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How harvest, cleaning and conservation good practices affect the quality of saffron: results of a research conducted in Italy

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Summary

Saffron (*Crocus sativus* L.) spice making requires time-spending manual operations: stigmas are separated from the flower picked in bud early in the morning, and once dried they are preserved protected from light. This study verified how the correct pursuing of these good practices affects saffron quality. Few hours of exposure of the flower to the sun determined a significant decrease in the colouring strength (239.66 ± 10.33 *versus* 255.35 ± 11.87). The correct cleaning of stigmas determined a very significant increase of colouring strength (247.12 ± 13.32 instead of 224.35 ± 14.88) and a significant increase of flavour strength (99.72 ±7.48 against 90.31 ± 6.32, $p < 0.05$). In 24 months, all the samples kept in the dark were still of first category of quality while the ones kept in the light dropped in second category. For all samples there was an increase of aroma strength and a decrease of the flavour and colouring strength, but only the colouring strength loss followed a significantly more sloping trendline. A detectable difference in the content of *trans*-crocin 4 correlated to the ageing or the way of conservation was not found, nevertheless, it was confirmed that the isomers tend towards a photostationary state where the *trans* isomer is more present.

Key words: Saffron production and conservation, food quality, aroma strength, flavour strength, colour strength, crocins

Introduction

Saffron is considered the most expensive spice worldwide (Winterhalter and Straubinger, 2000). This spice is made by the dried stigmas of the flower of *Crocus sativus* L., a geophyte plant of the Iridaceae family. Saffron is interesting not only as a spice but also as functional food and herbal product, and it is used for flavouring foods, dyeing industries and cosmetic (Basker and Negbi, 1983). In recent years the demand for this spice increased mainly because of Asian population growth (Arslanalp et al., 2019) and to the spread of Asian cooking worldwide. Nowadays, world saffron production is estimated about 200 tons per year (Fernández, 2004). The principal producers and exporting countries are Iran, Afghanistan, and India (OEC, 2019). Nevertheless, also in Europe there are some countries that produce saffron, and Spain, Italy and Greece are the most important (OEC, 2019). While in Iran and in other Asian countries there was an increasing of production (Yasmin et al., 2018), in Italy, where saffron is a traditional product since Middle Age (D'ascenzo, 2006), the cultivation followed the trend of land abandonment of Second World War. In central Italy the cultivation reduced from 300 ha in 1910 to 60 ha around the 2000s (Gresta et al., 2008). However, in recent years, there has been a renewed interest for saffron cultivation (Plodari, 2021), also in Italian areas where saffron production is not a tradition, as northern Italy (Giorgi et al., 2015; Giorgi et al., 2017; Manzo et al., 2015).

Saffron requires meticulous manual operations for its making, determining its high value. The flowers of *C. sativus* must be picked in bud early in the morning (Fig. 1), stigmas must be carefully separated from the rest of the flower (Fig. 2) and dried at 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 mostly by hand (Husaini et al., 2010). The process of separation of stigma from the rest of the flower is called "*mondatura*", literally "cleaning", and in Italy the most diffused practice is to leave the three stigma threads attached

