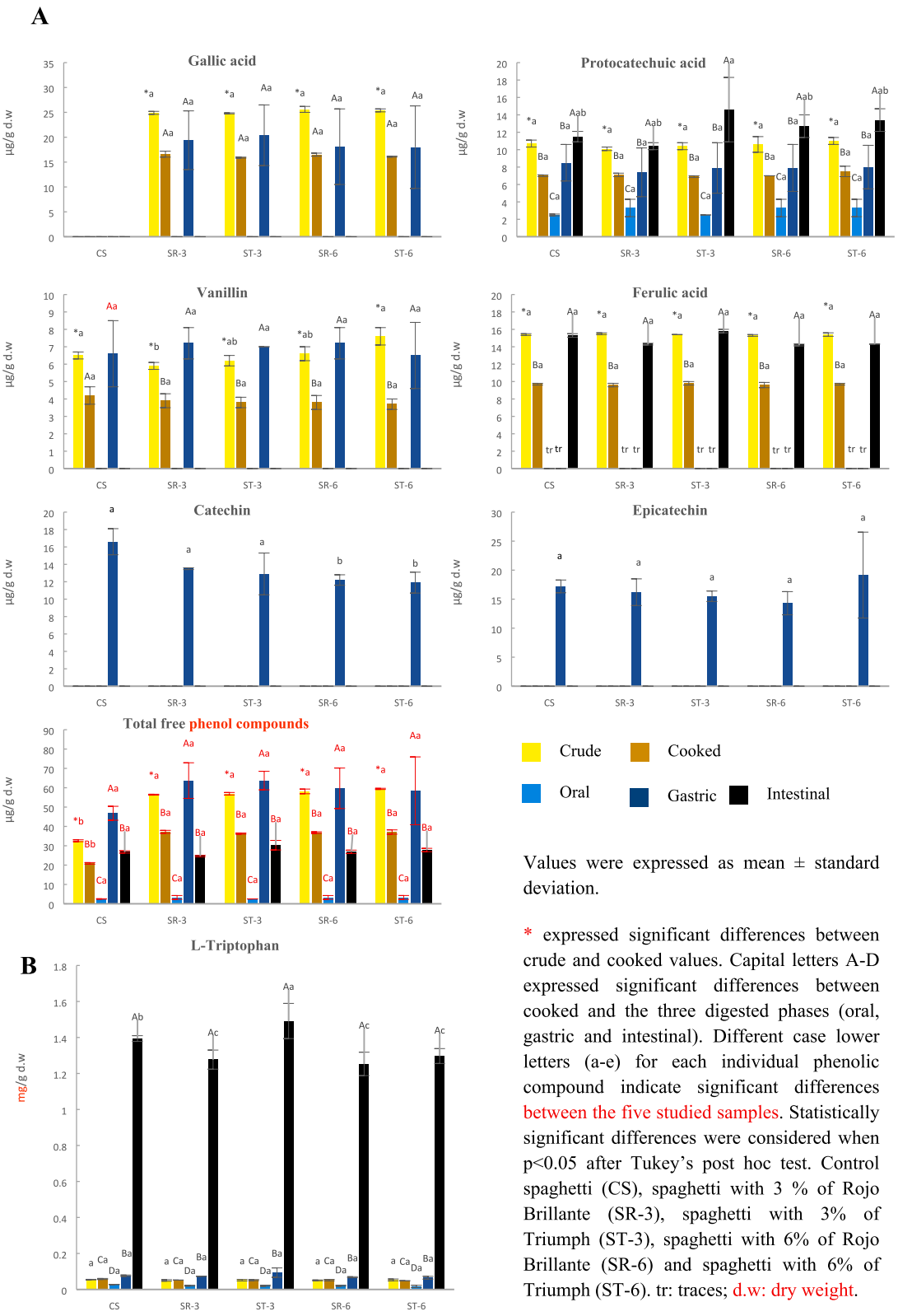

Fig. 2. Compounds detected in chyme soluble fraction of crude, cooked and digested spaghetti (after the oral, gastric and intestinal phase). A. Free polyphenol profile. B. Aromatic aminoacid.

