In vitro starch digestibility and fate of crocins in pasta enriched with saffron extract

Armellini, R.¹,², Peinado, I.*³, Asensio-Grau, A.³, Pittia, P.², Scampicchio, M. ¹, Heredia, A. ³ & Andres, A.³

Rita Armellini, ¹Free University of Bolzano, Faculty of Science and Technology, 39100, Bolzano, IT & ²University of Teramo, Faculty of Bioscience and Technology for Food Agriculture and Environment, 64100 Teramo, IT. rita.armellini@gmail.com

*Irene Peinado, ³Research Institute for Food Research and Development, Universitat Politècnica de València, P.O. Box 46022, Valencia, Spain. Corresponding author: irpeipar@gmail.com; tel: +34 963877365.

Andrea Asensio Grau, ³Research Institute for Food Research and Development, Universitat Politècnica de València, P.O. Box 46022, Valencia, Spain. anasgr@upv.es

Paola Pittia, ²University of Teramo, Faculty of Bioscience and Technology for Food Agriculture and Environment, 64100 Teramo, IT. ppittia@unite.it

Matteo Scampicchio, ¹Free University of Bolzano, Faculty of Science and Technology, 39100, Bolzano, IT. matteo.scampicchio@unibz.it

Ana Heredia, ³Research Institute for Food Research and Development, Universitat Politècnica de València, P.O. Box 46022, Valencia, Spain. anhegu@tal.upv.es

Ana Andrés, ³Research Institute for Food Research and Development, Universitat Politècnica de València, P.O. Box 46022, Valencia, Spain. aandres@tal.upv.es
Abstract
This work aims to study the effect of the addition of saffron extract on fresh pasta in-vitro digestibility. Fresh pasta was formulated with different concentrations of saffron extracts (0.2 and 0.4 %w/w), cooked at two different times (1.5 and 3 min), and in vitro digested (oral, gastric and intestinal stages). Oil was added to pasta before digestion to evaluate the presence of lipids on starch and crocin bioaccessibility. Saffron enrichment and oil addition slowed down the digestion of starch, thus, decreasing the glycemic index of pasta. Concentration of saffron and oil addition contributed to crocin release in the digestion fluids, with the opposite effect of cooking time. Isomerization from trans to cis was enhanced by both, cooking and oil addition. Bioaccessibility of total crocins varied from 2.9±1.1, to 97±3 %. Finally, the trans:cis isomers distribution was only close to 50:50 in enriched-pasta cooked during 3 min or with oil addition.

Chemical Compounds
Crocin I (Pubchem CID: 5281233); trans-crocetin (b-D-glucosyl)-(b-D-gentiobiosyl) ester (Pubchem CID: 9940690); Crocetin Digentiobiosyl Ester (Pubchem CID: 44630212); Safranal (Pubchem CID: 61041).

Key-words: saffron, pasta, oil, in vitro digestion, starch, crocin bioaccessibility
1. Introduction

Pasta is a worldwide known food product. It is an important carbohydrate source, composed mainly of starch and low content of fats, proteins, dietary fibre, minerals, vitamins and phenolic compounds. Starch, the most important digestible polysaccharide in human nutrition usually accounts for 20-50% of the total energy intake. It is made up of two different glucose polymers, amylose (15-20%) and amylopectin (80-85%), and it is mainly derived from plant seeds such as wheat, maize, rice, oats and rye. Starch is a major source of glucose that appears at relatively high concentrations in blood circulation during digestion, playing a predominant role in the human diet as it contributes to the glycaemic index (Bohn et al., 2017). The glycaemic index (GI) is associated with the presence and concentration of carbohydrates in a particular food, and it indicates their effect on a person's blood glucose level. Acute increments in postprandial plasma glucose and insulin levels after eating foods rich in carbohydrate (high glycaemic response), may increase the risk of metabolic diseases such as type 2 diabetes, cardiovascular disease, and obesity (Kim et al., 2008). Therefore, low glycemic responses are considered favorable to health, and ways are being sought to reduce the glycemic impact of food carbohydrates (Kim et al., 2008). Moreover, it is known that a series of factors including the type of carbohydrate, physical entrapment of the carbohydrate within the food, as well as fat and protein content of the food and organic acids or salt content of the meal may affect glycemic index (Bohn et al., 2017). Pasta is a popular and simple carbohydrate-based food with a relative low glycemic response (Singh, Dartois, & Kaur, 2010) that derives from its composition and structural properties determined by the wheat flour and processing.

In order to enhance nutritional value of pasta, several studies have focused on the possibility of adding functional ingredients into it (Bustos, Perez, & León, 2013; Fiorda, Soares, da Silva, Grosmann, & Souto, 2013). Design and development of fortified formulated bakery products,
is one of the latest trends in the specific industry sector with a growing interest due to their improved nutritional properties.

Among the wide variety of functional ingredients with potential health benefits, saffron, a spice derived from the dried stigmas of *Crocus sativus* L. flowers, has received increased attention in the past two decades in both, cookery and food industry, due to its colouring properties, pleasant bitter taste and alluring aroma (Serrano-Díaz, Sánchez, Maggi, Carmona, & Alonso, 2011). It owes its sensory properties mostly to the presence of three carotenoid derivates, mainly synthesized during flowering, but also during processing, which are crocins, picrocrocin and saffranal, responsible of the saffron colour, flavour and aroma, respectively. These secondary metabolites are produced by oxidative cleavage of zeaxanthin, followed by oxidative modifications and glycosylation (Namin et al., 2009). Crocetin digentiobiose ester (C₄₄H₆₄O₂₄), a natural and food-grade colouring agent, is considered one of the major bioactive constituents of saffron and has a wide spectrum of biological activities including antigenotoxic and cytotoxic effects, antioxidant, anti-inflammatory, anti-atherosclerosis, anti-diabetic, hypotensive, hypolipidaemic, hypoglycemic and antidepressant (Rahaiee, Moini, Hashemi, & Shojaosadati, 2015). However, in general, crocins have low stability and lose most of their functionality during processing and storage after exposure to heat, oxygen, light, acidic environment and/or due to presence of additives (Maggi et al., 2009).

