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Crocus sativus L. Cultivation in Alpine Environments: Stigmas and Tepals as Source of Bioactive Compounds

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Received: 31 July 2020; Accepted: 22 September 2020; Published: 25 September 2020

Abstract: Saffron is a well-known spice, produced for a long time, mainly in the Mediterranean area. In the last few years, saffron has been seen as an alternative crop for the diversification of agricultural production and a new source of income, due to its high price, including in Alpine areas. Apart from the spice, constituted by the stigmas, saffron production provides a high amount of fresh tepals, which have so far been considered agricultural waste. Recently, studies on the composition of saffron tepals have been revealing their potential as a rich source of bioactive compounds. In this study, we evaluated the productive traits and the quality of the spice of saffron cultivated in open fields in the north western Italian Alps, for 2 years. In addition, in order to valorize the crop residues, we evaluated the bioactive compound content in tepals by using maceration and ultrasound assisted extraction with different rates of water and methanol as solvents. Higher yields in spice were obtained in the second year of cultivation. However, in both years, the spice had a high quality (ISO 3632), and a very high total phenolic content and antioxidant activity. Thirteen bioactive compounds were identified, including flavonols, cinnamic acids, benzoic acids, catechins, and vitamin C, with few differences between sites and year of cultivation. In tepals, the extraction method and the solvent used influenced the evaluated parameters, i.e., total phenolics and total anthocyanins content, antioxidant activity, and the amount of the four phenolic compounds found (hyperoside, rutin, ellagic acid, and epicatechin). Overall, this study revealed that both saffron spice and its by-product, the fresh tepals, produced in the north western Italian Alps can be considered a source of bioactive compounds with nutraceutical properties, having an antioxidant capacity that is often similar or higher than those of some vegetables and fruits.

Keywords: antioxidant activity; carotenoids; polyphenols; saffron; ultrasound assisted extraction

1. Introduction

Saffron spice consists of dried red stigmas coming from the flowers of *Crocus sativus* L., an autumn-flowering geophyte. Iran, Spain, Morocco, India, Greece, and Italy are the major saffron producing countries in the world. Annual production exceeds 220,000 kg and circa 110,000–165,000 flowers are needed to produce 1 kg of dried stigmas [1,2]. The intensive hand labour required for flower picking and stigma separation make the saffron the world's highest-priced spice, and for this reason named as "red gold" [3].

Saffron flower induction is a complicated mechanism directly related to pedoclimatic conditions and field management [3]. As in most geophyte plants, both seasonal and daily thermoperiodism are

involved as the main environmental factors. Flower induction requires an incubation of the corms at high temperature (23–27 °C), followed by a period of exposure at circa 17 °C for flower emergence. In Mediterranean environments, flower induction occurs from early spring to midsummer, while flower emergence occurs from early- to late-autumn. Flowering is followed by a vegetative stage throughout the winter and formation of replacement corms at the base of shoots. At the end of spring, the leaves reach their highest length, start to senesce, and wither, and the bulbs go into dormancy [4].

In the last ten years, interest in saffron has increased, for using it as an alternative crop for the diversification of agricultural production and as an important, new source of income. Indeed, for many farms, economic diversification has become a keystone for obtaining an adequate income and, consequently, continuing business [5,6]. This trend is particularly evident in mountain areas such as in the north western Italian Alps, where saffron production has been recently started [4–6].

The quality of this merchandise is closely related to the concentration of three main components: crocin, picrocrocin, and safranal, which provide, respectively, the unique color, bitter taste, and aroma [4,7–9]. The concentration of these constituents concurs to determine the saffron quality, as defined by the International Organization for Standardization (ISO) [10]. The usage of this spice as a natural ingredient in food products is a commercial fact. In addition, saffron has long been considered a medicinal plant for its therapeutic properties. Recently, studies related to the quality of saffron have revealed the properties of several compounds present in the spice and their positive influence on human health. Phenolic and anthocyanin contents of plant material play a key role in preventing oxidative damage caused by free radicals, which are both responsible for degradation of dietary lipids, hence of nutritional value, and the cause for various human diseases [11]. Saffron active constituents, such as carotenoids (i.e., crocins), polyphenols, and vitamins, show significant antioxidant activity and could enhance the memory capability, and have antitumor and cancer-preventive properties [2,12–15].

Currently, agricultural residues, such as citrus peel [16] and cocoa husk [17], are the focus of research because they may contain large amounts of bioactive compounds that are beneficial to human health and can be exploited instead of being wasted [18]. Recently, there have been some efforts for the extraction of bioactive components not only from stigmas but also from tepals of *C. sativus* [19]. Tepals, compared with stigmas, are inexpensive; to harvest 1 kg of stigmas, around 350 kg of tepals are unused and thrown away as waste. However, they could be considered a high-quality by-product and a rich source of bioactive compounds, such as flavonoids, particularly anthocyanins [2,19–21]. Although flowers have been used in food traditions during the centuries, nowadays they represent a source of nutrients and phytochemicals with health benefits [22–24]. According to recent medical studies, saffron tepals can be used to treat depression [25] and had antinociceptive, anti-inflammatory, and antioxidant activities [26]. Saffron tepals can thus represent a significant cheap source of bioactive compounds for the development of potential functional foods and cosmetic formulations [27–29], as well as being used as a fertilizer for the soil or as ornamentation of dishes/products based on saffron [21,30].

In recent years, "green" techniques have been developed for obtaining natural extracts [31] to avoid the problems encountered when conventional methods are used (i.e., high energy and time consumption, use of chemical solvents) [18]. Ultrasound assisted (UA) extraction is considered as an "environmentally friendly" or "green technique" compared to classical maceration [32,33] because it consumes less fossil energy, is more effective, and also allows the reduction of solvent use, resulting in higher yields in a shorter extraction time [18]. The selection of a suitable solvent is crucial to improve the extraction efficiency. Water and organic solvents, such as methanol, are the most commonly used [34]. Nevertheless, water is only effective as an extraction solvent for polar compounds, while organic solvents are efficient to extract polar and weak polar compounds [35]. However, their toxicity, environmental hazardous, high cost, and low biodegradability extremely limit their applications. Thus, the research for sustainable and safe alternatives for replacing toxic organic solvents without compromising efficiency is of utmost importance. Aqueous methanol or water solutions are already used for the extraction of many bioactive constituents from saffron stigmas [4,9,36]. However, very

little information is available on the most environmentally safe and appropriate extraction method to obtain biomolecules for industrial purposes from saffron tepals [37,38].

This study aimed at first assessing the productive traits and the quality of the spice of saffron cultivated in Alpine environmental conditions. Then, in order to valorise the crop residues, green extractions using UA extraction with different rates of water and methanol as solvents were performed to extract bioactive compounds from tepals.

2. Materials and Methods

2.1. Plant Material and Site Characteristics

Crocus sativus corms (caliber size of 6–7 cm), kindly provided by the Azienda Agricola "La Branche di Diego Bovard" (Morgex, AO, Italy) were planted in August 2016 in three experimental sites of the Italian Western Alps, located in the municipalities of Chambave (45°45′65.1″ N; 7°02′18.1″ E; 560 m a.s.l.), Saint Cristophe (45°45′06.9″ N; 7°20′37.0″ E; 700 m a.s.l.), and Morgex (45°45′35.1″ N; 7°02′37.3″ E; 1000 m a.s.l.). In Table 1 are listed the physical and chemical properties of each site. The cultivation lasted two cycles (2016–2017 and 2017–2018). Nine experimental plot units (replications) per site were used. Each plot unit consisted of 56 corms, planted in a 1.44 m^2 (39 corms m^{-2}) area. Intra-row planting distance was 7 cm, while between-row distance was 25 cm. Plots were separated from each other with at least 4 m distance. In all the three sites, manual irrigation and weed control were performed during cultivation, while no treatments against fungal pathogens and pesticides were applied.

Table 1. Physical and chemical properties of the soils collected in the three saffron experimental fields located in the municipalities of Chambave, Saint Cristophe, and Morgex (AO, Italy).

		Chambave	S. Christophe	Morgex
Texture	Clay (%)	5.0	3.7	3.8
	Fine Silt (%)	18.0	21.8	21.0
	Coarse Silt (%)	21.6	20.2	14.4
	Fine Sand (%)	23.9	24.3	25.0
	Coarse Sand (%)	31.6	29.9	35.8
Bulk density (g L^{-1})		n.d.	1123.7	1075.6
Moisture (%)		13.7	17.3	20.2
P Olsen (mg Kg^{-1})		16.9	69.2	113.0
pH		6.7	6.9	7.4
Electrical conductivity (µS/cm^{-1})		162	316	243
N tot (%)		0.14	0.31	0.23
C tot (%)		1.88	3.50	3.79
Cation-exchange capacity (meq 100 g^{-1})		13.9	19.2	15.7
Exchangeable Ca (meq 100 g^{-1})		11.15	17.31	16.61
Exchangeable K (meq 100 g^{-1})		0.47	1.47	0.53
Exchangeable Mg (meq 100 g^{-1})		1.23	1.90	0.76

2.2. Site Environmental Conditions

In the first cultivation season (2016–2017), average temperatures ranged from −0.3 °C to 21.5 °C in Chambave (Figure 1a), from −3.5 °C to 23.4 °C in Saint Cristophe (Figure 1b), and from −2.7 °C to 20.0 °C in Morgex (Figure 1c). Overall, Morgex resulted in being the site with the superior precipitation rate (57.5 mm $month^{-1}$) and highest relative humidity (R.H.), with the peak in November (89.3%). Conversely, Saint Cristophe was the driest site. The total radiation was generally lower in Chambave than in the other locations, with the highest peak in April (218 KJm^{-2}). In the second cultivation season (2017–2018), average temperatures, ranged from 0.9 °C to 21.0 °C in Chambave, from −2.3 °C to 22.6 °C in Saint Cristophe, and from −1.2 °C to 19.5 °C in Morgex, and weather conditions were, in general, more wet with more rainfall. In particular, the highest precipitation rate and R.H. were measured in Morgex (74.5 mm/month and 84.0%, respectively).

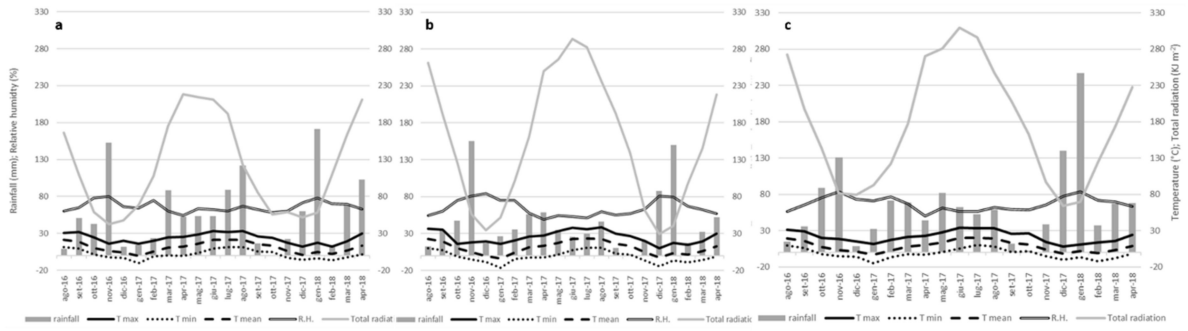

Figure 1. Climatic conditions of the *Crocus sativus* experimental sites located in the municipalities of Chambave (**a**), Saint Christophe (**b**), and Morgex (**c**) (AO, Italy).