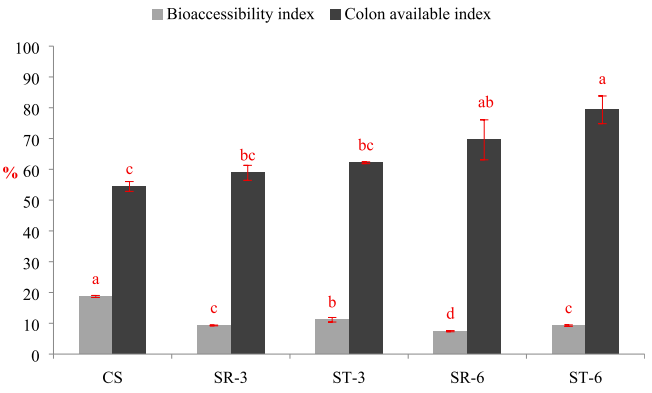

Fig. 3. Bioaccessibility index and colon available index of studied spaghetti. Values are expressed as mean ± standard deviation (%). Different case lower letters (a-e) for each studied parameter indicate significant differences. Statistically significances were considered when $p < 0.05$ after Tukey s post hoc test. Control spaghetti (CS), spaghetti with 3% of Rojo Brillante (SR-3), spaghetti with 3% of Triumph (ST-3), spaghetti with 6% of Rojo Brillante (SR-6) and spaghetti with 6% of Triumph (ST-6).

downed, but in a gradual way through gastrointestinal digestion. The protocatechuic acid content in the three digested samples (oral, gastric and intestinal) of the studied spaghetti was always lower than in cooked samples, however after gastric phase a light raise was showed ($p < 0.05$). The behavior of gallic acid was similar than reported for the ferulic acid, briefly, a significant increase was observed in oral phase, then its concentration decreased in gastric and intestinal phases, although these differences were only significant respect to the cooked samples at intestinal phase. Furthermore, 6% persimmon flour enriched spaghetti showed higher amounts of gallic acid than those showed in the 3% formulations ($p < 0.05$). In addition, a dose-dependent increase was showed in the content of coumaric acid-*O*-hexoxide, but only after intestinal phase. After digestion process, the most remarkable differences in the polyphenols content between CS and enriched spaghetti were for the ferulic acid content, showing higher concentration in the persimmon flours enriched spaghetti (SR-3, ST-3 SR-6 and ST-6) than in the CS at the end of intestinal phase, and also the presence of gallic acid and coumaric acid-*O*-hexoside. Therefore, the bound polyphenols release from spaghetti matrix after *in vitro* gastrointestinal digestion were poor. Chait, Gunenc, Bendali & Hosseinian (2020) reported low matrix release of bound polyphenols, like gallic acid, vanillic acid and protocatechuic acid content in carob after *in vitro* digestion process. Therefore, after digestion process a high number of polyphenols continue linked to cell walls and to indigestible polysaccharides. Spaghetti samples showed different colon available polyphenols index (Fig. 1), following this serie ST-6 > SR-6 > ST-3 > SR-3 > CS. So, a high amount of polyphenols could arrive to colon and could be used by gut microbiota. Polyphenols present in wheat can the ability to modulate gut microbiota in a positive way, since promote the grow of *Bifidobacterium* and *Lactobacillus*, and reduce the proliferation of *Escherichia coli* and *Clostridium* spp. (Costabile et al., 2008). Gallic acid has also b linked to promoting the grow of beneficial bacteria and the inhibition of harmful bacteria (Li et al., 2019). Actually, many scientists start to point that cancer colonic prevention derive to cereals consumption could be associated with their polyphenols content although these polyphenols have not a high bioaccessibility (Mileo, Nisticò & Miccadei, 2019). Regarding these investigations, it could be said that although the majority of bound polyphenols have not been released from their food matrix during gastrointestinal digestion, these can still have a health-promote action, which in the case of studied spaghetti samples could be enhanced by the presence of gallic acid.

3.3. Antioxidant activity of crude and cooked spaghetti

The antioxidant potential of crude spaghetti samples and their changes after cooked and digested process were studied using two different *in vitro* antioxidant essays, ABTS and FRAP.

