Economic and qualitative traits of Italian Alps saffron

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Abstract: Saffron, obtained from the flower stigmas of *Crocus sativus* L., is one of the most expensive food spices. The introduction of saffron in alpine areas could help to broaden and diversify the activities of mountain multifunctional farms, with a positive impact on economy and land management. According to ISO 3632 (2010/2011), saffron can be classified into three categories of quality (I, II, III) depending on the concentration of the three main metabolites responsible for its characteristic colour, flavor and aroma: Crocin, Picrocrocin and Safranal. This study represents the first investigation of the quality of saffron produced in the Italian Alps evaluated with spectrophotometry, HPLC, solid-phase microextraction (SPME), and gas chromatographic analysis combined with mass spectrometry (GC/MS). The experiments used *Crocus sativus* stigmas produced in 2012-2013 in different areas of the Central Italian Alps located at an altitude between 720 and 1200 m a.s.l.. Results obtained were compared to commercial saffron. The analyses confirmed that all samples can be classified in the first quality category according to the ISO classification. This high quality is also confirmed by HPLC analysis. Moreover, the SPME-GC/MS analysis identified some differences in the aromatic profile of saffron samples in particular regarding safranal concentration. A preliminary assessment of the economic viability of high quality saffron production for local markets was also performed. Our study provides valid information regarding the quality and economic sustainability of saffron production in the alpine area confirming this crop as a good candidate for a new source of income for multifunctional farms in mountain areas.

Keywords: *Crocus sativus* L.; Alps; ISO 3632; UV–Vis spectrophotometry; SPME-GC/MS; HPLC

Introduction

Saffron is one of the most expensive food spices obtained from the flower stigmas of *Crocus sativus* L., a member of the Iridaceae family (Grestaet al. 2009). It is an autumn-flowering geophyte plant, that can propagate vegetatively only by means of corms.

This plant is mainly used in the food, cosmetic and dyeing industries, but recent studies have also concentrated on its medical properties (Das et al. 2010). Saffron quality depends on the concentration of its three major metabolites providing the unique color, taste and aroma to the stigmas. Its main biologically active metabolites are crocins, a family of red-colored and water-soluble carotenoids. Additionally, picrocrocin (C16 H26 O7) is considered to be the main bitter principle of saffron. It is a monoterpene glycoside precursor of safranal (C10 H14 O), the major volatile oil responsible for the aroma (Lozano et al. 2000). Many methods of saffron component analysis have been described (Tarantilis et al. 1995) and the chemical composition of saffron samples from many countries indicates that the values reported are strongly influenced by the methods employed for drying, extraction and analysis (Lozano et al. 2000; Zareena et al. 2001; Kanakis et al. 2004).

For a long time, saffron cultivation was neglected by researchers and farmers since it was considered a minor crop (Gresta et al. 2008).Only in the last few years has interest increased in 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 the keystone for obtaining an adequate income and, consequently, continuing business (Grande 2011). This trend is particularly evident in mountain areas, where the economic results of conventional agriculture are negatively influenced by natural conditions and by higher production costs compared to the plain, and where CAP subsidies are quite limited. Van Der Ploeg and Roep (2003) identified three different diversification strategies, or pathways, which farmers can opt for: deepening, broadening and re-grounding. In particular, saffron production can be assimilated to a deepening strategy that concerns a reassessment of farm activities towards the improvement of product quality and the development of on-farm processing and, especially, farm-gate selling.

Selling at the farm-gate is a common experience in mountain areas. For example, in the Lombardy Region, where our research takes place, 60% of mountain farms sell their products directly to consumers, a percentage that falls to 14.2% among farms in the plain (Italian Agricultural Census 2010). However, despite the encouraging context, every form of farm diversification requires a business plan to carefully verify economic feasibility.

Our study aimed to provide valid information about the quality and economic sustainability of saffron production in the Central Italian Alps. In particular the quality of saffron produced in this area was evaluated by spectrophotometry, by HPLC and by solid-phase microextraction (SPME) followed by gas chromatographic analysis combined with mass spectrometry (GC/MS).

Additionally, a preliminary assessment of the economic viability of high quality saffron production for local markets was also presented.

1 Materials and Methods

1.1 Plant materials and chemicals

Samples of *Crocus sativus* L. were obtained directly from six producers of different areas of the Central Italian Alps. In particular all the saffron fields were located in Valle Camonica, Brescia, Italy (46° 00’ 00” N;

10° 20' 00" E) and high Val Trompia, Brescia, Italy, (45° 44' 00" N; 10° 12' 00" E) at an altitude between 600 and 1200 m a.s.l.. Samples from these two areas will hereafter be mentioned as samples A, B, C, D, E and F. A sample purchased from a local supplier, with a long experience in saffron cultivation (G), and commercial saffron were also analyzed in order to compare results (H) (Table 1).