In order to exert the target functionality in vivo, bioactive compounds need to be active after digestion. The fraction of a bioactive compound released from the food matrix following digestion, that is solubilised into the gut for intestinal uptake, is usually known as the bioaccessible fraction (Carbonell-Capella, Buniowska, Barba, Esteve, & Frigola, 2014; Rodriguez-Roque et al., 2015). The bioaccessibility of a particular compound depends, often to a significant extent, on interactions with other dietary constituents (O’Brien, 2013). Besides crocins and saffranal, saffron is also rich in many other bioactive compounds including...
phenols, that along with other phytochemicals, can be linked to macro-molecules via bonds of various nature depending on their molecular and chemical characteristics. In fact, phenols have been able to influence the starch digestibility both, in vitro and in vivo (Forester, Gu, & Lambert, 2012). Thus, food matrix interactions between bioactives and macronutrients in complex foods like pasta should be considered as potentially affecting the digestibility of the macronutrients (i.e. starch, gluten) and, in turn, the bioaccessibility and bioavailability of the micronutrients and bioactives.

The enrichment of pasta with other functional ingredients may have effects on its digestibility, and, in particular, on that related to the starch fraction. That is why, in this study, the main goal of this research was to study the effect of saffron extract addition (0.2 and 0.4 %w/w) on the digestibility of starch as well as the fate and release of bioactive compounds and in particular, crocins, of fresh pasta subjected to different cooking times (1.5 and 3 minutes). The impact of the addition of olive oil to cooked pasta, and prior to digestion, on crocins bioaccessibility was also evaluated.

2. Materials and methods

2.1. Chemicals

Crocin, saffranal, and methanol, were purchased from Sigma Sigma-Aldrich (Deisenhofen, Germany). Ammonium bicarbonate, potassium dihydrogen phosphate, porcine pepsin (3200-4500 U / mg), pancreatin (8 × USP) from porcine pancreas and bovine bile extract, were from Sigma-Aldrich (Deisenhofen, Germany). Sodium carbonate hydrogen was purchased from Scharlau (Barcelona, Spain). DNS (3-5’ dinitrosalicylic acid) reagent, glucose, Na-K tartrate/NaOH, ethanol, invertase and acetonitrile were purchased from Sigma-Aldrich (Deisenhofen, Germany). Amyloglucosidase was from Megazyme (E- AMGDF, Megazyme, Ireland). All solvents used for the determination of crocins were HPLC grade and the others of...
analytical grade. Bidistilled water was used for chromatographic analysis (Milli-Q, Millipore Corp., Bedford, MA). Standard solutions were stored at -20 °C.

Saffron powders (Hacendado, Valencia) and wheat flour (Hacendado, Valencia) were purchased in a local market. For pasta preparation and cooking, tap water of Valencia’s municipality (Valencia, Spain) was used.

2.2 Sample preparation

2.2.1. Pasta samples preparation

All samples (with and without saffron) were prepared by mixing wheat flour and water (70:30 (w/w)). Different batches of pasta were made and each of them was made of ca. 250 g. The following pasta formulations were prepared based on a previous study (Armellini et al., 2018) as follows: control dough (C) made of wheat flour (165 g) and tap water (70 ml), (no saffron); “saffron enriched pasta” was made of wheat flour (165 g) + 70 ml of a dispersion of saffron powder in tap water (either 0.2 or 0.4 %w/w saffron in dough). All the ingredients were mixed using a home professional equipment (Thermomix TM-31, Vorwerk, Wuppertal, Germany) for 2 min under ‘dough mode’. The mix was left to settle for 5 minutes at room temperature before to extrude it to a “pappardelle” shape (large and flat, width: 4 cm length: 24 cm) using a home scale pasta-shaper (Marcato SPA, Padova, Italy).

2.2.2. Cooking of the pasta

Aliquots of of pasta (60 g each) were cut into smaller pieces (8 cm x 4 cm) and cooked in boiling tap water (1.5 L) (Armellini et al., 2018). Samples were removed from the boiling water 1.5 minutes after boiling started, that corresponded, based on preliminary tests, to the optimum cooking time from the organoleptic point of view, as well as crocins preservation (Armellini et al., 2018). Additionally, pasta enriched with 0.4% of saffron was also cooked for 3.0 min in order to evaluate the effect of overcooking.
Samples were coded based on their saffron enrichment (0 (control), 0.2 or 0.4 % (w/w) and cooking time (1.5 or 3.0 min) as follows: 0%-1.5’ (control), 0.2%-1.5’,0.4%-1.5’, 0.4%-3’).

After cooking, samples were drained and cooled by soaking them in cold water for 10 s; excess water was removed by lightly patting the samples between paper towels. An aliquot of both raw and cooked pasta corresponding to half of the sample was immediately used for moisture measurements and in vitro digestion. The remaining part was freeze-dried (-45 °C, 0.8 Pa 48 h, Telstar, Spain) and used for the remaining chemical analyses.

Data reported are referred to at least three different batches of pasta samples prepared in different days. All measurements were performed in triplicate for each batch (n=9).

2.3 In vitro digestion process

The digestive process (oral, gastric and intestinal stages) and the simulated fluids (salival, gastric and intestinal) were prepared according to the protocol proposed by Minekus et al., (2014), with slight modifications. The simulated salival fluid (SSF), the simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared daily from stock solutions. The electrolyte composition of the different solutions is shown in Table 1. The enzymatic activity was tested before each experiment following the protocol proposed by Carrière et al., (2000). Each experiment was performed in triplicate. The in vitro digestion process was performed as follows:

For the oral stage, pasta samples were mixed with SSF (1:1 w/v). Firstly, three healthy volunteers, providing a signed consent form, chewed the tested pasta samples (5 g) 15 times for approximately 15 seconds and then expectorated it into a 50 mL Falcon tube. After that, the bolus was weighted again and the amount of SSF (pre-warmed at 37 °C; pH 8) required to complete the ratio (1:1 w/v) was added. The gastric stage, continued with the immediate addition to the oral bolus of 10 mL of SGF, 5 µL of CaCl₂ (0.3 M) and a sufficient volume of
HCl (1M) to adjust the pH to 2.8 ± 0.1. Porcine pepsin had been added into the SGF to a concentration of 25000 U pepsin / mL of SGF. Subsequently, the mixture was placed in a thermostatically controlled chamber (Selecta, Spain) at 37 °C coupled to a shaker (Intelli-Mixer RM 2, Elmi Ldt., Baltics and Russia) at a speed of 55 rpm and head-over-heels rotatory movements. The total duration of this stage was 120 min. Every 30 min, the pH was measured and re-adjusted (if necessary). For the intestinal stage, 20 mL of SIF were added to the gastric chime (ratio 1: 1 (v/v)), pH was adjusted to 7.0 ± 0.1 with NaOH 1M, right after 120 min of gastric stage. The final bile salts and pancreatin concentrations were 10 mM and 1260 LU (Lipase Units or FCC), respectively, in each tube.