As regard to crude samples, persimmon flour enriched spaghetti showed higher ABTS and FRAP values than CS samples in both polyphenol fractions ($p < 0.05$), as can be observed in Table 1. That increase was dose dependent ($p < 0.05$), except for the activity of the free fraction in the ABTS assay, where not differences were showed between persimmon flour enriched spaghetti ($p > 0.05$). These results agreed with those reported by Khan et al. (2013) and De Paula et al. (2017), who reported a dose-dependent increase in antioxidant activity in spaghetti enriched with other vegetable ingredients at increasing

Table 1
Values of antioxidant activity determined by ABTS and FRAP assays in both fractions (free and bound) of studied spaghetti samples (crude, cooked and digested).

			Crude	Cooked	Oral	Gastric	Intestinal
ABTS (μg TE/g d.w)	Free fraction	CS	24.2 ± 1.4^{Yb}	12.4 ± 0.5^{ZBa}	11.4 ± 1.7^{Ba}	15.2 ± 3.6^{Ba}	432.0 ± 138.9^{Aa}
		SR-3	31.1 ± 2.6^{Ya}	16.5 ± 1.8^{ZBa}	8.5 ± 1.4^{Ba}	15.3 ± 3.7^{Ba}	457.1 ± 29.0^{Aa}
		ST-3	26.4 ± 0.8^{Ya}	18.7 ± 1.9^{ZBa}	8.9 ± 0.7^{Ba}	15.4 ± 2.2^{Ba}	492.0 ± 6.5^{Aa}
		SR-6	30.7 ± 3.0^{Ya}	16.2 ± 1.1^{ZBa}	8.2 ± 0.8^{Ba}	15.6 ± 2.3^{Ba}	427.0 ± 9.0^{Aa}
		ST-6	33.6 ± 0.8^{Ya}	16.5 ± 0.6^{ZBa}	7.5 ± 1.5^{Ba}	16.3 ± 4.7^{Ba}	447.2 ± 32.1^{Aa}
	Bound fraction	CS	13.7 ± 1.5^{Yc}	16.4 ± 2.2^{AYc}	9.1 ± 0.8^{Ac}	9.1 ± 1.7^{Ac}	6.5 ± 0.5^{Bc}
		SR-3	22.9 ± 1.4^{Yb}	26.0 ± 6.2^{CYbc}	55.2 ± 3.1^{Ab}	51.0 ± 2.5^{Ab}	45.2 ± 1.5^{Bb}
		ST-3	28.9 ± 3.5^{Yb}	33.6 ± 4.9^{CYba}	52.1 ± 7.1^{Ab}	55.5 ± 8.4^{Ab}	47.1 ± 3.1^{Bb}
		SR-6	42.3 ± 3.2^{Ya}	43.9 ± 3.5^{CYa}	72.3 ± 10.1^{Aa}	68.8 ± 11.1^{Aa}	56.9 ± 2.6^{Ba}
		ST-6	40.2 ± 3.6^{Ya}	41.0 ± 7.5^{CYa}	64.2 ± 9.6^{Aa}	63.3 ± 1.1^{Aa}	53.7 ± 7.3^{Ba}
FRAP (mg TE/g d.w)	Free fraction	CS	0.69 ± 0.09^{Yc}	0.20 ± 0.01^{ZCc}	0.27 ± 0.03^{Ca}	0.77 ± 0.01^{Ba}	4.98 ± 0.19^{Ab}
		SR-3	1.05 ± 0.10^{Ybc}	0.42 ± 0.09^{ZCb}	0.32 ± 0.02^{Ca}	1.24 ± 0.06^{Ba}	5.21 ± 0.74^{Ab}
		ST-3	1.29 ± 0.14^{Yab}	0.37 ± 0.05^{ZCab}	0.36 ± 0.09^{Ca}	1.17 ± 0.08^{Ba}	6.18 ± 0.76^{Aab}
		SR-6	1.52 ± 0.15^{Ya}	0.52 ± 0.07^{ZCa}	0.47 ± 0.07^{Ca}	1.52 ± 0.25^{Ba}	5.83 ± 0.44^{Ab}
		ST-6	1.79 ± 0.13^{Ya}	0.40 ± 0.05^{ZCa}	0.37 ± 0.01^{Ca}	1.49 ± 0.17^{Ba}	7.79 ± 0.95^{Aa}
	Bound fraction	CS	0.14 ± 0.02^{Yc}	0.16 ± 0.04^{YBc}	0.25 ± 0.02^{Ab}	0.21 ± 0.06^{ABb}	0.15 ± 0.03^{Bb}
		SR-3	0.49 ± 0.04^{Yb}	0.55 ± 0.14^{YAb}	0.49 ± 0.03^{ABab}	0.49 ± 0.03^{ABa}	0.32 ± 0.05^{Bab}
		ST-3	0.69 ± 0.09^{Yb}	0.75 ± 0.16^{YAab}	0.51 ± 0.03^{ABa}	0.52 ± 0.03^{ABa}	0.46 ± 0.01^{Ba}
		SR-6	1.12 ± 0.09^{Ya}	1.07 ± 0.03^{YAa}	0.49 ± 0.03^{Bab}	0.50 ± 0.02^{Ba}	0.52 ± 0.00^{Ba}
		ST-6	1.10 ± 0.22^{Ya}	1.00 ± 0.09^{YAa}	0.48 ± 0.02^{Bab}	0.54 ± 0.01^{Ba}	0.52 ± 0.01^{Ba}