Sample collection was performed in 2012/2013. Flowers were picked by hand and then stigmas were separated manually and dried for 2-3 h at 50°C in a forced air oven. Dried stigmas were stored in dark glass jars at 4°C until analysis was performed.

Demineralised water (from Mili-Q system Model MiliporeElix 3; Milipore, France), acetonitrile and methanol of super gradient purity (Sigma-Aldrich, St Luis, MO) were used throughout the experiment. Pure reference standards were obtained from Aldrich (Sigma-Aldrich, St Luis, MO).

1.2 Determination of moisture and volatile matter content

This measure was determined according to the ISO 3632 trade standard (ISO/TS 3632, 2010/2011). Briefly, 2.5 ± 0.001 g of powdered samples were introduced in an oven set at 103°C for 16 h. The moisture and volatile matter content, w_{MV}, were then expressed as a percentage of the initial samples according to:

$$wMV = (\text{initial mass} - \text{final mass}) \times \frac{100}{\text{initial mass}}\%$$

1.3 Spectrophotometric Analysis

For UV-Vis determination all saffron samples were extracted and analyzed according to the ISO 3632 trade standard (ISO/TS 3632, 2010/2011) as follows:

- 500 mg of powdered samples were transferred into a 1000 ml volumetric flask and 900 ml of distilled water were added;
- The aqueous solution was stirred for 1 h away from the light and then brought to 1000 ml with distilled water;
- An aliquot of 20 ml of saffron extract was transferred into a 200 ml volumetric flask and 180 ml of distilled water were added;
- After filtration the aqueous saffron extract was analyzed

Spectral characteristics of aqueous saffron extracts were monitored using a UV-Vis spectrophotometer (model UV-240, Shimadzu, Milan, Italy). According to ISO, picrocrocin, safranal and crocins are expressed as direct readings of the absorbance of 1% aqueous solution of dried saffron at 257, 330 and 440 nm respectively, using a 1 cm pathway quartz cell. Results were obtained by direct reading of the absorbance, A, at three wavelengths, as follows:

E1%1cm 257 nm: absorbance at about 257 nm (maximum absorbance of picrocrocin);
E1%1cm 330 nm: absorbance at about 330 nm (maximum absorbance of safranal);
E1%1cm 440 nm: absorbance at about 440 nm (maximum absorbance of crocins);
Where E 1% 1cm = $(A \times 10000)/(m \times (100\text{-}mMV)$

Where *A* is the specific absorbance, 10000 is the total extract dilution and *m* is the mass of the test portion.

This method allows the determination of the main characteristics of saffron related to picrocrocin, safranal and crocin content. A higher amount of these components results in higher quality saffron (Lage and Cantrell 2009).

1.4 Sample preparation for HPLC analysis

For the determination of crocins, picrocrocin and safranal in saffron, 50 mg of powdered samples were extracted with 10 ml of methanol-water (50:50, v/v) and magnetically stirred for 24 h at 4°C in the dark.

After extraction, samples were centrifuged at 30,000g for 10 min and then the supernatant was collected and filtered through a 0.45 μm filter tip into an HPLC vial for HPLC analysis. Before quantitative chromatographic analysis, 1 ml of 2-nitroaniline (0.5 mg ml^{-1}) was added as an internal standard to 1 ml of each tested sample (Caballero-Ortega et al. 2007).

1.5 HPLC analysis

The HPLC system used was a Jasco LC/Net II/ADC consisting of a degasser, a quaternary gradient pump, an auto-sampler and a UV-Vis detector. A PhenomenexKinetexC18 , 4.6 × 250 mm, 5 μm column was used for this analysis with a column flow of 1 ml min^{-1}. Sample injections were made at 50 ml for all samples and standards. A linear gradient of methanol (10%–100%) in water (15% of acetonitrile) was used as a mobile phase.

The analyses were tripled for each sample. Safranal was detected at 310 nm, picrocrocin at 250 nm and crocin at 440 nm. A calibration curve was prepared within the range concentrations of 0.010, 0.10, 0.5, and 1.0 $mgml^{-1}$. Quantitative determinations were made taking into account the molecular coefficient absorbance of each peak obtained at the wavelength of maximum absorbance of the respective ingredient as previously reported and they are expressed in milligrams per gram of saffron stigmas (Caballero-Ortega et al. 2007). The R^2 range was from 0.9965 to 0.999.

