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Evaluation of *Verbascum* flower extracts as a natural source of pigments with potential health benefits†

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Crocins are bioactive glucosylated apocarotenoids that confer a yellow pigmentation. In addition to their coloring ability, crocins offer potential health benefits because of their antioxidant and anti-inflammatory properties. These compounds are present in the flowers and fruits of a few plant species, including saffron, gardenia, *Buddleja* and *Verbascum* species. Saffron extracts have been used for the formulation of functional foods. However, there is no evidence of the use of the other plants producing crocins in the food industry. This study evaluated the effect of the addition of ground dry flowers of two *Verbascum* species, with antioxidant activity, as well as dry fruit powder, from a recently engineered tomato plant producing fruits that accumulate high levels of crocins, as functional ingredients during the processing of rice, wheat cous–cous and maize noodles, providing a yellow pigmentation. Correlation analyses revealed that the increased antioxidant activity in the three food matrices was due to the presence of crocins, which showed no toxicity. Furthermore, *in vitro* digestion showed that crocins were more bioaccessible from rice than from cous–cous or maize noodles, inferring the importance of the food matrix in bio accessibility. The obtained results showed the commercial potential of *Verbascum*'s flowers, as a source of crocins, natural pigments with antioxidant activities.

1. Introduction

Pigments in nature are present in fruits and vegetables, but they are also incorporated into multiple food products for human and animal consumption. The current marketing trend is associated with the use of natural pigments, not just because of the concern of consumers about the safety of artificial food dyes, but also due to the health benefits of these compounds, particularly in the prevention and management of chronic diseases such as obesity, or diabetes, among others.[1] In this regard, studies on carotenoids are particularly extensive.[2]

In general, animals do not produce carotenoids *de novo*, and obtain them through their diet. There are species-specific carotenoids with a high economic value in the food sector such as astaxanthin, canthaxanthin, capsanthin and capsorubin.[3,4] Carotenoids are subjected to enzymatic cleavage reactions, generating apocarotenoids. The best studied pigmented apocarotenoids used in the food industry are bixin and crocin. Crocin is a yellow pigment that contains a variable number of sugar molecules that confer water solubility. Crocin mainly accumulates in the stigmas of *Crocus sativus* L.,[5] and also accumulates, but at lower levels, in other plants.[6] In addition to providing color, crocin exhibits various pharmacological benefits, due to its anti-inflammatory and anti-oxidative properties.[7] The dried stigma of saffron has been widely used in various food products, such as beverages and bakeries as a coloring agent and an aroma, increasing shelf life, and the antioxidant activities.[8]

Verbascum is a widespread genus of the family *Scrophulariaceae*, which comprises at least 325 Eurasiatic species, making it the largest genus in this family. Originating from the Eastern Mediterranean Basin, *Verbascum* has significant applications in traditional medicine.[9] Aerial parts of

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Verbascum species have mucolytic, expectorant, and demulcent properties, and they are used to treat respiratory disorders such as dry coughs, bronchitis, asthma, and tuberculosis. Additionally, these plants are utilized in the treatment of hemorrhoids, rheumatic pain, superficial fungal infections, wounds, and diarrhea.[10] Recently, the content of carotenoids and apocarotenoids was evaluated in the flowers of two *Verbascum* species, *V. sinuatum* and *V. giganteum* (Morote *et al.*, 2023a).[43] Crocins concentration in the flowers of both species are around 11 to 14 mg g^{-1} DW. Both species have a broad distribution in the Iberian Peninsula and grow in two completely different habitats with a preference for dry soils. In addition, they are considered as invasive plants that become naturalized in most temperate regions of the world, where they can be locally abundant on roadsides, old fields, pastures, and other open, disturbed areas. Sites with poor to average soil disturbed by fire, logging, and storms are also ideal. The seeds can remain viable for more than 100 years, making established populations of these species difficult.[11] Furthermore, *V. giganteum* and *V. sinuatum* can tolerate xenobiotic and abiotic stresses well.[12,13]

The combination of all these positive characteristics, positions *Verbascum* as plants with high potential for the exploitation of their crocin content in territories suffering the consequences of climate change. Utilizing crocins, as nutraceuticals for food fortification, not only enhances the nutritional properties of foods but also offers beneficial effects in various digestive diseases.[14] Bearing in mind all these considerations, the objective of this work was to evaluate the coloring and antioxidant capacities of *Verbascum* extracts added to different food matrices, as a sustainable source of crocins.

