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Chemical profile and bioaccessibility of polyphenols from wheat pasta supplemented with partially-deoiled chia flour

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ABSTRACT

The by-product obtained after the oil extraction from chia seeds has been used to produce supplemented wheat pasta, generating a food product with improved nutritional quality. In a previous work, we have demonstrated its technological feasibility, as well as its good acceptance by semi-trained judges. One of the enhanced nutritional properties of the supplemented pasta was the antioxidant capacity. In the present work, we characterize the phenolic composition of partially-deoiled chia flour and the supplemented pasta by HPLC-MS/MS. Fourteen polyphenols, mostly phenolic acids, have been identified in the acetone/water (4:1) extract. The two major components were rosmarinic acid and its glycoside (salviaflaside). The study of the effects of cooking on the phenolic profile showed that many bioactive components of chia seeds remain stable at the moment of intake. Finally, the bioaccessibility of the polyphenols has been assessed through an *in vitro* simulation of human gastrointestinal digestion.

Keywords:

Salvia hispanica L., Rosmarinic Acid, Phenolic Compounds, HPLC-MS, Digestion

1. Introduction

Chia seeds (*Salvia hispanica* L., Lamiaceae) are a popular and ancient food from Central America, being widely known by their high oil content (25-38%) rich in poly-unsaturated fatty acids (Martínez et al., 2012). During the oil extraction process, a residual by-product is generated. Some interesting nutritional characteristics have been attributed to this partially-deoiled portion, including protein and fiber content, as well as antioxidant components (de Falco, Amato, & Lanzotti, 2017). The use of chia flour has been significantly increased during recent years and it also has demonstrated to be valuable in the elaboration of gluten-free products (Menga et al., 2017; Moreira, Chenlo, & Torres, 2012). Altogether, these properties make this by-product a noteworthy ingredient of supplemented food products.

The knowledge of food composition is important for industry and consumers (Cubero-leon, Peñalver, & Maquet, 2014). Besides the interest in avoiding food fraud, there is also a tendency to understand the potential effects of foods on health and nutrition (Elmadfa & Meyer, 2010). Additionally, the assessment of stability of bioactive components throughout the manufacturing, cooking and digestive processes is fundamental to have an insight of the metabolites that remain bioavailable to exert their biological effects.

Wheat pasta is a very popular food, widely considered as a target to be fortified. Previously, we demonstrated the technological feasibility of using partially-deoiled chia flour (PDCF) in the manufacture of pasta. We evaluated a number of quality indicators and the antioxidant capacity of pasta supplemented with PDCF in different proportions (2.5%, 5% and 10%). Our results showed the improvement of nutritional properties and the enhancement of antioxidant capacity (Aranibar et al., 2018).

Antioxidant activity is generally associated with the content of phenolic compounds. It is widely accepted that even the simplest methods of food preparation can modify the composition of the bioactive ingredients of a food (Fares et al., 2008; Fares, Platani, Baiano, & Menga, 2010; Verardo et al., 2011). In the case of whole-wheat pasta, for instance, the cooking process causes an increase

in the total free polyphenols attributed to the release of bound polyphenols (Podio, Baroni, Pérez, & Wunderlin, 2019). Thus, the main goal of the present work was to determine the polyphenol composition of the functional ingredient (PDCF), and to evaluate how these components are affected by the manufacturing and cooking processes of the supplemented pasta, in order to understand which potential bioactive compounds are truly available at the moment of intake. Furthermore, as it is widely known, human digestion process involves many steps that modify the components initially ingested. The drastic changes in pH and the enzymes involved through the different stages could affect the stability of some compounds, or release some polyphenols from the food matrix making them more bioavailable (Lucas-González, Viuda-Martos, Pérez Álvarez, & Fernández-López, 2018). To exert its action, any bioactive component should firstly reach the bloodstream to be effectively distributed to the tissues. The absorption at the intestinal level is one of the main entrance ways. Particularly, polyphenols can be absorbed in the small intestine, or remain in the lumen to continue towards the colon where the local microflora plays an important role in their absorption (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014; Podio et al., 2019). In this study, we performed a simulated *in vitro* gastrointestinal digestion of the cooked pasta to analyze the variation of the antioxidant activity through the different stages, and to assess the absorption of individual polyphenols.

