Effect of saffron (*Crocus sativus* L.) enrichment on antioxidant and sensorial properties of wheat flour pasta

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Abstract
Saffron, used in cookery as flavouring and colouring agent, is well-known for its antioxidant and healthy properties. In the present work, the effect of saffron addition (0-control, 0.1, 0.2 and 0.4 %, w/w) in the formulation of fresh pasta was evaluated on textural, physical-chemical, and sensory properties of the cooked product. Content and retention of the bioactive molecules of saffron (crocins) were evaluated by HPLC along with the corresponding antioxidant activity of enriched pasta. The presence of saffron significantly influenced textural and physical-chemical properties of pasta. Higher saffron concentrations enhanced the antioxidant activity of pasta with the higher values of crocins in samples enriched with 0.4 % saffron extract even after 3 minutes of cooking (4.23-5.06 mg/ g db). Sensory analysis showed an increased acceptability of the saffron enriched pasta for all descriptors selected (visual aspect, colour, aroma, taste, chewiness, hardness, gumminess and overall acceptability).

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); PIlcocrocin (Pubchem CID: 130796).

Key words
Saffron; Crocins; Pasta formulation; Antioxidants; Sensory evaluation
1. Introduction

In the last few decades, the new nutritional trends have increased the request of foods with enhanced quality and ‘nutraceuticals’ properties for health promotion and disease prevention. One strategy to increase nutritional properties of foods is the incorporation of functional ingredients in staple foods. The challenge is to enhance the potential health benefits maintaining the consumer acceptability of the food (Foschia, Peressini, Sensidoni, & Brennan, 2013).

Pasta is a traditional cereal-based product accepted worldwide due to the low cost, easy production and sensory attributes (Chillo, Laverse, Falcone, Protopapa, & Del Nobile, 2008). The traditional Italian-style pasta is a rather simple product obtained by mixing durum wheat flour and water followed by kneading, shaping or extrusion and, depending on the desired final product, drying. Quality and nutritional characteristics of pasta, depending on the raw material and processing conditions, will be mainly attributed to the starch and protein fraction (Hirawan, Ser, Arntfield, & Beta, 2010). Several recent studies have focused on the role of whole-grain diets in preventing degenerative diseases. These beneficial health properties of whole-grain products have been associated with the presence of variable amounts of antioxidants, as compared to their corresponding refined flours. Many of these are scavengers of free radicals, including carotenoids (De Simone et al., 2010; Ficco et al., 2014) and anthocyanins (Abdel-Aal, Young, & Rabalski, 2006; Ficco et al., 2014).

Potentially, pasta is a useful carrier for substances acting as nutrition enhancers or providing specific physiological functions and has, thus, been the object of many functionalization strategies (Li, Zhu, Guo, Brijs, & Zhou, 2014).

Being a formulated product, its quality and health functionality could be further improved by the addition of ingredients rich in bioactive compounds. In fact, pasta has already been enriched with
several natural ingredients, such as artichokes extract (Pasqualone et al., 2017), grape marc powder (Sant’Anna, Christiano, Marczak, Tessaro, & Thys, 2014), and spirulina biomass (Rodríguez De Marco, Steffolani, Martínez, & León, 2014). In most of them, the enrichment of pasta affected the colour of the final product, but did not alter the main textural parameters and cooking performances.

Among the wide variety of functional ingredients with potential health benefits, saffron, the dried stigmas of *Crocus sativus* L., is a spice highly valued both in cookery and in the food industry due to its colouring properties, pleasant bitter taste and alluring aroma (Serrano-Diaz, Sánchez, Maggi, Carmona, & Alonso, 2011). The sensory properties of saffron spice are given by the presence of three carotenoid derivatives (crocins, picrocrocin and safranal, responsible of colour, flavour and aroma, respectively), mainly synthesized during flowering as well as during processing. These metabolites are produced by oxidative cleavage of zeaxanthin, followed by oxidative modifications and glycosylation (Namin et al., 2009). Crocin or crocetin digentiobiose ester (C_{44}H_{64}O_{24}), a natural and food-grade colouring agent, is considered one of the major bioactive constituents and has a wide spectrum of biological activities including antigenotoxic and cytotoxic effects, antioxidant, antinociceptive and anti-inflammatory, anti-atherosclerosis, anti-diabetic, hypotensive, hypolipidaemic, hypoglycemic and antidepressant (Rahaiee, Moini, Hashemi, & Shojaosadati, 2015).

In this context, pasta may be a meal-carrier to add saffron as ingredient and to enhance antioxidant properties of humans’ diet. To the best of our knowledge, there are no studies on pasta fortification with saffron or saffron extracts and the characterization of this product.
The aim of this work was, thus, to evaluate quality of pasta enriched with different concentrations of saffron powder dispersed in water (0, 0.1, 0.2 and 0.4 %w/w of saffron) through textural, physical-chemical, antioxidant and sensory impacts of saffron addition to pasta.

2. Materials and Methods

2.1. Chemicals

The standards crocins, safranal, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Tolox), and gallic acid were all analytical grade (> 90 %, Sigma Aldrich, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ABTS, potassium persulfate, Folin-Ciocalteau reagent, Na₂CO₃ and methanol were purchased from Sigma (Sigma Aldrich, USA).

Materials

Saffron powder (Hacendado, Novelda, Spain) and soft wheat flour (Hacendado, Novelda, Spain) were purchased from a local supermarket. For pasta preparation and cooking, tap water of Valencia municipality was used.

2.2. Sample preparation

2.2.1. Pasta samples preparation

All samples (with and without saffron) were prepared by mixing wheat flour (70 %w/w) and water (30 %w/w). Each batch of pasta was made of ca. 250 g.

Saffron was added in different concentrations as a solution or dispersion, by substituting the water; the following pasta samples were prepared: Control dough (C1) consisted in wheat flour and tap water (no saffron); “Non filtered saffron enriched pasta” (NF) was made of wheat flour (165 g) + 70 ml of a dispersion of saffron powder in tap water (0.1, 0.2 or 0.4 % of saffron (w/w)
in dough); “Filtered saffron enriched pasta” (F) consisted in wheat flour (165 g) + 70 ml of a dispersion of saffron powder in tap water in the same concentrations as in NF (0.1, 0.2 or 0.4 % of saffron (w/w) in dough), but the dispersion was preliminarily filtered (Whatman no. 40 paper filter) before mixing it with the flour. A summary of the pasta samples prepared in this study is reported in Table 1.

All the ingredients were mixed using a home professional equipment (Thermomix TM- 31, Vorwerk, Wuppertal, Germany) for 2 min (‘dough mode’). The mix was left to settle for 5 minutes at room temperature before to extrude it to a “pappardelle” shape (large and long slices) using a home scale pasta-shaper (Marcato SPA, Padova, Italy).