Furthermore, in order to evaluate the effect of the addition of a lipid component as excipient on starch digestibility and bioaccessibility of bioactive compounds, two samples (4 g) namely the pasta formulated with 0.2 and 0.4 % w/w of saffron cooked for 1.5 min were individually mixed with 1 g of olive oil, prior to the oral stage. These sample were coded as ‘0.2%-1.5’+oil’ and ‘0.4%-1.5’+oil’.

To stop the enzymatic reactions after the different sampling times along the gastric and intestinal simulated digestion, the tubes were placed in ice for 10 min and afterwards centrifuged at 1200 x G for 15 min, at 10° C, to separate the solid pellet and the liquid supernatant, called ‘micellar’ phase. The pellet of each sample was used to determine the matrix degradation index (MDI) while the micellar phase was divided into aliquots (1 mL) and frozen at -40°C until analysis including starch determination and carotenoids. For the latter analysis, samples were stored at -40 °C for 24 h before freeze-drying (-45 ºC, 0.8 Pa 48 h, Telstar, Spain).
2.4. Analytical determinations

2.4.1 Matrix Degradation Index

Matrix Degradation Index of each sample was determined from the proportion of solids that were finely dispersed in the digestion juices. After centrifugation, the pellet collected from the digestion tube was filtered on a metallic sieve (1.6 mm x 1.6 mm mesh) to separate out large food particles. The drained liquid or micellar phase was collected and used for starch determination as well as crocins analysis. The solid pasta particles were rinsed twice with 2 mL of digestion juice to remove any digested material and transferred to an aluminium dish previously exactly weighted, placed in a forced air oven at 60 °C for 48 h, and weighed again to determine the mass of large pasta solids. The matrix degradation index (MDI) corresponds to the proportion of pasta solids passing the metallic sieve (Asensio-Grau, Peinado, Heredia, & Andrés, 2018; Lamothe, Azimy, Bazinet, Couillard, & Britten, 2014; Lamothe, Rémillard, Tremblay, & Britten, 2017).

2.4.2. Digestible Starch

Sugars released during digestion were determined as monosaccharides by the dinitrosalicylic acid (DNS) colorimetric method after an invertase + amyloglucosidase secondary digestion (Mishra, Monro, & Hedderley, 2008) with a small-scale modification. An aliquot of the micellar phase (1 mL) was taken after 20 and 120 min of intestinal stage, mixed with absolute ethanol (4 mL), and then centrifuged at 1000 x G during 10 min, at 20 °C. Afterwards, 0.05 mL of the supernatant, or glucose standard (1 mg/mL glucose) was mixed with 0.25 mL of an enzymatic solution made of 1 % amyloglucosidase (E-AMGDF, Megazyme), 1 % invertase (Sigma Aldrich) in acetate buffer pH 5.2 and 0.75 mL of the DNS solution (1 % glucose, 1 % NaOH 1 M, 5 % DNS), and heated at 100 °C for 15 min. After heating, samples were cooled and diluted with 4 mL of distilled water.
Starch measurement was recorded at 530 nm using a spectrophotometer (UV/vis, Beckman Coulter). Rapid digested starch (RDS) was determined as reducing sugars measured after 20 min of intestinal stage while slowly digested starch (SDS) corresponded to the reducing sugars measured after 120 min (RDS + SDS). Resistant starch (RS) was estimated by the difference between RDS + SDS and total starch. Total starch was measured as glucose x 0.9 in a sample of freeze-dried pasta that had been milled to a powder. A sub-sample (100 mg) was gelatinized in dimethyl sulphoxide (2.0 mL, 100 ºC, 10 min) and digested for 30 min at 37 ºC after adding 8 mL acetate buffer pH 5.2 containing 0.1 mL of amyloglucosidase (Mishra et al., 2008).

2.4.3. Total crocin and crocin isomers

The content of crocin isomers was determined in agreement with the method reported in Armellini et al. (2018). Crocins from freeze-dried pasta samples, micellar phases and saffron powder (0.1 g) were extracted with methanol (5 mL, 80 % in water) using an Intell-Mixer RM-2 (Elmi Ltd, Riga, Latvia). Samples were rotated head-over-heels at 55 rpm for 2 h at room temperature and then centrifuged at 1200 x G for 10 minutes. The solid pellet was extracted again following the same procedure. The supernatants of the two extractions were collected and mixed (10 mL), and then they were used for HPLC analysis.

Crocetin esters were determined by HPLC and carried out by using a Waters instrument comprising a pump and DAD detector (Waters, USA) equipped with a Kromasil C18 (100 A, 4.60 x 250 mm, 5 µm) column. The eluents were water (A) and acetonitrile (B) with the following gradient: 95% to 5% A, 0-40 min; 5% A, 40-50 min; 5% to 95% A, 50-55 min at a flow rate of 1 mL/min. 20 µL sample injections were made for all samples and standards. The identification of crocin was carried out by comparing its retention time with that of the standard at 440 nm; quantification was performed by external calibration curves (10, 20, 50, 100 and 150 mg/L), with six replicates for each level (n=6), being 0.002 mmol/L of crocin (Trans-4-
the limit of quantification. Quantification of crocetin isomers was carried out referring to the most abundant isomer, expressed as mg of crocin/g dry base.