Values were expressed as mean ± standard deviation. Different capital letters ranged Y-Z expressed significant differences between crude and cooked values in the same row. Capital letters A-D expressed significant differences between cooked and the three digested phases (oral, gastric and intestinal) in the same row. Different case lower letters (a-e) in the same columns for each antioxidant method and fraction indicate significant differences. Statistically significant differences were considered when $p < 0.05$ after Tukey's post hoc test. Control spaghetti (CS), spaghetti with 3% of Rojo Brillante (SR-3), spaghetti with 3% of Triumph (ST-3), spaghetti with 6% of Rojo Brillante (SR-6) and spaghetti with 6% of Triumph (ST-6).

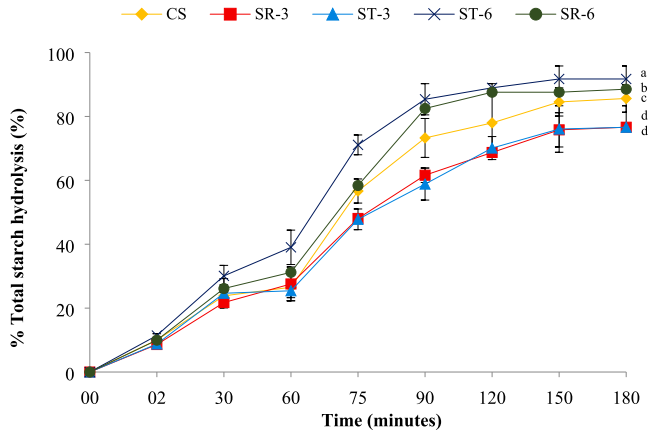

Fig. 4. Total starch hydrolysis rate of spaghetti during gastrointestinal digestion. Different case lower letters (a-e) indicated significant differences. Statistically significant differences were considered when p < 0.05 after Tukey's post hoc test. Control spaghetti (CS), spaghetti with 3% of Rojo Brillante (SR-3), spaghetti with 3% of Triumph (ST-3), spaghetti with 6% of Rojo Brillante (SR-6) and spaghetti with 6% of Triumph (ST-6).

concentrations.

Cooking treatment caused a significant loss in the ABTS radical scavenging ability and in the ability to reduce iron in the free fraction, respect to the crude CS sample, while these antioxidant activities were not modified in the bound fractions. Enriched spaghetti showed the same behavior than control during the cooking ($p < 0.05$), however the FRAP and ABTS values in both fractions were higher and dose-dependent than in control samples ($p < 0.05$). These results can be correlated with the results found in polyphenol profile (Fig. 2.A). Podio et al. (2019) reported no changes in the antioxidant activity of whole noodle samples make with whole wheat flour in free fractions and an increase in bound fraction while other authors (Tackás et al., 2018; Khan et al., 2013) have observed loses in wheat spaghetti and durum wheat spaghetti enriched with sorghum, respectively, after cooking process.