This work represents an important contribution, being the first report with a detailed profile of chia phenolic compounds as an ingredient of a supplemented pasta, including the evaluation of the effects of manufacture and cooking. Additionally, the analysis of bioaccessibility of the identified polyphenols through an *in vitro* simulated gastrointestinal digestion is included.

2. Materials and Methods

2.1. Standards and reagents

Ultra-pure water was obtained from Arium 61316-RO plus Arium 611 UV (Sartorius, Germany). Folin-Ciocalteu reagent, ABTS (2,2'-azino-bis-(3-thylbenzothiazolne-6-sulfonic acid) diammonium

salt), TTPZ (2,4,6-tripyridyl-S-triazine) and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) were obtained from Sigma Aldrich (Switzerland). Methanol (HPLC grade) and formic acid (puriss. p.a. for MS) were provided by J. T. Baker (Edo. de Mexico, Mexico) and Fluka (Steinheim, Germany), respectively. Commercial standards of ferulic acid and caffeic acid were obtained from Extrasynthese (Genay, France), quinic acid, rosmarinic acid and quercetin were purchased from Sigma-Aldrich (Steinheim, Germany). Filters (0.45 μm, HVLP04700) were obtained from Millipore (São Paulo, Brazil). Pepsin (P-7000, from porcine stomach mucosa), pancreatin (P-1750, from porcine pancreas), and bile extract (B-6831, porcine) were provided by Sigma-Aldrich (Buenos Aires, Argentina). Dialysis bags were SnakeSkin®, 10 KDa cut off.

2.2. Samples and pasta preparation

Commercial wheat flour was obtained from Molino San José, José Minetti & CIA Ltda. (Córdoba-Argentina). Chia seeds were obtained in a local market. PDCF was obtained by pressing. Manufacture and conservation of pasta supplemented with different proportions of PDCF (2.5%, 5.0% and 10%) is described in our previous work (Aranibar et al., 2018).

2.3. Extraction of phenolic compounds

The meal obtained after oil extraction of chia seeds was ground with a coffee grinder (Tecnodalvo, Santa Fe, Argentina) to obtain the PDCF. To avoid interferences in HPLC-MS analysis, PDCF was completely defatted using *n*-hexane before the extraction of polyphenols. On the other hand, dry raw and cooked pasta were prepared and ground according to the methodology described by Aranibar et al. (2018).

Subsequently, the extraction of the free fraction of phenolic compounds was performed with a solvent mixture of acetone/water (4:1). Five grams of PDCF, or pasta, were suspended in 20 mL of the solvent mixture. After 1 h agitation in darkness, the supernatant was filtered through a cellulose paper. This procedure was repeated twice. Finally, the supernatants were collected and dried in a

rotatory evaporator under reduced pressure at 50ºC, and recovered with 5 mL of methanol (HPLC grade). Samples were prepared in duplicate and stored at -80ºC until analysis.

2.4. *In vitro* simulation of gastrointestinal digestion of cooked pasta

In vitro digestion procedure was performed according to the methodology described by Podio et al. (2019) with some modifications. Three stages were performed:

Oral digestion (OD): 2 g of freshly cooked pasta were placed in a 15 mL Falcon plastic tube, adding 2 mL of freshly collected human saliva. To simulate the chewing process, the blend was homogenized in a T18 ultra-turrax tissue homogenizer (Ika-Labortechnik, Germany) at 22,000 rpm for 30 s, adding 2 mL of ultra-pure water. Finally, the pH was adjusted to 2 with 100 µL of 6 M HCl.

Gastric digestion (GD): 500 µL (12,640 units) of a pepsin solution (20 mg in 500 µL of 0.1 M HCl) was added to the homogenate obtained from OD. It was incubated with shaking for 2 h at 37°C.

Intestinal digestion (ID) and Dialysate (DIA): After incubation with pepsin, 3 mL of a pancreatin/porcine bile solution (15 mg of porcine pancreatin plus 75 mg of porcine bile extract in 3 mL of 0.1 M $NaHCO_3$, pH=7.5) was added to simulate intestinal digestion. The digestion product was placed in a dialysis bag to simulate the absorption of the compounds through the membrane of the small intestine (dialysate). The closed dialysis bag was placed in a 100-mL glass vial with 40 mL of 0.1 M $NaHCO_3$ solution (pH=7.5) and the completely submerged bag was allowed to dialyze for 3 h at 37°C.