2.2.2. Cooking of the pasta

Aliquots of 60 g of each pasta sample were cut into smaller pieces (8 cm x 4 cm) and cooked in boiling tap water (1.5 L) (Sozer, Dalgıç, & Kaya, 2007). Samples were removed from the boiling water at 1.5 and 3 minutes after boiling start, that corresponded, based on preliminary cooking tests, to the optimum and overcooking times, respectively.

An additional saffron enriched-pasta sample was obtained by cooking C1 (raw pasta) in boiling water with saffron (50 mg powder/100 g raw pasta). Pasta was, like in the other cases, subjected to both 1.5 and 3 minutes cooking time.

After cooking, samples were drained and cooled by soaking in cold water for 10 s; excess water was removed by lightly patting the samples between paper towels.

An aliquot corresponding to half of the samples (both raw and cooked) was immediately used for analytical and instrumental measurements (moisture, $a_w$, colour and texture). The remaining half was freeze dried (-45 °C, 0.8 Pa 48 h, Telstar, Spain) and used for 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.

### 2.3. Analytical measurements

#### 2.3.1. Saffron characterization

The commercial saffron used for this study was preliminarily characterized for its quality according to colour, aroma, flavour and moisture content, following the procedures reported by the ISO 3632-1:2011. For moisture content, an aliquot of 15 mg of saffron powder was dissolved in 10 mL of distilled water, then samples were dried at 103°C in oven (Heraus, Thermo Fisher) for 16 hours. Quality indices (colouring strength, aroma strength, flavour strength) were carried out on solutions obtained by dissolving 10 mg of saffron powder in 2 mL of distilled water. After centrifugation (10 min, 1200 x g), 5 µL of the supernatant were added in 500 µL of distilled water. UV-VIS spectrophotometric analyses were carried out and the absorbance values measured at 257, 330 and 440 nm were used to evaluate picrocrocin, safranal, and crocin respectively. Each sample was analysed in triplicate.

#### 2.3.2. Moisture content ($x_w$) and water activity ($a_w$) of pasta samples

Moisture content of raw and cooked samples was determined according to the Association of Official Analytical Chemists (AOAC, 2000). The $a_w$ of samples was measured by using a dew point hygrometer (Aqualab, Decagon Devices, Pullman, WA, USA).

#### 2.3.3. Antioxidant activity of pasta samples

Freeze-dried samples and saffron powder (0.25 g) were extracted with methanol (3 mL, 80 % in water) using an Intell-Mixer RM-2 (Elmi Ltd, Riga, LV-1006, Latvia). Samples were rotated head-over-heels at 55 rpm for 2 h at room temperature and then centrifuged at 1200 x g force for
10 min. The supernatant was then used for antioxidants analysis. DPPH, ABTS as well as TPC analyses were performed in a spectrophotometer (UV/vis, Beckman Coulter).

The radical scavenging activity (DPPH) was determined following the modified protocol of Brand-Williams, Cuvelier, & Berset (1995). A DPPH solution (0.024 g/L) was prepared freshly in methanol (80 % in water). An aliquot of this solution (3.9 mL) was pipetted into 4 mL plastic cuvette and the absorbance was measured at 515 nm. Thereafter, 100 µL of methanol sample extract (saffron extracts and pasta extracts), was added into the DPPH solution to start the reaction. The absorbance was measured at 515 nm just after sample addition and after 30 min, time necessary to reach a constant value of absorbance.

The ABTS method was performed as described by Re et al., (1999). The ABTS•⁺ radical was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1:1, v/v) and leaving the mixture for 16 h until the reaction was completed and the absorbance was stable. The ABTS•⁺ solution was diluted with 2.45 mM buffer solution to an absorbance 0.700 at 734 nm for measurements. An aliquot of this solution (0.9 mL) was pipetted into 1 mL plastic cuvette and the absorbance was measured at 734 nm. Thereafter, 100 µL of methanol sample extract (saffron extracts and pasta extracts), was added into the ABTS•⁺ solution to start the reaction. The absorbance was measured at 734 nm after 6 min, time necessary to reach a constant value of absorbance.

For both the DPPH and ABTS assays, calibration curves of Trolox (0–750 mM) were prepared and results were expressed as the number of equivalents of Trolox (µmol TE/g dry base).

2.3.4. Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined following the modified protocol of the Folin–Ciocalteu assay (Magalhães, Santos, Segundo, Reis, & Lima, 2010). Methanolic extracts (pasta
samples or saffron extracts) (125 µL) were added into a 4 ml plastic cuvette with distilled water (0.5 ml) and Folin-Ciocalteau reagent (125 µL). After 5 min, 1.25 ml of Na₂CO₃ solution (7 % [w/v]) and distilled water (1 mL) were added. Measurements were recorded at 15 and 30 min, at a wavelength of 660 nm. Gallic acid (0–750 mM) was used for TPC and results expressed as the number of equivalents of gallic acid (µmol GAE/g dry bases).

2.3.5. HPLC analysis

Crocin esters and safranal content of saffron extract and enriched pasta samples were determined by HPLC analysis. Freeze-dried samples 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 force for 10 min. The solid pellet was extracted again following the same procedure. The supernatants of the two extractions were collected together (10 mL); an aliquot (1.5 mL) was filtered (0.45 µm PTFE) and used for the HPLC analysis.

Analyses were carried out by 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 % A 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. Injections of 20 µL were made for all samples and standards.

The identification of safranal and crocins was carried out by comparing their retention times with those of their standards at 330 and 440 nm, respectively; quantification was performed by calibration curves in fortified C1 freeze dried powder, extracted with methanol 80 % of the standard compounds (10, 20, 50, 100 and 150 mg/L), with six replicates for each level (n=6),
being 0.0003 mmol/L of saffranal and 0.002 mmol/L of crocin (Trans-4-GG) the limit of quantification.

Crocetin isomers were labelled as follows: first, the name indicating the isomeric cis- or trans-form separated by the total number of glucose moieties at both extremes of the base molecule. Then, the glucose moieties are indicated with (G) gentiobioside or (g) glucoside. Quantification of crocetin isomers was carried out referring to the most abundant isomer, expressed as mg of crocin/g dry base.

2.3.6. Texture profile analysis (TPA)

The Texture Analyser TA/XT/PLUS (Stable Micro System, Godalming, UK) was used for TPA analyses (Sozer et al., 2007). A sample of pasta (8 cm x 4 cm) was placed at the centre of the base, under the compression plate of pasta firmness/ stickiness rig. The test and post-test speed was 1 mm/s. The resulting force-deformation curve was used to determine the following textural parameters: hardness, cohesiveness, elasticity and chewiness.