2. Materials and methods

2.1. Materials

Mize noodles, and cous–cous, were purchased from Comercial Gallo, SA (Barcelona, Spain), and rice was purchase from Arrocerias PONS SA (Valencia, Spain). Chemical reagents used were of analytical and/or HPLC grade, and include methanol, acetonitrile, and trifluoracetic acid, purchased from Sigma (Germany). Standard crocin (CAS no.: 42553-65-1) was purchased from PhytoLab (Germany).

2.2. Collection of plant tissues

Flowers from *Verbascum* species, *V. sinuatum* and *V. giganteum*, were collected from plants growing in the Botanical Garden of Castilla-La Mancha (Albacete, Spain). Immediately after collection, the tissues were placed in a −20° freezer, where they were stored before lyophilization. The dry tissues were ground using a mixer mill MM400 (Retsch GmbH, Haan, Germany) and kept at room temperature. Tomato plants used in this study were originally obtained from conventional breeding conducted between O1_9A (*Solanum lycopersicum* cv. MM) and hp3/Bsh (*S. lycopersicum* cv. M82)[17] F8 seeds were germinated and grown in 30-plug trays containing sterilized soil under controlled light and temperature conditions consisting of 16 h light (200 μE m^{-2} s^{-1}) at 25 °C and 8 h dark at 18 °C at constant 60% relative humidity in a phytotron. Tomato fruits were collected at ripe stage.

2.3. Apocarotenoid analyses

Apocarotenoids extraction from flowers of *Verbascum* species and fruits from tomato plants producing crocins[15] was performed as previously described[16] with modifications. In brief, 1 mL 75% MeOH was added for each 10 mg of dry material, mixed by vortex, and placed in a ultrasound bath for 10 min followed by centrifugation at 10 000*g* for 10 min at 4 °C. The aqueous phases were recuperated and stored at −80 °C until analysis by HPLC. All assays were performed in triplicate. HPLC methodology and crocins analyses have been previously described[15,17] using a Hewlett Packard 1100 HPLC (Palo Alto, CA) equipped with a Sugerlabor Inertsil ODS-2.5 μm C18 column (250 × 4.6 mm), thermostated at 30 °C and connected online to a photodiode array detector, with a range from ultraviolet to visible region (200–600 nm). A gradient elution mode was done using mixtures of 10% acetonitrile in water and 0.1% trifluoroacetic acid (A), 90% acetonitrile in water and 0.1% trifluoroacetic acid (B) and 100% MeOH as mobile phases, flowing at a rate of 1.0 mL min^{-1}. The separation gradient profile was as follows: 87% A and 13% B for 5 min; 87% A to 20% A in 15 min; 100% C for 5 min; and back to the original composition for 6 min. The detection wavelength selected for crocins was 440 nm. Quantitation was conducted with external calibration curves, calculated by lineal regression between the concentration of a crocin (CAS no.: 42553-65-1) standard and the chromatographic peak area. 10 mg of crocin was diluted in 1 mL to realize a stock solution and solutions between 1 and 200 mg L^{-1} (1 : 1000 and 1 : 5) were realized in water/acetonitrile, 90/10, v/v. Each solution was filtered through a 0.2 μm cellulose acetate membrane (VWR international, USA). All the samples and standards were analyzed in triplicate.

