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Quality traits of saffron (*Crocus sativus* L.) produced in the Italian Alps

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Abstract: Saffron (*Crocus sativus* L.) is a perennial herbaceous geophyte in the Iridaceae family. It propagates vegetatively by corm. All saffron production processes are generally conducted by hand: from bulb implantation, harvesting of flowers to stigma separation. Saffron is the most expensive spice in the world because of the intensive hand labour required for production. The increasing interest in *Crocus sativus* cultivation and production in the Italian Alpine area could increase revenues for the rural farming economy. Twenty eight dried saffron samples were collected from different farmers of the Italian Alpine area (Lombardia, Trentino Alto Adige, Piemonte and Veneto) between November 2015 and March 2016. Each sample was processed to determine their moisture content and amount of picrocrocin, crocins and safranal using the methods established by the International Organization for Standardization for saffron (ISO 3632 1,2:2010-2011). Over 82.1 % of the samples analyzed were ranked in the highest quality category of the ISO 3632. A high quality saffron product can be produced in the Italian Alpine area suggesting that this crop could serve as a sustainable source of economic revenues to diversified farms in the Alps.

Keywords: Saffron, *Crocus sativus* L.; ISO 3632 1,2:2010-2011; picrocrocin; crocins; safranal; UV-Vis Spectrophotometry, Alps

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1 Introduction

Saffron (*Crocus sativus* L.) is a perennial herbaceous geophyte in the Iridaceae family. Crocus L. genus, which includes around 80 species mainly distributed in the Mediterranean area and in the south-east of Asia (Giorgi A. et al., 2015).

It propagates vegetatively by means of a corm (Gresta F. et al., 2009). Harvesting of flowers and stigma separation are still carried out by hand in most areas. The intensive hand labour is why saffron is widely known as the most expensive spice in the world (Melnyk J.P. et al., 2010).

In Italy, besides *Crocus sativus*, there are several spontaneous *Crocus* species, some of which are distributed in the Alpine area (Aeschimann D. et al., 2004). In particular *Crocus imperati* Ten., *Crocus albiflorus* Kit. and *Crocus vernus* Auct. are spring bloom species while *Crocus longiflorus* Raf. and *Crocus thomasii* Ten. are autumnal species (Giorgi A. et al., 2015).

The increasing interest in *C. sativus* cultivation in the Italian Alps led to expanded production of this spice in recent years as reported in the sector plan of officinal plants 2014-2016 drawn up by the Italian Ministry of Food, Forestry and Agriculture politics (https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/7562). This phenomenon has been mostly evident in fields abandoned by people who moved to the cities during the second half of XX century. Saffron cultivation could represent a valid means for increasing incomes of multifunctional farms in the Alpine area playing a crucial role in the sustainable recovery and development of these areas (Giorgi A. & Scheurer T., 2015).

Saffron, the dried crocus stigmas, is mainly used in food preparation for colouring, flavouring and aromatic properties. In addition, various medicinal properties have been attributed to saffron.

Several scientific studies have reported that saffron and its chemical components are potential anti-ulcer agents (Kianbakht S. & Mozaffari K., 2009), they improve digestion (Nabavizadeh F. et al., 2009), they play a role as anticancerogenic (Abdullaev F.I., 2002), they reduce

atherosclerosis (He S.Y. et al., 2005; He S.Y. et al., 2007; Zheng S.G. et al., 2005), they are used as anti-depressant in the traditional medicine (Akhondzadeh S. et al., 2005).

The main compounds that contribute to the sensory profile of saffron are crocin (mono-glycosyl or di-glycosyl polyene esters), crocetin (a natural carotenoid dicarboxylic acid precursor of crocin), picrocrocin (monoterpene glycoside precursor of safranal) and safranal (aldehyde) (Melnyk J.P. et al., 2010).

Guidelines for the analyses of these major bioactive compounds have been established by the International Standards Organization (ISO 3632 1,2:2010-2011). They have defined procedures to determine these compounds by spectrophotometric analyses and have established the limits by which saffron quality is classified. According to ISO 3632, picrocrocin, safranal and crocins express the flavour or bitterness, the aroma and the colouring respectively. These values are defined as direct reading of the absorbance of a 1% aqueous solution of dried saffron at 257, 330 and 440 nm using a 1 cm pathway quartz cell (Lage M. & Cantrell C.L., 2009).