Sub-samples were collected throughout the described procedure in triplicate. Blanks of reagents were made in each stage. OD, GD and ID sub-samples were centrifuged at 13,000X*g* for 15 min, and the ID and DIA sub-samples were acidified to pH=2 with formic acid. All sub-samples were filtered through 0.45 µm filters, fractionated and stored at -80 °C until analysis.

2.5. Total phenolic content and antioxidant activity

Total polyphenol content (TPC) was measured by Folin-Ciocalteu method according to the methodology previously described (Podio et al., 2019). Appropriate dilutions of the samples were mixed with 1.68 mL of ultra-pure water and the corresponding volume of methanol to reach a total of 110 μL (sample + methanol). Then, 100 μL of Folin-Ciocalteu reagent were added and vortexed. After 1 min, 300 μL of aqueous sodium carbonate (20 g 100 mL^{-1}) were added, vortexed and allowed to stand 120 min at room temperature in darkness. The absorbance was read at 750 nm. TPC was calculated by linear regression using gallic acid as standard. Results are expressed in mg of gallic acid equivalents (GAE) per 100 g of pasta.

Antioxidant activity was measured using the TEAC assay (Re et al., 1999). Briefly, ABTS radical cation was prepared by reacting 7 mM ABTS and 2.45 mM potassium persulfate in 10 mL of water. The mixture was stored in darkness 16 h before use. The aqueous ABTS*+ solution was diluted with methanol to an absorbance of 0.80 ± 0.02 at 734 nm (dilution 1/70 ca.). For sub-samples from the digestion the dilution of working reagent was prepared in PBS buffer (pH=7.5). An appropriate dilution of each sample was added to 3 mL of TEAC solution, adding a corresponding volume of methanol. The mixture was incubated for 30 min in darkness and the absorbance measured at 734 nm. The concentration of each sample was calculated by linear regression using Trolox as standard. Results are expressed in mmol of Trolox equivalents (TE) per 100 g of pasta.

All samples were analyzed in triplicate and blanks of reagents were used for each type of sample.

2.6. Polyphenols profile by HPLC-MS

Polyphenols were identified by HPLC-DAD-ESI-qTOF (MS/MS) using an Agilent Series 1200 LC System (Agilent, Santa Clara, CA, USA), coupled to a DAD detector (Agilent Series 1200) in tandem with an ESI source, connected to a mass spectrometer (Micro-QTOF II; Bruker Daltonics, Billerica, MA, USA). The analyses were performed on a LUNA (Phenomenex, Torrance, CA) C18 column with the same conditions described by Podio et al. (2015). Briefly, a mobile phase consisting on 0.5% formic acid (v/v, solvent A) and 0.5% formic acid in methanol (v/v, solvent B)

was used. The gradient started at 20% B and changed to 50% B along 3 min (held for 5 min), followed by a second ramp to 70% B along 7 min, maintained 5 min, and a third ramp to 80% B along 1 min, held for 9 min. Flow rate was 0.4 mL min^{-1} and injection volume was 40 µL. Polyphenols were identified according to their retention times, exact mass, MS and MS/MS spectra, comparing with compounds reported in the literature or with authentic standards when available. Quantification was based on external calibration curves from available phenolic standards, using the mass peak areas from the base peak chromatograms, at concentrations between 0.1 and 100 mg L^{-1}. When corresponding standards were not available, an external standard with a similar structure to the tentative compound was used. All samples were filtered (0.45 µm) before injection, and analyzed in triplicate together with blanks of reagents. Results were expressed in µg of standard equivalent per gram of dry pasta. Limits of detection (LoD) and quantification (LoQ) of the method were calculated from the calibration curves. Depending on the specific standard, LoQ ranged from 0.31 to 0.95 mg L^{-1}, and LoD from 0.09 to 0.28 mg L^{-1}.

2.7. Statistical analysis

Results were analyzed using the Infostat software package (Di Rienzo et al., 2008). Differences between samples were evaluated with ANOVA; in the case of significance ($p < 0.05$), a DGC (Di Rienzo, Guzmán, & Casanoves, 2002) comparison test was performed to reveal paired differences among means.