3.4. Antioxidant activity during in vitro gastrointestinal digestion

As regard to the antioxidant activity in the chyme soluble fractions of studied spaghetti, their ABTS and FRAP values showed a slight increase after oral and gastric digestion that was higher after intestinal phase, in comparison with values reported for liquid fractions in cooked samples ($p < 0.05$). Furthermore, no differences in ABTS radical scavenging activity and ferrous-ion chelating capacity between the CS and persimmon flour enriched spaghetti ($p > 0.05$) were found for any simulated digestion step, with the exception of the Triumph flour enriched spaghetti, whose FRAP values after intestinal digestion were the highest ($p < 0.05$). Podio et al. (2019) also reported a progressive increase in the FRAP values of free fractions in durum wheat noodles after simulated gastrointestinal digestion with the highest values observed in the intestinal step. It is important to note that the antioxidant activity in chyme soluble fractions measured by ABTS and FRAP assays was strongly correlated with the L-tryptophan content in the spaghetti samples ($R^2 = 0.984 p < 0.001$; $R^2 = 0.917$, $p < 0.001$, respectively). ABTS assay seem to be more sensitive to the antioxidant activity of L-tryptophan than FRAP assay, since a strong positive correlation was also found between protocatechuic acid content and FRAP values in free fraction ($R^2 = 0.999\, p < 0.001$). These results were in agreement whit those reported by Pešić et al. (2019), who attributed the increase in ABTS radical scavenging activity and ferrous-ion chelating capacity observed in digested meat food matrix enriched with grape extracts, to the presence of meat protein hydrolysates and carnosine, being ABTS the most affected.

In the case of the ferrous-ion chelating capacity showed by CS in the pellet fraction, the oral digestion improved it ($p < 0.05$), when compared with the antioxidant activity showed after cooking, but then, the subsequent digestions caused a gradual decrease in their antioxidant activity ($p < 0.05$). Whereas, in the case of persimmon enriched spaghetti samples (SR-3, ST-3, SR-6 and ST-6), the losses in their ferrous-ion chelating ability caused by digestion process ($p < 0.05$) were similar between the three simulated digestion phases ($p > 0.05$). However, in spite of the mentioned reduction in their antioxidant activity, their FRAP values were always higher than those of CS ($p < 0.05$). These results were correlated with the polyphenol content in studied spaghetti.

As regard to the $ABTS^+$ radical scavenging activity in the pellet fraction of persimmon enriched spaghetti, their ABTS values increased significantly after digestion process, with respect to the values showed in the bound fractions of the respective cooked spaghetti ($p < 0.05$). However, their antioxidant activity was gradually reduced through the gastrointestinal digestion, so the lowest values were showed after intestinal phase ($p < 0.05$). Furthermore, the enrichment with persimmon flours (Rojo Brillante and Triumph) improved their $ABTS^+$ radical scavenging ability after digestion process, in a concentration-dependent manner ($p < 0.05$). These increases could be due to the presence of other bioactive compounds, like carotenes or terpenes in the digested bound extract. Other authors (Chait, Gunenc, Bendali & Hosseinian, 2020) in studies about the polyphenol bioaccessibility in several foods reported increases in the antioxidant activity measured in the free fractions and reductions in the bound fractions, as occurred in the current work.

3.5. Kinetic of starch digestion

As can be seen in Fig. 4, the starch hydrolysis of the five studied spaghetti started in the oral phase by the action of alpha-amylase, where around 10% of starch was released from the spaghetti matrix ($p < 0.05$). The starch hydrolysis continued in gastric phase, where, after 30 min, around 13–19% of starch was transformed into glucose, then, the hydrolysis process remained without significant changes ($p > 0.05$) until the end of gastric phase. Although the action of salivary alpha-amylase has been usually ignored in the past, due to the short contact with the food and the optimal pH action (6.9), it seems that during gastric phase it have residual activity (Bustos, Vignola, Pérez & León, 2017; Freitas & Le Feunten, 2019). The highest ratios of starch hydrolysis (around 50–60%) were observed during the intestinal phase. The released glucose continuously increased until 120 min and then a plateau was observed until the end of digestion process, since not differences were observed between the starch digestibility at 120, 150 and 180 min of *in vitro* digestion models ($p < 0.05$). As regard to kinetic of starch digestion of white bread, the highest starch hydrolysis rate was observed in gastric phase, where of 66% of starch was releases from white bread matrix after 30 min. These results were similar to that reported by Bustos et al. (2017) for both, white bread and whole-grain pasta. The kinetic of starch digestion was similar for the 3% enriched persimmon flour spaghetti ($p > 0.05$) and was different from the rest of studied spaghetti ($p < 0.05$). Therefore, persimmon flours (Rojo Brillante and Triumph) enrichment to durum wheat spaghetti modified their kinetic of starch hydrolysis, being these modifications concentration-dependents and in the highest studied concentrations (6%), also to persimmon flour type. Lu et al. (2018) reported changes in the kinetic of starch digestion in an *in vitro* digestion model of fresh pasta (durum wheat semolina) with different amounts (5, 10 and 15%) of mushroom powders (white button, shiitake and porcini mushrooms) depending on the concentration and type of mushroom, however these results showed an inverse proportionality between concentration and starch digestibility. Chillo et al. (2011) studied the effect of two barley β-glucan concentrates (Glucagel™ and Barley Balance™) on *in vitro* glycaemic impact of durum wheat spaghetti, reporting that the starch