2.4. DPPH radical scavenging ability

The antioxidant capacity of the petals extracts was measured using the radical scavenging capacity method with the 2-2, diphenyl-1-picryl-hydrazide (DPPH) stable radical, were carried out basically as previously described.[18] In brief, 5 mg of DPPH was dissolved in EtOH to a final volume of 50 mL. 100 μL of this DPPH solution was added to 100 μL of the extracts (0.05 g in 2 mL 2% v/v in EtOH) and then the same procedure was followed for the dilutions of the extracts. The samples were incubated at room temperature in the dark for 30 min. A calibration curve constructed with DPPH between 0.1 and 2.0 mg L^{-1} was used to calculate the remaining concentration of DPPH in the reaction medium. The absorbance was measured at 515 nm, and the percentage of inhibition was calculated as: %Inhibition = $[(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$, where A_{blank} is the absorbance of the sample containing no extract. The concentration of the sample required for a 50% inhibition of DPPH was determined (IC50), expressed as mg DW of petal extracts per mL of sample. The measures were performed in triplicate.

For the antioxidant activity of the polar extracts obtained from the processed food, the DPPH procedure previously described was used.[15]

2.5. Cooking experiments using *Verbascum* tepals

As a cooking model, 0.05 g DW petals of both *Verbascum* species and 0.05 g DW of tomato fruits (containing 0.15 mg total crocins) were added to the water for cooking long grain white rice, maize noodles and wheat cous–cous as follows: 0.05 g DW of each sample was added to 50 mL water, and polished basmati rice (10 g) was added and boiled for 20 min. For pasta, 0.05 g DW of each sample was added to 50 mL water, and maize noodles (10 g) were added and boiled for 5 min. Four cous–cous cooking, 0.05 g DW of each sample were added to 40 mL water and boiled, the pan was taking off the heat, the cous–cous (15 g) was added to the water, the pan covered, and the cous–cous was allowed to steam for 5 minutes. After cooking, the samples were drained, frozen and subjected to lyophilization.

2.6. Extraction of crocins from cooked materials and HPLC–DAD analysis

The lyophilized material was pulverized using a mill and used for crocins extraction as follows: 5 g of maize noodles and basmati rice, and 7.5 g of cous–cous were extracted in 50 mL 75% MeOH by stirring for 30 min at 20 °C, followed by sonication in a water-bath for 10 min and centrifugation to eliminate solid material. All the extracts were concentrated to dryness by lyophilization. Each extract was dissolved in 1 mL 75% MeOH, and 20 μL were analyzed by HPLC–DAD as previously described.[15]

2.7. Light microscopy

Powders of cooked and dried food samples were dispersed in H_2O and examined (×10 and ×40) under a light microscope (Nikon H600L, DS-Fi1c). Images were taken using a Nikon Digital Sight camera.

2.8. *In vitro* digestion experiments of cooked materials

The digestive process was performed according to the protocol proposed by Minekus *et al.*[19] using 1 g of each sample. All the working solutions were prepared daily from stock solutions. Each experiment was performed in triplicate. Bioaccessibility (%) was calculated as follows: $(A/B) \times 100$. Where A is the total crocin content (μg g^{-1} DW) determined in the supernatant at the end of the *in vitro* digestion and B is the total crocin content in the cooked samples before digestion and expressed in the same units. To estimate the Carotenoid Bioaccessible Content (BCB), which is being currently proposed to designate the actual quantity of carotenoid that is potentially absorbable per weight/volume or a typical food serving, we consider 75 g in the calculation.

2.9. Toxicity test of *Drosophila melanogaster*

The toxicity test was carried out by exposing flies to the *V. sinuatum* and *V. giganteum* petals powder. In the mortality test, twenty adult flies, four days old, were isolated in pots four days old, were isolated in pots containing porridge based on yeast, corn and agar. The final concentration of petals powder was of 1 mg ml^{-1}. This test was repeated four times for each extract. Then, mortality readings were taken at time intervals of 3, 6, 9, 12, 24, 36 and 48 h. A 12 h light/dark cycle and temperature controlled at 25 °C were respected during the tests. To determine if the ingestion of the extracts has any effect on the locomotor system, negative geotaxis tests were carried out in parallel. The glass tube in which the flies were contained was tapped so that the flies were positioned at the bottom of the tube. Then, the number of flies that were able to climb up to the edges to a height of 6 cm was counted. The reading was performed twice for each tube.