The aim of this study was to analyze several saffron samples produced during the 2015-2016 season by small farmers of the Italian Alpine area and determine their ISO category.

2 Methods

2.1 Sample preparation

A total of 28 saffron dried samples, which had been grown in 2015, were collected from different small farms of the Italian Alpine area in the regions of Lombardia, Trentino Alto Adige, Piemonte and Veneto (Figure 1) between November 2015 and March 2016 (Table 1).

To obtain a representative saffron sample, a superfine powder (1 g) was prepared using mechanical grinding-activation in an energy intensive vibrational mill MM 400 (Retsch. Haan, Germany). Each sample was introduced into a 50 ml jars with a 20 mm stainless steel ball and let vibrating at a frequency of 30 Hz for 1 minute.

2.2 Analytical methods

Following procedures established by ISO 3632 1,2:2010-2011, moisture content and the amount of picrocrocin, crocins and safranal for each sample was determined.

To measure moisture content, 500 mg of each dried powder saffron sample was incubated in an oven for 16 hours at 103 ± 2 ° C and then weighted again.

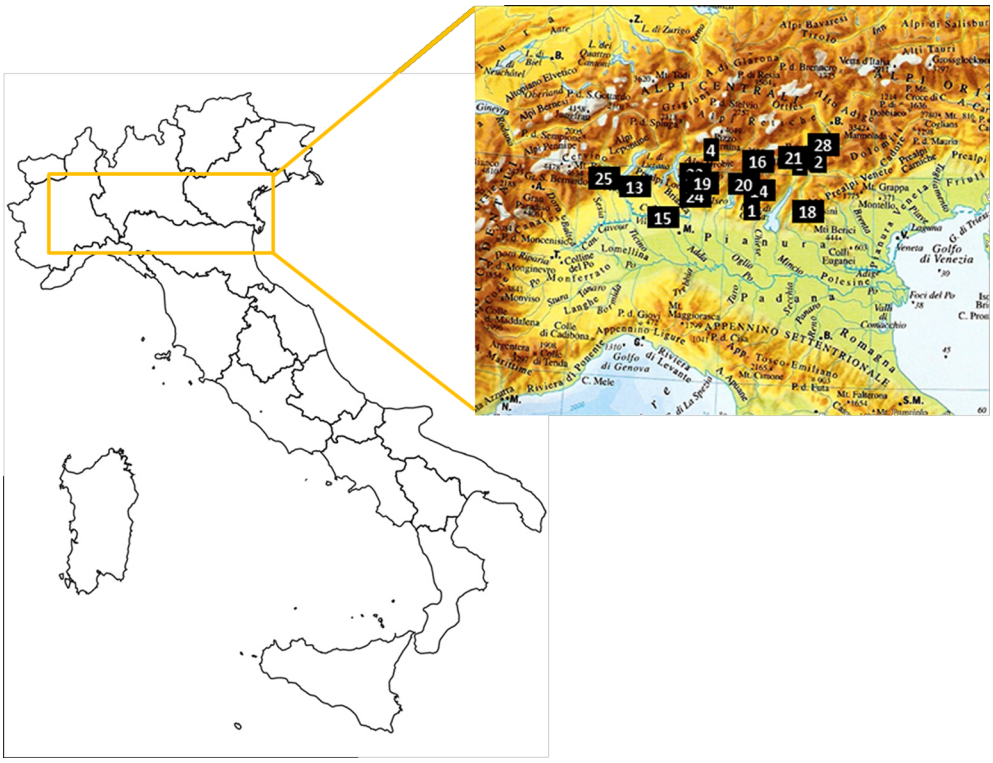

Figure 1 Sample's collection

Table 1. Locations where samples were collected

Sample	Municipality	Province	Region	Latitude N	Longitude E	Elevation (m. a.s.l.)
1	Gardone Val Trompia	Brescia	Lombardia	45°41'28"	10°11'10"	332
2	Rocca Pietore	Belluno	Veneto	46°26'02"	11°58'36"	1143
3	Laives	Bolzano	Trentino Alto Adige	46°25'36"	11°20'18"	258
4	Sondrio	Sondrio	Lombardia	46°10'06"	09°52'16"	307
5	Sondrio	Sondrio	Lombardia	46°10'06"	09°52'16"	307
6	Sondrio	Sondrio	Lombardia	46°10'06"	09°52'16"	307
7	Sondrio	Sondrio	Lombardia	46°10'06"	09°52'16"	307
8	Sondrio	Sondrio	Lombardia	46°10'06"	09°52'16"	307
9	Sondrio	Sondrio	Lombardia	46°10'06"	09°52'16"	307
10	Sondrio	Sondrio	Lombardia	46°10'06"	09°52'16"	307
11	Sondrio	Sondrio	Lombardia	46°10'06"	09°52'16"	307
12	Sondrio	Sondrio	Lombardia	46°10'06"	09°52'16"	307
13	Vedano Olona	Varese	Lombardia	45°46'32"	08°53'15"	360
14	Bovegno	Brescia	Lombardia	45°47'28"	10°16'12"	680
15	Triuggio	Monza e Brianza	Lombardia	45°39'41"	09°16'00"	231
16	Edolo	Brescia	Lombardia	46°10'40"	10°19'59"	720
17	Edolo	Brescia	Lombardia	46°10'40"	10°19'59"	720
18	Vestenanova	Verona	Veneto	45°32'27"	11°13'43"	510
19	Locatello	Bergamo	Lombardia	45°50'10"	09°32'06"	557
20	Darfo Boario Terme	Brescia	Lombardia	45°53'41"	10°11'12"	220
21	Salorno	Bolzano	Trentino Alto Adige	46°14'26"	11°12'42"	224
22	Darfo Boario Terme	Brescia	Lombardia	45°53'41"	10°11'12"	220