hydrolysis of the spaghetti was reduced by Barley Balance™ (reduction dose-dependent) but not by Glucagel™.

The differences found in the starch hydrolysis between the 6% persimmon flour enriched spaghetti and the rest of studied samples (SR-3, ST-3 and CS) could be related with insufficient semolina hydration during spaghetti processing, derived from the competition for water between semolina and the fiber and sugars present in persimmon flours. The consequence of a low semolina hydration is the supramolecular modification in the gluten network (Lucas-González et al., 2020). In this sense many researchers have pointed that the defects in the gluten network induce by fiber, especially insoluble fiber, causes open pores in their structure which could promote the greater accessibility of enzyme attack to starch granules (Bustos et al., 2017; Padalino et al., 2017). Although the water-holding ability of both studied persimmon flours was taking into account when pasta where produced, Lucas-Gonzalez et al. (2020) reported a weaker structure in SR-6 and ST-6 samples. Since these samples were easily fracturable in comparison with the other studied spaghetti, being ST-6 which showed lower fracturabilty value (defined as the force needs to break an uncooked strand of spaghetti when mechanical strength is applied). Furthermore, this sample also showed the lower value of uncooked diameter, which are related to shrinkage phenomena during drying of spaghetti. As regards to the total fiber content in spaghetti samples, this content increased depending on the persimmon flour concentration and type, so the ST-6 showed the highest value, followed by SR-6 > ST-3 > SR-3 > SC (Lucas-González et al. 2020). Furthermore, insoluble fiber content is higher in Triumph flour than Rojo Brillante flour (Lucas-González et al., 2017). These facts point out the negative effect of the high fiber content contributed by persimmon flours to the durum wheat semolina. However, to improve the final structure of spaghetti with a higher concentration of persimmon flours and to reduce the starch hydrolysis index, the effect of proper hydration could be studied, perhaps increasing the water in the spaghetti formulation or even mixing the flours (semolina and persimmon flours) with the water separately.

3.6. Predicted glycemic index

The glycemic index is defined by FAO/WHO experts as “the incremental area under the blood glucose response curve of a 50 g carbohydrate portion of a test food, expressed as a percent of the response to the same amount of carbohydrate from a standard food taken by the same subject” (FAO/WHO, 1998). It is considered a useful nutritional concept to classify foods in function of its ability to increase postprandial insulin respond. Nowadays, it is known that the post-prandial insulin respond is a good indicator of lipid anabolism and consequently a weight increase indicator in form to visceral fat. The predicted glycemic index (pGI) of studied spaghetti ranged from 79.3 to 91.8 (Table 2), being the 3% persimmon enriched formulations which showed the lower value ($p < 0.05$), and the 6% persimmon enriched formulations the highest, although these differences were not significant respect to the control samples. The differences between pGI among enriched spaghetti could be due to gluten network modification and the greater amylase attack to starch as previously have been discussed. All values were into the range of *in vivo* spaghetti GI (Foster-Powell et al., 2002) and following the carbohydrate food classification in base of their GI, mentioned before, all formulations showed a high glycemic index. These results were higher than that reported by other authors (Goñi et al., 1997; Simonato et al., 2019). These difference could be due to *i*) differences in the *in vitro* methods used, since these authors did not carry out oral phase; *ii*) different size of spaghetti strand, since a less size is more easy to be attacked by digestive enzymes, so in the present work small spaghetti strand was used (2–5 mm) following the recommendations of Minekus et al. (2014), however other authors used higher sizes, which are more close to real mastication of spaghetti, and iii) spaghetti processing (extrude, dry temperature, etc.).