3. Results and Discussion

3.1. Polyphenols profile of PDCF

The HPLC-MS/MS analysis of the acetone/water (4:1) extract from PDCF allowed us to determine the presence of 14 phenolic compounds (**Table 1**). Twelve of them were hydroxycinnamic acid derivatives, one was quinic acid, and one corresponded to the flavonoid methylquercetin. Additionally, the amino acid tryptophan has been detected. Among the hidroxycinnamic acid

derivatives, ten compounds are structurally related to caffeic acid, while the other two are ferulic acid and fertaric acid. The extract was characterized by the prominent abundance of two main compounds identified as rosmarinic acid and its glycosylated derivative (salviaflaside). Both components are approximately ten times more abundant than the rest of the detected compounds.

Our results are in agreement with those reported by Oliveira-Alves et al. (2017), who identified 29 phenolic acids considering also the hydrolyzed extract of chia seeds. Rosmarinic acid was reported as the major compound from chia seeds according to Martínez-Cruz & Paredes-López (2014). On the other hand, despite the reported presence of flavonoids such as quercetin, myricetin, daidzein or kaempferol (Ayerza, 2013; Marineli et al., 2014; Martínez-Cruz & Paredes-López, 2014; Rahman, de Camargo, & Shahidi, 2017), none of these structures were detected, except by a very low quantity of methylquercetin (< 1 μg/g).

Variations in the reported profiles can be attributed to: the solvent mixture used to obtain the extracts (Alcântara et al., 2019; Scapin, Schmidt, Prestes, & Rosa, 2016); the differential origin of seeds (de Falco, Fiore, Rossi, Amato, & Lanzotti, 2018); and the methodology used in the identification, exact masses and MS patterns are more reliable than retention times and UV spectra alone.

3.2. Polyphenols profile of pasta supplemented with PDCF

In order to assess the effects of manufacture and cooking on the phenolic compounds, we performed HPLC-MS quantitative analysis of the pasta extracts. **Table 2** shows the phenolic compounds quantified in raw and boiled pasta (μg/g of dry pasta). Two of the 14 polyphenols of PDCF were not detected in supplemented pasta: methylquercetin, probably due to its extremely low concentration; and caffeic acid hexoside, which could be degraded by the manufacturing process.

Almost all the polyphenols quantified in pasta extracts, as well as the sum of them (**Figure 1**), showed a significant rise correlated with the increasing content of PDCF (**Table 2**). The only exception was ferulic acid which did not show significant differences between the control and

supplemented pasta, neither in raw nor cooked samples. Interestingly, ferulic acid and its derivatives are the most characteristic components of whole-wheat pasta (Podio et al., 2019). Thus, despite its presence in PDCF (17.39 μg/g), it is possible that the ferulic acid quantified in the pasta comes mainly from the wheat flour and the addition of PDCF does not affect its levels.

In the following analysis, we only consider the values of 10% supplemented pasta to illustrate the comparison more clearly. The sum of individual polyphenols showed an increase of around 3 times the theoretical value expected for a 10% PDCF contribution in raw pasta, indicating that the manufacture of pasta causes a release of polyphenols. The analysis of individual components reveals that the most important contribution is due to the increase of rosmarinic acid and salviaflaside, which are around 3 times higher than the expected value (**Figure 2**). Also, the amounts of caffeic acid, salvianolic acids I/H and E/B/L, and caftaric acid are increased. On the other hand, the levels of some compounds are slightly lower than the expected value (quinic acid, danshensu, ferulic acid and methylrosmarinate), while the quantities of fertaric acid and salvianolic acid C remain unaffected. It has been demonstrated that pasta manufacture process can affect the levels of polyphenols from the original ingredients (Fares et al., 2010). In many cases, some compounds are degraded due to oxidation caused by water, oxygen, and heat, as in whole-wheat pasta where polyphenols showed a marked decrease (Podio et al., 2019). While sometimes, such as in berry-enriched pasta, TPC is increased after kneading and sheeting processes (Bustos, Vignola, Paesani, & León, 2019). Noteworthy, the main bioactive components of chia seeds seem to be released from the matrix along the elaboration process.