2.3.7. Colour determination of pasta samples

Colour was determined by a spectrophotocolorimeter CM-3600d (Minolta Co. Ltd., Osaka, Japan), placing the pasta sample at room temperature directly into the cell using a black plate as the background to standardize the measurements. Visible absorption spectra were recorded between 380 and 770 nm by reflectance to obtain tristimulus values of CIE L*a*b*, using illuminant D65 and standard observer (10° visual field) as references. The yellowness index (YI) was calculated with the equation (1) often referenced to Francis & Clydesdale (1975).

\[ YI_{FC} = 142.86 \frac{b^*}{L^*} \]

Equation (1)
2.3.8. Sensory evaluation

Sensory characteristics of pasta were evaluated by descriptive sensory analysis on selected cooked samples without any additional seasoning (AENOR). According to the colour and antioxidant results of previous analysis, the following samples of pasta were chosen: C1-1.5, 0.2-F-1.5, 0.2-F-3, 0.2-NF-1.5, 0.4-NF-1.5 and 0.4-NF-3 (n=6). The sensory test was carried out by a panel of 23 non-trained judges, but accustomed consumers of pasta.

The size of the panel used in this test is lower than that generally required by a conventional descriptive sensory analysis, but sufficient to obtain reliable sensory results on the perception of the attributes by consumers familiar with pasta dishes (Clapperton & Plggott, 1979; Husson, Le Dien, & Pagès, 2001; Husson & Pagès, 2003).

In particular, panellists were asked to evaluate six descriptors of pasta: visual aspect, colour, flavour, taste, chewiness, hardness and stickiness. Moreover, they were asked to also assess the overall acceptability. To this aim they were asked to rank the 6 samples from 1 (dislike) to 6 (like), according to the differences among them for each parameter. All samples were administrated in white dishes in a randomized order. The Friedman test was carried out to establish the existence of significant differences between samples. The statistical function T Friedman was calculated by means of equation 2. These results were compared with the tabulated critical value (t-Friedman) $X^2=11.97$ (significance $\alpha = 0.05$) with (t-1) degrees of freedom. Afterwards, equation 3 was used to establish between which samples these differences lay (Meilgaard, Civille, & Carr, 2007):

$$F = \frac{12}{J \times P (P + 1)} (R_1^2 + R_2^2 + \cdots + R_6^2) - 3J (P + 1)$$

Equation (2)
where $P$ is the number of panellists ($n=23$), $J$ is the number of samples ($6$) and $R_i$ is the rank sum for each evaluated attribute.

2.4. Statistical Analysis

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

3. Results and Discussion

3.1 Saffron characterization

The commercial saffron used in this study was classified as II category (ISO 3632-1:2011), being characterised by the following quality parameters: colour ($A_{1\%_{1cm}, 440 \text{ nm}} = 169$; aroma ($A_{1\%_{1cm}, 330 \text{ nm}} = 41$; flavour ($A_{1\%_{1cm}, 257 \text{ nm}} = 67$ and a moisture content (%) = 11.86.

3.2 Water activity and moisture content

Table 2 reports the moisture content (%) and $a_w$ values of all pasta types. Data reported are in agreement with those typical of fresh pasta products with a moisture content higher than 30 % and $a_w$ values > 0.97.
The fresh, uncooked products (controls C1 and C2, as well as those enriched with saffron) presented similar values of moisture and $a_w$ before cooking. During cooking swelling of pasta occurs along with a water uptake that indicates how well pasta responds to cooking with a significant increase of moisture and $a_w$ of the samples ($p$-value < 0.05) (Table 2). Moreover, as expected, a longer cooking time caused an increase in water absorption, since more water can diffuse and interact with both gluten and starch. Dried gluten acts as a sponge for water during cooking; it opens its structure and embeds the starch granules inside this network (Sozer et al., 2007).

The addition of higher concentrations of saffron (0.2 and 0.4 %) in the pasta resulted to reduce the water uptake during cooking in respect to that observed in the control samples, and this effect was even more noticeable when saffron was not filtered, so likely the presence of small non-soluble saffron particles may have hindered the water diffusion into the gluten matrix.

### 3.3 Colour analysis

The values of the CIELAB colour coordinates of the differently formulated pasta are reported in Table 2. Fresh pasta colour is a very important quality attribute that greatly influences consumer acceptance, being the first property that consumers can evaluate when selecting a product in the market (Carini, Vittadini, Curti, & Antoniazzi, 2009). Due to various enrichment levels, there was a considerable difference in colour among the different pasta samples. $L^*$ values decreased with the incorporation of saffron powder in the pasta dough, indicating that samples became darker due to the presence of the spice to the overall colour of the product with an effect that was significant even for the very low saffron concentration (0.1 %, both filtered and non-filtered) and increasing at higher saffron concentration added in the pasta ($p$-value < 0.05).
The increase of saffron concentration (0.2 and 0.4 %) resulted in a gradual increase of the $a^*$ and $a^*/b^*$ values; this was an expected result, since saffron powder is a carotenoids-rich product characterized by a dark orange colour and the $a^*$ parameter is related to the redness. However, the $a^*$-value increased less in the F-pasta than in NF-samples, probably due to an additional contribution of the small particles undissolved to the overall surface colour of the pasta.

All samples showed positive $b^*$ (yellow) values with similar trends to those observed for the $a^*$ parameter due to filtration and saffron concentration. The Yellow Index (YI) was evaluated to better understand the effect of saffron addition and cooking time on the yellowness degree. In all samples, the YI increased in samples cooked at 1.5 minutes, followed by a decrease at higher cooking time, as noticed for the individual chromatic parameters.

In general, after cooking, saffron enriched pasta samples showed values significantly different to the control, which indicates an ability of the pasta matrix to retain the chromatic components of the saffron extracts even if a decrease of the colorimetric coordinates ($L^*$, $a^*$ and $b^*$) values was observed.

This latter result might be partly due to the moisture increase that, overall resulted in a decreased in the final concentration of pigment in the sample. On the other hand, some components from the saffron extract might have been degraded during cooking and/or can be lost in the cooking water due to leaching processes. Selim, Tsimidou & Biliaderis, (2000) demonstrated an increase in the decolouring rate of saffron with increasing $a_{ws}$, particularly above the intermediate-moisture regime. This behaviour may be related to a higher water solubility of saffron carotenoids, favouring a greater access of dissolved oxygen to the pigments. Eventually, changes in the physical properties of the pasta induced by the heat treatment and water uptake (swelling
and gelatinisation, protein denaturation, gelation) can also have contributed to the change of the colour parameters data due to a different light reflectance during the measure.