2.3. Plant Performance and Saffron Yield

At flowering (November 2016 and 2017), the daily number of picked flowers per corm and the yield per m^2 in spice (i.e., stigmas dried at 40 °C for 8 h in oven) were measured. At the end of the vegetative period (May 2017 and 2018), the wilted rate, the shoot size, the leaf length, and the relative quantity of chlorophyll (Chlorophyll Meter SPAD-502, Konica Minolta Sensing Inc., Osaka, Japan) were measured in 20 randomly selected plants per plot. Then, leaves were cut and oven-dried at 65 °C for one week and the dry biomass was recorded. At the same time point, 20 plants per plot were lifted, corms rid of topsoil, cleaned, and de-tunicated, then the number, the size, and the weight of replacement corms were determined.

2.4. Stigmas Extract Preparation and Determination of Quality by ISO 3632

The saffron aqueous extracts were prepared according to Caser et al. [4]. Briefly, 50 mg of powdered dried saffron were suspended into 5 mL of deionised water. After stirring (1000 rpm) for 1 h at room temperature (circa 21 °C) in the dark, the solution was filtered with polytetrafluoroethylene (PTFE, VWR International, Milano, Italy) filters, with a 25 mm diameter and 0.45 µm pore size. The saffron extract was then diluted 1:10 with deionised water to obtain the working solution for future analyses. Each sample was prepared in triplicate.

Saffron aqueous extracts were analyzed with a spectrophotometer (Ultrospec 2100 Pro, GE Healthcare, Chicago, IL, USA) to determine crocin, picrocrocin, and safranal content, which provide respectively the unique color, bitter taste, and aroma. Data were related to the dry matter percentage and expressed as the absorbance of a 1% aqueous solution of dried saffron at 257, 330, and 440 nm respectively, using a 1 cm pathway quartz cell (*A1% 1 cm (λ max)*) and calculated according to the following formula:

$$A1\%\ 1\ cm\ (\lambda\ max) = D \times 10000/m \times (100 - wMV)$$

where D is the specific absorbance, m is the mass of the evaluated solution in grams, and wMV is the moisture expressed as a percentage mass fraction of the sample. Moisture content *(wMV)* was determined using the following formula:

$$wMV = (m0 - m1) \times (100/m0)\%$$

where *m0* is the mass, in grams, of the saffron portion before drying, and *m1* is the mass, in grams, of the dry residue after incubation, performed in an oven for 16 h at 103 ± 2 °C. All analytical steps were conducted in the dark to prevent analyte degradation. Stigma extracts were maintained at −20 °C for further analyses.

2.5. Tepal Extract Preparation

Fresh tepals picked in the second growing season, in the three experimental fields previously described, were mixed and grinded in liquid nitrogen. Then, 1 g was put into a glass tube with 25 mL of extractive solution. Four different extractive solutions were used: (1) deionised water; (2) deionised water:methanol (80:20 *v*/*v*); (3) deionised water:methanol (50:50 *v*/*v*); (4) deionised water:methanol (20:80 *v*/*v*). Two different extraction procedures were followed: (1) the glass tubes were put into the ultrasound extractor (23 kHz; Reus sarl, Drap, France) for 15 min at room temperature (UA method), or (2) maceration in the dark for 1 h at room temperature (circa 21 °C, M method). Three repetitions were carried out for each extractive solution and extraction procedure. Each extract obtained was filtered through paper filters (Whatman filter papers No. 1, Whatman, Maidstone, UK) and then with polytetrafluoroethylene (PTFE, VWR International, Milano, Italy) filters of 25 mm diameter and 0.45 µm pore size. Samples were maintained at −20 °C for the following analyses.

2.6. Bioactive Compounds

2.6.1. Total Polyphenols

The total phenolic content was determined following the Folin–Ciocalteu method, as indicated by Caser et al. [39]. The analysis was performed as follows: 1000 µL of diluted (1:10) Folin reagent was mixed with 200 µL of phytoextract in each plastic tube. The samples were left in the dark at room temperature for 10 min, then 800 µL of Na_2CO_3 (7.5%) was added to each tube. Samples were left in the dark at room temperature for 30 min. Absorbance was then measured at 765 nm by means of a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA), and the results were expressed in milligrams of gallic acid equivalents per 100 g (mg GAE 100 g^{-1}). In the whole manuscript, results were expressed as mg 100 g^{-1} of dry weight (DW) for stigmas and mg 100 g^{-1} of fresh weight (FW) for tepals.

2.6.2. Total Anthocyanins

The total anthocyanin content in the extracts was determined through the pH-differential method [40]. The analysis was performed as follows: 1 mL of phytoextract was put into a 10 mL flask, and then made up to volume with a buffer solution at pH 1 (4.026 g KCl + 12.45 mL HCl 37% in a 1 L water volume). The same was made in a second flask with a buffer solution at pH 4.5 (32.82 g $C_2H_3NaO_2$ + 18 mL $C_2H_4O_2$ in a 1 L water volume). Samples were put in the dark at room temperature for 20 min. Absorbance of both flasks was measured at 515 nm and 700 nm by means of a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA), and the results were expressed in milligrams of cyanidin-3-O-glucoside equivalents per 100 g (mg C3GE 100 g^{-1}). In the whole manuscript, results were expressed as mg 100 g^{-1} of dry weight (DW) for stigmas and mg 100 g^{-1} of fresh weight (FW) for tepals.

2.6.3. Antioxidant Activity

DPPH Assay

The first procedure adopted was the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method [41]. The working solution of DPPH radical cation ($DPPH^{\cdot}$, 100 µM) was obtained by dissolving 2 mg of DPPH in 50 mL of MeOH. The solution must have an absorbance of 1.000 (±0.05) at 515 nm. To prepare the samples, 40 µL of phytoextract was mixed with 3 mL of $DPPH^{\cdot}$. Samples were then left in the dark at room temperature for 30 min. Absorbance was measured at 515 nm by means of a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA). The DPPH radical-scavenging activity was calculated as:

$$[(Abs0 - Abs1/Abs0) \times 100]$$

where *Abs0* is the absorbance of the control (solution without phytoextract) and *Abs1* is the absorbance of the sample. The antioxidant capacity was plotted against a Trolox calibration curve and results were expressed as μmol of Trolox equivalents per gram (μmol TE g^{-1}). In the whole manuscript, results were expressed as μmol TE g^{-1} of dry weight (DW) for stigmas and μmol TE g^{-1} of fresh weight (FW) for tepals.

ABTS Assay

The second procedure adopted was the 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method [42]. The working solution of ABTS radical cation ($ABTS^{\cdot}$) was obtained by the reaction of 7.0 mM ABTS stock solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) solution. After the incubation for 12–16 h in the dark at room temperature, the working solution was diluted with distilled water to obtain an absorbance of 0.70 (±0.02) at 734 nm. The antioxidant activity was assessed mixing 30 μL of phytoextract with 2 mL of diluted $ABTS^{\cdot}$. Samples were left in the dark at room temperature for 10 min. Absorbance was then measured at 734 nm by means of a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA). The ABTS radical-scavenging activity was calculated as:

$$[(Abs0 - Abs1/Abs0) \times 100]$$

where *Abs0* is the absorbance of the control (solution without phytoextract) and *Abs1* is the absorbance of the sample. The antioxidant activity was plotted against a Trolox calibration curve and results were expressed as μmol of Trolox equivalents per gram (μmol TE g^{-1}). In the whole manuscript, results were expressed as μmol TE g^{-1} of dry weight (DW) for stigmas, and μmol TE g^{-1} of fresh weight (FW) for tepals.

FRAP Assay

The third procedure was the ferric ion reducing antioxidant power (FRAP) method [43]. The FRAP solution was obtained by mixing a buffer solution at pH 3.6 ($C_2H_3NaO_2$ + $C_2H_4O_2$ in water), 2,4,6-tripyridyltriazine (TPTZ, 10 mM in HCl 40 mM), and $FeCl_3 \cdot 6H_2O$ (20 mM). The antioxidant activity was determined mixing 30 μL of phytoextract with 90 μL of deionised water and 900 μL of FRAP reagent. The samples were then placed at 37 °C for 30 min. Absorbance was measured at 595 nm by means of a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA). The antioxidant activity was plotted against a $FeSO_4 \cdot 7H_2O$ calibration curve. Solution without phytoextract was used as a control sample. Results were expressed as millimoles of ferrous iron equivalents per kilogram (mmol Fe^{2+} kg^{-1}). In the whole manuscript, results were expressed as mmol Fe^{2+} kg^{-1} of dry weight (DW) for stigmas and mmol Fe^{2+} kg^{-1} of fresh weight (FW) for tepals.

2.6.4. Identification and Quantification of Bioactive Compounds by HPLC

The bioactive compounds contained in the spice and tepal extracts were determined by means of four high performance liquid chromatography-diode array detection (HPLC–DAD) methods [4], using an Agilent 1200 High-Performance Liquid Chromatograph coupled to an Agilent UV-Vis diode array detector (Agilent Technologies, Santa Clara, CA, USA). Detailed chromatographic methods are reported in Table 2. Phytochemical separation was achieved with a Kinetex C18 column (4.6 × 150 mm, 5 μm, Phenomenex, Torrance, CA, USA), using several mobile phases for compound identification and recording of UV spectra at different wavelengths, based on HPLC methods, as previously tested and validated [9,44], with some modifications. UV spectra were recorded at 330 nm, 280 nm, 310 and 441 nm, and 261 and 348 nm. The following bioactive compounds were determined: phenolic acids (cinnamic acids–caffeic, chlorogenic, coumaric, and ferulic acid; benzoic acids–ellagic and gallic acid); flavonols (hyperoside, isoquercitrin, quercetin, quercitrin, and rutin); flavanols (catechin and epicatechin); vitamin C (as the sum of ascorbic and dehydroascorbic acid); carotenoids (safranal and crocins). All single compounds were identified by a comparison and combination of their retention

times and UV spectra with those of authentic standards under the same chromatographic conditions. Results were expressed as mg 100 g^{-1} of dry weight (DW) for stigmas and mg 100 g^{-1} of fresh weight (FW) for tepals.

Table 2. HPLC methods and relative chromatographic conditions.

Method	Classes of Interest	Stationary Phase	Mobile Phase	Wavelength (nm)
A	cinnamic acids, flavonols	KINETEX—C18 column (4.6 × 150 mm, 5 µm)	A: 10 mM KH_2PO_4/H_3PO_4, pH = 2.8 B: CH_3CN	330
B	benzoic acids, catechins	KINETEX—C18 column (4.6 × 150 mm, 5 µm)	A: H_2O/CH_3OH/HCOOH (5:95:0.1 *v/v/v*), pH = 2.5 B: CH_3OH/HCOOH (100:0.1 *v/v*)	280
C	vitamins	KINETEX—C18 column (4.6 × 150 mm, 5 µm)	A: 5 mM $C_{16}H_{33}N(CH_3)_3Br$/50 mM KH_2PO_4, pH = 2.5 B: CH_3OH	261, 348
D	carotenoids	KINETEX—C18 column (4.6 × 150 mm, 5 µm)	A: H_2O B: CH_3CN	310, 441

Elution conditions. Method A, gradient analysis: 5% B to 21% B in 17 min + 21% B in 3 min (2 min conditioning time); flow: 1.5 mL min^{-1}; Method B, gradient analysis: 3% B to 85% B in 22 min + 85% B in 1 min (2 min conditioning time); flow: 0.6 mL min^{-1}; Method C, isocratic analysis: ratio of phase A and B: 95:5 in 10 min (5 min conditioning time); flow: 0.9 mL min^{-1}; Method D, gradient analysis: 5% B to 95% B in 30 min + 95% B to 5% B in 5 min (10 min conditioning time); flow: 0.6 mL min^{-1}.