Regarding the general effects of boiling, the sum of individual polyphenols of the supplemented pasta did not show significant differences between raw and cooked. However, a significant increase is observed in the total of phenolic compounds of the non-supplemented pasta (0% PDCF) (**Table 2, Figure 1**). These results are in accordance with the analyses of the antioxidant capacity, in which the boiling process affected mainly the TPC of control pasta, without significant effects on pasta with PDCF (Aranibar et al., 2018). The increase of polyphenols caused by the cooking of wheat

pasta have been attributed to the release of the polyphenols bound to macromolecules facilitated by the high temperatures of boiling water (Fares et al., 2010; Podio et al., 2019). These results indicate that the compounds released by boiling are mainly the components provided by wheat, while the bioactive compounds from PDCF are not significantly affected.

Analyzing the changes of individual polyphenols, three components showed a significant increase after cooking: caffeic acid, ferulic acid and danshensu (the hydrated form of caffeic acid). The increase of caffeic acid is the most remarkable, being 4 times higher than in raw pasta. The non-supplemented pasta is mainly characterized by the increase of ferulic acid, which is in agreement with the reported increase of ferulic acid derivatives in cooked whole-wheat pasta (Podio et al., 2019). Conversely, three components showed a significant decrease in the supplemented pasta: caftaric acid, fertaric acid and salvianolic acid E/B/L. Both caftaric and fertaric acid (esters of tartaric acid with caffeic and ferulic acid, respectively) were not detected in boiled pasta, indicating their decomposition. These compounds, including salvianolic acid E/B/L (a tetramer of caffeic acid), have ester bonds susceptible to be broken under high temperature conditions. The increase of caffeic and ferulic acids described above is probably related to the decomposition of these derivatives.

Finally, the remaining six polyphenols analyzed did not show significant changes in their abundances after cooking. Interestingly, the two main components of PDCF (rosmarinic acid and salviaflaside) are not significantly affected by the cooking process, indicating their availability at the moment of intake (**Figure 2**).

3.3. In vitro simulation of gastrointestinal digestion

To gain insight into the extent in which the proposed bioactive components enter to the organism to produce their effect, a simulation of human digestion stages of cooked pasta has been performed. For a clearer comparison, in this section we only report the results of the control (0%) and supplemented pasta with 10% PDCF.

The evaluation of the TPC (Folin-Ciocalteu) of samples from the different steps of gastrointestinal digestion showed the same tendency for control (0%) and supplemented pasta (**Figure 3**). The results are similar to those reported for whole-wheat pasta (Podio et al., 2019). Apparently, the OD step allows the release of 37% and 50% of the TPC found in cooked control and supplemented pasta, respectively. Moreover, GD and ID cause a much higher increase (300-500%) indicating that the action of enzymes (pepsin, pancreatin) and pH at these stages effectively release polyphenols from the food matrix, including the components of PDCF and wheat (Podio et al., 2019). Finally, samples representing the fraction absorbed in the intestine (DIA), showed an increase of around 50% compared with the values of boiled pasta. However, it should be pointed out that the TPC test is not specific for polyphenols, since other reducing agents can also react with Folin-Ciocalteu reagent (Amorati & Valgimigli, 2015).

The levels of antioxidant activity (TEAC) are not significantly modified by the two first stages of digestion (OD and GD) (**Figure 3**), but the conditions of the intestinal phase cause a marked increase in the antioxidant capacity (ID). Moreover, the absorbed fraction (DIA) shows even higher values than ID, suggesting that compounds with radical scavenging capacity are significantly released at this phase.

Intestinal absorption is one of the main entrance ways of food components. Thus, to assess if the components from our pasta are most likely to be absorbed in the small intestine (DIA), or to remain in the intestinal lumen (ID) to continue towards the colon, we performed the HPLC-MS analysis of these sub-samples.

From the 10 components quantified in boiled pasta, only 2 were above the LoD and LoQ in the intestinal sub-samples of pasta with 10% PDCF: rosmarinic acid and salviaflaside. Both of them were below the LoQ (< 0.31 mg L^{-1}) in the DIA sub-samples, while their presence in the ID sub-samples accounted for: 1.70 ± 0.16 μg/g of dry pasta for rosmarinic acid, and 3.31 ± 0.33 μg/g of dry pasta for salviaflaside. These values represent a relative percentage with respect to the cooked pasta extract of 1.6% and 4.7%, respectively. The detection of these components in the DIA sub-

samples, although they were below LoQ, indicates that at least a small fraction is being absorbed at this stage. In addition, a higher proportion of them is likely to remain in the intestine to pass towards the colon (ID). Given that rosmarinic acid and salviaflaside are the major components of PDCF, this is an interesting result highlighting their bioaccessibility. In agreement with our results, rosmarinic acid from chia seeds has been reported to be absorbed and bioaccessible through the intestinal phase of digestion (Pellegrini et al., 2018). Rosmarinic acid has been widely studied due to its remarkable bioactivity; besides its antioxidants properties, it has shown many other beneficial effects on human health, such as anti-inflamatory and anti-cancer activities (Nunes et al., 2017).