4. Conclusions

Polyphenol profile of spaghetti (control and persimmon enriched) was affected by cooking treatment, being these changes stronger when persimmon flours were added at higher concentrations and depending on the polyphenol form (free or bound) and on the individual compound.

The enrichment of durum wheat semolina with both persimmon flours (3, 6%) increases the total polyphenol content in a dose-dependent way and apports gallic acid and coumaric acid-o-hexoside, which are not present in the control spaghetti. The polyphenols bioaccessibility in the spaghetti was poor and it was not improved by the addition of persimmon flours. After *in vitro* digestion a high number of polyphenols continued link to cell wall or indigestible polysaccharides and so they could be used by gut microbiota. The enrichment of durum wheat spaghetti with both persimmon flours (Rojo Brillante and Triumph) modified the kinetic of starch digestion, decreasing it, only in the case of the 3% enriched persimmon spaghetti. However, the predicted glycemic index was similar between control and enriched spaghetti. Spaghetti with 3% persimmon flours showed glycemic index lower than spaghetti with 6% persimmon flours. In conclusion, both persimmon flours (Rojo Brillante and Triumph) added at 3% could be used to develop spaghetti, with more polyphenol content and less starch digestibility than traditional spaghetti. Nevertheless, further *in vivo* assays are needed to confirm these *in vitro* results.

CRediT authorship contribution statement

Raquel Lucas-González: Investigation, Methodology, Writing - original draft. **José Ángel Pérez-Álvarez:** Formal analysis. **Salvatore Moscaritolo:** Resources. **Juana Fernández-López:** Writing - review & editing. **Giampiero Sacchetti:** Data curation, Formal analysis. **Manuel Viuda-Martos:** Conceptualization, Methodology, Supervision.

Table 2
Total starch content (TS), total starch hydrolyzed at 120 and 180 min (SH_{120} and SH_{180}), kinetic constant (k), area under de curve (AUC), hydrolysis index (HI) and predicted glycemic index (pGI).

	TS (%)	SH_{180} (%)	SH_{120} (%)	k_{120}	AUC_{120}	HI_{120}	pGI_{120}
CS	74.5 ± 1.0^{a}	85.6 ± 4.3^{ab}	78.0 ± 9.0^{ab}	0.036 ± 0.001	7239 ± 889	82.5 ± 10.1^{ab}	85.0 ± 5.6^{ab}
SR-3	73.4 ± 0.5^{a}	76.6 ± 1.3^{b}	68.7 ± 1.5^{b}	0.035 ± 0.000	6326 ± 146	72.1 ± 1.7^{bc}	79.3 ± 0.9^{b}
ST-3	72.7 ± 0.7^{ab}	76.6 ± 6.7^{b}	70.1 ± 3.6^{b}	0.035 ± 0.000	6462 ± 354	73.7 ± 4.0^{c}	80.2 ± 2.2^{b}
SR-6	68.4 ± 2.7^{bc}	88.5 ± 0.2^{ab}	87.6 ± 0.8^{a}	0.037 ± 0.000	887 ± 78	93.3 ± 0.9^{a}	91.0 ± 0.5^{a}
ST-6	65.3 ± 1.1^{c}	91.7 ± 4.1^{a}	89.0 ± 1.3^{a}	0.037 ± 0.000	8324 ± 29	94.9 ± 1.5^{a}	91.8 ± 0.8^{a}
WB	65.2 ± 2.1	93.5 ± 2.0	93.5 ± 2.0	0.038 ± 0.000	877 ± 196	100	–

Different case lower letters (a-e) in the same columns indicated significant differences. Statistically significant differences were considered when $p < 0.05$ after Tukey’s post hoc test. Control spaghetti (CS), spaghetti with 3% of Rojo Brillante (SR-3), spaghetti with 3% of Triumph (ST-3), spaghetti with 6% of Rojo Brillante (SR-6) and spaghetti with 6% of Triumph (ST-6).

Declaration of Competing Interest

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

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