The crocetin isomers were determined in cooked pasta (before digestion) and different digestion times (120 min of gastric stage and 10, 20, 30, 45, 60, 90 and 120 min of intestinal stage). One tube was used for each different sampling time.

In addition, bioaccessibility at the end of the intestinal stage (%) was calculated according to Eq. (1) (Asensio-Grau et al., 2018):

\[
\text{Bioaccessibility (\%)} = \frac{A}{B} \times 100
\]

where A is total crocin content (µg/g dry basis of pasta) determined in the supernatant at the end of gastrointestinal digestion and B is total crocin in pasta before digestion and expressed in the same units.

2.5. Statistical analysis

Results are reported as average ± standard deviation of nine measurements. Analysis of Variance (Multivariate ANOVA) followed by Fisher LSD post-hoc tests was performed using IBM SPSS Statistics 23 (IBM, USA), differences were considered statistically significant when p-value < 0.05.

3. Results and Discussion

3.1. Effect of saffron enrichment, oil addition and cooking time on Matrix Degradation Index (MDI (%)) of digested fresh pasta

During digestion, the absorption of a significant amount of water by the matrix, combined with the action of digestive enzymes, promotes the softening of the food matrix and the reduction
of cohesive forces that hold the matrix together, resulting in the different degradation profiles
(Kong & Singh, 2009).

The matrix degradation index (MDI), corresponding to the amount (as %) of finely dispersed
pasta solids, was used as an estimation of mechanical disruption during the gastro-intestinal
digestion. **Figure 1** reports the values of the MDI after gastric and intestinal stages of control
and saffron-enriched pasta, with or without addition of oil. Results show that digestion time as
well as stage (gastric vs. intestinal) affected significantly the MDI.

Gastric stage (120 min) induced a MDI of ca. 55 % in the control sample after 1.5 minutes of
cooking (0%-1.5’). A significant increase of MDI, up to ca. 60 % (p-value < 0.05), was
observed in saffron enriched samples (0.2 or 0.4 %w/w), being more noticeable in the 0.4%-enriched pasta cooked for 3 min (0.4%-3’). Furthermore, the addition of oil prior to digestion
(0.2+1.5’+oil 0.4+1.5’+oil), led to an increase of the MDI up to 65 %, compared to the control
(0%-1.5’).

This degradation is the result of the activity of α-amylase present in the saliva that starts
breaking the starch branches into maltose and dextrin, followed by that of the gastric pepsin
which, in combination with the mechanical forces acting during the gastric stage, contributes
to break the food matrix. The degradation degree of pasta will depend on the characteristics
and composition of the food matrix. Saffron addition into the pasta did not have a significant
effect on the textural parameters of the raw pasta while a main effect was observed as a
consequence of cooking time. Food matrix became softer and less cohesive after 3 min of
cooking compared to that boiled for just 1.5 min (Armellini et al., 2018). Food matrix
characteristics such as hardness, cohesiveness, and elasticity have been previously associated
with resistance of other foods, such cheese, to the gastric environment (Lamothe et al., 2017).
Other factors, such as the composition of the matrix, the nature of bonds, and the permeability
of the matrix to small molecules could also influence matrix degradation (McClements,
Decker, Park, & Weiss, 2008). In addition, this resistance of the food matrix to degradation might have an effect on the antioxidant release from the matrix into the digestion juices (Lamothe et al., 2017).

During the intestinal stage, the entity of digestion significantly increased, reaching values above 80% after 120 min in all samples regardless saffron addition or cooking time. The increase of MDI after the intestinal stage is the result of an enhanced enzymatic activity. The action of the amylases, as well as the proteolytic enzymes (trypsin and chymotrypsin), present in duodenal juices, contributed to the degradation of the gluten matrix (Lamothe et al., 2014).

### 3.2. Effect of saffron enrichment, oil addition and cooking time on Starch digestibility of fresh pasta

Pasta samples’ starch digestibility was evaluated by determining the amount of monosaccharides released after the in vitro gastrointestinal digestion. An increase of glucose concentration occurs along the digestion process, indicating that a progressive enzymatic activity proceeds during the whole digestion process being this the result of the α-amylase in saliva (oral stage), as well as pancreatic amylase activity (intestinal stage).

Table 2 reports the values of the different fractions of starch (Rapidly Digested Starch (RDS), Slowly Digested Starch (SDS), and Resistant Starch (RS)) for each pasta sample. The addition of saffron to pasta (0.2%-1.5’ and 0.4%-1.5’) led to a slightly increase of SDS compared to the control (0%-1.5’) corresponding to an increase of 3.8% of the total starch (TS). This increase was statistically significant for the sample with the highest saffron concentration (0.4%-1.5’) in respect to the lower ones (i.e.3.8% and 6.5% of the TS respectively, for 0.2 and 0.4% of added saffron).

A slow digestion of starch contributes to limit the increase of the glycaemic index, which is related to the blood glucose concentration after food consumption (Bohn et al., 2017). Based
on these results, it can be supposed that saffron enrichment might have a hindering effect on starch degradation and glucose release, as it has been previously reported for other ingredients added in pasta formulation (Heo et al., 2013).

By comparing the results obtained for pasta enriched with 0.4 % of saffron extract after 1.5 and 3 min of cooking, longer cooking time led to a significant decrease of the RDS fraction (19 % vs 8 % for 1.5 and 3 min, respectively). A similar effect was noticed by Ye & Sui, (2016), who studied the effect of cooking time on starch digestibility of Chinese noodles. In their study, they concluded that cooking enlarged the voids around swelling starch granules and decreased the amount of continuity of intervening proteins, leading to the greater accessibility of α-amylase to the starch polymers (Fardet et al., 1998). The transformation of protein structure could increase the accessibility of α-amylase to the swollen starch granules, resulting in an increased starch digestion. In contrast, when noodles were conducted from an optimal cooked state to an overcooked state, proteins were likely to refold and aggregate with inter- and intra-molecular interactions, in turn containing strongly linked starch polymers leading to a lower digestion effect (Zhang & Hamaker, 2009).