2.10. Statistical analysis

All the experiments were performed with triplicate samples. Mean values for each sample were calculated and the results were expressed as mean ± standard error (SE). Statistical analyses were carried out using ANOVA and differences among the individual means were compared by Tukey's *post hoc* test. The significance of differences was set at the $P < 0.05$ level.

3. Results

3.1. Antioxidant properties of the flowers of *Verbascum*

The antioxidant activities of polar extracts of *V. sinuatum* and *V. giganteum* were evaluated by the DPPH assay. The best DPPH radical scavenging ability was shown by the extract of *V. sinuatum* (IC50 = 12.5 ± 0.91 mg mL^{-1}), compared with that of *V. giganteum* (IC50 = 18.65 ± 1.43 mg mL^{-1}). These differences could be linked to the higher levels of crocins present in the extracts of *V. sinuatum* (Fig. S1†). Therefore, *Verbascum* flower extracts presented a potential antioxidant activity that may be related to their crocins content and could be used as new food supplements or to fortify food products, improving their functional properties. To test these possibilities, a cooking approach was designed to study the applications of *Verbascum* flowers as edible flowers with antioxidant properties. Dry flowers of both *Verbascum* species were used to prepare rice, cous–cous, and pasta (Fig. 1).

3.2. Coloring capacity of flower extracts in different foods is associated with the crocin content

Three food products were used in the coloring experiments using dry flowers from the two *Verbascum* species. In addition, we used powder obtained from dry fruits of tomato plants engineered to produce crocins,[15] due to the similar levels of crocins accumulation in these fruits compared with those present in the flowers of *Verbascum*. The use of these extracts increased the coloration in all cooked products to which were added (Fig. 1). The levels of crocins were determined in all the

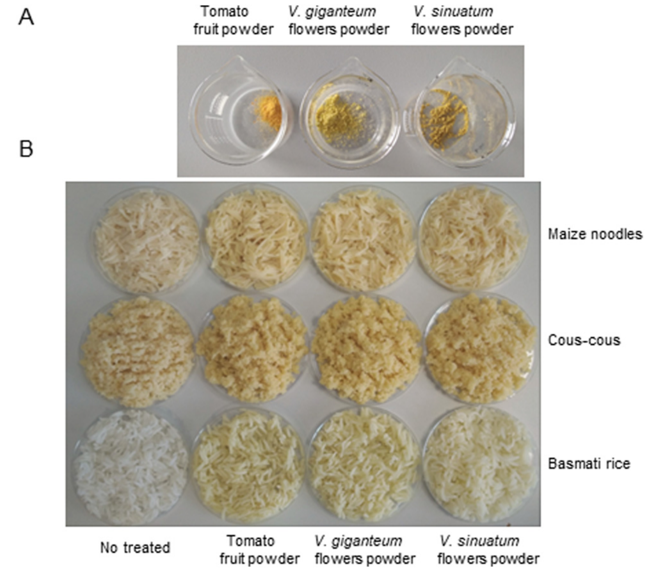

Fig. 1 Pigmented extracts and cooked samples. (A) Aspect of the dry powder directly obtained from tomato fruits, and flowers from *Verbascum*. (B) Cooked matrix in the absence or presence of the different extracts.

