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Quality traits of saffron produced in Italy: geographical area effect and good practices

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Summary

Saffron (*Crocus sativus* L.) is the most expensive spice in the world and is used in food, cosmetic and dyeing industries. Considering that the production of saffron is increasingly widespread in medium-small Italian farms as well as the scarceness of information and studies regarding the quality of the saffron produced in Italy, the principal aim of this study was to investigate the quality of Italian saffron. Qualitative analysis was conducted in accordance with ISO 3632 1,2:2010-2011 considering 484 samples collected over four years (2015-2018). In particular, moisture content, aroma strength (safranal), colouring strength (crocin) and flavour strength (picrocrocin) were assessed for each sample, and whether spice quality varied according to the geographical area where the spice was produced was also investigated. Qualitative analysis showed that the majority (84-93%) of the samples analysed are of the first quality category, regardless of the year of production. Moisture content and colouring strength are the factors that influence the quality of the spice most. Principal component analysis showed that quality is not influenced by the geographical area where the spice was produced. Finally, some best agricultural practices to obtain a high quality saffron spice are reported.

Keywords: *Crocus sativus*, picrocrocin, crocin, safranal, ISO 3632, food quality

Introduction

Dried stigmas of saffron (*Crocus sativus* L., Iridaceae) are considered the most expensive spice by weight worldwide (WINTERHALTER and STRAUBINGER, 2000). *Crocus sativus* is an autumn-flowering geophyte extensively grown in the Near East and the Mediterranean basin since the Late Bronze Age (ZOHARY and HOPF, 1994; NEGBI, 1999). Saffron is a male-sterile triploid lineage that ever since its origin has been propagated vegetatively only. More precisely, it has been demonstrated that *Crocus sativus* is an autotriploid that evolved in Attica by combining two different genotypes of *Crocus cartwrightianus* Herb., a species occurring in southeastern mainland of Greece and on the Aegean islands (NEMATI et al., 2019). Triploid sterility and vegetative propagation prevented afterwards segregation of the favorable traits of *C. sativus*, resulting in worldwide cultivation of a unique clonal lineage (NEMATI et al., 2019).

Its long scarlet stigmas are highly valued for flavouring foods and for textiles and cosmetic dyeing industries (BASKER and NEGBI, 1983). In addition, several studies have demonstrated that the chemical compounds of this spice act as anti-ulcer agent (TAMADDONFARD et al., 2019), play a role as anti-cancerogens (MILAJERDI et al., 2016), re-

duce arteriosclerosis (GHAFFARI and ROSHANRAVAN, 2019) and have antidiabetic potential (YARIBEYGI et al., 2019), making saffron an interesting matter not only as a spice but also as functional food or as herbal product.

In recent years demand for this spice has increased due to Asian population growth (ARSLANALP et al., 2019) and to the spread of Asian cooking worldwide. Today, world saffron production is estimated at about 200 tons per year (FERNANDEZ, 2004), and the principal producers and exporting countries (Iran, Afghanistan and India) are located in Asia (OEC, 2019). Nevertheless, also in Europe there are some countries that produce this spice and Spain, Italy and Greece are the most important of these. However, in the last century, European saffron cultivation areas have decreased production: in Spain, saffron dropped from 6,000 ha in 1971 to 200 ha in 2004 (FERNANDEZ, 2004), in Greece from 1,600 ha in 1982 to 860 ha according to the most recent information (FERNANDEZ, 2004), and in central Italy they reduced from 300 ha in 1910 to 60 ha some years ago (GRESTA et al., 2008). On the contrary, an enormous increase has been registered in Iran and in other Asian countries (YASMIN et al., 2018). It has been estimated that Iran produces 76% of total world saffron production annually (MILAJERDI et al., 2016). Iran has the widest area cultivated (47,000 ha), most of which is located in the Khorasan province (EHSANZADEH et al., 2004). In Italy, the traditional cultivation areas of saffron are mostly concentrated in Sardinia (about 25 ha) and Abruzzo (about 6 ha) (GRESTA et al., 2008) and, although cultivated areas have decreased compared to past centuries in recent years, there has been renewed interest in saffron cultivation which has increased recently also in northern Italy (GIORGI et al., 2015a; GIORGI et al., 2017; MANZO et al., 2015) in the Alpine macro-Region (EUSALP). Nowadays, Italy produce about 400 kg of saffron per year and is one of major saffron-importing countries together with Spain, France, Sweden, Germany, the United Kingdom and Switzerland (OEC, 2019).