2.7. Chemicals and Reagents

Sodium carbonate, Folin–Ciocalteu phenol reagent, sodium acetate, citric acid, hydrochloric acid, iron(III) chloride hexahydrate, 2,4,6-tripyridyl-S-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium chloride, potassium persulfate, safranal, and 1,2-phenylenediamine dihydrochloride (OPDA) were purchased from Sigma Aldrich (St. Louis, MO, USA), whereas acetic acid was purchased from Fluka Biochemika (Buchs, Switzerland). Ethylenediaminetetraacetic acid (EDTA) disodium salt was purchased from AMRESCO (Solon, OH, USA), whereas sodium fluoride was purchased from Riedel-de Haen (Seelze, Germany). Ethanol, acetone, and sodium citrate were purchased from Fluka Biochemika. Analytic HPLC grade solvents, methanol/acetonitrile and formic acid were purchased from Sigma Aldrich and Fluka Biochemika, respectively; potassium dihydrogen phosphate, ammonium dihydrogen phosphate, and phosphoric acid were also purchased from Sigma Aldrich. Milli-Q ultrapure water was produced by Sartorius Stedium Biotech mod. Arium (Sartorius, Goettingen, Germany). Cetyltrimethylammonium bromide (cetrimide) was purchased from Extrasynthése (Genay, France). Crocin I and crocin II were purchased from Phytolab. All the polyphenolic standards were purchased from Sigma Aldrich, whereas ascorbic acid (AA) and dehydroascorbic acid (DHAA) were purchased from Extrasynthése.

2.8. Statistical Analysis

An arcsin transformation was performed on all percentage incidence data before statistical analysis in order to improve the homogeneity of the variance (Levene test, $p < 0.05$). All the analyzed data were checked for the normality of variance (Shapiro test, $p > 0.05$). For all the analysed parameters, mean differences were computed using a one-way or two-way ANOVA with a Tukey post hoc test ($p \leq 0.05$). Mean differences of the three sites over the two years were computed by "between-subjects effects" tests. All these analyses were performed with SPSS 25.0 Inc. software (USA). Principal coordinate analysis (PCA)–biplot was performed using PAST 3.20. Eigenvalues were calculated using a covariance matrix among 30 traits as input, and the two-dimensional PCA biplot (including both altitude, morphological, productive, and biochemical constituents) was constructed. Pearson correlations among methanol

content in the extractive solutions and the studied parameters in fresh saffron tepals were computed by using PAST 3.20.

3. Results

3.1. Stigma Yield and Growing Performance

Environment and growing season affected stigma yield, flower production, and plant growth (Table 3). In particular, moldy corms (wilted) occurred more in the first cultivation season (36.8%) than in the second (16.8%), and more in the field of Morgex (52.4%) than at Saint Cristophe (39.1%). The elevated percentage of wilted corms was probably due to the absence of corm antifungal treatments and to the high relative humidity and precipitation rate (more than 550 mm year^{-1}), mainly occurring in Morgex (Figure 1c). Even if a characterization of plant pathogens was not conducted, the high wilting rate could be favored by *Fusarium* species transmission as suggested by Di Primo et al. [45]. In fact, the optimal environmental conditions for saffron are: (1) a dry season (April through June), in which the leaves senesce and wither, and corms enter into dormancy, and (2) annual rainfall less than 400 mm [46,47]. Recently, Dastranj et al. [48], on the base of a dataset from an eight-year open field saffron experiment conducted in Iran, showed that saffron yield cannot be predicted with adequate accuracy by using the annual rainfall. Instead, these authors concluded that a combination of saffron age, mean and maximum daily air temperature in the given season, and fall and winter weather characteristics can be used to predict the saffron yield. However, based on these observations, in the Alpine environment, antifungal treatments would be recommended to protect against disease agents, as already suggested by Gresta et al. [49].

Flower yield is a difficult parameter to forecast in saffron, as it is influenced by a combination of agronomic, biological, and environmental factors [50]. In our two-year-long experiment, plants produced more flowers per square meter and flower per corm in the second cultivation cycle in all the sites (66.4 vs. 28.8 flowers m^{-2} and 3.8 and 1.2 flower corm^{-1}) (Table 3a). Furthermore, the environment influenced the flower yield, which was significantly higher in Saint Cristophe and Chambave with respect to Morgex (58.6, 53.6, and 30.7 flowers m^{-2}, respectively) (Table 3b). The stigma yield was highest in the second year (with a mean of 0.48 g m^{-2}) (Table 3a) and in the fields of Saint Cristophe and Chambave (0.40 and 0.35 g m^{-2}, respectively) (Table 3b). Generally, a saffron field may produce 0.2 to 3.0 g m^{-2} of spice, depending on the abovementioned factors [4,50] and obviously by the planting density, which may vary considerably.

At similar density conditions (39 corms m^{-2}), Mzabri et al. [51] in Eastern Moroccan areas obtained similar saffron yields (0.37 g m^{-2}). By planting at a 55 corms m^{-2} density in Southern Italy (Sicily), Gresta et al. [49] obtained more than 1.2 g m^{-2}. While, in the area of Navelli (Italy) [46], with a similar corm density, the average yield ranged between 1.0 and 1.6 g m^{-2}. In Iranian fields with a density of 150 and 100 corms m^{-2}, Mollafilabi et al. [52] and Koocheki et al. [53] obtained 0.74 and 0.37 g m^{-2} of saffron, respectively. Regarding the amount of saffron per flower, our data resulted in agreement with Gresta et al. [50], ranging between 6 and 7 mg per flower, but was much superior to different provinces of Iran (3.6–4.3 mg per flower) [54].

The corm size (ranging between 20.47 and 25.99 mm) and the number of replacement corms (ranging between 1.67 and 3.67) were not affected either by the year or the environment (Table 3a). Conversely, the corm weight was higher in Chambave than in Saint Cristophe (6.65 and 5.95 g, respectively) (Table 3b). Koocheki et al. [53] in Iran found that the number of replacement corms ranged between one and three, depending on agronomic practices. Gresta et al. [49] in south Italy (Sicily) found a superior result (i.e., more than five replacement corms per corm). Data about the leaf length, SPAD value, and shoot size showed a decrease from the first to the second year. Probably these parameters were negatively affected by the higher relative humidity and precipitation rate registered in 2018, particularly in Morgex.

Table 3. Effects of cultivation season (Year 1, Y1, and Year 2, Y2, a.) and experimental site (Morgex, M, Saint Cristophe, SC, and Chambave, C, b.) on saffron plant growth and performance traits. Data in Table 3a are presented as mean ± standard deviation for each site in each year and statistical comparisons were conducted among mean values of Y1 and Y2.

a.	**Values**								
Traits	**Year 1**			**Year 2**			**Mean Values**		
	M	**SC**	**C**	**M**	**SC**	**C**	**Y1**	**Y2**	***p***
Wilting rate (%)	40.70 ± 5.50	30.90 ± 9.10	38.90 ± 13.40	23.40 ± 6.73	16.50 ± 5.86	10.50 ± 6.82	36.80 ± 9.68	16.80 ± 5.67	***
Flower (n m^{-2})	23.12 ± 16.97	39.11 ± 5.20	24.30 ± 10.90	38.19 ± 3.18	78.00 ± 23.91	82.86 ± 23.09	28.84 ± 18.66	66.35 ± 17.64	***
Flower/corm (n)	1.10 ± 0.86	1.51 ± 0.12	1.00 ± 0.22	2.90 ± 0.15	4.10 ± 1.31	4.50 ± 0.92	1.20 ± 0.51	3.83 ± 1.08	***
Stigma yield (g m^{-2})	0.13 ± 0.09	0.20 ± 0.03	0.11 ± 0.04	0.28 ± 0.02	0.60 ± 0.18	0.56 ± 0.11	0.15 ± 0.04	0.48 ± 0.11	***
Stigma/flower (mg)	6.00 ± 0.42	5.20 ± 0.53	5.20 ± 1.22	7.40 ± 1.30	7.60 ± 0.61	6.80 ± 0.62	5.46 ± 1.13	7.23 ± 0.75	***
Leaf length (cm)	36.39 ± 2.73	37.33 ± 3.87	43.44 ± 2.03	20.73 ± 2.25	24.77 ± 0.05	30.80 ± 0.57	39.06 ± 5.76	24.09 ± 4.98	***
SPAD unit	74.66 ± 6.00	75.91 ± 6.04	74.72 ± 3.20	33.33 ± 10.68	54.97 ± 4.47	49.64 ± 4.94	75.09 ± 12.53	45.98 ± 10.28	***
Shoot size (mm)	5.13 ± 1.51	5.07 ± 0.33	5.10 ± 0.25	3.41 ± 1.01	3.70 ± 0.77	5.40 ± 0.46	5.10 ± 0.13	4.17 ± 0.28	**
Corm size (mm)	20.47 ± 4.42	20.76 ± 3.58	20.62 ± 3.87	26.01 ± 3.63	21.49 ± 3.83	25.99 ± 1.45	20.62 ± 3.60	24.50 ± 3.43	ns
Replacement corm/corm (n)	1.67 ± 0.57	2.50 ± 1.32	2.08 ± 0.94	2.67 ± 2.08	3.67 ± 2.88	2.33 ± 1.20	2.08 ± 1.02	2.89 ± 1.97	ns
Corm weight (g)	6.84 ± 2.55	6.29 ± 2.22	6.57 ± 2.34	6.25 ± 0.25	5.63 ± 0.44	10.65 ± 1.76	6.57 ± 2.16	7.51 ± 2.54	ns

b.				
Traits	**Morgex**	**Saint Cristophe**	**Chambave**	***p***
Wilting rate (%)	52.42 ± 8.32 ^{a}	39.15 ± 5.75 ^{b}	44.15 ± 7.25 ^{a,b}	***
Flower (n m^{-2})	30.66 ± 14.76 ^{b}	58.56 ± 10.42 ^{a}	53.58 ± 8.68 ^{a}	**
Flower/corm (n)	2.00 ± 1.15	2.81 ± 1.65	2.80 ± 2.02	ns
Stigma yield (g m^{-2})	0.21 ± 0.05 ^{b}	0.40 ± 0.10 ^{a}	0.35 ± 0.09 ^{a}	**
Stigma/flower (mg)	6.71 ± 1.15	6.36 ± 1.41	6.06 ± 1.24	ns
Leaf length (cm)	28.56 ± 2.15 ^{b}	31.05 ± 3.09 ^{a}	30.83 ± 1.86 ^{a}	***
SPAD unit	53.90 ± 5.82 ^{b}	65.40 ± 4.98 ^{a}	62.10 ± 3.79 ^{a}	**
Shoot size (mm)	4.27 ± 1.49	4.39 ± 0.91	5.40 ± 0.46	ns
Corm size (mm)	23.24 ± 4.72	21.12 ± 3.14	25.99 ± 1.95	ns
Replacement corm/corm (n)	2.17 ± 1.47	3.08 ± 2.10	2.33 ± 1.20	ns
Corm weight (g)	6.54 ± 0.78 ^{a,b}	5.95 ± 1.10 ^{b}	6.65 ± 0.56 ^{a}	**

Same letter denotes no significant differences according to Tukey post-hoc test ($p < 0.05$). The statistical relevance is provided (** $p < 0.01$; *** $p < 0.001$). The statistical relevance of "between-subjects effects" tests for Y1 and Y2 is provided (** = $p < 0.01$; *** = $p < 0.001$).

3.2. Quality Traits of Stigmas Produced in the Alpine Environment

Few spices are able to provide the combination of color, taste, and aroma to foods, and possess several nutraceutical properties for human health, like saffron [4]. In the present study, spectrophotometric and chromatographic methods were used to determine spice quality and bioactive compound content of the saffron produced in the three sites located in the north-west Italian Alps (Table 4).