3.4. Texture analyses

Determination of the textural parameters after pasta cooking is of great importance from the consumers’ acceptability point view. There are several factors that might affect the characteristics of cooked pasta such for instance protein quality and quantity, drying conditions and/or composition of cooking water (Sozer et al., 2007).

The results of the TPA analysis as data of hardness, cohesiveness, elasticity and chewiness, are shown in Table 3.

The addition of saffron did not significantly affect the textural parameters of the raw pasta, while a main effect was observed as a consequence of cooking time. Hardness values (index that could be associated to the force required to compress pasta between teeth) decreased as cooking time increased, especially in non-filtered samples (Table 3). Cohesiveness, that according to Sozer et al., (2007) is a good indicator of how the sample holds together upon cooking, seemed to be slightly influenced by cooking time, highlighting the negative effect of cooking time on cooked pasta quality. Elasticity, or springiness, (the elastic recovery occurring when a compressive force is removed), showed the highest values in cooked samples after 1.5 minutes, except for the NF-samples with the highest concentration of saffron, although the differences were not significant (p-value >0.05). Finally, chewiness, as cohesiveness and hardness did, decreased with increasing cooking time; chewiness is related to the elastic strength of the protein matrix and defined as the effort required to masticate pasta to the point of swallowing (Sozer et al., 2007).
As regards the effect of saffron on uncooked/raw pasta samples, no effect was observed in the majority of the studied samples, while a significant decrease (p<0.05) was observed in the 0.1 and 0.2 % NF-pasta which could be associated to a weakening effect of the added, insoluble components of the no-filtered saffron extracts on the macroscopic gluten matrix of the pasta products (Carini, Curti, Spotti, & Vittadini, 2012; Sant’Anna et al., 2014), thereby a dilution of the gluten strength occurs. However, this effect was not observed in the sample added with the higher concentration (0.4 %, NF) and in this case other effects of the saffron extracts could have counteracted the disrupting effects on the gluten matrix by favouring an increased gluten structuring and corresponding firmness. Saffron is a rather complex spice and aqueous extracts may contain, besides crocins, other compounds like proteins and phenolic compounds. Cross-linking abilities of phenolic compounds have been already observed while no scientific evidences are available in the literature for saffron extracts and additional investigations are needed.

As regards the cooked samples, the saffron extracts showed a lower water uptake than control samples at the fixed cooking time (1.5 and 3.0 min), index of a lower water diffusion ability of the pasta matrix due to the spice components (soluble and insoluble) and a higher firmness than reference samples at equal cooking time. Cooked pasta firmness is partly related to the hydration of starch granules during cooking and the subsequent embedding of gelatinised starch granules in the pasta protein matrix (Li et al., 2012; Sun-Waterhouse, Jin, & Waterhouse, 2013). A lower water uptake (Table 1) could be associated to a lower swelling of starch granules and to a higher firmness of the corresponding samples.
3.5. Antioxidant activity of enriched pasta

The antioxidant properties of *C. sativus* stigma are generally attributed to its phenolic content as well as to its active ingredients such as safranal, crocins, crocetin and carotene, all of which have been reported to exert antioxidant properties (Armellini, Compagnone, Scampicchio, & Pittia, 2016; Assimopoulou, Sinakos, & Papageorgiou, 2005; Karimi, Feizy, Mehrjo, & Farrokhnia, 2016; Namin et al., 2009; Rahaiee et al., 2015). If antioxidant activity varies directly as the number of the double bonds, crocin (Trans-4-GG) which has seven double bonds in a molecule should be as effective antioxidant as α-tocopherol (Bathaie & Mousavi, 2010; Soeda et al., 2007).

**Figure 1** shows the Total Phenolic Content (TPC) and the antioxidant activity data evaluated by DPPH and ABTS methods of pasta before (raw) and after cooking. All pasta samples showed, before cooking, similar values of TPC regardless the addition of saffron powder (**Figure 1a**). The cooking procedure however, slightly affected the total phenolic content (p-value < 0.05), which decreased as cooking time increased for samples enriched with 0.1 % of saffron, while samples enriched with 0.2 and 0.4 % did not have significant losses of TPC. The TPC decrease in pasta samples with the lower saffron concentration may be due to the relatively low thermo-stability of phenolic compounds as well as to the leaching of these compounds into the cooking water (Hirawan et al., 2010; Verardo, Gómez-Caravaca, Messia, Marconi, & Caboni, 2011). However, this behaviour was reduced in samples with higher concentration of saffron that resulted in a higher content of antioxidant compounds, whose presence could be due to interactions with other pasta matrix compounds contributing to reduce their loss.

It is worth to note that C2 pasta samples (i.e. C1 pasta cooked in a boiling saffron dispersion), showed similar TPC values than C1 (p-value > 0.05) at similar cooking time. This could indicate
that under these conditions (very short cooking time) only a limited amount of saffron could
have diffused into the pasta during cooking, likely mainly adsorbed at surface level thereby only
determining a significant colour change (Table 2) but to a very little and not significant quantity
to contribute significantly to the overall phenolic content of the pasta after heat treatment.
It is remarkable that despite the results on TPC and the effect of cooking, the decrease of
phenolic compounds did not affect negatively the antioxidant activity (DPPH and ABTS values)
of saffron enriched pasta, which, in general, was significantly higher than both controls (p-value
< 0.05) (Figures 1b and 1c).
This is an interesting result since one of the main aims in pasta supplementation is the increase of
its antioxidant activity (Boroski et al., 2011; Sęczyk, Świeca, & Gawlik-Dziki, 2016). Actually, fresh, uncooked pasta control (C1) presented significant lower antioxidant values, for both DPPH
and ABTS analysis, when compared to saffron enriched samples (p-value < 0.05). Pasta enriched
with saffron showed higher values of DPPH compared to C1 with a significant effect due to the
added saffron concentration up to 0.2 %, while a higher saffron addition (up to 0.4 %) did not
determine any further increase in the antioxidant activity.
Cooking time influenced the results of the antioxidant activity as evaluated by the DPPH test in a
different way depending on the sample. It could be noticed that DPPH value of C1 significantly
increased after cooking, and this result could be related to an increased “availability” or
extractability of antioxidant compounds that influence the DPPH analysis (i.e. those with a free
radical scavenging capacity, which are present in the flour and are made available from the pasta
matrix due to cooking). A similar effect was observed for 0.1-NF and 0.2-F samples during
cooking, starting, however, by an initial TE value higher than C1 and no effect due to cooking
time.
On the other hand, ABTS values increased for all samples after cooking. These results suggest the contribution of different compounds that influence differently on the total antioxidant activity of the enriched pasta. Similar effect was noticed by Pasqualone et al., (2016), that evaluated lipophilic antioxidant activity (LAA), with ABTS reaction, of pasta enriched with lyophilized tomato. They reported higher values of ABTS in tomato enriched pasta compared to the control, with a positive effect due to cooking time. It is likely that this is related to partial solubilisation of lycopene crystals during cooking, which increased solubility in the solvent used for LAA determination or to the formation of lipophilic degradation products with higher antioxidant activity than lycopene. In our case, some antioxidant compounds from the saffron might be released with cooking time, contributing to the ABTS values (Pasqualone et al., 2016; Pham, Cormier, Farnworth, Tong, & Van Calsteren, 2000).