The main reason why these developed countries produce a small amount of saffron is due to the high requirement of meticulous manual operations for its production and the high cost of labor. In fact, the flowers of *C. sativus* must be picked during the early hours of the day, tepals must be carefully separated from stigmas and dried slowly at a low temperature. Furthermore, this work is concentrated on a few days a year and on a few hours a day, and all the other activities (field preparation, corm planting, weeding, etc.) are performed by hand (HUSAINI et al., 2010). No irrigation, chemical fertilization or chemical weed treatments are applied in some geographical areas in which it is cultivated, making it a very interesting option for farms working according to an organic regime, which, in Italy and in Europe, are in continuous expansion (WILLER and LERNOUD, 2017). Due to its biological, physiological and agronomic traits, saffron can exploit

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marginal land and can be included in low input cropping systems, representing a viable alternative crop for sustainable agriculture in fragile agroecosystems such as those in rural and mountain areas (GRESTA et al., 2008). Italy has many abandoned rural areas, mostly in mountain areas, where *C. sativus* is and can be grown (GIORGI et al., 2015a; GIORGI et al., 2017). Moreover, it is a country of important food traditions, and has many high quality foods that are appealing for the development of tourism (CORIGLIANO, 2003). Some Italian traditional dishes are prepared with saffron (such as “risotto alla milanese” and “bagoss” cheese (GIORGI et al., 2015a; GIORGI et al., 2017; MANZO et al., 2015) but currently there are few and fragmentary data about the quality of saffron produced in Italy today, despite the fact that this aspect is of fundamental importance for the start-up and promotion of unique and successful local agro-food chains (GIUPPONI et al., 2018a). For this reason, the aims of this research are:

- To evaluate the quality of saffron produced in Italy during the last four years (2015–2018) according to the procedures established by ISO 3632 1,2:2010–2011 that evaluate the concentration of the three main metabolites responsible for saffron colour, flavour and aroma: crocin ($C_{44}H_{64}O_{24}$), picrocrocin ($C_{16}H_{26}O_7$) and safranal ($C_{10}H_{15}O$) respectively. These analyses were conducted considering about 100 samples for each year as well as their geographical area of production in order to highlight possible qualitative differences (PARIZAD et al., 2019).
- To estimate the average amount of spice produced by Italian farms in 2018 in order to show how much farmers invest in saffron production nowadays.
- To suggest advice and/or best practices useful for farmers to improve saffron quality.

This research was carried out in collaboration with many Italian farms, farmer associations and three Italian Universities.

Materials and methods

Saffron samples

During the four production seasons 2015–2018, 484 saffron samples were collected throughout Italy. In detail, 88 samples were collected in 2015, 126 in 2016, 127 in 2017 and 143 in 2018 (Fig. 1). Each sample, consisting of about 1.5–2 g of dry stigmas, was stored in the laboratory in the dark and at 20 °C, inside a sterile airtight container, in order to avoid altering its chemical characteristics, before being analysed. The geographic coordinates (latitude and longitude) and the altitude of each sampling field were known. The saffron producers from whom the 2018 samples were collected were also asked to

report, on a voluntary basis and using an online questionnaire created using Google Form, the annual quantity of spice produced in order to estimate the average saffron production of the Italian farms involved in this study.

Quality evaluation

In order to determine the quality of saffron produced in Italy the samples collected were pulverized using MM400 vibrational mill (frequency: 30 Hz; time: 1 minute), then moisture content and the amount of picrocrocin (flavour strength), safranal (aroma strength) and crocin (colouring strength) were measured according to procedures established by ISO 3632 1,2:2010–2011. These analyses were performed each year (from 2015 to 2018) two months after the collection of the flowers and drying of the stigmas. Moisture content was determined by weighing 500 mg of dried powder saffron and incubating it in an oven for 16 hours at 103 ± 2 °C. Each sample was later weighed and moisture content (wMV) was calculated by the following formula:

$$wMV = m_0 - m \times 100 / m_0$$

where m_0 is the mass, in grams, of the test portion before incubation and m is the mass, in grams, of the dry residue after incubation.