1.6HS-SPMEvolatile compound sampling of powdered saffron

All samples were prepared by weighing exactly 100 mg of powdered saffron samples in a 20 ml glass vial, fitted with a cap equipped with silicon/PTFE septa (Supelco, Bellefonte, PA, USA), and by adding 1 ml of the internal standard solution (IS) in water (1,4-cineol, 1 μg ml^{-1}, CAS 470-67-7) to check the quality of the fibres. Sample pulverization was performed in order to obtain greater representativeness and homogeneity.

At the end of the sample equilibration period (1 h), a conditioned (1.5 h at 250°C) 50/30 μm Divinylbenzene/Carboxen™/polydimethylsiloxane (CAR/PDMS/DVB) StableFlex™fiber (Supelco; Bellefonte, PA) was exposed to the headspace of the sample for extraction (3 h) by CombiPAL system injector autosampler (CTC analytics, Switzerland). 25°C was selected as the extraction temperature in order to prevent possible matrix alterations (oxidation of some compounds, particularly aldehydes and furans) (Panseri et al. 2013a; 2013b). The vials were maintained on a heater plate (CTC Analytics, Zwingen, Switzerland) to maintain a constant temperature during analysis.

1.7 Gas chromatography-mass spectrometry analysis of VOCs

HS-SPME analysis was performed using a Trace GC Ultra (Thermo-Fisher Scientific; Waltham, MA, USA) Gas Chromatograph coupled to a quadrupole Mass Spectrometer Trace DSQ (Thermo-Fisher Scientific; Waltham, MA, USA) and equipped with an Rtx-Wax column (30 m; 0.25 mmi.d.; 0.25 μm film thickness, Restek, USA).

The oven temperature program was: from 35°C, hold 8 min, to 60°C at 4°C min^{-1}, then from 60°C to 160°C at 6°C min^{-1} and finally from 160°C to 200°C at 20°C min^{-1}. Carry over and peaks originating from the fibre were regularly assessed by running blank samples. After each analysis fibres were immediately thermally desorbed in the GC injector for 5 min at 250°C to prevent contamination. The injections were performed in splitless mode (5 min). The carrier gas was helium at a constant flow of 1 ml $^{-1}$. An *n*-Alkanes mixture (C_8-C_{22}, Sigma R 8769, Saint Louis, MO, USA) was run under the same chromatographic conditions as the samples to calculate the Kovats retention indices (KI) of the detected compounds (Kovats 1958). All KI indices were also compared with NIST library search database for polar capillary column.

The transfer line to the mass spectrometer was maintained at 230°C, and the ion source temperature was set at 250°C. The mass spectra were obtained by using a mass selective detector with the electronic impact at 70 eV, a multiplier voltage of 1456 V, and by collecting the data at a rate of 1 scan s^{-1} over the *m/z* range of 30-350. Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds analyzed under the same conditions. The identification of MS fragmentation patterns was performed either by comparison with those of pure compounds or using the National Institute of Standards and Technology (NIST) MS spectral database. Volatile compound measurements from each headspace of saffron extracts were carried out by peak area normalization (expressed in percentage). All analyses were done in duplicate.

1.8 A preliminary economic analysis of saffron production in the Central Italian Alps

A preliminary cost-benefit analysis of saffron cultivation in the Lombardy mountain area was performed, collecting technical and economic data with a very detailed survey of ten producers located in the study area. It should be noted that saffron is not traditionally cultivated in the Italian Alpine area, and therefore the number of growers and crop areas are very limited and, in nearly every case, activity started only a few years ago. In addition to the lack of reliable long-term data, soil and weather conditions determine an extremely high variability in crop yields, making a reliable economic evaluation difficult. As a consequence the following estimate has to be considered as representing an ordinary situation. All technical and economical parameters used to perform the simulation represent the average value observed in the sample. In a few cases we dropped some outliers from the dataset. For each saffron producer the surveys gathered information about:

- Cultivation method (cultivated area, length of planting cycle, planting density, agronomic practices, year of corms uprooting);
- Average yields per year of cultivation and yields of uprooted corms;
- Market prices of saffron and corms;

- Production costs (corms, nets for crop protection, manure, pots, labels, energy, etc)
- Work hours (detailing all cultivation, processing and packaging phases).

In our simulation we hypothesize a crop cycle of five years and a cultivated surface of 500 square meters that is a size two family farm workers can manage without hired labor. Saffron cultivation is projected as a secondary activity in a conventional mountain farm, together with other crops or breeding. For example, in the Alps, this situation is common not only among Italian producers, but also in the Swiss, Austrian and French Alps, where saffron is cultivated near Mund and Wachauer and in the area of Mount Ventoux. For example, the President of the guild of Mund saffron producers declares that viable saffron production is feasible only as a side activity without hired workers (Maurer 2004).