23	San Giovanni Bianco	Bergamo	Lombardia	45°52'24"	09°39'15"	448
24	Caprino Bergamasco	Bergamo	Lombardia	45°44'56"	09°29'32"	315
25	Briga Novarese	Novara	Piemonte	45°43'45"	08°27'21"	345
26	Briga Novarese	Novara	Piemonte	45°43'45"	08°27'21"	345
27	Briga Novarese	Novara	Piemonte	45°43'45"	08°27'21"	345
28	Cavalese	Trento	Trentino Alto Adige	46°17'25"	11°27'31"	1000

Moisture content (*wMV*) was determined for each sample using the following formula:

$$wMV = (\mathrm{m0} - \mathrm{m4}) \times \frac{100}{\mathrm{m0}}\%$$

where

m0 is the mass, in grams, of the test portion before incubation;

m4 is the mass, in grams, of the dry residue after incubation.

The amount of picrocrocin, safranal and crocins was determined for each sample following the methods described by Manzo et al. (Manzo A. et al., 2015). Briefly, 500 mg of the powdered saffron was transferred into a 1000 ml volumetric flask and 900 ml of distilled water was added. After stirring with an electromagnetic agitator (Falc 60, Falc Instruments S.R.L., Treviglio, Bergamo, Italy) for 1 hour at room temperature (20°C approx.), the solution was made up to 1000 ml with distilled water and filtered. The extract was diluted (1:10) with distilled water. Extracts were directly analyzed by Varian Cary 50 UV-Vis Spectrophotometer (Varian, Palo Alto, California, USA) to determine the amount of picrocrocin, crocins and safranal 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.

Picrocrocin, safranal and crocins determination ($A^{1\%}_{1cm}$ (λ max)) of each sample was calculated by the following formula:

$A^{1\%}_{1cm}(\lambda\ max) = D \times 10000/m \times (100\text{-}wMV)$

where

D is the specific absorbance

m is the mass, in grams, of the test portion

wMV is the moisture expressed as percentage mass fraction of the sample

Each sample was analyzed in duplicate. All analytical steps were conducted in the dark to keep the saffron solution away from all lights.

3 Results

Results of picrocrocin (flavor strength), safranal (aroma strength) and crocins (colouring strength) amount and moisture content (expressed as percentage) of each sample are reported in Table 2. According to the limits established by the ISO 3632 1,2:2010-2011, a quality category has been also attributed to them. The three quality categories defined by ISO are: first, second and third. The first one is the highest one and it refers to saffron of high quality.

Table 2. Mean absorbance values for picrocrocin, safranal and crocins and moisture content of analyzed samples.