To support the results of the TPC, DPPH and ABTS analyses, the individual content of crocin (trans-4-GG), and its isomers, picrocrocin, as well as safranal content in enriched pasta samples was evaluated by HPLC-DAD. Safranal was detected but not quantified because its range of concentration was below the LOQ (Limit of Quantification) evaluated in this experiments (0.00003 µM). Similarly, picrocrocin was identified but not quantified.

In Table 4 the concentration of crocetin isomers (expressed as mg crocin / g dry base) of pasta enriched samples, is shown. Although all the isomers were also identified in C2, the concentration of the main isomer (trans-4-GG) was below the LOQ, and therefore the concentration could not be quantified.

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-4-ng), and *cis*-crocetin (b-D-glucosyl)-(b-D-gentiobiosyl) ester (cis-3-Gg) according to literature (García-Rodríguez et al., 2017).

All uncooked samples (filtered and non-filtered), presented a difference higher than 50 % between initial and final concentration in the final product: these differences may be due to the manipulation during preparation and kneading in the mixer, as well as to the extraction process, being some of the antioxidants still entrapped into the food matrix (Pineda-Vadillo et al., 2017).

Concentration of crocin and its isomers seemed to be dependent on pasta formulation as well as cooking time. As expected, the highest values of crocin concentration held to the highest concentration of added saffron powder in the samples; indeed, filtration of the saffron solution, before the mixing with the dough, led to differences of about 50 % of total crocin between samples with the same theoretical concentration of saffron (0.1 and 0.2 %), underlining a higher concentration in non-filtered samples (*Table 4*). Moreover, cooking had, as expected, a negative effect on total crocins concentration, which decreased after cooking. Furthermore, in the same way that observed for the antioxidant activity values, cooking time did not significantly affect crocins concentration, resulting both times, 1.5 and 3 minutes, in similar values of crocins (*Table 4*). The same behaviour was observed for all crocetin isomers except for trans-2-gg and cis-4-ng, both showing an increase after cooking. These results confirm the different contribution of the different antioxidant compounds, depending on their nature, to the total antioxidant activity of the enriched pasta. Thus, the specific isomers trans-2-gg and cis-4-ng, might contribute to the ABTS values, whereas, other isomers, negatively influenced by cooking time, may scavenge free radicals, contributing to the DPPH values.
Crocins are considered to be thermal-resistant compounds, and the different crocetin glycosylation degree does not affect their heat resistance; contrary to that which occurs when light irradiation is applied to crocins, showing crocins containing gentiobiose more stability than the ones with a glucose extreme (Carmona, Zalacain, Sánchez, Novella, & Alonso, 2006). On the other hand, few previous studies of saffron aqueous extracts have shown that it is sensitive upon exposure to light, thermal treatment, and acidic environment as well as to the presence of additives following, its colour degradation, a first-order kinetics (Sánchez, Carmona, Campo, & Alonso, 2009). In our case, the lower concentrations found in cooked samples, might be mostly due to the hydro-soluble nature of crocin, which leaches into the cooking water. While in a minor degree, some of the isomers might be affected by temperature.

3.6 Sensory analysis

Sensory analysis was carried out taking into account different attributes of pasta samples enriched with saffron. For the ordination, samples with the same score were assigned an average value of the corresponding ordination number. Figure 2 shows the results of the scores for each individual sensory attribute, together with the t-Friedman values. Panellists found significant differences (t-Friedman > 11.97) between C1-1.5 and the tested samples in terms of acceptability, colour, aroma and chewiness. In addition, they found significant differences between 0.2-1.5 and 0.4- (1.5 and 3 minutes) based on aroma intensity and chewiness. According to our score plan, overall, high scores are related to the higher acceptance of the evaluated attribute. Results of the sensory evaluation of saffron enriched pasta showed that, in general, the enrichment with saffron powder was highly accepted as shown by the marked difference between control and enriched samples. In particular, panellists gave the
highest scores to samples added with NF-saffron extract, aspect that could be of interest for future real applications.

4. Conclusions

In this study pasta enriched with saffron extracts have been produced by different formulations. Results showed that significant effects due to saffron addition were obtained in all quality parameters investigated and, in particular, TPC, colour and antioxidants content and their bioactivity. Moreover, new formulated samples showed higher scores in sensory evaluation, especially those formulated with non-filtered saffron extracts with higher saffron concentration (0.2 and 0.4 %). Results showed that presence of saffron extracts significantly influenced antioxidant activity of the pasta samples. Interesting further studies have to focus on the effects of the likely interactions of saffron secondary metabolites and bioactives with flour compounds on structural, textural and antioxidant properties as well as digestibility of bioactives.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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References


Carini, E., Vittadini, E., Curti, E., & Antoniazzi, F. (2009). Effects of different shaping modes on


Pasqualone, A., Punzi, R., Trani, A., Summo, C., Paradiso, V. M., Caponio, F., & Gambacorta, G. (2017). Enrichment of fresh pasta with antioxidant extracts obtained from artichoke canning by-products by ultrasound-assisted technology and quality characterisation of the


Figure 1. Total Phenolic Content of different freeze-dried samples of pasta (TPC, µmol equivalents of gallic acid (GAE) /g dry base). Antioxidant activity of different freeze-dried samples of pasta analyzed by DPPH (µmol Trolox Equivalents /g dry base) and ABTS+ (µmol Trolox Equivalents /g dry base). Sample code description: Control samples: letters C1 and C2 indicate for controls without saffron (C1) and with saffron powder added into the cooking water (C2). For the non-control: the first number indicates de saffron concentration in pasta dough (0.1, 0.2 and 0.4 %). NF refers to saffron addition without filtration and F with filtration of the dispersion; Numbers below X axe indicate the cooking time in minutes (0, 1.5 or 3).
Figure 2. F-value (t-Friedman) probability 5 % (significance α = 0.05) together with the spider plot of sensory parameters of the six different pasta samples selected for the sensory evaluation. Sample code description: Control samples: C1 indicates for control without saffron. For the non-control: the first number indicates de saffron concentration in pasta dough (0.2 and 0.4 %). NF refers to saffron addition without filtration and F with filtration of the dispersion; Numbers after F and NF indicate the cooking time in minutes (1.5 or 3).
Table 1. Saffron concentration, cooking medium and acronyms of samples.