Amounts of picrocrocin, safranal and crocin were determined by UV-Vis spectrophotometric analyses. 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) for 1 hour at room temperature (20 °C), 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 spectrophotometer (Varian Cary 50 UV-Vis) to determine the amount of picrocrocin, crocin 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 [$A^{1\%}_{1cm}(\lambda_{max})$] of each sample was calculated using the following formula:

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

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. All analytical steps were conducted in the dark to keep the saffron solution away from all light. Spectrophotometric analyses were performed in duplicate. Values of moisture content, picrocrocin, safranal and crocin were used to evaluate the quality category of saffron samples according to the quality category limits established by ISO 3632 (Tab. 1).

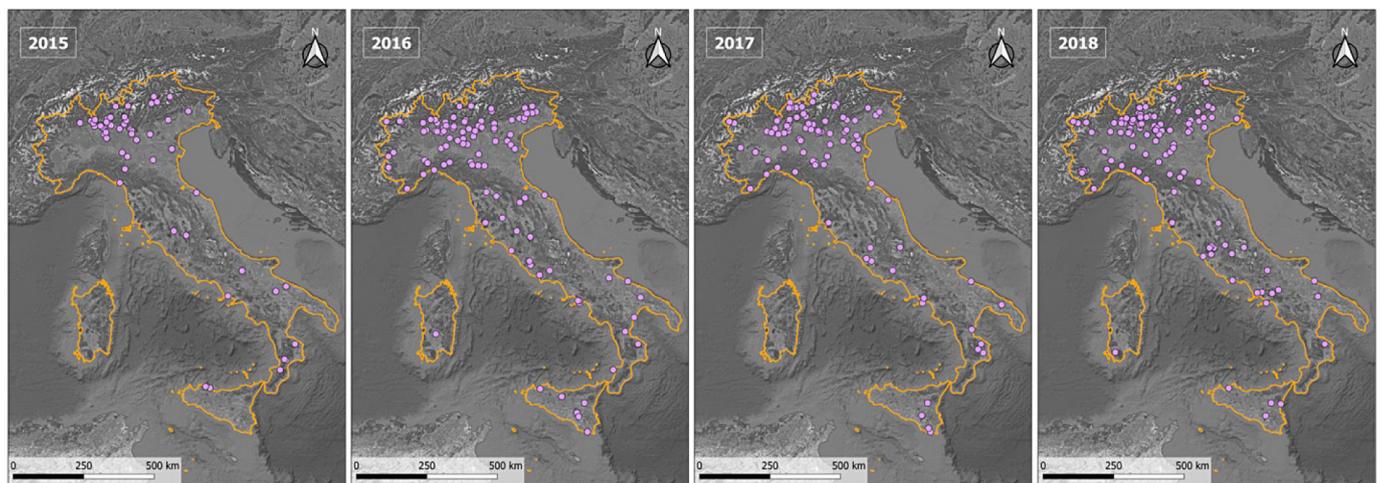


Fig. 1: Geographical origin of the saffron samples (purple dots) divided by year of production/analysis. Orange line shows the borders of Italy.

Tab. 1: Quality category limits for saffron in filaments according to ISO 3632 1,2:2010-2011.

Characteristics	Specifications Categories		
	I	II	III
Moisture and volatile matter content (%)	≤12	≤12	≤12
Flavour strength (picrocrocin) A ₁ ^{1%} cm 257 nm	≥70	≥55	≥40
Aroma strength (safranal) A ₁ ^{1%} cm 330 nm	20-50	20-50	20-50
Colouring strength (crocin) A ₁ ^{1%} cm 440 nm	≥200	≥170	≥120

Statistical analysis

Boxplots were used to visualize the data regarding moisture content, picrocrocin, safranal and crocin amounts of samples according to different years of production. In addition, one-way analysis of variance (ANOVA) was performed considering moisture content, picrocrocin, safranal and crocin as dependent variables and year of production as the independent variable. The saffron samples were also sorted according to principal component analysis (PCA) in order to highlight the main variables that differentiated the samples. PCA was performed considering moisture content, picrocrocin, safranal and crocin amount of each sample and the data referring to their geographical area of production: latitude, longitude and altitude. Statistical analyses were performed using “ggplot2” package (WICKHAM, 2016) of R 3.5.2 software (R DEVELOPMENT CORE TEAM, 2019).