cooked samples by HPLC–DAD (Table 1 and Fig. S1†). Interestingly, the crocins profiles in the rice and noodle samples closely resembled those in the original tissue extracts (Fig. S1†). However, the profiles in the cooked cous–cous samples differed, showing fewer resolutive peaks. Notably, cooking time did not appear to be implicated in the observed differences. In fact, couscous was only processed for 5 min, as in the case of maize noodles. Therefore, the observed differences could be related to the composition of the matrix itself because no clear structural differences could be observed at the microscopic level (Fig. S2†). The levels of crocins incorporated in the cooked samples varied, ranging from the 6% for cous–cous and 40% for maize noodles (Table 1). Initially, higher levels were not expected, partly due to the anticipated susceptibility of crocins to thermal and oxidative degradation during cooking. Nevertheless, the crocins that remained in the different food products, retained their ability to impart color (Fig. 1 and Fig. S3†). This not only makes them valuable as natural colorants but also contributes to the health benefits associated with their antioxidant properties.[20,21]

Table 1 Crocins content (μg g^{-1} dry sample) of extracts obtained in the different food samples cooked with 30 mg of crocins

	Crocins μg g^{-1} DW food matrix		
Food samples	Tomato fruit	*V. giganteum*	*V. sinuatum*
Rice	9.86 ± 1.29^{a}	7.64 ± 0.87^{b}	6.46 ± 0.46^{b}
Cous–cous	2.03 ± 0.28^{b}	1.71 ± 0.21^{c}	2.21 ± 0.34^{c}
Maize noodles	8.85 ± 1.53^{a}	11.69 ± 2.03^{a}	12.30 ± 2.11^{a}

All the experiments were performed with triplicate samples. Mean values for each sample were calculated and the results were expressed as mean ± standard error (SE). Values in the same column with different superscripts are significantly different (P < 0.05).

3.3. Antioxidant activity of the cooked samples

The antioxidant properties of crocins have been previously demonstrated.[22] Therefore, the antioxidant activity of the cooked samples was determined in the pigmented food by the potential scavenging of artificial radical DPPH. In this assay, the highest antioxidant capacity was obtained in rice and noodles samples (Fig. 2), while cous–cous samples showed reduced antioxidant activity (Fig. 2). These findings align with the crocins content in the respective samples (Table 1). In fact, according to Pearson correlation, DPPH scavenging ability was significantly correlated with the content of crocins ($r^2 = 0.8342$, $p < 0.05$).

3.4. *In vitro* digestion

This part of the study was focused on the evaluation of the food matrix effect on the release, and stability of crocins during the *in vitro* digestion. To achieve this goal, the crocin-enriched matrices were subjected to *in vitro* digestion, and crocins were quantified in the soluble fractions of the intestinal phase of digestion. The results, expressed as recovery percentages, are presented in Fig. 3. After the *in vitro* digestion process, the proportion of crocins detected was significantly higher in rice samples (58.8% and 49.7%) than in cous–cous and maize-noodles (44 and 45%, respectively). Regarding the crocins from the tomato extract, major bio-accessibility was found in rice, followed by cous–cous and noodles. In the case of the crocins from the extracts of *Verbascum*, the recovery of crocins was reduced compared with the tomato extract, and in rice and noodles, the *V. sinuatum* extracts showed higher crocin recovery (35–45%).