All farming operations, with the exception of soil preparation, are performed by means of manual labor. The planting density is 25 corms per square meter (corresponding to 0.36 t per 500 square meters). In this scenario high quality saffron production is entirely devoted to a short supply chain (private consumers and restaurateurs). The market price of saffron, depending on the quantity sold, is quoted on average at 27.45 US$ per gram of stigmas (average value in the sample). Stigmas are sold in pots. The 5-year yield of dried saffron stigmas is prudentially evaluated at 700 grams per 500 square meters, ranging from 70 grams per year for the first year to 200 grams per year for the fifth year. Further revenue comes from corms which are uprooted at the 5th year; 50% of uprooted corms are hypothesized to be marketable.

In evaluating the economic feasibility of our saffron cultivation scenario, we adopted Sharma et al (2012) and Shah and Tripathi (2009) methodology. Particularly for the entire cultivation cycle and for each year of cultivation we reported the value of output, the sum of production costs and the net return. Successively, we calculated the Net Present Value (NPV), the Benefit Cost Ratio (BCR), the Internal Rate of Return (IRR), the Modified Internal Rate of Return (MIRR) and the Payback Period.

2 Results and Discussion

2.1 Moisture and spectrophotometric analysis

All saffron samples analyzed fulfilled the ISO specifications for category I regarding moisture and the main spectrophotometric characteristics. As can be seen in Table 2 the moisture of all saffron samples ranged from 6% to 9%, values lower than 12%, the maximum limit established by ISO 3632 (2010/2011) for *Crocus sativus* L.. Del Campo et al. (2010) showed that, if the processing temperature was 40°C - 55°C, the lowest and the greatest value for this parameter were respectively 7% and 9%. Carmona et al. (2006) have pointed out that, unless a heat source is used, changes in the chemical composition of the spice, due to the degradation of some compounds, and their fermentation, due to the high water activity, may be observed.

Saffron quality depends on the concentration of its major metabolites and the results of the spectrophotometric analysis are presented in Table 2. As reported, in the samples from ValleCamonica and high Val Trompia E $^{1\%}_{1cm}$ 440 nm ranged from 203.4 to 234.95, E $^{1\%}_{1cm}$ 257 nm ranged from 81.3 to113 and E $^{1\%}_{1cm}$ 257 nm ranged from 23.2 to 36.15.

Comparing all results, sample G showed the best results. The high quality of this saffron can be attributed to the long experience in saffron cultivation of the Pozzolengo farm (Picci 1986).In commercial sample H we observed a high safranal content. The different values among the sites and between the sites and the commercial sample are normally due to the different environmental conditions and cultivation practices (Zarinkamar et al. 2011).

Moreover, the difference in safranal content between the sites and the commercial sample depends on the decomposition of picrocrocin that gives rise to safranal and this process takes place during the processing (drying, storage) of saffron (Rios et al. 1996; Straubinger et al. 1998). The degree of degradation depends on temperature, humidity, light irradiation and other climatic conditions.

2.2 HPLC analysis

Table 3 shows the results obtained by HPLC determination of crocins, picrocrocin and safranal in the 8 samples tested. The use of HPLC for the qualitative and quantitative determination of saffron secondary metabolites has been extensively applied (Caballero-Ortega et al. 2007; Carmona et al. 2006, 2007; Lozano et al. 1999).

The results obtained from HPLC analysis confirm those obtained from ISO measurements. In fact sample G, coming from Pozzolengo farm, showed the best results. In particular, this sample contained a higher amount of crocin (37.3 mg g^{-1}), while the commercial sample H contained a high amount of safranal (0.32 mg g^{-1}). The concentration of crocin, picrocrocin and safranal in Valle Camonica and high Val Trompia samples ranged respectively between28.1-32.5 mg g^{-1}, 4.87-6.62 mg $g^{-1}$and 0.15-0.23 mg g^{-1}. A broad range of values is reported for these saffron components and the amount varies greatly from country to country. Literature data reported values for crocin as varying from 29 mg $g^{-1}$to 45.99 mg $g^{-1}$for Iranian saffron and 67.3 mg g^{-1} for Indian samples (Caballero-Ortega et al. 2004; Sujata et al. 1992). Previously obtained results from Spanish and Iran saffron reported a picrocrocin concentration between 3.69 and 8.14 mg g^{-1} (Caballero-Ortega et al. 2007). Finally, safranal levels reported by some researchers are around a minimum of 0.06 mg g^{-1} and a maximum of 0.29 mg g^{-1}(Hadizadehet al. 2007). Saffron is dried differently (shade, heating system, electric ovens, sunlight, etc.) in various regions of the world, and drying practices are known to affect the final composition of saffron (Straubinger et al. 1998).