Results

The quality analysis of saffron samples collected from 2015 to 2018 shows that over 80% of samples are in the first quality category (Fig. 2). In particular, 2018 first category samples increased by over 90% and those of the second quality category are in the lower percentage (3-11%) over the four-year period. In all four years the percentage of third category samples is low (1-2%) while the percentage of samples which could not be classified in any quality category increased (3-8%). Considering aroma strength (Fig. 3) and flavour strength (Fig. 4) most samples are in the first category range (Tab. 1) and there are no significant differences between years as shown by the ANOVA results (Tab. 2). Instead, when taking into consideration moisture content (Fig. 5) and colouring strength (Fig. 6), a larger number of samples are outside the first category limit in all years considered. In fact, as reported in Figure 7, the 64 samples (12.8%)

not assigned to the first category have low colouring strength and/or high moisture content. PCA biplot (Fig. 8) shows that the samples are arranged along the first axis (PC1) according to quality category, in particular, from the left to the right of the PC1 the quality of the spice increases, as do flavor strength and colouring strength, while moisture and aroma strength decrease. The elevation of the areas where the saffron samples analyzed were produced (which varies from 0 to 1500 m a.s.l.), as well as latitude and longitude, do not influence the quality category (Fig. 8). According to the 53 data items collected from questionnaires filled out by farmers in the year 2018 it turns out that, on average, Italian farmers produce small amounts of saffron: 144.6 ± 166 g.

Discussion

According to the results obtained in the four-year period (2015-2018), the quality of saffron produced on Italian farms is, mostly, of the first category of quality according to ISO 3632. Samples that are not first quality have, in most cases, high moisture and/or low colouring strength. These are therefore the two main variables that influence the quality of saffron produced in Italy and to which particular attention must be paid. Drying of the stigmas is a particularly delicate and

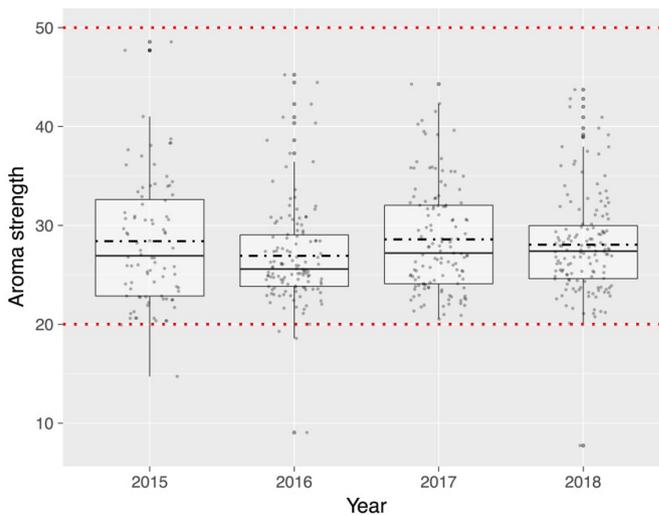


Fig. 3: Boxplots of aroma strength (safranal) of the saffron samples collected/analyzed during the four years (2015-2018). Red broken lines show the limits of the three quality categories (ISO 3632 1,2:2010-2011). For each boxplot, mean (black broken line) and median (black solid line) were highlighted.

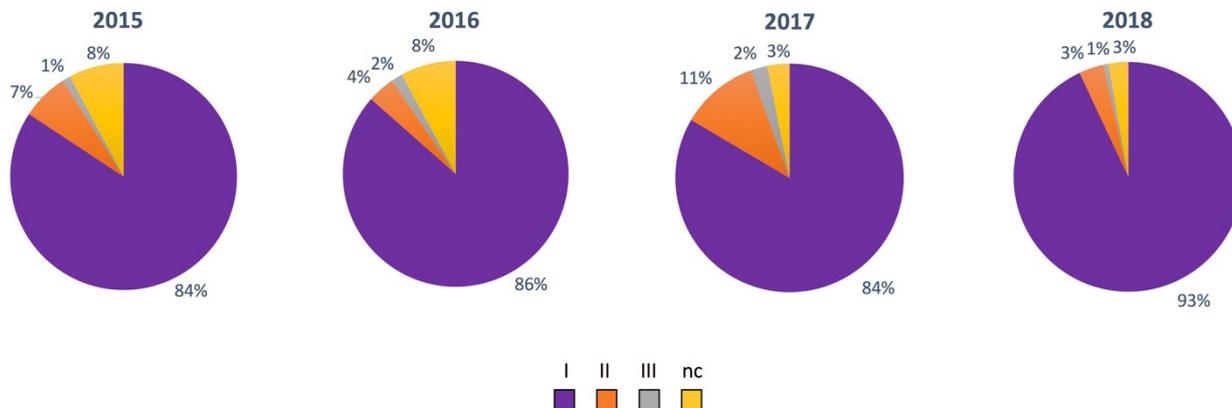


Fig. 2: Quality of Italian saffron produced from 2015 to 2018 according to ISO 3632 1,2:2010-2011. Key: I, first quality category; II, second quality category; III, third quality category; nc, not classifiable.