Sample	Flavour/Picrocrocin $A^{1\%}_{1cm}$ (λ 257) = D x 10000/m x (100- wMV)	Aroma/Safranal $A^{1\%}_{1cm}$ (λ 330) = D x 10000/m x (100- wMV)	Colouring/Crocins $A^{1\%}_{1cm}$ (λ 440) = D x 10000/m x (100- wMV)	Moisture (%)	Quality Category*
1	93.59	23.82	234.91	8.41	1
2	76.43	19.95	177.47	7.52	n.c.**
3	112.14	23.58	259.29	6.19	1
4	80.57	48.56	163.10	7.74	3
5	98.01	34.96	240.76	6.70	1
6	106.65	26.02	277.02	9.37	1
7	89.11	20.35	218.94	5.35	1
8	93.65	33.08	196.06	8.11	2
9	90.56	31.29	225.46	8.53	1
10	104.87	21.28	234.17	5.27	1
11	91.89	26.25	231.16	6.21	1
12	103.12	22.74	244.22	9.14	1
13	98.05	25.11	234.06	7.51	1
14	90.55	23.34	214.26	7.96	1
15	96.02	23.59	229.16	8.79	1
16	107.23	25.05	260.86	6.01	1
17	102.89	22.19	230.10	6.44	1
18	98.92	38.32	241.45	6.69	1
19	98.63	30.93	220.32	6.25	1
20	105.10	24.08	267.16	6.39	1
21	86.95	28.22	217.27	8.29	1
22	103.62	34.42	245.90	6.10	1
23	107.89	27.05	253.73	6.69	1
24	90.30	34.20	210.68	7.24	1
25	85.91	36.17	209.11	7.76	1
26	73.19	33.83	175.36	8.90	2
27	82.70	37.65	196.32	7.25	2
28	95.04	41.01	202.19	7.83	1

* ISO 3632 limits for the first quality category (saffron filaments) are: Picrocrocin >70, Safranal 20-50, Crocins >200

* ISO 3632 limits for the second quality category (saffron filaments) are: Picrocrocin >55, Safranal 20-50, Crocins >170

* ISO 3632 limits for the third quality category (saffron filaments) are: Picrocrocin >40, Safranal 20-50, Crocins >120

* ISO 3632 limit for moisture for all quality categories (saffron filaments) is >12%

** n.c.: not classifiable in any of the three quality categories

Concerning flavour strength, aroma strength and coulouring strength determined by UV-Vis Spectrophotometry and according to ISO 3632 1,2:2010-2011, the average observed was: 95.13, 29.18, and 225.37 respectively. Moisture average was instead 7.31 %.

Among the 28 samples, 82.1 % fell within the ISO category 1, 10.7 % in category 2 and 3.6 % for category 3. One sample fell outside the range for ISO categorization.

4 Discussion

Based on the limits fixed by the ISO for the first quality category, saffron can be declared belonging to this category if the amount of picrocrocin is equal or higher than 70, safranal is between 20 and 50 and crocins are equal or higher than 200. Moisture limits for the first quality of category fix this value below 12%. These results indicate that high quality saffron can be produced in the Italian Alp region.

Samples not belonging to the first category had crocins and safranal levels that excluded them from the high quality category as observed in our previous study (Manzo A. et al., 2015). The reason of such result could be due to some factors that have to be considered while producing saffron. The first one is the harvesting of flowers. Normally this step is conducted during the first hours of the day while blossoms are still closed and light is not degrading crocins that are inside stigmas. Light would indeed cause a loss of these compounds. The second aspect to consider is the drying process. This process has to be conducted in an accurate way. Final product has to be dry enough in order to guarantee the preservation of the sample and the stability of metabolites that characterize saffron quality. In the Alpine area there are various methods for getting saffron dry: wood stove, airy electric oven and fireplace are the most used. Depending on temperature and time program, saffron quality could vary due to formation of certain secondary metabolites rather than others (Pardo J.E. et al., 2002; Carmona M. et al., 2005; Gregory M.J. et al., 2005). The third aspect to consider is that saffron quality control has to be conducted at least one month after packaging. During such time and in general during storage time, the chemical compounds of saffron reach stability toward the aroma profile of this spice (Maggi et al., 2010; data not showed). Sample number 2 resulted not classifiable just for this reason.

Our results give interesting information about saffron quality produced in the Alpine area. 82,1% of samples analyzed belong to the first category. In terms of quality it can compete with the world's finest saffron while low income countries might produce cheaper. Nevertheless, saffron profile from the Alps can be strategic resource for mountain economy. It is a high quality luxury good that has the potential to become a more known cultural good. Moreover, it brings sustainable economic value to multifunctional mountain farms in the Alps.

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