4. Conclusions

These results represent an important contribution to the knowledge of phenolic composition and bioactivity of wheat pasta supplemented with the by-product of chia seeds (PDCF), providing data to understand the effects of manufacture, cooking and digestion processes.

The HPLC-MS analysis of the free phenolic fraction of PDCF and the enriched pasta revealed the presence of 14 phenolic compounds, most of them hydroxycinnamic acids structurally related to caffeic and ferulic acids. Rosmarinic acid and its glycoside are the major components.

Regarding the effects of boiling process, the main antioxidant compounds of the supplemented pasta are not strongly affected by cooking, suggesting that these bioactive components provided by PDCF remain available at the moment of intake.

Moreover, the results of the simulated gastrointestinal digestion model showed that only a small proportion of the free polyphenols studied are likely to be absorbed in the small intestine, while a higher proportion of the same compounds continues towards the colon. Interestingly, despite the low percentage of supplementation with PDCF (10%), rosmarinic acid and its glycoside are detected at the intestinal level.

Altogether, these results reveal promising nutritional properties of the by-product from chia seeds oil extraction, allowing us to promote the use of this ancient grain as a functional ingredient to enrich one of the most popular sources of carbohydrates around the world, wheat pasta.

Conflicts of Interest

The authors declare no conflict of interest.

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Table 1. HPLC-MS/MS analysis of the free phenolic fraction of PDCF. Solvent mixture: acetone/water (4:1).

	RT (min)	Tentative ID	Abrev.	Molecular formula	$[M-H]^-$ (m/z) experimental	$[M-H]^-$ (m/z) calculated	Error ppm	MS/MS	[mg/100g PDCF]*
1	7.8	Quinic acid	QA	$C_7H_{11}O_6$	191.0572	191.0561	-5.5	-	2.19 ± 0.49
2	13.4	Danshensu	D	$C_9H_{10}O_5$	197.0441	197.0455	-7.3	179	1.76 ± 0.10
3	13.8	Caftaric acid	CTA	$C_{13}H_{12}O_9$	311.0385	311.0409	-7.5	179	0.95 ± 0.32
4	14.8	Tryptophan	Try	$C_{11}H_{12}N_2O_2$	203.0831	203.0826	2.6	-	NQ[a]
5	15.0	Caffeic acid hexoside	CAH	$C_{15}H_{18}O_9$	341.0911	341.0878	9.6	179	1.44 ± 0.32
6	15.3	Salvianolic acid I/H	SA I/H	$C_{27}H_{22}O_{12}$	537.1016	537.1038	-4.3	339; 295; 197	0.73 ± 0.01
7	15.4	Fertaric acid	FTA	$C_{14}H_{14}O_9$	325.0547	325.0565	5.6	193	3.90 ± 1.41
8	17.4	Caffeic acid	CA	$C_9H_8O_4$	179.0339	179.0350	-5.9	-	0.69 ± 0.13
9	17.8	Salvianolic acid E/B/L	SA E/B/L	$C_{36}H_{30}O_{16}$	717.1467	717.1461	0.9	519; 475; 339	2.83 ± 0.53
10	20.4	Ferulic acid	FA	$C_{10}H_{10}O_4$	193.0487	193.0506	-10.3	-	1.74 ± 0.32
11	20.6	Salviaflaside	SF	$C_{24}H_{26}O_{13}$	521.1354	521.1301	10.3	359; 323; 197	30.69 ± 3.15
12	22.8	Rosmarinic acid	RA	$C_{18}H_{16}O_8$	359.0732	359.0772	-11.2	197; 179; 161	33.97 ± 3.19
13	24.1	Salvianolic acid C	SA C	$C_{26}H_{20}O_{10}$	491.1011	491.0984	5.5	311	2.30 ± 0.07
14	24.5	Methylrosmarinate	MeRA	$C_{19}H_{18}O_8$	373.0961	373.0929	8.5	281; 197; 179	2.39 ± 0.39
15	25.2	Methylquercetin	MeQ	$C_{16}H_{12}O_7$	315.0464	315.0510	-14.8	300	< LoQ[b]