Generally, the quality of the spice is related to the content of crocins, picrocrocin, and safranal [2,13,36]. These compounds were determined spectrophotometrically following ISO 3632 [10], which defines three categories of quality, with category I (ISO 3632 [10] limits are: crocins >200, picrocrocin >70, and safranal 20–50) as the best. Saffron quality may vary greatly from country to country, on the basis of several factors, among which are climatic conditions [55]. In our study, the saffron produced in all the three experimental sites belonged to the quality category I, and this was

true for both cultivation cycles (Table 4a). This is mostly in line with the four-year (2015–2018) research period conducted by Giupponi et al. [56], in which the quality of saffron produced in 484 farms located in different regions of Italy was generally of the first quality category according to ISO 3632 [10].

Table 4. Effects of cultivation season (Year 1, Y1 and Year 2, Y2; a.) and experimental site (Morgex, M, Saint Cristophe, SC, and Chambave, C; b.) on the mean values of bioactive compounds and International Organization for Standardization (ISO) 3632 [10] traits on the studied saffron. Data in Table 3a are presented as mean ± standard deviation for each site in each year and statistical comparisons were conducted among mean values of Y1 and Y2.

a.	**Values**								
	Year 1			**Year 2**			**Mean values**		
ISO 3632 [10] traits (E1% abs)	**M**	**SC**	**C**	**M**	**SC**	**C**	**Y1**	**Y2**	***p***
Flavour/Picrocrocin	130.60 ± 5.02	129.10 ± 7.18	140.90 ± 3.53	136.50 ± 0.55	135.70 ± 9.32	129.90 ± 4.89	133.5 ± 7.27	134.00 ± 6.11	ns
Aroma/Safranal	34.70 ± 3.06	35.90 ± 6.22	44.60 ± 3.67	47.30 ± 8.18	45.10 ± 0.47	40.60 ± 6.25	38.40 ± 3.14	44.40 ± 2.65	**
Colouring/Crocins	207.60 ± 3.66	204.10 ± 8.83	198.00 ± 5.69	390.30 ± 73.95	334.10 ± 34.57	420.30 ± 76.53	207.10 ± 56.72	368.50 ± 43.98	***
Bioactive compounds (mg 100 g^{-1} DW)									
Coumaric acid	23.57 ± 0.15	23.66 ± 0.18	23.39 ± 0,23	23.76 ± 0.02	23.60 ± 0.23	23.34 ± 0.33	23.54 ± 0.20	23.57 ± 0.27	ns
Isoquercitrin	2.58 ± 0.02	2.53 ± 0.03	2.53 ± 0.02	2.40 ± 0.19	2.57 ± 0.02	2.43 ± 0.18	2.55 ± 0.33	2.47 ± 0.15	ns
Quercitrin	25.68 ± 5.60	19.47 ± 9.73	18.68 ± 3.88	16.50 ± 3.41	13.79 ± 2.36	18.61 ± 2.82	21.28 ± 2.65	16.30 ± 3.12	**
Gallic acid	5.63 ± 0.07	4.92 ± 0.57	4.59 ± 0.24	4.95 ± 0.28	4.92 ± 0.21	4.64 ± 0.15	5.05 ± 0.55	4.83 ± 0.24	ns
Ellagic acid	1.61 ± 0.80	2.10 ± 0.29	1.57 ± 1.16	0.93 ± 0.63	0.55 ± 0.37	0.93 ± 1.08	1.76 ± 0.76	0.81 ± 0.68	ns
Catechin	5.01 ± 1.28	5.56 ± 1.03	6.58 ± 2.59	2.80 ± 0.98	3.56 ± 2.40	4.58 ± 0.28	5.72 ± 3.90	3.64 ± 1.52	ns
Epicatechin	6.84 ± 0.83	6.46 ± 2.04	6.68 ± 0.74	8.13 ± 2.79	14.36 ± 7.37	8.86 ± 1.30	6.66 ± 1.64	10.45 ± 2.06	**
Safranal	4.06 ± 0.08	3.99 ± 0.01	4.05 ± 0.02	4.31 ± 0.04	4.34 ± 0.20	4.41 ± 0.21	4.03 ± 0.08	4.35 ± 0.25	**
Crocin I	53.98 ± 29.30	35.43 ± 26.31	19.34 ± 6.87	65.09 ± 41.55	101.55 ± 37.29	129.61 ± 27.78	35.98 ± 24.79	98.75 ± 41.89	ns
Crocin II	23.22 ± 7.23	25.95 ± 8.34	36.37 ± 2.97	30.03 ± 16.26	31.73 ± 8.68	42.37 ± 6.98	28.51 ± 8.29	34.71 ± 11.42	ns
Dehydroascorbic acid	30.10 ± 0.16	29.47 ± 1.12	30.11 ± 1.66	30.43 ± 0.51	31.41 ± 1.54	28.08 ± 2.30	29.89 ± 1.05	29.98 ± 0.82	ns
Ascorbic acid	45.18 ± 5.15	49.75 ± 13.21	30.75 ± 3.24	35.27 ± 4.86	41.93 ± 1.51	36.56 ± 8.86	41.90 ± 11.95	37.59 ± 6.14	ns
Total vitamin C	755.28 ± 5.00	79.22 ± 13.25	60.86 ± 1.58	64.70 ± 5.38	73.34 ± 2.90	64.65 ± 6.62	71.79 ± 10.99	67.56 ± 6.25	ns
TPC (mgGAE 100 g^{-1} DW)	1191.86 ± 77.98	947.44 ± 233.05	1744.08 ± 320.85	1295.55 ± 538.14	3642.95 ± 2448.05	888.35 ± 284.18	1294.46 ± 673.86	1942.28 ± 1802.48	ns
Anthocyanins (mgC3G 100 g^{-1} DW)	577.20 ± 235.16	3900.06 ± 2870.32	641.35 ± 76.52	504.42 ± 380.23	2487.52 ± 1027.65	1074.52 ± 519.76	1706.20 ± 336.76	1355.49 ± 221.5	ns
Antioxidant activity									
FRAP (mmol Fe^{2+} kg^{-1})	328.31 ± 47.70	348.01 ± 83.00	554.63 ± 20.76	2327.30 ± 1222.59	2366.65 ± 1877.61	3245.38 ± 1252.34	410.32 ± 119.11	2646.44 ± 2017.86	ns
ABTS (μmolTE g^{-1})	4.56 ± 0.44	4.10 ± 0.32	3.95 ± 0.04	4.94 ± 0.61	4.63 ± 0.27	4.34 ± 0.55	4.21 ± 0.38	4.64 ± 0.50	ns

Table 4. *Cont.*

b.				
ISO 3632 [10]traits (E1% abs)	**Morgex**	**Saint Cristophe**	**Chambave**	***p***
Flavour/Picrocrocin	133.5 ± 4.53	132.4 ± 8.26	135.4 ± 7.09	ns
Aroma/Safranal	41.00 ± 8.82	40.5 ± 6.41	42.6 ± 5.08	ns
Colouring/Crocins	298.9 ± 110.46	269.10 ± 74.66	309.10 ± 131.04	ns
Coumaric acid	23.67 ± 0.14	23.63 ± 0.18	23.36 ± 0.26	ns
Isoquercitrin	2.49 ± 0.15	2.55 ± 0.03	2.48 ± 0.12	ns
Quercitrin	21.09 ± 6.51	16.63 ± 7.06	18.64 ± 3.03	ns
Gallic acid	5.28 ± 0.25 ^{a}	4.91 ± 0,18 ^{a,b}	4.63 ± 0.34 ^{b}	*
Ellagic acid	1.27 ± 0.74	1.33 ± 0.89	1.25 ± 1.06	ns
Catechin	3.90 ± 1.58	4.56 ± 4.25	5.58 ± 3.11	ns
Epicatechin	7.49 ± 1.97	10.41 ± 9.58	7.77 ± 1.52	ns
Safranal	4.18 ± 0.14	4.16 ± 0.22	4.23 ± 0.24	ns
Crocin I	59.13 ± 21.54 ^{b}	68.48 ± 15.78 ^{b}	129.60 ± 27.65 ^{a}	***
Crocin II	26.62 ± 8.45 ^{b}	28.84 ± 5.96 ^{b}	42.36 ± 6.73 ^{a}	*
Dehydroascorbic acid	30.26 ± 1.45 ^{ab}	30.44 ± 0.74 ^{a}	28.08 ± 1.46 ^{b}	*
Ascorbic acid	39.73 ± 7.47	45.84 ± 9.43	33.66 ± 6.76	ns
Total vitamin C	69.99 ± 7.42	76.28 ± 9.16	62.75 ± 4.78	ns
TPC (mgGAE 100 g^{-1} DW)	1243.70 ± 348.56	2295.19 ± 214.44	1316.21 ± 567.85	ns
Anthocyanins (mgC3G 100 g^{-1} DW)	540.81 ± 340.53	319.79 ± 153.75	857.94 ± 330.67	ns
Antioxidant activity				
FRAP (mmol Fe^{2+} kg^{-1})	1327.80 ± 313.86	1357.33 ± 323.45	1900.00 ± 687.95	ns
ABTS (µmolTE g^{-1})	4.75 ± 0.52	4.37 ± 0.39	4.15 ± 0.40	ns

Same letter denotes no significant differences according to Tukey post-hoc test ($p < 0.05$). The statistical relevance is provided (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$). The statistical relevance of "between-subjects effects" tests for Y1 and Y2 is provided (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

Most of the beneficial effects of saffron have been recognized since ancient times and are mainly due to its phenolic content (TPC) and antioxidant activity (FRAP and ABTS assays). Overall, the saffron produced in the north west Italian Alps showed a very high TPC (ranging between 888.35–3642.95 mgGAE 100 g^{-1} DW) with no differences between years or sites (Table 4a). Results are slightly higher than saffron cultivated in different areas of Lebanon (160 mgGAE 100 g^{-1} DW) [57], in different provinces of Iran (296—605 mgGAE 100 g^{-1} DW) [54], and much superior if compared with other common food additives and spices, such as *Eugenia caryophyllata* Thunb., *Lavandula* spp., *Curcuma domestica* Val, and *Curcuma longa* L. (0.26, 0.22, 23, and 36 mgGAE 100 g^{-1} DW, respectively [58,59]. Apart from crocins, Asdaq and Inamdar [60] highlighted that polyphenols, such as flavonols, are responsible for the synergistic antihyperlipidemic and antioxidant potential of saffron. Results of ABTS and FRAP assays also showed an elevated antioxidant activity regardless of the year or site of cultivation. ABTS assay values were comparable to those found in Greek saffron by Ordoudi et al. [61]. FRAP assay values (328.31–3245.38 mmol Fe^{2+} kg^{-1}) (Table 4a) were higher compared to the Iranian samples (circa 570 mmol Fe^{2+} kg^{-1}) analyzed by Karimi et al. [62]. Environmental conditions (i.e., altitude and rainfall) can influence the content of flavonols [63]. As an example, the content of crocins in the spice was seen to range from 25 and 35% of dried saffron on the base of the spice quality and production site [64].

Although the bioactive properties of saffron are reported in several manuscripts [65], the identification and quantification of the responsible compounds in saffron are present only in a few [4,9], making comparisons difficult to perform. The composition and relative range values of the 13 bioactive compounds detected in the saffron samples from the three experimental sites and the two cultivation cycles are listed in Table 4. Overall, the saffron produced in the north west Italian Alps showed a lower content of crocin II (23.22–42.37 mg 100 g^{-1} DW) (Table 4a) than the saffron produced in Sardinia (75 mg 100 g^{-1} DW; Italy, DOP Zafferano di Sardegna) [65], while it presented a higher content of gallic acid compared to that found in Iranian and Greek saffron (2 mg and 1.2 100 g^{-1} DW)

by Karimi et al. [62] and Proestos et al. [66]. Thus, the saffron produced in Alpine environments could be of particular interest for its elevated antioxidant properties.