<table>
<thead>
<tr>
<th>Saffron concentration (% w/w* of saffron in the final dough)</th>
<th>Cooking medium</th>
<th>Acronym fresh pasta</th>
<th>Acronym cooked fresh pasta (after 1.5 or 3 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (control)</td>
<td>Water</td>
<td>C1</td>
<td>C1-1.5, C1-3</td>
</tr>
<tr>
<td></td>
<td>Saffron enriched water</td>
<td>C2</td>
<td>C2-1.5, C2-3</td>
</tr>
<tr>
<td>Non filtered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>Water</td>
<td>NF-0.1</td>
<td>NF-0.1-1.5, NF-0.1-3</td>
</tr>
<tr>
<td>0.2</td>
<td>Water</td>
<td>NF-0.2</td>
<td>NF-0.2-1.5, NF-0.2-3</td>
</tr>
<tr>
<td>0.4</td>
<td>Water</td>
<td>NF-0.4</td>
<td>NF-0.4-1.5, NF-0.4-3</td>
</tr>
<tr>
<td>Filtered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>Water</td>
<td>F-0.1</td>
<td>F-0.1-1.5, F-0.1-3</td>
</tr>
<tr>
<td>0.2</td>
<td>Water</td>
<td>F-0.2</td>
<td>F-0.2-1.5, F-0.2-3</td>
</tr>
<tr>
<td>0.4</td>
<td>Water</td>
<td>F-0.4</td>
<td>F-0.4-1.5, F-0.4-3</td>
</tr>
</tbody>
</table>
### Table 2. Mean values and standard deviations of moisture content (x^w^) and water activity (a_w) of pasta samples as well as their colorimetric coordinates (L*, a*, b*, Yellow Index and a^*/b^* ratio) by CIELAB method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>x^w^ (g / 100 g)</th>
<th>a_w</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>a^<em>/b^</em></th>
<th>YI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1-0</td>
<td>32.7 ± 0.9^b</td>
<td>0.988 ± 0.002^ef</td>
<td>79.2 ± 0.5^f</td>
<td>2.67 ± 0.05^h</td>
<td>22.2 ± 0.2^a</td>
<td>0.120 ± 0.002^bc</td>
<td>40.1 ± 0.5^a</td>
</tr>
<tr>
<td>C1-1.5</td>
<td>53 ± 0.2^b</td>
<td>0.995 ± 0.002^ghi</td>
<td>73.0 ± 0.3^b</td>
<td>2.08 ± 0.07^ab</td>
<td>21.7 ± 0.4^a</td>
<td>0.096 ± 0.002^b</td>
<td>42.6 ± 0.5^a</td>
</tr>
<tr>
<td>C1-3</td>
<td>59.8 ± 0.4^i</td>
<td>0.998 ± 0.002^h</td>
<td>72.0 ± 0.9^b</td>
<td>1.32 ± 0.09^a</td>
<td>19.26 ± 0.14^a</td>
<td>0.068 ± 0.005^a</td>
<td>38.3 ± 0.7^a</td>
</tr>
<tr>
<td>C2-1.5</td>
<td>47.1 ± 1^de</td>
<td>0.989 ± 0.002^e</td>
<td>71.0 ± 0.4^g</td>
<td>2.7 ± 0.4^b</td>
<td>29.4 ± 0.2^b</td>
<td>0.092 ± 0.012^b</td>
<td>59.1 ± 0.3^b</td>
</tr>
<tr>
<td>C2-3</td>
<td>54.5 ± 0.2^h</td>
<td>0.991 ± 0.002^def</td>
<td>71.0 ± 0.3^g</td>
<td>1.9 ± 0.2^ab</td>
<td>30.5 ± 0.6^b</td>
<td>0.062 ± 0.005^a</td>
<td>61 ± 1^b</td>
</tr>
<tr>
<td>0.1-NF-0</td>
<td>33.9 ± 0.2^b</td>
<td>0.988 ± 0.002^e</td>
<td>72.3 ± 0.4^d</td>
<td>11.6 ± 0.5^f</td>
<td>62.0 ± 1.7^g</td>
<td>0.187 ± 0.006^de</td>
<td>123 ± 3^e</td>
</tr>
<tr>
<td>0.1-NF-1.5</td>
<td>51.6 ± 0.9^g</td>
<td>0.993 ± 0.002^d</td>
<td>66.67 ± 0.13^ede</td>
<td>10.8 ± 0.6^f</td>
<td>59.5 ± 0.6^k</td>
<td>0.181 ± 0.009^de</td>
<td>128 ± 2^e</td>
</tr>
<tr>
<td>0.1-NF-3</td>
<td>57.7 ± 0.9^i</td>
<td>0.996 ± 0.002^h</td>
<td>66.1 ± 0.5^d</td>
<td>9.1 ± 0.6^de</td>
<td>54.9 ± 3.0^g</td>
<td>0.165 ± 0.002^d</td>
<td>119 ± 6^d</td>
</tr>
<tr>
<td>0.1-F-0</td>
<td>31.9 ± 0.3^b</td>
<td>0.986 ± 0.002^b</td>
<td>73.46 ± 0.06^h</td>
<td>9.60 ± 0.12^e</td>
<td>70.0 ± 0^g</td>
<td>0.137 ± 0.002^c</td>
<td>136.3 ± 0.9^cd</td>
</tr>
<tr>
<td>0.1-F-1.5</td>
<td>49.3 ± 0.7^e</td>
<td>0.991 ± 0.002^def</td>
<td>67.6 ± 0.4^e</td>
<td>8.2 ± 0.3^sd</td>
<td>62 ± 1^c</td>
<td>0.133 ± 0.006^c</td>
<td>131 ± 3^cd</td>
</tr>
<tr>
<td>0.1-F-3</td>
<td>52.9 ± 0.2^gh</td>
<td>0.992 ± 0.002^f</td>
<td>68 ± 1^e</td>
<td>7.2 ± 0.7^c</td>
<td>58 ± 1^d</td>
<td>0.125 ± 0.013^bc</td>
<td>122 ± 1^c</td>
</tr>
<tr>
<td>0.2-NF-0</td>
<td>32.2 ± 0.3^b</td>
<td>0.984 ± 0.002^b</td>
<td>67.2 ± 0.6^e</td>
<td>18.6 ± 0.7^f</td>
<td>71.4 ± 0.9^de</td>
<td>0.261 ± 0.006^f</td>
<td>152 ± 3^e</td>
</tr>
<tr>
<td>0.2-NF-1.5</td>
<td>46.81 ± 0.12^cd</td>
<td>0.989 ± 0.002^de</td>
<td>66.2 ± 0.5^cd</td>
<td>15.54 ± 0.12^b</td>
<td>67.0 ± 0.8^f</td>
<td>0.232 ± 0.002^f</td>
<td>145 ± 3^de</td>
</tr>
<tr>
<td>0.2-NF-3</td>
<td>50.9 ± 0.7^f,g</td>
<td>0.990 ± 0.002^def</td>
<td>64.12 ± 0.12^a</td>
<td>14.8 ± 0.2^h</td>
<td>59.4 ± 0.7^de</td>
<td>0.250 ± 0.002^f</td>
<td>132 ± 2^cd</td>
</tr>
<tr>
<td>0.2-F-0</td>
<td>29.5 ± 0.5^a</td>
<td>0.980 ± 0.002^a</td>
<td>73.14 ± 0.09^h</td>
<td>13.9 ± 0.2^f</td>
<td>69.9 ± 0.2^g</td>
<td>0.199 ± 0.003^e</td>
<td>136.7 ± 0.4^cd</td>
</tr>
<tr>
<td>0.2-F-1.5</td>
<td>45.7 ± 0.7^e</td>
<td>0.988 ± 0.002^def</td>
<td>69 ± 1^f</td>
<td>14.1 ± 0.6^e</td>
<td>75.2 ± 0.3^f</td>
<td>0.187 ± 0.007^de</td>
<td>156 ± 3^e</td>
</tr>
<tr>
<td>0.2-F-3</td>
<td>52.6 ± 0.8^gh</td>
<td>0.992 ± 0.002^gh</td>
<td>69.2 ± 0.2^f</td>
<td>11.86 ± 0.13^f</td>
<td>68.4 ± 0.9^f</td>
<td>0.173 ± 0.002^de</td>
<td>141 ± 1^de</td>
</tr>
<tr>
<td>0.4-NF-0</td>
<td>33.2 ± 0.2^b</td>
<td>0.983 ± 0.002^ab</td>
<td>66.45 ± 0.09^de</td>
<td>23.38 ± 0.07^a</td>
<td>71.8 ± 0.2^h</td>
<td>0.326 ± 0.003^l</td>
<td>154.4 ± 3^e</td>
</tr>
<tr>
<td>0.4-NF-1.5</td>
<td>48.6 ± 0.5^e</td>
<td>0.993 ± 0.002^gh</td>
<td>65.48 ± 0.09^bc</td>
<td>19.4 ± 0.2^h</td>
<td>72.2 ± 0.7^h</td>
<td>0.269 ± 0.006^g</td>
<td>158 ± 2^e</td>
</tr>
<tr>
<td>0.4-NF-3</td>
<td>53.2 ± 0.3^gh</td>
<td>0.992 ± 0.002^gh</td>
<td>63.1 ± 0.2^a</td>
<td>18.17 ± 0.13^f</td>
<td>60.3 ± 0.8^k</td>
<td>0.301 ± 0.004^h</td>
<td>137 ± 2^de</td>
</tr>
</tbody>
</table>