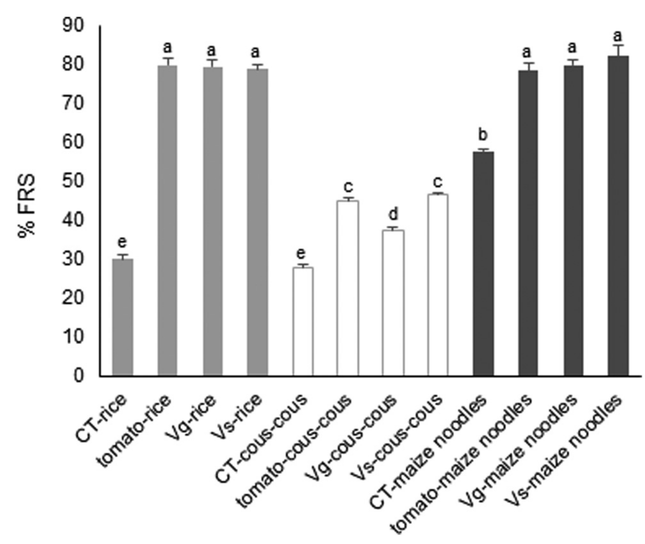

Fig. 2 Antioxidant activity of the different cooked food samples by scavenging of 1.1-diphenyl-2-picrylhydrazyl (DPPH). The results were expressed as a free radical scavenging percentage (% FRS).

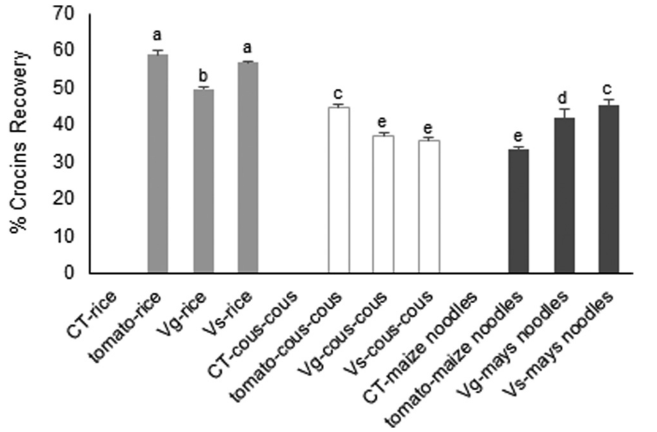

Fig. 3 Percentage of crocins recovered after *in vitro* digestion of the different food samples. The different lowercase indicates significant differences ($P < 0.05$).

Table 2 Bioaccessible crocin content (μg per 75 g) of different cooked food samples

Food sample	Tomato fruit	*V. giganteum*	*V. sinuatum*
Rice	435.00 ± 24.00	285.00 ± 51.00	274.00 ± 38.25
Cous–cous	68.25 ± 27.00	47.35 ± 18.13	60.57 ± 15.37
Maize noodles	222.75 ± 30.75	360.75 ± 39.00	419.25 ± 27.00

All the experiments were performed with triplicate samples. Mean values for each sample were calculated and the results were expressed as mean ± standard error (SE).

The bioaccessible carotenoid content is summarized in Table 2. The intake of approximately one portion of rice or noodles, *i.e.* 75 g, provided more crocins that the intake of any of the cous–cous samples supplemented with any of the plant's extracts. The bioaccessible amount of crocins in the case of the food samples supplemented with the tomato extracts in rice > noodle > cous–cous, and in the food samples supplemented with the extracts from *V. giganteum* and *V. sinuatum*, the bioaccessible amount of crocins in noodles > rice > cous–cous.

3.5. Toxicity test of *Verbascum* flower extracts

The toxicity test on *Drosophila melanogaster* flies is a widely used and viable model for verifying the toxic activities of substances.[23] Toxicity was tested through mortality, which was verified according to the number of fly deaths when they were in contact with 1 mg of extracts per ml of fly food matrix. In the mortality test, the extract had no potential to kill flies (Fig. 4A). From the results presented, we can infer that the tested extracts do not exhibit high toxicity. In addition, we used the negative geotaxis (climbing) assay[44] as a Drosophila health metrics of its locomotor capacity. In this method, flies are gently knocked to the bottom of a vial and are recorded by eye as they instinctively climb upward. With the doses used, we did not observe damage of the locomotor system, corroborating the absence of mortality after 48 h of exposure (Fig. 4B). In addition, the statistical analysis of the negative geotaxis tests showed that there were no significant differences between the groups.