As resulted by the computed two-way ANOVA, only quercitrin, epicatechin, and safranal content varied in the two cultivation cycles (Table 4a), while the site affected the concentration of gallic acid, crocin I, crocin II, and dehydroascorbic acid biosynthesis (Table 4b). Specifically, the content of quercitrin significantly decreased (21.28 and 16.30 mg 100 g^{-1} DW in the first and second year, respectively), while the mean of the other two compounds significantly increased (6.66 and 10.45 mg 100 g^{-1} DW, and 4.03 and 4.35 mg 100 g^{-1} DW, respectively) (Table 4a). The saffron produced in Morgex and Saint Cristophe was significantly superior in gallic acid and dehydroascorbic acid than in Chambave. On the other hand, the content of crocin I and crocin II was significantly higher in the saffron produced in Chambave (Table 4b). Differences in saffron plants secondary metabolites, in quantitative and qualitative terms, and in relation to the environmental conditions of growth, were already supposed by different authors, particularly regarding safranal content [56,67].

The relationships between the studied parameters and the cultivation sites in the two growing seasons were evaluated through a PCA and represented in a two-dimensional PCA scatter plot (based on the first principal components (PCs)), reported in Figure 2.

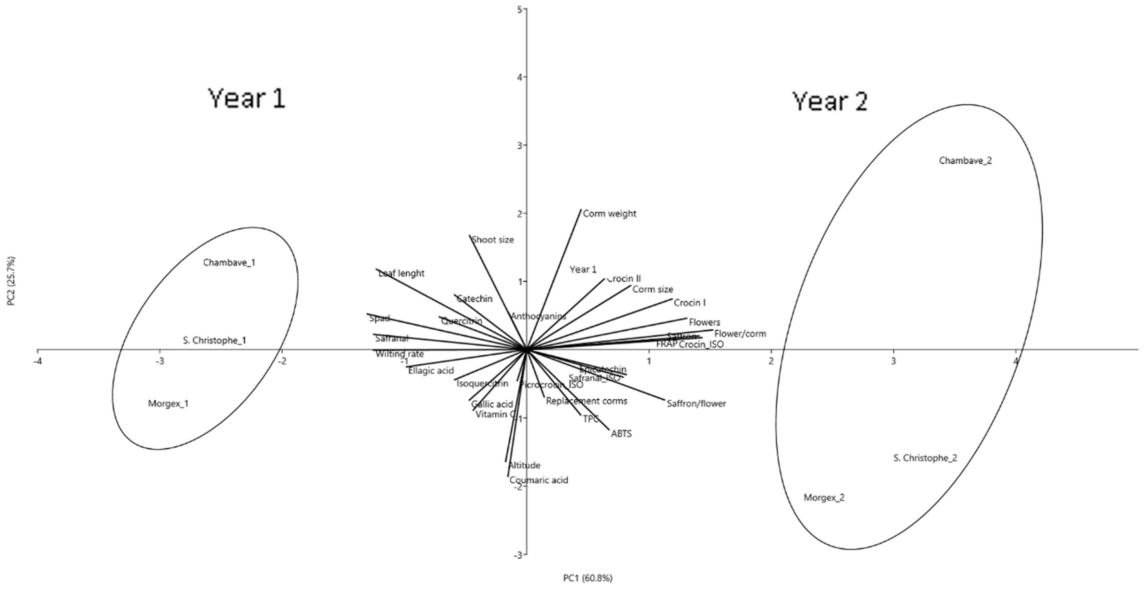

Figure 2. Principal component analysis (PCA)-biplot of the *C. sativus* corms and saffron traits cultivated during the first and second growing seasons (Years 1 and 2) in the experimental fields located in the municipalities of Morgex (AO, Italy), Saint Christophe (AO, Italy), and Chambave (AO, Italy), according to the first two principal components.

As depicted, the first two PCs explained 85.5% of total variation. The first PC accounted for 60.8% of the total variance and was positively correlated with flower $corm^{-1}$ (0.302) and saffron g m^{-2} (0.274). The plot clearly divided Year 1 and Year 2, with the first growing season mainly linked to wilting rate, SPAD values, leaf length, and the content of safranal, quercitrin, catechin, and ellagic acid. While the corms from the second growing season (Year 2) presented higher agronomic performances, and the produced saffron contained higher polyphenol content and antioxidant activities. Specifically, corms in the fields of Morgex and S. Christophe produced more replacement corms, mg of saffron per flower, and the spice was of high quality, based on picrocrocin and safranal measurement [10]; it also possessed higher antioxidant activities (ABTS method), and was rich in TPC, picrocrocin, and epicatechin. While, in the field of Chambave, larger and heavier corms were produced and more flowers m^{-2}, flowers per corm, and a greater quantity of saffron were obtained. In this site, the spice resulted in a higher quality, based on the presence of crocins [10], and with high antioxidant activities (FRAP method).

Thus, the obtained PCA clearly demonstrated that the largest difference in the flower-related traits and corm properties were found between the growing seasons (Years 1 and 2). Saffron is a perennial crop which is propagated through corms [47]. During the corm-formation in the first growing season, new replacement corms will be produced and grown, which results in an increment in flower production in the next year [47,50,68]. Whereas the bioactive traits were not strongly affected by the growing seasons, as indicated in Tables 4 and 5. These results are in agreement with the findings of Lage and Cantrell [68], Ghanbari et al. [54] and Cardone et al. [69] who reported that bioactive compounds greatly depend upon the growing conditions in Morocco, Iran, and Italy, respectively. However, the Chambave field turned out to be more productive, while those of Morgex and S. Cristophe produced a qualitatively superior spice.

3.3. Bioactive Compounds in Fresh Tepal Extracts

The relative phenolic and anthocyanin content of fresh saffron tepal extracts varied from 374.8 to 677.7 mgGAE 100 g^{-1} FW, and from 88.8 to 143.3 mgC3G 100 g^{-1} FW (Table 5). No significant differences between the extraction methods were found in both parameters. While the extracting solution containing 80% methanol extracted a significantly higher quantity of TPC compared to the other solutions. The same solution was also most effective in extracting anthocyanins compared to the solution with 50% of methanol. The extraction method and solvent type influenced the extract antioxidant activity measured with FRAP and ABTS assays, while no differences were observed for DPPH assay (Table 5). The maceration method with 100% water had a superior antioxidant activity on the basis of the FRAP method. While the UA method with 50% methanol was more effective for the ABTS method.

Based on our knowledge, only a few studies have been conducted on different extraction methods and on the nutraceutical properties of similar fresh saffron tepal extracts. Tuberoso et al. [70] showed that in aqueous extracts obtained by maceration of *C. sativus* fresh tepals the content of TPC was in line with our findings (461.6–742.1 mgGAE 100 g^{-1} FW). Through the applied extractive method, the authors obtained an antioxidant activity equal to 35.5 and 55.4 mmol Fe^{2+} kg^{-1} in FRAP assay, much inferior to the present work, and slightly superior values with DPPH assay (7 and 12.3 µmolTE g^{-1}, respectively).

In the tepal extracts, only four compounds out of 16 were found, namely hyperoside, rutin, ellagic acid, and epicatechin. The extract obtained with the ultrasound assisted method contained more ellagic acid (+87%) and epicatechin (+67%) in comparison to the maceration method, while no differences were observed for the other two compounds. Solvents with a low percentage of methanol (<20%) allowed the extraction of a high amount of hyperoside, while a concentration superior of 20% better extracted ellagic acid. A high content of methanol (>50%) was significantly effective for epicatechin. Chromatographic profile of all the considered samples (maceration vs. ultrasound-assisted extraction at each methanol percentage) were reported for each phenolic class (cinnamic acids and flavonols in Figure 3; benzoic acids and catechins in Figure 4).

Table 5. Total polyphenol content (TPC, mgGAE 100 g^{-1} FW), anthocyanins (mgC3G 100 g^{-1} FW), antioxidant activity (FRAP, mmol Fe^{2+} kg^{-1}; DPPH, µmolTE g^{-1}; ABTS, µmolTE g^{-1} assays), and bioactive compounds (mg 100 g^{-1} FW) in fresh saffron tepal extracts obtained with maceration (M) and ultrasound assisted (UA) extraction, varying percentage of methanol in aqueous solvents (0%, 20%, 50%, and 80%). Data are presented as mean ± standard deviation.

			Antioxidant Activity			Flavonols		Benzoic Acids	Catechins
Extraction Method	**TPC**	**Anthocyanins**	**FRAP**	**DPPH**	**ABTS**	**Hyperoside**	**Rutin**	**Ellagic Acid**	**Epicatechin**
M	465.7 ± 36.4	119.7 ± 15.6	167.3 ± 10.2	5.53 ± 0.79	5.84 ± 1.1	2.00 ± 0.72	0.15 ± 0.04	1.63 ± 0.35	3.13 ± 0.72
UA	486.9 ± 41.8	119.3 ± 12.8	141.1 ± 7.3	6.06 ± 1.12	8.88 ± 0.97	2.42 ± 0.37	0.13 ± 0.05	3.06 ± 0.66	5.27 ± 0.42
p	ns	ns	**	ns	***	ns	ns	***	*
Solvent (% methanol)									
0%	449.8 ± 35.5 ^{b}	142.5 ± 23.5 ^{a,b}	253.5 ± 5.3 ^{a}	4.61 ± 1.27	5.56 ± 1.03 ^{b}	2.96 ± 0.64 ^{a}	0.16 ± 0.07	1.42 ± 0.35 ^{b}	0.00 ± 0.00 ^{b}
20%	374.8 ± 47.2 ^{b}	103.3 ± 18.9 ^{a,b}	108.8 ± 11.7 ^{c}	4.82 ± 1.86	6.45 ± 0.98 ^{b}	2.98 ± 0.59 $^{a.}$	0.09 ± 0.02	2.52 ± 0.18 ^{a}	0.00 ± 0.00 ^{b}
50%	402.9 ± 28.0 ^{b}	88.8 ± 15.9 ^{b}	105.9 ± 15.2 ^{c}	6.99 ± 1.43	10.53 ± 1.23 ^{a}	1.34 ± 0.79 ^{b}	0.16 ± 0.10	2.71 ± 0.31 ^{a}	9.18 ± 0.97 ^{a}
80%	677.7 ± 23.4 ^{a}	143.3 ± 22.3 ^{a}	148.6 ± 10.4 ^{b}	6.77 ± 1.76	6.79 ± 0.72 ^{b}	1.53 ± 0.68 ^{b}	0.21 ± 0.09	2.72 ± 0.22 ^{a}	7.62 ± 1.65 ^{a}
p	***	*	***	ns	***	***	ns	**	***
Interaction									
Extraction × Solvent	ns	ns	**	ns	***	***	ns	ns	*

Same letter denotes no significant differences according to Tukey post-hoc test ($p < 0.05$). The statistical relevance is provided (ns = non-significant; ** = $p < 0.01$; *** = $p < 0.001$). The statistical relevance of "between-subjects effects" tests is provided (ns = non-significant; * $p < 0.05$; ** $p < 0.01$; *** = $p < 0.001$).