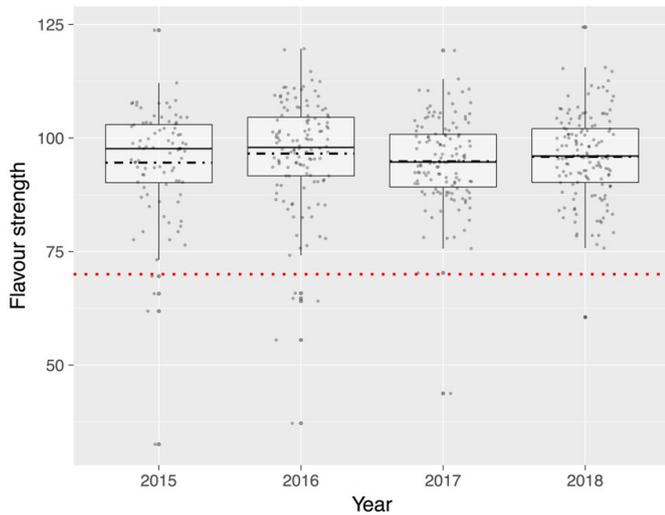


Fig. 4: Boxplots of flavour strength (picrocrocin) of the saffron samples collected/analyzed during the four years (2015-2018). Red broken lines show the minimum limit of the first quality category (ISO 3632 1,2:2010-2011). For each boxplot, mean (black broken line) and median (black line) were highlighted.

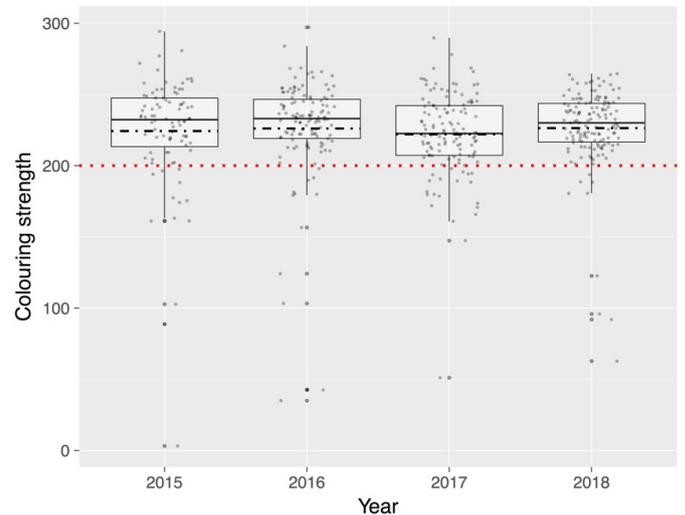


Fig. 6: Boxplots of coloring strength (crocin) of the saffron samples collected/analyzed during the four years (2015-2018). Red broken line shows the minimum limit of the first quality category (ISO 3632 1,2:2010-2011). For each boxplot, mean (black broken line) and median (black line) were highlighted.

Tab. 2: One-way ANOVA results. The effect of year of production on moisture, crocin, picrocrocin and safranal is shown. Key: ns, not significant ($p > 0.05$).

Source of variance	Degrees of freedom	Sum of squares	Mean of squares	F-value	p-value
Moisture	3	21.6	7.186	2.203	0.087 ns
Crocin	3	5477	1826	1.441	0.230 ns
Picrocrocin	3	752	250.7	1.411	0.239 ns
Safranal	3	32	10.75	0.349	0.790 ns