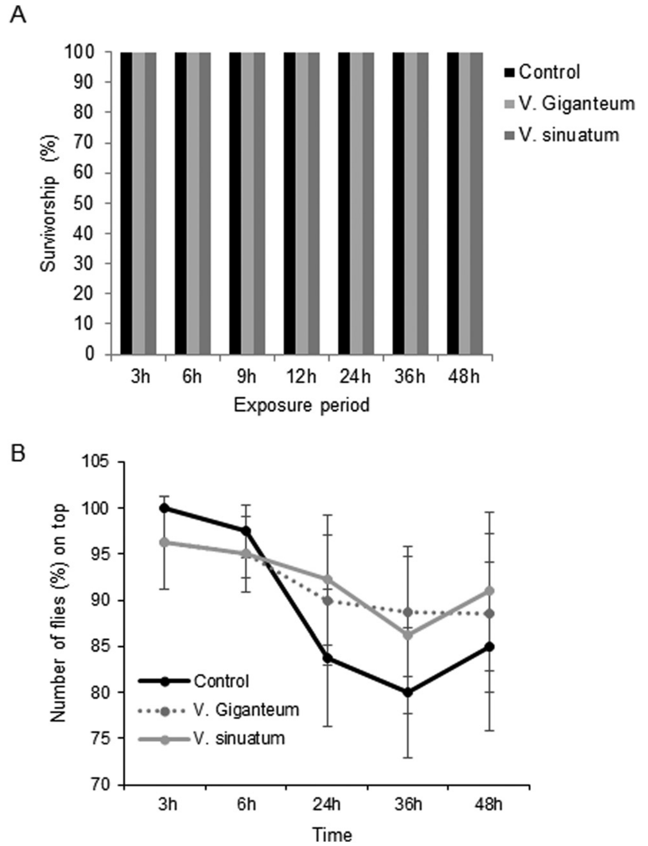

Fig. 4 Toxicity is verified through mortality and the effect of the extract on the flies' locomotor system. (A) Toxicity test caused by the powder obtained from *Verbascum* flowers on *Drosophila melanogaster*. (B) Damage caused by the powder obtained from *Verbascum* flowers to the locomotor apparatus of the flies.

4. Discussion

The demand for natural pigments, which provide health benefits, has significantly increased among consumers due to concerns about the toxicity of synthetic colors.[24,25] Food pigments with health promoting effects such as antioxidant, and anti-inflammatory effects may also be used as an alternative to dietary supplements in terms of safety, consumption, and effectiveness.[26] Crocins are key bioactive compounds that dissolve easily in water and aqueous solutions, providing a distinctive yellow to orange color depending on the concentration used. Besides their coloring capacity, in the last years many studies have demonstrated that crocins are powerful pharmacologically active compounds due mainly to their antioxidant and anti-inflammatory properties.[20]

In various food products, such as bakeries, pasta and beverages, extracts from the stigma of saffron, are widely used as a coloring agent and to provide flavor and aroma,[21,27] as well as used for the development of functional food products.[8] The development of cookies enriched with extracts of the stigma of saffron showed special attributes such as increased shelf life, and high antioxidant activities.[28] In addition, pasta enriched with saffron extracts, exhibit a higher antioxidant activity and low glycemic index due to resistant starch digestibility.[29,30]

In this study, we have tested the pigmentation and antioxidant effects of flower extracts of two *Verbascum* species, and tomato fruits engineered to accumulate crocins, on three food matrices from different cereals. Compared to vegetables and fruits, cereals contain a relatively small quantity of carotenoids, and are almost exclusively concentrated in the grain bran layer. Unfortunately, the common practice of milling results in the loss of these bioactive compounds.[31] We tested rice as being recognized as one of the most sustainable and important foods for a high percentage of the population (**https://www.fao.org/**), and due to the use of different yellow pigments during the cooking of rice in the traditional cuisine of several countries such as Spain, Italy, Iran or India, among others. Another yellow-pigmented dish is cous–cous, a traditional dish of Berber cuisine. Maize noodles were also used because the increasing prevalence of gluten-related disorders has led to a higher consumer demand for gluten-free pasta products.[32]