2.3 Volatile organic compounds (VOCs) analysis

Safranal, 2,2,6-trimethyl-1,4-cyclohexanedione, 2(5H)-furanone, 3,5,5-Trimethyl,2-cyclohexandione (isophorone), acetic acid and safranal isomer were the six main volatile compounds detected in all saffron headspace (Table 4). As can be seen in Table4, safranal and isophorone represented the main constituent of saffron volatile composition. These two molecules contribute greatly to saffron aroma and are responsible for its typical spicy aromatic and floral notes (Cadwallader 2002). As previously reported, safranal, a monoterpene aldehyde formed by hydrolysis from picrocrocin, is not present in fresh stigmas and its concentration in saffron depends strongly on both drying and storage conditions (Castellar et al. 1993). Carmona et al. (2007) showed that, during the first 2 years of storage, safranal content increased and then decreased again.

The secondary components of the saffron aroma are primary alcohols and aldehydes. Among these chemical classes the detection of some compounds such as safranal isomer, 2,6,6-trimethyl,1,4-cyclohexandione and 2(5H)-furanone was also reported by other authors (D'Auria et al. 2004, 2006; Anastasaki et al. 2009; Maggi et al. 2009). In particular Rodel and Petrzika (1991) attributed respectively to safranal isomer and 2,6,6-trimethyl,1,4-cyclohexandione the citrus and spicy notes of saffron.

2.4 Cost-benefit analysis of saffron cultivation in the Italian Alps

Table 5 shows the results of the cost-benefit analysis, while Table 6 presents the estimated measures of economic feasibility of the investment. Referring to the entire 5-year cycle, the total cost of saffron production amounts to 29,695US$. 64.7% of these costs depend on family labor, that is paid 13.72US$ per hour, a value in line with local agriculture employment contracts. 42.3 % of costs arise in the 1st year, largely due to corm cost and planting work. Total returns account for 32,254US$, split into saffron (59.6%) and uprooted corms (40.4%). Net return is 2,560US$ that corresponds to the remuneration of working capital interests and risks. The Internal Rate of Return of the investment is 5.4% - that is a value greater than 2.37%, the opportunity cost of the working capital – but not to a greater extent, indicating a low residual remuneration of risk, of about 3%.

That result is only apparently unsatisfying. In fact, in agriculture, and even more in mountain agriculture, the full reward of workforce and working capital cannot absolutely be taken for granted. From the household point of view, the farmer's family obtains a net farm income (inclusive of family labor cost and working capital reward that are implicit costs) of 21,775US$ in 5 years, equal to 4,355US$ per year and 15.55US$per hour on average. This amount represents undoubtedly a reasonable supplementary income.

In any case, there are adequate margins to enhance the economic performance of saffron by improving the efficiency of manual operations, above all weeding. Furthermore a longer cycle – up to 10 years – must also be taken into account.

3 Conclusions

A preliminary economic analysis suggests that the cultivation of saffron represents a viable opportunity to diversify agricultural income in multifunctional farms in mountain areas. This opportunity should be seen within a context of reduced generalized public support for agriculture and a focus on specific targets and priorities. Not surprisingly, one of the priorities of the recently approved Rural Development Policy 2014-2020 of the European Union explicitly refers to agricultural diversification development. Particularly in mountain areas farm diversification and a greater integration between agricultural activity and tourism appear necessary in order to maintain farm businesses in the future. From that viewpoint a high value added product such as saffron could reasonably be integrated in a mountain farm short supply chain. Indeed, valid information about the high quality of the saffron produced in alpine areas has been provided by the results obtained from spectrophotometric, HPLC and VOCs analysis. This high quality saffron production obviously cannot compete in the world market with saffron from low-cost manual labor-intensive countries, but should aim at a potential high quality niche market. The process must be accompanied by traceability and quality marking in order to attract more consumer interest while, on the supply side, a well-considered planning of cultivation management techniques is required in order to contain the significant manual labor costs and to prevent rather frequent production losses.

Acknowledgements

This study was partly supported by "Accordo di Programma, affermazione in Edolo del Centro di Eccellenza Università della Montagna" MIUR-Università degli Studi di Milano, prot. no. 386 1293-05/08/2011 and by Fondazione della Comunità Bresciana- Onlus.

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Conflict of Interest: The authors declare that they have no conflict of interest