Sample code description: Control samples: letters C1 and C2 indicate for controls without saffron (C1) and with saffron powder added into the cooking water (C2). Numbers after C1 and C2 indicate the cooking time in minutes (0, 1.5 or 3). For the non-control: the first number indicates de saffron concentration in pasta dough (0.1, 0.2 and 0.4 %); NF refers to saffron addition without filtration and F with filtration of the dispersion; Numbers after F and NF indicate the cooking time in minutes (0, 1.5 or 3). Superscript letters refer to the homogeneous groups obtained by the ANOVA (p<0.05).
Table 3. Mean values and standard deviations of texture parameters obtained by the TPA analysis for pasta samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hardness (N)</th>
<th>Cohesiveness</th>
<th>Elasticity</th>
<th>Chewiness (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1-0</td>
<td>407 ± 16</td>
<td>0.96 ± 0.002</td>
<td>0.94 ± 0.03</td>
<td>365 ± 27</td>
</tr>
<tr>
<td>C1-1.5</td>
<td>331 ± 21</td>
<td>0.93 ± 0.02</td>
<td>0.97 ± 0.05</td>
<td>300 ± 37</td>
</tr>
<tr>
<td>C1-3</td>
<td>256 ± 21</td>
<td>0.91 ± 0.02</td>
<td>0.88 ± 0.05</td>
<td>206 ± 18</td>
</tr>
<tr>
<td>C2 - 1.5</td>
<td>362 ± 16</td>
<td>0.94 ± 0.04</td>
<td>1.00 ± 0.03</td>
<td>305 ± 79</td>
</tr>
<tr>
<td>C2 - 3</td>
<td>213 ± 12</td>
<td>0.90 ± 0.02</td>
<td>1.00 ± 0.02</td>
<td>193 ± 14</td>
</tr>
<tr>
<td>0.1-NF-0</td>
<td>317 ± 25</td>
<td>0.96 ± 0.02</td>
<td>0.92 ± 0.02</td>
<td>283 ± 27</td>
</tr>
<tr>
<td>0.1-NF-1.5</td>
<td>287 ± 36</td>
<td>0.91 ± 0.04</td>
<td>0.95 ± 0.09</td>
<td>252 ± 49</td>
</tr>
<tr>
<td>0.1-NF-3</td>
<td>263 ± 30</td>
<td>0.90 ± 0.04</td>
<td>0.92 ± 0.12</td>
<td>220 ± 58</td>
</tr>
<tr>
<td>0.1-F-0</td>
<td>422 ± 4</td>
<td>0.95 ± 0.02</td>
<td>0.90 ± 0.02</td>
<td>362 ± 5</td>
</tr>
<tr>
<td>0.1-F-1.5</td>
<td>373 ± 16</td>
<td>0.89 ± 0.03</td>
<td>0.98 ± 0.09</td>
<td>323 ± 32</td>
</tr>
<tr>
<td>0.1-F-3</td>
<td>282 ± 38</td>
<td>0.88 ± 0.03</td>
<td>0.87 ± 0.12</td>
<td>217 ± 54</td>
</tr>
<tr>
<td>0.2-NF-0</td>
<td>306 ± 16</td>
<td>0.96 ± 0.02</td>
<td>0.93 ± 0.03</td>
<td>261 ± 31</td>
</tr>
<tr>
<td>0.2-NF-1.5</td>
<td>262 ± 17</td>
<td>0.94 ± 0.03</td>
<td>0.96 ± 0.05</td>
<td>235 ± 27</td>
</tr>
<tr>
<td>0.2-NF-3</td>
<td>237 ± 20</td>
<td>0.87 ± 0.02</td>
<td>0.87 ± 0.02</td>
<td>179 ± 22</td>
</tr>
<tr>
<td>0.2-F-0</td>
<td>433 ± 8</td>
<td>0.97 ± 0.02</td>
<td>0.89 ± 0.02</td>
<td>376 ± 9</td>
</tr>
<tr>
<td>0.2-F-1.5</td>
<td>402 ± 6</td>
<td>0.86 ± 0.05</td>
<td>0.95 ± 0.09</td>
<td>327 ± 51</td>
</tr>
<tr>
<td>0.2-F-3</td>
<td>277 ± 17</td>
<td>0.88 ± 0.02</td>
<td>0.89 ± 0.07</td>
<td>218 ± 33</td>
</tr>
<tr>
<td>0.4 -NF-0</td>
<td>418 ± 9</td>
<td>0.96 ± 0.02</td>
<td>0.93 ± 0.03</td>
<td>375 ± 22</td>
</tr>
<tr>
<td>0.4 -NF-1.5</td>
<td>332 ± 36</td>
<td>0.85 ± 0.04</td>
<td>0.88 ± 0.08</td>
<td>248 ± 37</td>
</tr>
</tbody>
</table>