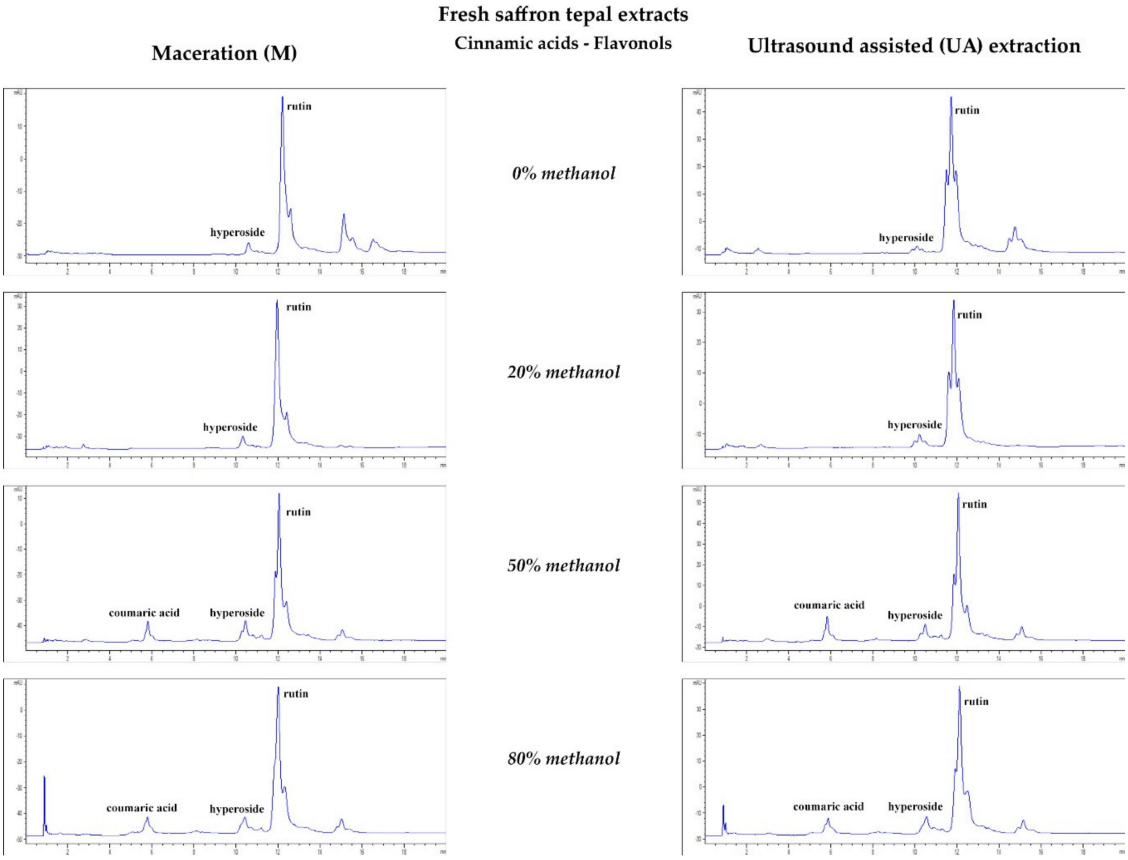

Figure 3. Chromatographic profile of cinnamic acids and flavonols in aqueous (0% methanol) and hydroalcoholic (20%, 50%, and 80% of methanol) extracts of fresh saffron tepals obtained by maceration (M) and ultrasound assisted (UA) extraction. Coumaric acid was not detected in aqueous (0% methanol) and hydroalcoholic (20%) samples, however it was identified but not quantified in hydroalcoholic (50% and 80% of methanol) extracts.

The best extractions were performed with a methanol range from 50% to 80%. In addition, ultrasonic-assisted extraction greatly reduces the composition differences between extracts with different percentage of extraction solvents. In general, our study is the first that has identified these compounds in saffron tepals. Within the flavonoid group, hyperoside characterizes plants of the family of Hypericaceae, Rosaceae, and Labiatae, and the genus Crataegus [71]. This molecule has been suggested to attenuate oxidative stress-related diseases. Catechins and flavonols are known to be extremely important for human health. Several studies support the health benefits of the consumption of food rich in catechins, such as mint and tea, considered to be responsible for preventing cardiovascular diseases, improving blood flow, eliminating various toxins and improving resistance to various illnesses thanks to their antioxidative, anti-inflammatory, antidiabetic and antimicrobial properties [72].In the present work, Pearson correlations indicated significant positive correlations ($p \leq 0.05$) between increasing methanol content and ellagic acid, epicatechin, TPC and DPPH assay. On the contrary, hyperoside content and FRAP assay were reduced.Given the few studies conducted on fresh saffron tepals, a comparison was made with the nutraceutical properties of the main edible flowers commonly studied. If compared with data on anthocyanin content, saffron tepals contained much superior amounts than fresh flowers of Calendula officinalis L., Tagetes erecta L., Tropaeolum majus L., Viola × wittrockiana Gams., and Viola cornuta L. as reported by Benvenuti et al. [73], Pires et al. [23], and Demasi et al. [24]. The authors obtained 0.47, 0.75, 8.27, 12.4, and 27.76 mgC3G 100 g−1 FW, respectively, by extracting with 50–80% methanol solution. Regarding antioxidant activity, our results are in line with those obtained in the same species (36.8, 704.2, 100.5, 365.5, and 391.89

mmol Fe^{2+} kg^{-1} by FRAP assay, respectively). Furthermore, our findings indicated that the antioxidant power of fresh saffron tepals (FRAP assay) was very high compared with literature data on leaves of red and white Brassica oleracea var. capitata "Autoro" and "Bartolo", and Brassica oleracea var. sabauda "Thaler" (0.2–24.4 mmol Fe^{2+} kg^{-1}) [74,75], tissues of five apple cultivars (16–210 mmol Fe^{2+} kg^{-1}) [76], goji fruits (19 mmol Fe^{2+} kg^{-1}) [77], and Citrullus lanatus pulp (16 mmol Fe^{2+} kg^{-1}) [78].

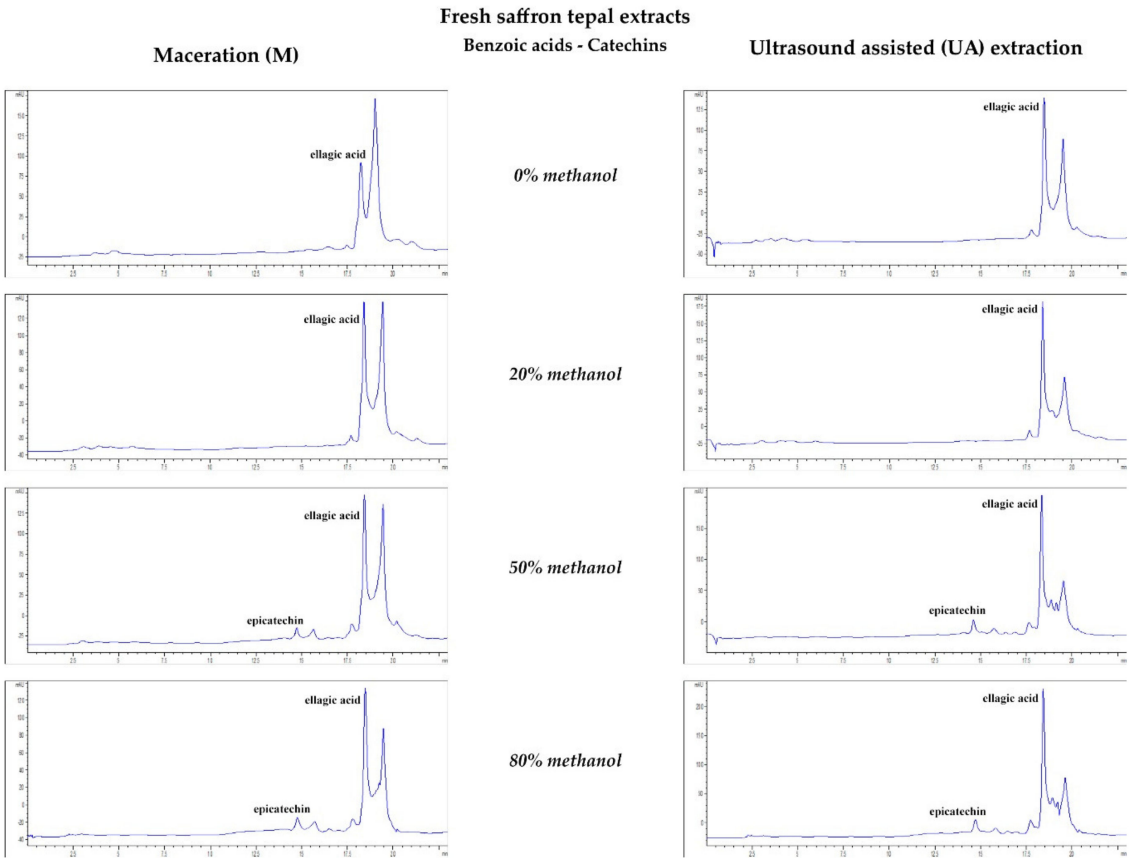

Figure 4. Chromatographic profile of benzoic acids and catechins in aqueous (0% methanol) and hydroalcoholic (20%, 50%, and 80% of methanol) extracts of fresh saffron tepals obtained by maceration (M) and ultrasound assisted (UA) extraction. Epicatechin was not detected in aqueous (0% methanol) and hydroalcoholic (20%) samples, but it was identified and quantified in hydroalcoholic (50% and 80% of methanol) extracts.

In general, a combination of organic solvent and water facilitates the extraction of all compounds that were soluble in both water and organic solvents [79]. Differences in the structure of phenolic compounds also determine their solubility in solvents of different polarity. Regarding the single tested molecules, water solutions were significantly effective in the extraction of both studied flavonols (hyperoside and rutin). Therefore, type of extraction as well as the solvent used may have a significant impact on the yield of polyphenols, anthocyanins, and the extent of antioxidant activity from plant materials. There are some reports concerning the optimization of extraction conditions of phenolic compound content and antioxidant activities of some plant foods, but as some researches indicated, the optimal procedure is usually different for different plant matrices [80,81].

4. Conclusions

This research assessed the quality of saffron produced in the north western Italian Alps, complementing the data currently available on this crop. The spice produced and analysed over the two years of study was found to be of high quality and a rich source of bioactive compounds,

with nutraceutical properties, regardless of the cultivation site. In addition, fresh saffron tepals were found to be a source of nutraceutical components, with an antioxidant activity that is often similar or higher than that of some vegetables and fruits. Therefore, tepals could be utilized for the recovery of valuable compounds and not be considered as waste, contributing toward the zero-waste concept. The zero-waste concept is an effective strategy that allows effective valorization of generated agro-industrial wastes to value-added products, with applications in the food sector as colouring agents, antioxidative agents, preservatives, and many more.

Most industrial processes are basically scaled-up from the processes developed at laboratory scale, with a need for more efficient processes that can increase yields and the overall quality of natural products at a feasible cost. Ultrasound assisted extraction represents one of the most promising technologies for sustainable "green" extraction. This extraction system presents high reproducibility, reducing the consumption of solvent, simplifying the manipulation and the processing, and conferring a greater degree of purity to the final product. The studied parameters improved the hydromethanolic extraction of biocompounds and allowed the characterization of the saffron residue. The bioactive profile indicated differences in the polyphenolic composition, such as flavanols, anthocyanins, flavonols, and phenolic acids, which may guide technological and functional applications. In conclusion, the extractive approaches proposed in this work provide insights for the reutilization of this vegetal residue as a viable alternative to develop quality products with added value.

Author Contributions: Conceptualization, M.C., S.D., D.D. and V.S.; data curation, M.C. and S.D.; formal analysis, M.C., S.D., S.S., and D.D.; resources, V.S.; supervision, V.S.; validation, V.S.; writing—original draft, M.C.; writing—review and editing, M.C., S.D. and V.S.; project administration, V.S.; funding acquisition, V.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the program Interreg V-A Francia Italia Alcotra "Attività innovative per lo sviluppo della filiera transfrontaliera del fiore edule–Antea' n. 1139.