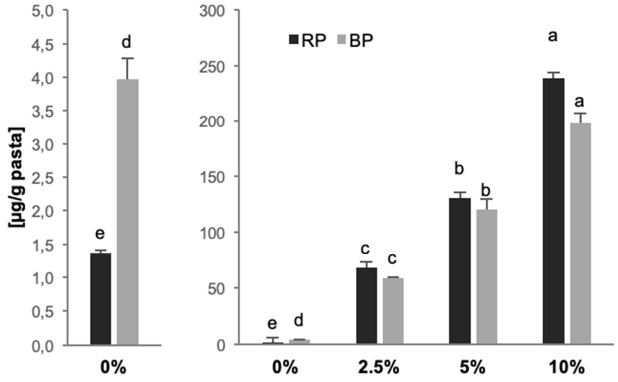
RT, retention time; $[M-H]^-$ (*m/z*), negatively charged molecular ion; MS/MS, main peaks of the fragmentation pattern; *Quantitative results are expressed as a mean ± SD of 4 measurements; [a]Not quantified; [b]Below the limit of quantification.

Table 2. Polyphenol profile in raw and cooked pasta.

		Raw Pasta				Cooked Pasta			
Comp.	**PDCF**	**0%**	**2.5%**	**5%**	**10%**	**0%**	**2.5%**	**5%**	**10%**
QA	21.90 ± 4.91	< LoQ	0.66 ± 0.09 **a,B**	0.81 ± 0.10 **a,B**	1.24 ± 0.05 **a,C**	0.55 ± 0.05 **A**	0.79 ± 0.07 **a,B**	0.93 ± 0.07 **a,B**	1.32 ± 0.08 **a,C**
D	17.58 ± 1.03		0.55 ± 0.00 **a,B**	0.80 ± 0.14 **a,B**	1.32 ± 0.05 **a,C**		1.64 ± 0.00 **b,B**	2.89 ± 0.28 **b,C**	3.87 ± 0.54 **b,C**
CTA	9.80 ± 3.23	< LoQ	< LoQ	0.58 **C**	1.38 ± 0.02 **D**				
SA I/H	7.34 ± 0.09		< LoQ	0.74 ± 0.08 **a,C**	1.54 ± 0.11 **a,D**		< LoQ	0.65 ± 0.08 **a,C**	1.16 ± 0.15 **a,C**
FTA	38.96 ± 14.13		1.12 ± 0.11**B**	1.89 ± 0.59 **B**	3.98 ± 0.47 **C**				
CA	6.86 ± 1.25		0.78 ± 0.10**a,B**	1.03 ± 0.13 **a,B**	1.52 ± 0.10 **a,C**	< LoQ	3.12 ± 0.14 **b,B**	4.50 ± 0.44 **b,C**	5.77 ± 0.35 **b,D**
SA E/B/L	28.34 ± 5.33		1.15 ± 0.15 **B**	2.30 ± 0.54 **a,B**	4.44 ± 0.33 **a,C**		< LoQ	0.99 ± 0.02 **a,C**	1.59 ± 0.15 **b,D**
FA	17.39 ± 3.19	1.37 ± 0.04 **a,A**	1.51 ± 0.16 **a,A**	1.24 ± 0.16 **a,A**	1.35 ± 0.09 **a,A**	3.43 ± 0.25 **b,A**	3.19 ± 0.20 **b,A**	3.35 ± 0.46 **b,A**	2.51 ± 0.04 **b,A**
SF	306.93 ± 31.47		20.91 ± 2.62 **a,B**	46.53 ± 5.62 **a,C**	89.96 ± 10.29 **a,D**		14.63 ± 0.61 **a,B**	34.81 ± 1.76 **a,C**	70.41 ± 5.31 **a,D**
RA	339.68 ± 31.94		40.84 ± 4.71 **a,B**	73.36 ± 9.92 **a,B**	128.63 ± 16.08 **a,C**		34.52 ± 1.88 **a,B**	70.01 ± 6.14 **a,C**	106.94 ± 2.76 **a,D**
SA C	23.05 ± 0.69		0.76 ± 0.02 **a,B**	1.16 ± 0.25 **a,B**	2.28 ± 0.30 **a,C**		0.85 ± 0.01 **b,B**	1.68 ± 0.05 **a,C**	2.70 ± 0.13 **a,D**
MeRA	23.93 ± 3.91		< LoQ	0.59 ± 0.11 **a,C**	1.18 ± 0.16 **a,C**		< LoQ	0.62 ± 0.03 **a,C**	1.23 ± 0.01 **a,D**
TOTAL	841.43 ± 74.37	1.37 ± 0.04 **a,A**	68.28 ± 7.73 **a,B**	130.72 ± 17.84 **a,C**	238.81 ± 27,92 **a,D**	3.98 ± 0.31 **b,A**	58.75 ± 0.84 **a,B**	120.43 ± 9.29 **a,C**	197.50 ± 9.51 **a,D**