However, the mechanisms by which the gluten network slows digestion rates of entrapped starch are not fully understood. The most common explanation is that the gluten network, entrapping starch granules, acts as a barrier to inhibit the accessibility of enzymes. It may also limit water absorption by starch granules, limiting the degree to which the starch is able to swell and hence gelatinise during pasta cooking in excess water; this might limit as well the ability of enzymes to access available starch and therefore, decrease the rate of starch digestion (Colonna et al., 1990). Some authors have suggested that the low starch digestion rates of pasta may be attributed to the tortuosity of the gluten network, which lengthens the pathway that α-amylase must take to reach its substrate (Fardet et al., 1998).
Moreover, our results highlight that the addition of oil prior digestion seems to modify starch digestibility with a decrease in RDS fraction. Concretely, an increase of SDS was noticed for 0.2%-1.5'+oil and a significant increase of RS for 0.4%-1.5'+oil compared to the same samples without oil addition. The addition of a lipid phase seems to have an important impact on the conformation of starch fractions. Rodríguez-Huezo et al., (2018), analysed the effect of the addition of different animal and vegetal fats on Tamales, Mexican traditional maize-based food, formulation. In this study, X-ray and DSC analyses revealed the formation of stable starch-lipid complexes, which are structures with the ability of opposing the attack of digestive enzymes. Apparently, the formation of additional SDS observed in 0.2%-1.5'+oil sample and RS in 0.4%-1.5'+ oil sample were obtained at the expenses of rapid digestible starch (RDS) fraction. Therefore, it may be concluded that the oil addition may exert a hypoglycaemic effect.

3.3. Crocin, crocin isomers and their bioaccessibility after digestion

The commercial saffron used for the present study to enrich pasta was characterized in a previous study (Armellini et al., 2018) and classified as II category (ISO 3632-1:2011), with the following quality parameters: colour (A 1% 1cm, 440 nm)=169; aroma (A 1% 1cm, 330 nm)=41; flavour (A 1% 1cm, 257 nm)=67 and a moisture content (%)=11.86.

The main cis- and trans-crocetin esters were identified at 440 nm: trans-crocetin di-(b-D-gentiobiosyl) ester (trans-4-GG), was identified as crocin by comparison with its standard, as well as with literature; the other isomers tentatively identified were: trans-crocetin (b-D-glucosyl)- (b-D-gentiobiosyl) ester (trans-3-Gg); trans-crocetin di-(b-D-glucosyl) ester (trans-2-gg); cis-crocetin di-(b-D-gentiobiosyl) ester (cis-4-GG); cis- crocetin (b-D-neapolitanosyl)- (b-D-glucosyl) ester (cis-3-Gg) according to the literature (Armellini et al., 2018; García-Rodríguez et al., 2017).

Table 3 shows the concentration of total crocin (µg/g pasta (d.b.)) and trans/cis-isomers
distribution (%) of saffron-enriched pasta before digestion, after gastric stage (GS) and at different times of the intestinal stage (IS).

Crocin content in samples (before digestion) was not only dependant on saffron addition (0.2 or 0.4%), but also on cooking time and oil presence during dish preparation. Of note, the higher whichever of three factors, the higher the total crocin content in pasta (0.4%-3’ ≈ 0.4%-1.5’+oil > 0.4%-1.5’+oil ≈ 0.4%-1.5’ > 0.2%-1.5’).

It could be observed that cooking and oil addition enhanced solubility of crocin from the food matrix during solvent extraction leading therefore, to a higher content quantified by chromatography. Isomerization trans-cis was also highly affected by cooking as it can be noticed from the results obtained for 0.4’-3% pasta, which presented the higher cis-isomers percentage (53 %) compared to the samples cooked for a shorter time, even when oil was added. It should be pointed out, that trans-4-GG and cis-3-Gg were, in general, the most abundant isomers before and after digestion regardless pasta formulation or trans-cis isomers distribution (%).

Although safranal was identified in the different samples, its concentration was below the LOQ, and therefore the amount in samples could not be quantified.

During gastrointestinal simulation, total crocin was clearly affected by the concentration of saffron extract added to the pasta dough (0.2 vs 0.4 %). The higher the saffron powder concentration, the higher the amount of crocin released into the digestion fluids at both, gastric and intestinal, stages. As regards the cooking time, the sample cooked for a longer time (0.4%-3’) showed a lower release of crocins into the digestion fluids despite of its highest crocin content before digestion. This is in agreement with the results of the starch digestibility already discussed, where inter- and intra-molecular interactions resulting from the overcooking of pasta, led to a lower digestion, and, thus, to a lower bioactives release from the food matrix (Lamothe et al., 2017; Ye & Sui, 2016). Finally, the addition of oil to pasta after cooking
seemed to significantly influence crocin release during digestion, and especially during the intestinal stage due to the liposoluble nature of this compound. To be absorbable in the human body, carotenoids need to be released from the food matrix and dispersed into the lipid phase in the stomach, where a fine emulsion structure is created. In a next step, the carotenoids need to be incorporated into mixed micelles in the intestine (Pasqualone et al., 2016). The presence of lipids in the food/dish preparation are essential for the emulsification and micellisation. The initiated trans to cis isomerization in samples progressed during digestion as well; and it was also enhanced by both cooking time (0.4%-3’) and oil addition (0.2%-1.5’+oil and 0.4%-1.5’+oil), as it can be deduced by comparing the cis-isomers (%) in these samples compared to that value in 0.2%-1.5’ and 0.4%-1.5’. Nevertheless, an increase of saffron powder in the dough pasta from 0.2 to 0.4% (w/w), without an increase of cooking time, implied an increase of total crocin content but not an activation of trans-cis isomerization during digestion. In fact, trans-isomers represented more than 80% of total crocin in 0.2%-1.5 and 0.4%-1.5’ at 120 min of the intestinal stage. Bioaccessibility (%) of each pasta estimated as total crocin content at the end of gastrointestinal digestion with respect of total crocin in pasta before digestion, was equal to 2.9 ±1.1, 73 ± 10, 25 ± 7, 62 ± 9 and 97 ± 3 % in 0.2%-1.5’, 0.4%-1.5’, 0.4%-3, 0.2%-1.5’+oil and 0.4%-1.5’+oil, respectively. Based on the results of the crocins content the enrichment of pasta with saffron extracts especially when the highest concentration of extract (0.4%) is used and pasta overcooked (3 min) may lead to obtain levels of crocins (ca. 1g/100 g pasta) that may have potential toxic effects or adverse effects on humans. Over the decades, several studies have focused on the toxic effects of saffron, which might include vomiting, uterine bleeding, bloody diarrhoea, haematuria, bleeding from the nose, lips and eyelids, vertigo, numbness and yellowing of the skin and mucous membranes. However, most of those reports are old and the respective...
amounts have been in times questioned (Lymperopoulou & Lamari, 2015; Smith & Zeeman, 2006). Some studies report that oral administration of doses up to 3 g crocin /kg of weight, within 2 days in mice did not cause mortality, and similar results were observed after intra peritoneal (IP) exposure at the same dose (Bostan, Mehri, & Hosseinzadeh, 2017; Hosseinzadeh, Sadeghi Shakib, Khadem Sameni, & Taghiabadi, 2011). Furthermore, different studies have proved crocin to be a practically low-toxic substance, which according to the toxicity classification, corresponds to a LD50 (lethal dose) value within the range of 1–5 g/kg (Hosseinzadeh, Sadeghi Shakib, Khadem Sameni, & Taghiabadi, 2011; Kennedy, Ferenz, & Burgess, 1986). The consumption of saffron enriched pasta, even in the case of the product with the highest content of crocins will, thus, not represent a risk.