Acknowledgments: The authors acknowledge Alessandro Putzolu for technical assistance, and Azienda Agricola "La Branche" di Diego Bovard, Azienda Agricola "Rosset", and Azienda Agricola "SteLo" for providing plant material and hosting cultivation.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Husaini, A.; Shafiq, A.; Parvaze, S.; Rather, G.; Parray, A.; Shikari, A.; Javid, M. Bioinformatics for saffron (*Crocus sativus* L.) improvement. *Commun. Biometry Crop Sci.* **2009**, *4*, 3–8.
2. Shahi, T.; Assadpour, E.; Jafari, S.M. Pharmacological activities of stigmas and tepals of 'red gold'; saffron. *Trends Food Sci. Technol.* **2016**, *58*, 69–78. [CrossRef]
3. Molina, R.; Valero, M.; Navarro, Y.; Guardiola, J.; García-Luis, A. Temperature effects on flower formation in saffron (*Crocus sativus* L.). *Sci. Hortic.* **2005**, *103*, 361–379. [CrossRef]
4. Caser, M.; Maxaieie Victorino, Í.M.; Demasi, S.; Berruti, A.; Donno, D.; Lumini, E.; Bianciotto, V.; Scariot, V. Saffron cultivation in marginal Alpine environments: How AMF inoculation modulates yield and bioactive compounds. *Agronomy* **2019**, *9*, 12. [CrossRef]
5. Grande, J. New venture creation in the farm sector—critical resources and capabilities. *J. Rural Stud.* **2011**, *27*, 220–233. [CrossRef]
6. Manzo, A.; Panseri, S.; Bertoni, D.; Giorgi, A. Economic and qualitative traits of Italian Alps saffron. *J. Mt. Sci.* **2015**, *12*, 1542–1550. [CrossRef]
7. Giorgi, A.; Pentimalli, D.; Giupponi, L.; Panseri, S. Quality traits of saffron (*Crocus sativus* L.) produced in the Italian Alps. *Open Agric.* **2017**, *2*, 52–57. [CrossRef]
8. Milajerdi, A.; Djafarian, K.; Hosseini, B. The toxicity of saffron (*Crocus sativus* L.) and its constituents against normal and cancer cells. *J. Nutr. Intermed. Metab.* **2016**, *3*, 23–32. [CrossRef]

9. Caser, M.; Demasi, S.; Victorino, I.M.M.; Donno, D.; Faccio, A.; Lumini, E.; Bianciotto, V.; Scariot, V. Arbuscular mycorrhizal fungi modulate the crop performance and metabolic profile of saffron in soilless cultivation. *Agronomy* **2019**, *9*, 232. [CrossRef]
10. ISO 3632-1:2011. *Spices—Saffron (Crocus Sativus L.). Food Products SC 7, Spices, Culinary Herbs and Condiments*; International Organization for Standardization: Geneva, Switzerland, 2011.
11. Lahmass, I.; Touria, L.; Delporte, C.; Sikdar, S.; Antwerpen, P.; Saalaoui, E.; Mégalizzi, V. The waste of saffron crop, a cheap source of bioactive compounds. *J. Funct. Foods* **2017**, *35*, 341–351. [CrossRef]
12. Rodriguez-Ruiz, V.; Barzegari, A.; Zuluaga, M.; Zunooni-Vahed, S.; Rahbar-Saadat, Y.; Letourneur, D.; Gueguen, V.; Pavon-Djavid, G. Potential of aqueous extract of saffron (*Crocus sativus* L.) in blocking the oxidative stress by modulation of signal transduction in human vascular endothelial cells. *J. Funct. Foods* **2016**, *26*, 123–134. [CrossRef]
13. Bagur, M.; Salinas, G.; Jiménez-Monreal, A.; Chaouqi, S.; Llorens, S.; Martinez-Tomé, M.; Alonso, G. Saffron: An old medicinal plant and a potential novel functional food. *Molecules* **2018**, *23*, 30. [CrossRef] [PubMed]
14. Liu, Y.; Qin, X.; Lu, X. Crocin improves endometriosis by inhibiting cell proliferation and the release of inflammatory factors. *Biom. Pharm.* **2018**, *106*, 1678–1685. [CrossRef] [PubMed]
15. Leone, S.; Recinella, L.; Chiavaroli, A.; Orlando, G.; Ferrante, C.; Leporini, L.; Brunetti, L.; Menghini, L. Phytotherapeutic use of the *Crocus sativus* L. (Saffron) and its potential applications: A brief overview. *Phytother. Res.* **2018**, *32*, 2364–2375. [CrossRef]
16. Singh, B.; Singh, J.P.; Kaur, A.; Singh, N. Phenolic composition, antioxidant potential and health benefits of citrus peel. *Food Res. Int.* **2020**, *132*, 109114. [CrossRef]
17. Figueroa, K.H.N.; García, N.V.M.; Campos-Vega, R. Cocoa by-products. In *Food Wastes and By-Products: Nutraceutical and Health Potential*; Campos-Vega, R., Dave Oomah, B., Vergara-Castañeda, H.A., Eds.; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2020; ISBN 9781119534105.
18. Varo, M.A.; Jacotet-Navarro, M.; Serratosa, M.P.; Mérida, J.; Fabiano-Tixier, A.S.; Bily, A.; Chemat, F. Green ultrasound-assisted extraction of antioxidant phenolic compounds determined by high performance liquid chromatography from bilberry (*Vaccinium myrtillus* L.) juice by-products. *Waste Biomass Valorization* **2019**, *10*, 1945–1955. [CrossRef]
19. Mahdavee Khazaei, K.; Jafari, S.M.; Ghorbani, M.; Hemmati Kakhki, A.; Sarfarazi, M. Optimization of Anthocyanin Extraction from Saffron Petals with Response Surface Methodology. *Food Anal. Methods* **2016**, *9*, 1993–2001. [CrossRef]
20. Mahdavee Khazaei, K.; Jafari, S.M.; Ghorbani, M.; Hemmati Kakhki, A. Application of maltodextrin and gum Arabic in microencapsulation of saffron petal's anthocyanins and evaluating their storage stability and color. *Carbohydr. Polym.* **2014**, *105*, 57–62. [CrossRef]
21. Moratalla-López, N.; Bagur, M.J.; Lorenzo, C.; Martínez-Navarro, M.; Salinas, M.R.; Alonso, G.L. Bioactivity and bioavailability of the major metabolites of *Crocus sativus* L. Flower. *Molecules* **2019**, *24*, 2827. [CrossRef]
22. Scariot, V.; Gaino, W.; Demasi, S.; Caser, M.; Ruffoni, B. Flowers for edible gardens: Combinations of species and colours for northwestern Italy. *Acta Hortic.* **2018**, *1215*, 363–368.
23. Pires, T.C.S.P.; Barros, L.; Santos-Buelga, C.; Ferreira, I.C.F.R. Edible flowers: Emerging components in the diet. *Trends Food Sci. Technol.* **2019**, *93*, 244–258.
24. Demasi, S.; Falla, N.M.; Caser, M.; Scariot, V. Postharvest aptitude of *Begonia semperflorens* and *Viola cornuta* edible flowers. *Adv. Hortic. Sci.* **2020**, *34*, 13–20.
25. Haudenblas, H.A.; Saha, D.; Dubyak, P.J.; Anton, S.D. Saffron (*Crocus sativus* L.) and majpr depressive disorder: A meta-analysis of randomized clinical trials. *J. Integr. Med.* **2013**, *11*, 377–383.
26. Sánchez-Vioque, R.; Santana-Méridas, O.; Polissiou, M.; Vioque, J.; Astraka, K.; Alaiz, M.; Herraiz-Peñalver, D.; Tarantilis, P.A.; Girón-Calle, J. Polyphenol composition and in vitro antiproliferative effect of corm, tepal and leaf from *Crocus sativus* L. on human colon adenocarcinoma cells (Caco-2). *J. Funct. Foods* **2016**, *24*, 18–25.
27. Kakouri, E.; Daferera, D.; Paramithiotis, S.; Astraka, K.; Drosinos, E.H.; Polissiou, M.G. *Crocus sativus* L. tepals: The natural source of antioxidant and antimicrobial factors. *J. Appl. Res. Med. Aromat. Plants* **2017**, *4*, 66–74.
28. Menghini, L.; Leporini, L.; Vecchiotti, G.; Locatelli, M.; Carradori, S.; Ferrante, C.; Zengin, G.; Recinella, L.; Chiavaroli, A.; Leone, S.; et al. *Crocus sativus* L. stigmas and byproducts: Qualitative fingerprint, antioxidant potentials and enzyme inhibitory activities. *Food Res. Int.* **2018**, *109*, 91–98. [PubMed]

29. Hosseini, S.I.; Farrokhi, N.; Shokri, K.; Khani, M.R.; Shokri, B. Cold low pressure O_2 plasma treatment of *Crocus sativus*: An efficient way to eliminate toxicogenic fungi with minor effect on molecular and cellular properties of saffron. *Food Chem.* **2018**, *257*, 310–315. [PubMed]
30. Mottaghipisheh, J.; Sourestani, M.M.; Kiss, T.; Horváth, A.; Tóth, B.; Ayanmanesh, M.; Khamushi, A.; Csupor, D. Comprehensive chemotaxonomic analysis of saffron crocus tepal and stamen samples, as raw materials with potential antidepressant activity. *J. Pharm. Biomed. Anal.* **2020**, *184*, 113183. [PubMed]
31. Jacotet-Navarro, M.; Rombaut, N.; Fabiano-Tixier, A.S.; Danguien, M.; Bily, A.; Chemat, F. Ultrasound versus microwave as green processes for extraction of rosmarinic, carnosic and ursolic acids from rosemary. *Ultrason. Sonochemistry* **2015**, *27*, 102–109.
32. Chemat, F.; Rombaut, N.; Fabiano-Tixier, A.S.; Pierson, J.T.; Bily, A. Chapter 1. Green extraction: From concepts to research, education, and economical opportunities. In *Green Extraction of Natural Products: Theory and Practice*; Chemat, F., Strube, J., Eds.; Wiley: Hoboken, NJ, USA, 2015; pp. 1–36.
33. Tiwari, B.K. Ultrasound: A clean, green extraction technology. *Trends Anal. Chem.* **2015**, *71*, 100–109.
34. Espino, M.; Fernández, M.A.; Gomez, F.J.V.; Boiteux, J.; Silva, M.F. Green analytical chemistry metrics: Towards a sustainable phenolics extraction from medicinal plants. *Microchem. J.* **2018**, *141*, 438–443. [CrossRef]
35. Turrini, F.; Donno, D.; Beccaro, G.L.; Zunin, P.; Pittaluga, A.; Boggia, R. Pulsed ultrasound-assisted extraction as an alternative method to conventional maceration for the extraction of the polyphenolic fraction of ribes nigrum buds: A new category of food supplements proposed by the finnover project. *Foods* **2019**, *8*, 466.
36. Rahaiee, S.; Moini, S.; Hashemi, M.; Shojaosadati, S. Evaluation of antioxidant activities of bioactive compounds and various extracts obtained from saffron (*Crocus sativus* L.): A review. *J. Food Sci. Technol.* **2015**, *52*, 1881–1888. [PubMed]
37. Da Porto, C.; Natolino, A. Extraction kinetic modelling of total polyphenols and total anthocyanins from saffron floral bio-residues: Comparison of extraction methods. *Food Chem.* **2018**, *258*, 137–143. [PubMed]
38. Jafari, S.M.; Khazaei, K.M.; Assadpour, E. Production of a natural color through microwave-assisted extraction of saffron tepal's anthocyanins. *Food Sci. Nutr.* **2019**, *7*, 1438–1445. [PubMed]
39. Caser, M.; Chitarra, W.; D'Angiolillo, F.; Perrone, I.; Demasi, S.; Lovisolo, C.; Pistelli, L.; Pistelli, L.; Scariot, V. Drought stress adaptation modulates plant secondary metabolite production in *Salvia dolomitica* Codd. *Ind. Crop. Prod.* **2019**, *129*, 85–96.
40. Donno, D.; Mellano, M.G.; Raimondo, E.; Cerutti, A.K.; Prgomet, Z.; Beccaro, G.L. Influence of applied drying methods on phytochemical composition in fresh and fried goji fruits by HPLC fingerprint. *Eur. Food Res. Technol.* **2016**, *242*, 1961–1974.
41. Wong, S.P.; Leong, L.P.; Koh, J.H.W. Antioxidant activities of aqueous extracts of selected plants. *Food Chem.* **2006**, *99*, 775–783.
42. Urbani, E.; Blasi, F.; Simonetti, M.S.; Chiesi, C.; Cossignani, L. Investigation on secondary metabolite content and antioxidant activity of commercial saffron powder. *Eur. Food Res. Technol.* **2016**, *242*, 987–993.
43. Benzie, I.F.; Strain, J.J. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol.* **1999**, *299*, 15–27.
44. Donno, D.; Mellano, M.G.; Riondato, I.; De Biaggi, M.; Andriamaniraka, H.; Gamba, G.; Beccaro, G.L. Traditional and unconventional dried fruit snacks as a source of health-promoting compounds. *Antioxidants* **2019**, *8*, 396. [CrossRef] [PubMed]
45. Di Primo, P.; Cappelli, C.; Katan, T. Vegetative compatibility grouping of *Fusarium oxysporum* f. sp. *gladioli* from Saffron. *Eur. J. Plant Pathol.* **2002**, *108*, 869–875.
46. Tammaro, F. Saffron (*Crocus sativus* L.) in Italy. In *Saffron: Crocus Sativus L.*; Negbi, M., Ed.; Harwood Academic Publishers: Reading, UK, 1999; Volume 8, pp. 53–62.
47. Kumar, R.; Singh, V.; Devi, K.; Sharma, M.; Singh, M.K.; Ahuja, P.S. State of art of saffron (*Crocus sativus* L.) agronomy: A comprehensive review. *Food Rev. Int.* **2008**, *25*, 44–85.
48. Dastranj, M.; Sepaskhah, A.R.; Kamgar-Haghighi, A.A. Rainfall and its distribution influences on rain-fed saffron yield and economic analysis. *Theor. Appl. Climatol.* **2019**, *137*, 3139–3147. [CrossRef]
49. Gresta, F.; Santonoceto, C.; Avola, G. Crop rotation as an effective strategy for saffron (*Crocus sativus* L.) cultivation. *Sci. Hortic.* **2016**, *211*, 34–39. [CrossRef]