The addition of the three extracts during the cooking process to the different food matrices allowed the acquisition of a yellowish pigmentation, due to the presence of crocins in the extracted added. Crocins were detected in all cooked samples, with a notable reduction observed in cous–cous samples for all the extracts tested for coloring, indicating that the matrix itself could affect the levels of these metabolites, which can be affected by enzymatic activities. Cous–cous is prepared from durum wheat semolina (*Triticum durum*), and durum wheat varieties showed differences at the level of lipoxygenase activities, which are directly related to the carotenoid content in the derived products.[33,34]

The DPPH scavenging activity of cooked samples increased significantly ($p < 0.01$) with the addition of *Verbascum* and tomato extracts. The observed increase in DPPH scavenging activity may be attributed to the crocins present in the extracts, which have been reported to have potent antioxidant activity.[35] In fact, the antioxidant activity observed in the three food samples was associated with the levels of crocins.

In order to exert the target functionality *in vivo*, the bioactive compounds need to be active after digestion. The fraction of bioactive compounds that are released from the food matrix after digestion and are solubilized for intestinal uptake is usually known as the bioaccessible fraction.[36] In addition, food matrix interactions between the bioactive compounds and macronutrients in foods such as rice or pasta should be considered as potentially affecting the bio-accessibility and bioavailability of the micronutrients and bioactive compounds.[37] Previously, we determined the bio-accessibility of crocins from raw tomato fruits to be 46%.[38] Similar values, 55%, were obtained with aqueous saffron extracts (50 mg L^{-1}).[39,40] In the present study, bio-accessibility values of crocins range from 33–58%, with rice performing as the best food matrix compared with the cous–cous and maize noodles, but the highest content of bioaccessible crocins was obtained for noodles treated with the *Verbascum* extracts and the rice treated with the extract from tomato. The observed differences may be associated with the dietary components of the matrix specifications (*e.g.* amylose content, protein, fibers), which can differentially interact with crocins, as observed before for other secondary metabolites.[41] In addition, the geometries (shape and size of the food particles) that have been shown to affect the diffusivity of the acid of the gastric solutions into the food matrix.[42]

Carotenoids are recognized as safe by several regulatory agencies such as the European Food Safety Authority (EFSA), and the Food and Drug Administration (FDA), with no specific recommendations regarding the intake of these compounds. However, recommendations for dietary carotenoid intake face obstacles. Firstly, carotenoid bioavailability is influenced by various dietary and host-related factors, leading to inter-individual variability in absorption and metabolism, and secondly, there is a scarcity of information on the metabolic conversion of carotenoids (Rodriguez *et al.*, 2018[2]). In addition, their use as colorant additives and functional ingredients in the food industry presents the inconvenient of water insolubility in aqueous formulations.[24] However, such restriction is not presented by crocins, which have a good coloring capacity together with important pharmacological actions.[21] Therefore, plant species that accumulate these apocarotenoids constitute a natural source of soluble pigments with medicinal properties for the food industry.

5. Conclusion

In this study, we have shown that among plant species accumulating crocins, *V. giganteum* and *V. sinuatum* have a considerable potential for producing nutraceuticals intended to be used as food preservatives or functional ingredients in the processing of functional foods. With an increasingly competitive food market, it is essential to obtain relatively cheap raw materials rich in compounds that can have pigment properties and exert bioactive functions. Moreover, new nutraceuticals might be formulated with *Verbascum* flower extracts for the prevention of diseases associated with oxidative stress.

Author contributions

Conceptualization, writing, review and editing: O. A. and L. G.-G. Extracts preparation, cooking experiments and *in vitro* digestion L. M., E. P., C. M., A. S. Metabolites extraction and analyses: L. M., E. P., antioxidant assays: L. M., A. R.-M. Toxicity test: C. M. All the authors have read and approved the manuscript.

Conflicts of interest

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

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