**Figure 3** shows the evolution of crocetin isomers in the digestion fluids during the intestinal stage of the in vitro digestion process for the saffron-enriched pasta samples. Intestinal conditions led to an increase *trans-cis* isomerization, being the final concentration dependent on cooking time and added oil. Among the identified *trans* and *cis*-isomers, *trans*-4-GG and *cis*-3-Gg presented the highest concentrations, respectively.

Additionally, *trans*-crocin isomers concentration (*trans*-4-GG, *trans*-3-GG, *trans*-2-gg) reached a maximum concentration after 20-30 min of the intestinal stage, and it decreased from this time onwards. An exception of the trend was observed in overcooked pasta (0.4%-3’), for which *trans* isomers concentration continuously augmented with respect to intestinal time. The observed decrease of *trans* isomers concentration after 30 min of intestinal digestion might be probably due to a pH change in the environment. In fact, according to Pineda-Vadillo et al., (2017), the change from the acidic gastric media to the mild alkaline intestinal environment, could induce physico-chemical modifications of molecules that influence their transfer to digestion fluids (solubility), and motivate degradation reactions along with the isomerization mechanism *trans-cis*. The same phenomenon has been reported for tomato-lycopene. Garcia-
Hernández, et al., (2018) reported a trans-cis isomerization during frying and during the further gastrointestinal digestion, being the final distribution of lycopene in micelles close to 50%. Similarly, cis-isomers of lycopene have been detected in blood samples reaching 60-80% of total lycopene even though lycopene occurs in food mainly in the trans form (Wu et al., 2003). Degradation reactions during digestive process have been also reported to occur also for other antioxidant molecules, such as polyphenols. A decrease in the amount of total flavonoids and total phenols in the bioaccessible fraction, was found due to the conversion of flavanones into chalcones, which are less soluble than flavones, and therefore less available for absorption (Gil-Izquierdo, Gil, Ferreres, & Tomás-Barberán, 2001). In addition to pH, the interaction between polyphenols and other components of the in vitro gastro-intestinal digestion like enzymes or other dietary components released during digestion (e.g. iron, other minerals, dietary fiber, proteins) might affect polyphenols solubility and bioaccessibility (Rodríguez-Roque et al., 2015). The same phenomenon might, thus, occur to crocin, negatively affecting its bioaccessibility.

On the other hand, the higher the amount of added saffron, the higher content of crocetin isomers in the digestive juices was observed with a significant effect of oil addition on isomers release, and particularly that of the cis-3-Gg isomer. These results highlight the affinity of the different isomers for the lipidic phase depending on their molecular structure, since some of them were detected in higher amounts than others in the digested fluids when oil was added. A number of studies have been conducted on the effect of the presence of oil on carotenoids bioaccessibility. Most of them report the enhancement of carotenoids bioaccessibility in fruit- and vegetable-based food products, when olive oil, or to a minor extent sunflower oil and soybean oil, are added. Olive oil contains mainly oleic acid, a monounsaturated long chain fatty acid (C18:1). The fatty acyl chain length has been identified as an important lipid feature with consequence on carotenoid bioaccessibility.
Eventually, the molecular size and structure of carotenoids, which are related to isomeric configuration, also seem to influence bioaccessibility (Nagao, Kotake-Nara, & Hase, 2013).

4. Conclusions

The effect of saffron extract addition on fresh pasta in-vitro starch digestibility and the fate and release of bioactives and, in particular, crocins during digestion has been investigated. Results evidenced that saffron extract seems to effect starch digestibility, with a likely decrease of starch digestibility and corresponding glycaemic index of the saffron-enriched pasta.

Concentration of saffron extract as well as cooking time significantly influenced crocin release in the digested fluids. The higher the saffron powder concentration and the shorter the cooking time, the higher is the amount of crocins released into the digestion fluids. Moreover, the addition of oil to the pasta prior to digestion significantly enhanced crocins release into the micellar phase during intestinal stage. Additionally, the isomerization from trans to cis occurred mainly during digestion, in the 3 min-cooked enriched saffron pasta likely favoured again by the presence of a lipidic dispersed phase that lead to a higher concentration of the cis isomers in the bioaccessible fraction of this system.

This preliminary study on the use of saffron as ingredient in formulated pasta products and its results could be a reference to be exploited in further investigations aimed to deepen the knowledge and applications of this spice and related bioactive compounds, their technological functionality, and their impact on food quality as well as human health and wellbeing.