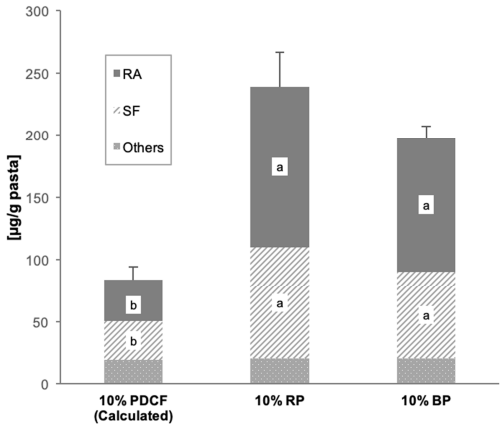
Results are expressed as the mean ± SD in μg/g of dry pasta. Different small letters (a,b) indicate significant differences between raw and cooked pasta of the same percentage (DGC, $p < 0.05$). Different capital letters (A-D) indicate significant differences between raw or cooked pasta with different content of PDCF (DGC, $p < 0.05$).

Figure 1. Total of quantified polyphenols in raw and boiled pasta.



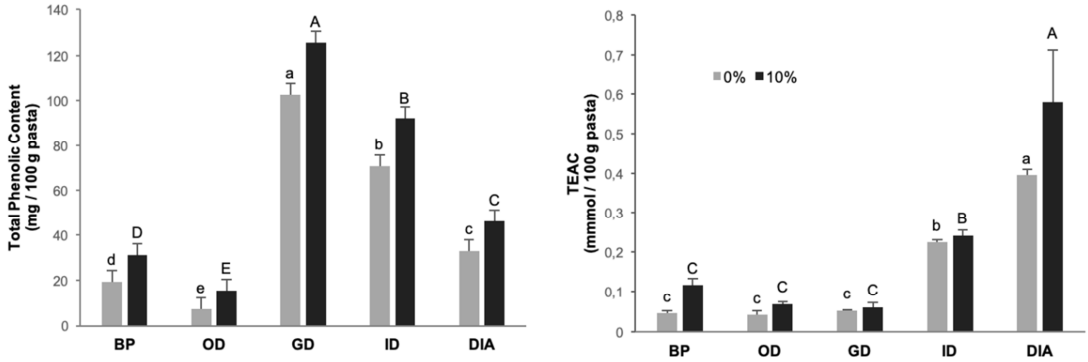
Different letters indicate significant differences ($p < 0.05$). On the left, amplified data of control pasta.

Figure 2. Variation of total quantified polyphenols after elaboration and cooking processes, highlighting the contribution of rosmarinic acid (RA) and salviaflaside (SF).



Different letters indicate significant differences among the samples ($p < 0.05$). The calculated value represents the theoretical contribution of 10% PDCF, according to PDCF quantification.

Figure 3. Total phenolic content and antioxidant activity of pasta along the stages of gastrointestinal digestion.



Different letters indicate significant differences ($p < 0.05$) between bars of the same color. The differences among control pasta (0%) and 10% pasta in each stage are significant, except for TEAC values of GD.

HIGHLIGHTS:

- Wheat pasta enriched with chia flour has shown improved antioxidant properties
- The polyphenols profile of supplemented pasta has been analyzed through HPLC-MS/MS
- Effects of manufacture, cooking and digestion on polyphenols has been assessed
- The main bioactive compounds of chia remain stable until the moment of intake
- Rosmarinic acid and salviaflaside are detected at the intestinal stage of digestion

Conflict of Interest Declaration

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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