Sample code description: Control samples: letters C1 and C2 indicate for controls without saffron (C1) and with saffron powder added into the cooking water (C2). Numbers after C1 and C2 indicate the cooking time in minutes (0 1.5 or 3). For the non-control: the first number indicates de saffron concentration in pasta dough (0.1 0.2 and 0.4 %); NF refers to saffron addition without filtration and F with filtration of the dispersion; Numbers after F and NF indicate the cooking time in minutes (0 1.5 or 3). Superscript letters refer to the homogeneous groups obtained by the ANOVA (p< 0.05).
Table 4. Concentration of the principal crocetin isomers identify by HPLC expressed as mg /g dry matter. The first number indicates the saffron concentration in pasta dough (0.1, 0.2 and 0.4 %). NF refers to saffron addition without filtration and F with filtration of the dispersion. Numbers after NF/F indicate the cooking time in minutes (0 1.5 or 3). Superscript letters (a-j) indicate de homogeneous groups obtained by the analysis of variance (ANOVA p-value < 0.05).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Trans-4-GG</th>
<th>Trans-3-Gg</th>
<th>Trans-2-gg</th>
<th>Cis-4-GG</th>
<th>Cis-4-ng</th>
<th>Cis-3-Gg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1-NF-0</td>
<td>2.25 ± 0.04</td>
<td>0.014 ± 0.002</td>
<td>0.132 ± 0.012</td>
<td>0.019 ± 0.002</td>
<td>0.101 ± 0.008</td>
<td>0.564 ± 0.106</td>
</tr>
<tr>
<td>0.1-NF-1.5</td>
<td>1.656 ± 0.102</td>
<td>0.012 ± 0.004</td>
<td>0.411 ± 0.012</td>
<td>0.001 ± 0.002</td>
<td>0.08 ± 0.002</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>0.1-NF-3</td>
<td>1.56 ± 0.16</td>
<td>0.013 ± 0.004</td>
<td>0.19 ± 0.02</td>
<td>0.003 ± 0.002</td>
<td>0.035 ± 0.007</td>
<td>0.165 ± 0.009</td>
</tr>
<tr>
<td>0.1-F-0</td>
<td>0.99 ± 0.02</td>
<td>0.02 3 ± 0.012</td>
<td>0.083 ± 0.005</td>
<td>0.011 ± 0.003</td>
<td>0.10 ± 0.03</td>
<td>0.337 ± 0.004</td>
</tr>
<tr>
<td>0.1-F-1.5</td>
<td>0.803 ± 0.012</td>
<td>0.015 ± 0.002</td>
<td>0.09 ± 0.02</td>
<td>0.016 ± 0.002</td>
<td>0.046 ± 0.002</td>
<td>0.190 ± 0.002</td>
</tr>
<tr>
<td>0.1-F-3</td>
<td>0.77 ± 0.08</td>
<td>0.015 ± 0.012</td>
<td>0.049 ± 0.006</td>
<td>0.003 ± 0.002</td>
<td>0.032 ± 0.002</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td>0.2-NF-0</td>
<td>3.38 ± 0.07</td>
<td>0.02 0 ± 0.007</td>
<td>0.36 ± 0.06</td>
<td>0.006 ± 0.002</td>
<td>0.21 ± 0.05</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>0.2-NF-1.5</td>
<td>2.85 ± 0.08</td>
<td>0.009 ± 0.008</td>
<td>0.806 ± 0.002</td>
<td>0.009 ± 0.002</td>
<td>0.35 ± 0.05</td>
<td>0.67 ± 0.06</td>
</tr>
<tr>
<td>0.2-NF-3</td>
<td>2.8 ± 0.3</td>
<td>0.021 ± 0.008</td>
<td>0.7 ± 0.2</td>
<td>0.010 ± 0.002</td>
<td>0.249 ± 0.003</td>
<td>0.89 ± 0.14</td>
</tr>
<tr>
<td>0.2-F-0</td>
<td>1.80 ± 0.08</td>
<td>0.014 ± 0.001</td>
<td>0.083 ± 0.004</td>
<td>0.003 ± 0.002</td>
<td>0.12 ± 0.03</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>0.2-F-1.5</td>
<td>1.44 ± 0.08</td>
<td>0.009 ± 0.009</td>
<td>0.18 ± 0.03</td>
<td>0.015 ± 0.002</td>
<td>0.11 ± 0.02</td>
<td>0.398 ± 0.006</td>
</tr>
<tr>
<td>0.2-F-3</td>
<td>1.50 ± 0.03</td>
<td>0.012 ± 0.004</td>
<td>0.15 ± 0.02</td>
<td>0.011 ± 0.002</td>
<td>0.15 ± 0.02</td>
<td>0.61 ± 0.02</td>
</tr>
<tr>
<td>0.4 -NF-0</td>
<td>5.1 ± 0.9</td>
<td>0.03 ± 0.02</td>
<td>0.45 ± 0.09</td>
<td>0.001 ± 0.002</td>
<td>0.48 ± 0.07</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>0.4-NF-1.5</td>
<td>4.2 ± 0.2</td>
<td>0.015 ± 0.001</td>
<td>0.7 ± 0.2</td>
<td>0.003 ± 0.002</td>
<td>0.38 ± 0.09</td>
<td>1.13 ± 0.07</td>
</tr>
<tr>
<td>0.4-NF-3</td>
<td>4.9 ± 0.2</td>
<td>0.015 ± 0.001</td>
<td>0.8 ± 0.2</td>
<td>0.008 ± 0.002</td>
<td>0.44 ± 0.04</td>
<td>1.56 ± 0.13</td>
</tr>
</tbody>
</table>