Conflicts of interest

There are no conflicts of interest to declare.
Acknowledgements

Authors are thankful the Province of Bolzano for financial support (Landesregierung mittels Beschluss Nr. 1472, 07.10.2013) and also the Research Institute for Food Research and Development at the Universitat Politècnica de València (UPV), for laboratory facilities.

References


Pasqualone, A., Gambacorta, G., Summo, C., Caponio, F., Di Miceli, G., Flagella, Z., …


Table 1. Composition of simulated digestion fluids

<table>
<thead>
<tr>
<th>Constituent</th>
<th>SSF</th>
<th>SGF</th>
<th>SIF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol · L⁻¹</td>
<td>mmol · L⁻¹</td>
<td>mmol · L⁻¹</td>
</tr>
<tr>
<td>KCl</td>
<td>15.1</td>
<td>6.9</td>
<td>6.8</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>3.7</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>13.6</td>
<td>25</td>
<td>85</td>
</tr>
<tr>
<td>NaCl</td>
<td>-</td>
<td>47.2</td>
<td>38.4</td>
</tr>
<tr>
<td>MgCl₂(H₂O)₆</td>
<td>0.15</td>
<td>0.1</td>
<td>0.33</td>
</tr>
<tr>
<td>(NH₄)₂CO₃</td>
<td>0.06</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1.5</td>
<td>0.15</td>
<td>0.6</td>
</tr>
</tbody>
</table>

The addition of pepsin, Ca²⁺ solution and water will result in the correct electrolyte concentration in the final digestion mixture.
Table 2. Starch fractions of digested saffron-enriched fresh pasta (0, 0.2 or 0.4 % of saffron (w/w)) cooked at different times (1.5 and 3 minutes) and with or without oil addition. RDS: Rapidly Digested Starch; SDS: Slowly Digested Starch; RS: Resistant Starch are expressed as percentage (%) on total starch. Samples are codified as follows: saffron content in dough pasta (0.2 or 0.4 %)-cooking time (1.5 or 3 min) + oil addition.

<table>
<thead>
<tr>
<th></th>
<th>RDS(%)</th>
<th>SDS(%)</th>
<th>RS(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%-1.5’ (control)</td>
<td>21.9 ± 1.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.00 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.8 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.2%-1.5’</td>
<td>17.4 ± 1.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.6 ± 1.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>79.1 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4%-1.5’</td>
<td>26 ± 5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.7 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.0 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4%-3’</td>
<td>8.9 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.4 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.2 ± 2.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.2%-1.5’ + oil</td>
<td>14.5 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.0 ± 1.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75.6 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4%-1.5’+ oil</td>
<td>14.22 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.27 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>85.1 ± 4.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-d</sup> Different letters indicate the homogeneous groups obtained by the analysis of variance (ANOVA p-value < 0.05)
Table 3. Total crocin content (µg/g pasta (dry base)) and trans and cis-isomers distribution (%)
in saffron-enriched pasta before and during the gastrointestinal digestion (GS: Gastric Stage; IS: Intestinal Stage). Samples are codified as follows: saffron content in dough pasta (0.2 or 0.4 %)-cooking time (1.5 or 3 min) + oil addition.