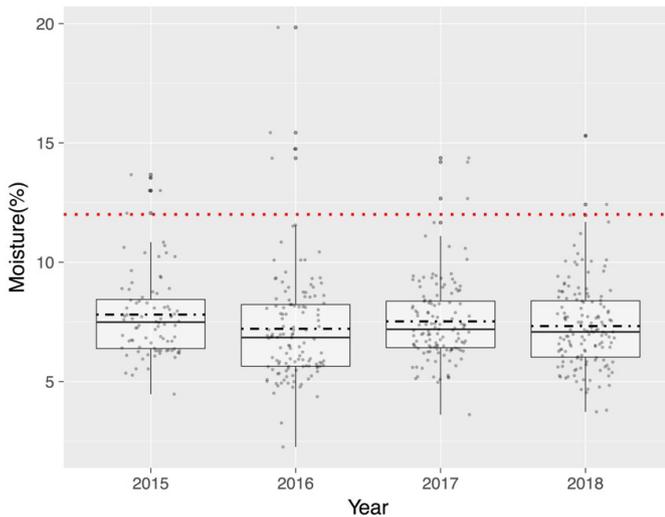


Fig. 5: Boxplots of moisture content in the saffron samples collected/analyzed during the four years (2015-2018). Red broken line shows the maximum limit (12%) of the three quality categories (ISO 3632 1,2:2010-2011). For each boxplot, mean (black broken line) and median (black line) were highlighted.

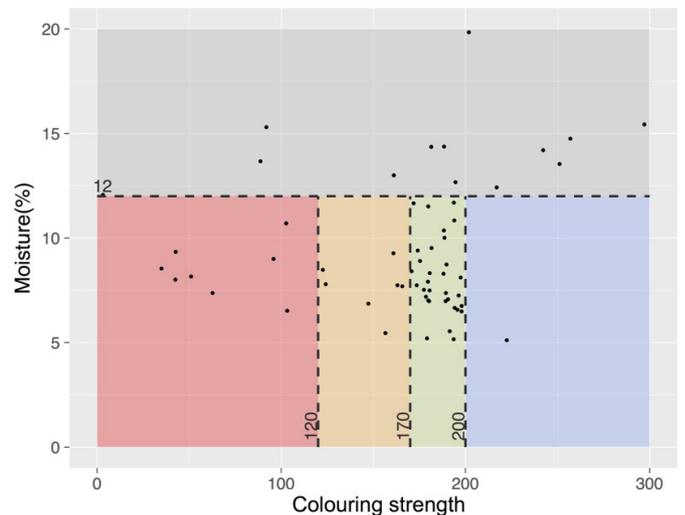


Fig. 7: Moisture content and coloring strength of saffron samples (black dots) not belonging to the first quality category. The broken lines and the background colors define the limits of the three quality categories according to ISO 3632 1,2:2010-2011.

subjective step since farmers perform the procedure using different methods and tools (wood embers, stove, oven, microwave, dehydrator etc.) which can be equally effective but not all easy to use. The fact is that, regardless of the drying method used, saffron must have a moisture content below 12% (Tab. 1), to be classified into one of the three ISO 3632 quality categories. Although the results of this research did not reveal qualitative differences related to the four production years and differences due to the latitude, longitude and altitude of the area where the saffron was produced, these aspects could be better investigated using new approaches and innovative systems for saffron quality assessment (KIANI et al., 2018) and/or by analyzing the volatile organic composition (VOCs) of saffron (MAGGI et al., 2010). In fact, plant secondary metabolites vary, in quantitative and qualitative terms, in relation to the environmental conditions of growth (GIORGI et al., 2015b; GIUPPONI et al., 2018b; PAVLOVIC et al., 2019) and Italy is a country that, due to its geographical characteristics, presents

