1 Introduction

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

*Corresponding author: Daniela Pentimalli, Centre for Applied Studies in the Sustainable Management and Protection of the Mountain Environment-Ge.S.Di.Mont.- University of Milan, Via Morino 8, 25048 Edolo, Brescia, Italy, E-mail: daniela.pentimalli@unimi.it

Annamaria Giorgi, Luca Giupponi, Centre for Applied Studies in the Sustainable Management and Protection of the Mountain Environment-Ge.S.Di.Mont.- University of Milan, Via Morino 8, 25048 Edolo, Brescia, Italy

Sara Panseri, Department of Health, Animal Science and Food Safety (VESPA)-University of Milan, Via Celoria 10, 20133 Milan, Italy
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).

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.

Figure 1 Sample’s collection
A. Giorgi, et al.

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 (A1%1cm (λ max)) of each sample was calculated by the following formula:

\[ wMV = \left( m_0 - m_4 \right) \times \frac{100}{m_0} \%
\]

where

- \( m_0 \) is the mass, in grams, of the test portion before incubation;
- \( m_4 \) 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

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:
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.

| Sample | Flavour/Picrocrocin | A1% \(\text{cm}^{-1}(\lambda 257) = D \times 10000/m \times (100-wMV)\) | Aroma/Safranal | A1% \(\text{cm}^{-1}(\lambda 330) = D \times 10000/m \times (100-wMV)\) | Colouring/Crocins | 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 colouring strength determined by UV-Vis Spectrophotometry and according to ISO 3632 1:2010-2011, 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.

Acknowledgments: This study was supported by “Accordo di Programma affermazione in Edolo del Centro di Eccellenza Università della Montagna” MIUR - Università degli Studi di Milano, prot. n. 386 1293 - 05/08/2011.

References

Abdullaev F.I. Cancer chemopreventive and tumoricidal properties of saffron (Crocus sativus L.). Experimental Biology and Medicine, 2002, 227, 20-25
Aeschimann D., Lauber K., Moser D.M. & Theurillat J.-P. Flora Alpina. Haupt Verlag, Bern, Stuttgart, Wien, 2004
Akhoundzadeh S., Tahmacebi-Pour N., Noorbala A., Amini H., Fallah-Pour H., Jamshidi A., et al. Crocus sativus L. in the treatment of mild to moderate depression: A double-blind, randomized and placebo controlled trial. Phytotherapy Research, 2005, 19, 148-151
Carmona M., Zalacain A., Pardo J.E., Alvarruiz A., and Alonso G.L. Influence of different drying and aging conditions on saffron constituents. J. Agric. Food Chem., 2005, 53, 3974-3979
Giorgi A., Bertoni D., Manzo A., Panseri S. L’oro Rosso delle Alpi. Biblione Edizioni, 2015
Giorgi A. and Scheurer T. Alpine Resources: Assets for a Promising Future-Conclusions from the Forum Alpinum 2014. Mountain Research and Development, 2015, 35(4), 414-415
Gregory M.J., Menary R.C. and Davies N.W. Effect of drying temperature and air flow on the production and retention of secondary metabolites in saffron. J. Agric. Food Chem., 2005, 53, 5969-5975
Gresta F., Avola G., Lombardo G.M., Siracusa L., Ruberto G. Analysis of flowering, stigmas yield and quantitative traits of Saffron (Crocus sativus L.) as affected by environmental conditions. Scientia Horticulturae, 2009, 119, 320-324
He S.Y., Qian Z.Y., Tang F.T., Wen N., Xu G.L., & Sheng L. Effect of crocin on experimental atherosclerosis in quails and its mechanisms. Life Science, 2005, 77(8), 907-921
He S.Y., Qian Z.Y., Wen N., Tang F.T., Xu G.L., & Zhou C.H. Influence of crocetin on experimental atherosclerosis in hyperlipidemic diet quails. European Journal of Pharmacology, 2007, 554(2-3), 191-195.
Kianbakht S., & Mozaffari K. Effects of saffron and its active constituents, crocin and safranal on prevention of indomethacin induced gastric ulcers in diabetic and non-diabetic rats. Journal of Medicinal Plants, 2009, 8(5), 30-38
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. Scientia Horticulture, 2009, 121, 366-373
Maggi L., Carmona M., Zalacain A., Kanakis C.D., Anastasakis E., Tarantilis P.A., Polissiou M.G., Alonso G.L. Changes in saffron
volatile profile according to its storage time. Food Research International, 2010, 43: 1329-1334
Manzo A., Panseri S., Bertoni D., Giorgi A. Economic and qualitative traits of Italian Alps saffron. Journal of Mountain Science, 2015, 12(6), 1542-1550
Melnyk J.P., Wang S., Marcone M.F. Chemical and biological properties of the world’s most expensive spice: Saffron. Food Research International, 2010, 43, 1981-1989
Nabavizadeh F., Salimi E., Sadroleslami Z., Karimian S. M., & Vahedian J. Saffron (Crocus sativus) increases gastric acid and pepsin secretions in rats: Role of nitric oxide (NO). African Journal of Pharmacy and Pharmacology, 2009, 3(5), 181-184
Pardo J.E., Zalacain A., Carmona M., Lopez E., Alvarruiz A. and Alonso G.L. Influence of the type of dehydration process on the sensory properties of saffron spice. Ital J. Food Sci., 2002, 14, 413-422
Zheng S.G., Qian Z.Y., Tang F.T., & Sheng L. Suppression of vascular cell adhesion molecule-1 expression by crocetin contributes to attenuation of atherosclerosis in hypercholesterolemic rabbits. Biochemical Pharmacology, 2005, 70(8), 1192-1199