<table>
<thead>
<tr>
<th></th>
<th>Total crocin (µg/g pasta (d.b.))</th>
<th>Trans-isomers (%)</th>
<th>cis-isomers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2%-1.5'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td>3549.6 ± 0.3&lt;sup&gt;AE&lt;/sup&gt;</td>
<td>80.772 ± 0.003&lt;sup&gt;DA&lt;/sup&gt;</td>
<td>19.23 ± 0.02&lt;sup&gt;DA&lt;/sup&gt;</td>
</tr>
<tr>
<td>GS-120 min</td>
<td>625 ± 188&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>93.5 ± 0.5&lt;sup&gt;C&lt;/sup&gt;</td>
<td>6.5 ± 0.5&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-10 min</td>
<td>773 ± 114&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>93.5 ± 0.7&lt;sup&gt;C&lt;/sup&gt;</td>
<td>6.5 ± 0.7&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-20 min</td>
<td>630 ± 150&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>99.03 ± 0.07&lt;sup&gt;DD&lt;/sup&gt;</td>
<td>0.97 ± 0.07&lt;sup&gt;DD&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-30 min</td>
<td>429 ± 98&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>96 ± 2&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>4 ± 2&lt;sup&gt;CD&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-60 min</td>
<td>188 ± 54&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>96 ± 2&lt;sup&gt;C&lt;/sup&gt;</td>
<td>5 ± 2&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-90 min</td>
<td>322 ± 97&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>95.4 ± 0.2&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>4.6 ± 0.2&lt;sup&gt;AC&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-120 min</td>
<td>102 ± 39&lt;sup&gt;AE&lt;/sup&gt;</td>
<td>88 ± 2&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>12 ± 2&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4%-1.5'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td>4236 ± 13&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>99.7 ± 0.3&lt;sup&gt;DE&lt;/sup&gt;</td>
<td>0.3 ± 0.3&lt;sup&gt;DE&lt;/sup&gt;</td>
</tr>
<tr>
<td>GS-120 min</td>
<td>1824 ± 290&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>59.0 ± 1.3&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>41.0 ± 1.3&lt;sup&gt;AA&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-10 min</td>
<td>1846 ± 216&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>87.169 ± 1.105&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>12.831 ± 1.105&lt;sup&gt;CD&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-20 min</td>
<td>4901 ± 1405&lt;sup&gt;ABCD&lt;/sup&gt;</td>
<td>90.80 ± 1.12&lt;sup&gt;ED&lt;/sup&gt;</td>
<td>9.20 ± 1.12&lt;sup&gt;ED&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-30 min</td>
<td>3194 ± 143&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>86 ± 3&lt;sup&gt;IE&lt;/sup&gt;</td>
<td>14 ± 3&lt;sup&gt;IE&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-60 min</td>
<td>2663 ± 412&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>87 ± 4&lt;sup&gt;ACD&lt;/sup&gt;</td>
<td>13 ± 4&lt;sup&gt;ACD&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-90 min</td>
<td>3562 ± 749&lt;sup&gt;ABCD&lt;/sup&gt;</td>
<td>88 ± 3&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>12 ± 3&lt;sup&gt;CD&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-120 min</td>
<td>3099 ± 431&lt;sup&gt;ABCD&lt;/sup&gt;</td>
<td>80.8 ± 0.6&lt;sup&gt;BB&lt;/sup&gt;</td>
<td>19.2 ± 0.6&lt;sup&gt;BB&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4%-3'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td>10483.1 ± 0.8&lt;sup&gt;DE&lt;/sup&gt;</td>
<td>46.961 ± 0.002&lt;sup&gt;AE&lt;/sup&gt;</td>
<td>53.039 ± 0.002&lt;sup&gt;AE&lt;/sup&gt;</td>
</tr>
<tr>
<td>GS-120 min</td>
<td>1183 ± 427&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>92.6 ± 0.9&lt;sup&gt;EF&lt;/sup&gt;</td>
<td>7.4 ± 0.9&lt;sup&gt;EF&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-10 min</td>
<td>1738 ± 208&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>80.8 ± 0.6&lt;sup&gt;BE&lt;/sup&gt;</td>
<td>19.2 ± 0.6&lt;sup&gt;BE&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-20 min</td>
<td>3985 ± 620&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>53.6 ± 2.7&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>46.4 ± 2.7&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-30 min</td>
<td>1444 ± 79&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>89.9 ± 0.7&lt;sup&gt;EF&lt;/sup&gt;</td>
<td>10.1 ± 0.7&lt;sup&gt;EF&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-60 min</td>
<td>2322 ± 920&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>53.71 ± 0.06&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>46.29 ± 0.06&lt;sup&gt;ABC&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-90 min</td>
<td>2724 ± 54&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>58 ± 2&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>42 ± 2&lt;sup&gt;BD&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-120 min</td>
<td>2618 ± 742&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>57 ± 2&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>43 ± 2&lt;sup&gt;CD&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.2%-1.5'+oil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td>4341 ± 40&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>60.6 ± 0.6&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>39.4 ± 0.6&lt;sup&gt;BD&lt;/sup&gt;</td>
</tr>
<tr>
<td>GS-120 min</td>
<td>919 ± 36&lt;sup&gt;AE&lt;/sup&gt;</td>
<td>95 ± 2&lt;sup&gt;E&lt;/sup&gt;</td>
<td>5 ± 2&lt;sup&gt;E&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-10 min</td>
<td>1880 ± 358&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>83 ± 0.2&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>16.7 ± 0.2&lt;sup&gt;BD&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-20 min</td>
<td>3901 ± 190&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>53.4 ± 1.3&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>46.6 ± 1.3&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-30 min</td>
<td>1965 ± 109&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>42 ± 2&lt;sup&gt;AE&lt;/sup&gt;</td>
<td>58.2 ± 2.3&lt;sup&gt;AE&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-60 min</td>
<td>1838 ± 427&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>43 ± 2&lt;sup&gt;AE&lt;/sup&gt;</td>
<td>57 ± 3&lt;sup&gt;AE&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-90 min</td>
<td>2153 ± 601&lt;sup&gt;ABC&lt;/sup&gt;</td>
<td>73 ± 7&lt;sup&gt;C&lt;/sup&gt;</td>
<td>28 ± 7&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-120 min</td>
<td>2675 ± 408&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>57 ± 2&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>43 ± 2&lt;sup&gt;BD&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.4%-1.5'+oil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td>9420 ± 3&lt;sup&gt;DEF&lt;/sup&gt;</td>
<td>73.470 ± 0.007&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>26.530 ± 0.007&lt;sup&gt;CD&lt;/sup&gt;</td>
</tr>
<tr>
<td>GS-120 min</td>
<td>3016 ± 259&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>84.4 ± 0.7&lt;sup&gt;BE&lt;/sup&gt;</td>
<td>15.6 ± 0.7&lt;sup&gt;BE&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-10 min</td>
<td>7094 ± 339&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>76 ± 2&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>24 ± 2&lt;sup&gt;BD&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-20 min</td>
<td>5006 ± 186&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>86 ± 3&lt;sup&gt;BE&lt;/sup&gt;</td>
<td>14 ± 3&lt;sup&gt;BE&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-30 min</td>
<td>5973 ± 632&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>75 ± 2&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>25 ± 2&lt;sup&gt;BD&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-60 min</td>
<td>4861 ± 469&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>65 ± 3&lt;sup&gt;C&lt;/sup&gt;</td>
<td>35 ± 3&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-90 min</td>
<td>10190 ± 687&lt;sup&gt;EF&lt;/sup&gt;</td>
<td>29.67 ± 1.04&lt;sup&gt;AE&lt;/sup&gt;</td>
<td>70.33 ± 1.04&lt;sup&gt;AE&lt;/sup&gt;</td>
</tr>
<tr>
<td>IS-120 min</td>
<td>9118 ± 279&lt;sup&gt;BE&lt;/sup&gt;</td>
<td>34.4 ± 0.5&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>65.6 ± 0.5&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-d</sup> Different letters refer to the homogenous groups obtained for different pasta formulations (0.2%-1.5’, 0.4%-1.5’, 0.4%-3’, 0.2%-1.5’+oil, 0.4%-1.5’+oil) at a statistical significance of 95% (p-value < 0.05). <sup>A-F</sup> Different letters refer to the homogenous groups obtained after digestion (pasta) and for the different times of digestion (GS-120 min, IS-10min, IS-20 min, IS-30 min, IS-60 min, IS-90 min and IS-120 min) at a statistical significance of 95% (p-value < 0.05).
Figure 1. Matrix degradation index (MDI (%)) achieved after the in vitro gastrointestinal digestion of saffron-enriched pasta with or without oil addition. Samples are codified as follows: saffron content in dough pasta (0.2 or 0.4 %)-cooking time (1.5 or 3 min) + oil addition.
Figure 2. Concentration of the principal crocetin isomers (mg/g d.b.) identified by HPLC in the different saffron-enriched pasta at different interval times of intestinal digestion. Samples are codified as follows: saffron content in dough pasta (0.2 or 0.4 %)-cooking time (1.5 or 3 min) + oil addition.