50. Gresta, F.; Lombardo, G.; Siracusa, L.; Ruberto, G. Saffron an alternative crop for sustainable agricultural systems. A review. *Agron. Sustain. Dev.* **2008**, *28*, 95–112.
51. Mzabri, I.; Legsayer, M.; Kouddane, N.; Boukroute, A.; Berrichi, A. Salt stress effects on some morphological, physiological and biochemical parameters of saffron plant (*Crocus sativus* L) in Eastern Morocco. *J. Mater. Environ. Sci.* **2017**, *8*, 4894–4901.
52. Mollafilabi, A.; Koocheki, A.; Moeinerad, H.; Kooshki, L. Effect of plant density and weight of corm on yield and yield components of saffron (*Crocus sativus* L.) under soil, hydroponic and plastic tunnel cultivation. *Acta Hortic.* **2013**, *997*, 51–58. [CrossRef]
53. Koocheki, A.; Seyyedi, S. How irrigation rounds and mother corm size control saffron yield, quality, daughter corms behavior and phosphorus uptake. *Sci. Hortic.* **2016**, *213*, 132–143. [CrossRef]
54. Ghanbari, J.; Khajoei-Nejad, G.; van Ruth, S.M. Effect of saffron (*Crocus sativus* L.) corm provenance on its agro-morphological traits and bioactive compounds. *Sci. Hortic.* **2019**, *256*, 108605. [CrossRef]
55. Carmona, M.; Sánchez, A.M.; Ferreres, F.; Zalacain, A.; Tomás-Barberán, F.; Alonso, G.L. Identification of the flavonoid fraction in saffron spice by LC/DAD/MS/MS: Comparative study of samples from different geographical origins. *Food Chem.* **2007**, *100*, 445–450. [CrossRef]
56. Giupponi, L.; Ceciliani, G.; Leoni, V.; Panseri, S.; Pavlovic, R.; Lingua, G.; Di Filippo, A.; Giorgi, A. Quality traits of saffron produced in Italy: Geographical area effect and good practices. *J. Appl. Bot. Food Qual.* **2019**, *92*, 336–342.
57. Makhlouf, H.; Saksouk, M.; Habib, J.; Chahine, R. Determination of antioxidant activity of saffron taken from the flower of *Crocus sativus* grown in Lebanon. *Afr. J. Biotechnol.* **2011**, *10*, 8093–8100.
58. Gülçin, I.; Güngör Şat, I.; Beydemir, S.; Elmastaş, M.; Küfrevioğlu, O.I. Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). *Food Chem.* **2004**, *87*, 393–400.
59. Chen, I.N.; Chang, C.C.; Ng, C.C.; Wang, C.Y.; Shyu, Y.T.; Chang, T.L. Antioxidant and antimicrobial activity of Zingiberaceae plants in Taiwan. *Plant Foods Hum. Nutr.* **2008**, *63*, 15–20. [CrossRef]
60. Asdaq, S.M.B.; Inamdar, M.N. Potential of *Crocus sativus* (saffron) and its constituent, crocin, as hypolipidemic and antioxidant in rats. *Appl. Biochem. Biotechnol.* **2010**, *162*, 358–372. [CrossRef]
61. Ordoudi, S.; Befani, C.; Nenadis, N.; Koliakos, G.; Tsimidou, M. Further examination of antiradical properties of *crocus sativus* stigmas extract rich in crocins. *J. Agric. Food Chem.* **2009**, *57*, 3080–3086.
62. Karimi, E.; Oskoueian, E.; Hendra, R.; Jaafar, H. Evaluation of Crocus sativus L. stigma phenolic and flavonoid compounds and its antioxidant activity. *Molecules* **2010**, *15*, 6244–6256. [CrossRef]
63. Fatemeh, Z.; Somaye, T.; Saeideh, S. Effects of altitude on anatomy and concentration of crocin, picrocrocin and safranal in '*Crocus sativus*' L. *Aust. J. Crop Sci.* **2011**, *5*, 831–838.
64. Garcia-Rodríguez, M.V.; Serrano-Diaz, J.; Tarantilis, P.A.; López-Córcoles, H.; Carmona, M.; Alonso, G.L. Determination of saffron quality by high-performace liquid chromatography. *J. Agric. Food Chem.* **2014**, *62*, 8068–8074.
65. Del Campo, G.P.; Carmona, M.; Maggi, L.; Kanakis, C.D.; Anastasaki, E.G.; Tarantilis, P.A.; Polissiou, M.G.; Alonso, G.L. Picrocrocin content and quality categories in different (345) worldwide samples of saffron (*Crocus sativus* L.). *J. Agric. Food Chem.* **2010**, *58*, 1305–1312. [CrossRef]
66. Proestos, C.; Chorianopoulos, N.; Nychas, G.J.E.; Komaitis, M. RP-HPLC analysis of the phenolic compounds of plant extracts. investigation of their antioxidant capacity and antimicrobial activity. *J. Agric. Food Chem.* **2005**, *53*, 1190–1195. [CrossRef]
67. Giorgi, A.; Bertoni, D.; Manzo, A.; Panseri, S. *L'oro Rosso Delle Alpi—Manuale Tecnico-Scientifico di Produzione Dello Zafferano*; Biblion Edizioni: Milano, Italy, 2015.
68. Lage, M.; Cantrell, C.L. Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions. *Sci. Hortic.* **2009**, *121*, 366–373. [CrossRef]
69. Cardone, L.; Castronuovo, D.; Perniola, M.; Cicco, N.; Candido, V. Evaluation of corm origin and climatic conditions on saffron (*Crocus sativus* L.) yield and quality. *J. Sci. Food Agric.* **2019**, *99*, 5858–5869. [CrossRef] [PubMed]
70. Tuberoso, C.I.G.; Rosa, A.; Montoro, P.; Fenu, M.A.; Pizza, C. Antioxidant activity, cytotoxic activity and metabolic profiling of juices obtained from saffron (*Crocus sativus* L.) floral by-products. *Food Chem.* **2016**, *199*, 18–27. [CrossRef] [PubMed]

71. Sagaradze, V.A.; Babaeva, E.Y.; Ufimov, R.A.; Trusov, N.A.; Kalenikova, E.I. Study of the variability of rutin, vitexin, hyperoside, quercetin in "Crataegi folium cum flore" of hawthorn (*Crataegus* L.) species from Russian flora. *J. Appl. Res. Med. Aromat. Plants* **2019**, *15*, 100217. [CrossRef]
72. Ananingsih, V.K.; Sharma, A.; Zhou, W. Green tea catechins during food processing and storage: A review on stability and detection. *Food Res. Int.* **2013**, *50*, 469–479. [CrossRef]
73. Benvenuti, S.; Bortolotti, E.; Maggini, R. Antioxidant power, anthocyanin content and organoleptic performance of edible flowers. *Sci. Hortic.* **2016**, *199*, 170–177. [CrossRef]
74. Llorach, R.; Martínez-Sánchez, A.; Tomás-Barberán, F.A.; Gil, M.I.; Ferreres, F. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. *Food Chem.* **2008**, *108*, 1028–1038. [CrossRef]
75. Wold, A.B.; Rosenfeld, H.; Lea, P.; Baugerød, H. The effect of CA and conventional storage on antioxidant activity and vitamin c in red and white cabbage (*Brassica oleracea* var. *capitata* L.) and savoy (Brassica oleracea var. sabauda L.). *Eur. J. Hortic. Sci.* **2006**, *71*, 212–216.
76. Henríquez, C.; Speisky, H.; Chiffelle, I.; Valenzuela, T.; Araya, M.; Simpson, R.; Almonacid, S. Development of an ingredient containing apple peel, as a source of polyphenols and dietary fiber. *J. Food Sci.* **2010**, *75*, 172–181. [CrossRef] [PubMed]
77. Donno, D.; Beccaro, G.; Mellano, M.; Cerutti, A.; Bounous, G. Goji berry fruit (*Lycium* spp.): Antioxidant compound fingerprint and bioactivity evaluation. *J. Funct. Foods* **2015**, *18*, 1070–1085. [CrossRef]
78. Guo, C.; Yang, J.; Wei, J.; Li, Y.; Xu, J.; Jiang, Y. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutr. Res.* **2003**, *23*, 1719–1726. [CrossRef]
79. Turkmen, N.; Sari, F.; Velioglu, S. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. *Food Chem.* **2006**, *99*, 835–841. [CrossRef]
80. Rababah, T.M.; Banat, F.; Rababah, A.; Ereifej, K.; Yang, W. Optimization of extraction conditions of total phenolics, antioxidant activities, and anthocyanin of oregano, thyme, terebinth, and pomegranate. *J. Food Sci.* **2010**, *75*, 626–632. [CrossRef]
81. Pellegrini, N.; Colombi, B.; Salvatore, S.; Brenna, O.V.; Galaverna, G.; Del Rio, D.; Bianchi, M.; Bennett, R.N.; Brighenti, F. Evaluation of antioxidant capacity of some fruit and vegetable foods: Efficiency of extraction of a sequence of solvents. *J. Sci. Food Agric.* **2007**, *87*, 103–111. [CrossRef]