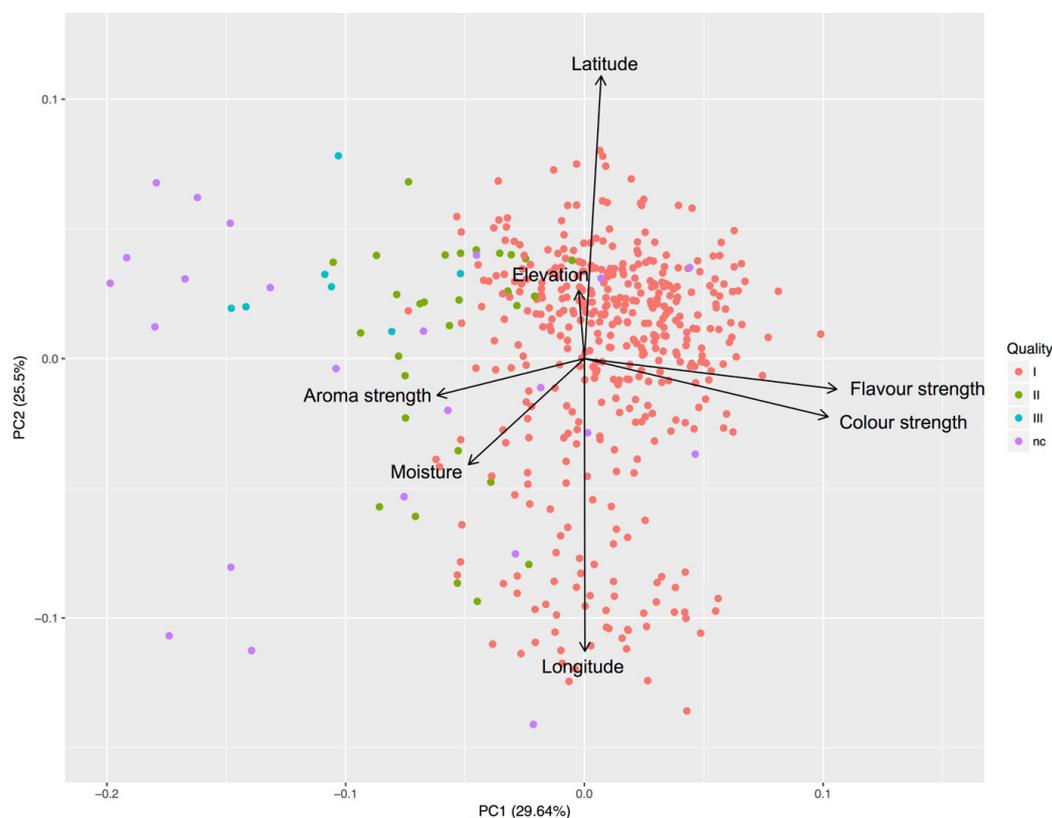


Fig. 8: PCA order biplot of saffron samples associated with geographical (latitude, longitude and elevation) and chemical-physical variables (moisture, flavour strength, color strength and aroma strength). The dots were colored according to the quality category of the samples: I, first quality category; II, second quality category; III, third quality category; nc, not classifiable. The first two principal components (PCs) explain 55.14% of the total variance in the dataset.

areas with somewhat different climatic and environmental conditions (BLASI et al., 2014). The variation of some metabolites produced by plants in relation to the environment was also found for saffron as regards safranal (LAGE and CANTRELL, 2009) but it would be interesting to investigate whether other chemical compounds that make up the aroma profile of saffron vary with the variation of the cultivation environment and spice processing methods. In fact the aroma profile of saffron is complex: as well as safranal, 3,5,5-trimethyl-2-cyclohexen-1-one (isophorone), 2,6,6-trimethyl-2-cyclohexene-1,4-dione (4-ketoisophorone), 3,5,5-trimethyl-3-cyclohexen-1-one (an isomer of isophorone), 2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde (an isomer of safranal), 2,2,6-trimethyl-1,4-cyclohexanedione, 4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-carboxaldehyde (HTCC) and 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one are also responsible for saffron aroma (WINTERHALTER and STRAUBINGER, 2000; MAGGI et al., 2010).

It has been already demonstrated that Italian saffron differs significantly from the same spice from other regions of the world, using different techniques and approaches: PTR-TOF-MS and HPLC analysis showed a sharp separation between Iranian and Italian samples and a higher content of total crocins and safranal in Italian saffron (MASI et al., 2016). The analysis of VOCs showed also to be a potential tool to discriminate saffron samples with different geographical origins (D'AURIA et al., 2004; ANASTASAKI et al., 2009). Also, by a basic analysis as the ISO3632:2011 it is possible to determine if edaphoclimatic and cultivation conditions significantly influence the quality of the spice (PARIZAD et al., 2019). The results of this research are comparable with the ones analysed by the research team for the only Italian Alpine Region (GIORGI et al., 2017) and no significative difference in the quality parameters was found according the area in the Italian Peninsula.

PCA results (Fig. 8) showed that most of the low quality samples are those that have high moisture content and/or those that have low colouring strength, low flavour strength and high aroma strength. While the high moisture content is due to poor drying of the stigmas and/or poor storage, the low colouring strength may be due to prolonged exposure of the stigmas to light during the collection phase and/or that of storage. In fact, crocin, the digentiobiosyl ester of crocetin, has high instability to light (TSIMIDOU and TSATSARONI, 1993). Furthermore, the high aroma strength of some poor quality samples may be related to their low flavour strength. In fact, picrocrocin is a monoterpene glycoside precursor of safranal: β -glucosidase action on picrocrocin liberates aglycone (HTCC) which is then transformed to safranal by dehydration during the drying process of the stigmas (CABALLERO-ORTEGA et al., 2007). Therefore, drying and correct storage of the spice are of fundamental importance for saffron quality. This is also confirmed by BOLANDI et al. (2008) according to whom, after 12 weeks of storage, the colour strength of saffron had a noticeable decrease and bitterness also decreased whereas the aroma increased especially in storage conditions with high temperature and humidity. Given that, based on the results obtained, good quality saffron can be produced in Italy throughout the peninsula and at very variable altitudes (from 0 to 1500 m a.s.l.), this spice could be a resource for rural and/or mountain areas (GIORGI and SCHEURER, 2015) and to develop profitable agricultural systems with low environmental impact.

The sustainable development of marginalized areas is essential to rediscover the relationship with nature, with territorial knowledge and consequently with land conservation. The low environmental impact that this cultivation produces is in agreement with European regulations about organic agriculture (Regulation 2018/848) and the recent 17 Sustainable Development Goals (SDG) established by the United

Nations for the global agenda 2030 (UNITED NATIONS, 2015). In addition, from the perspective of a circular economy, saffron could be a resource because tepals of *Crocus sativus* (the main waste product during the production phases of saffron) can be used not only as a fertilizer for the soil or as ornamentation of dishes/products based on saffron, but also as a raw material for the cosmetic and pharmaceutical industry since saffron petals have substances (flavonoids and anthocyanins) with antioxidant, anti-inflammatory and antidepressant activity (AKHONDZADEH BASTI et al., 2007; HOSSEINZADEH et al., 2007; BABAEI et al., 2014). From the analysis of the data referring to the quantity of spice that the Italian farms involved in this research produced in 2018, it emerged that on average they produce small quantities (145 g) even though some companies produce up to 700 g. The low production of spice per farm is justified by the fact that saffron production involves meticulous and almost exclusively manual work. To encourage greater production in developed countries such as Italy where labour costs are high, it would be appropriate improve the mechanization of different production steps, as HUSAINI et al. (2017) report in their study in the Kashmir area. Furthermore, scientific research should be encouraged so that it is possible to find new low-cost methodologies with low environmental impact to improve production processes (AGHAEI et al., 2018). But above all to maximize flower yield per bulb (without altering the quality). This objective could be achieved with the selection of healthy and productive bulbs or with the use of arbuscular mycorrhizal fungi as recently experimented by CASER et al. (2019). It would also be interesting to develop saffron-based functional cookies as proposed by BHAT et al. (2018), perhaps combined with other typical food products of high nutritional value such as, for Italy, “Mais Nero Spinoso” (CASSANI et al., 2017) and “Grano Siberiano Valtellinese” (GIUPPONI et al., 2019) recently characterized. This would allow the creation of unique, quality and low environmental impact food chains that are in line with the European directives for smart, sustainable and inclusive growth (EU COMMISSION, 2019). Considering that most of the Italian farmers are small producers, to valorise the quality of Italian saffron it could be convenient to organize associations and/or consortiums to better increase the visibility of this precious cultivation. Some associations are already working on this such as the national association “Zafferano italiano”, and the local associations “Oltre la Goggia” and “Zafferano Terra di Lavoro”. Another innovative way to “network” can be the use of social networks like the recent Facebook group (<https://www.facebook.com/groups/zafferanoUNIMONT/>) created to give visibility to the various farmers but also to allow sharing of ideas and information among farmers, researchers, agronomists, restaurateurs and others interested in the saffron chain.

Conclusion

This research assessed the quality of saffron produced in Italy and implemented the scarce data currently available on this topic. A large proportion of the samples produced and analysed over the four years of study were found to be of good quality, regardless of the environment/geographical area of cultivation. Nevertheless, some good practices to improve the quality of saffron are listed below:

- to remove the basal parts of the stigmas, which are often white or less coloured, in order to obtain a spice with high colouring strength;
- to dry the stigmas carefully (moisture must be less than 12% according to ISO 3632) and at temperatures below 55 °C (GIORGI et al., 2015a) to avoid alteration of the thermolabile molecules;
- to preserve the spice in a cool, dark and dry place, inside a sterile airtight container, in order to avoid altering its chemical characteristics (TSIMIDOU and TSATSARONI, 1993; BOLANDI et al., 2008). These suggestions may be useful not only for Italian farmers but also for all those who produce “red golden saffron” and want to improve

its quality since to produce food of high nutritional quality must be a strategic objective for intelligent growth not only in Italy and Europe (EU COMMISSION, 2019) but also throughout the whole world.

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