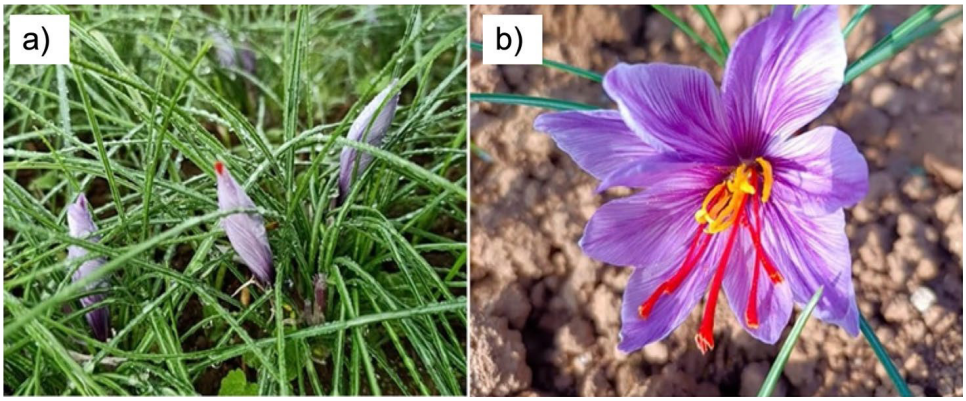

Fig. 1: Flower of saffron collected following the Italian good practices, in bud early in the morning (a), before the flower blooms and stigmas are exposed to sunlight (b).

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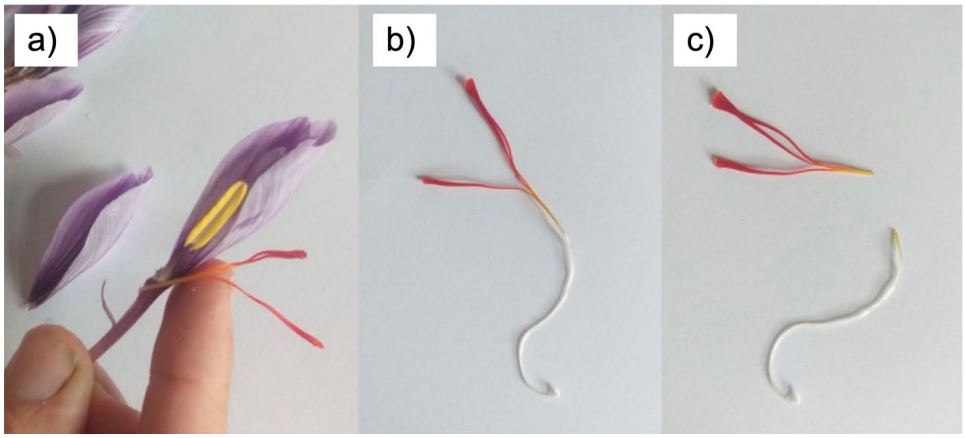

Fig. 2: The manual/traditional process of cleaning saffron ("*mondatura*"): entire saffron flower (a), saffron stigmas and stylus (b), stigmas removed from the stylus (c).

together, forming a cluster, because it has an aesthetically pleasing shape (Fig. 2). In Iran the corresponding procedure is called Negin and Negin saffron is one of the best compared to other types as the "bunch" saffron, where the entire pistil is kept, and stigmas are even tie in a bunch, therefore this quality category is called "Bunch" saffron. Instead, saffron called "Sargol", where just the red top of stigmas is kept, is considered the top quality.

Negin saffron is a subcategory of the first product category following the ISO 3632 1,2:2010/2011, the universally recognized analysis for saffron quality category definition. ISO 3632 1,2:2010/2011 categorizes saffron following the colour, aroma and taste strength through a spectrophotometric analysis, considering additionally the presence of extraneous substances. The analysis considers the characteristic flavour, bitter taste, and pigment of saffron. Aroma strength is linked to the concentration in the matrix of a volatile molecule, called safranal, and its isomers. The taste strength is linked to the presence of picrocrocin, a safranal precursor (Bathaie et al., 2014) that change in safranal during drying and conservation (Chaouqi et al., 2018; Tarantilis and Polissiou, 1997). The colour strength is linked to the presence of a group of molecules named crocins, responsible of the yellow-red colour of saffron. *Cis*- and *trans*-crocins are a family of water-soluble carotenoids. These molecules are known photolabile compounds prone to *trans*-to-*cis* isomerization (Vickackaite et al., 2004) and *cis* forms appear less intensely coloured (Raina et al., 1996).

The fact that saffron is sensitive to light and that the compounds responsible for saffron quality are concentrated in the red segments of the stigmas finds confirmation, in practical terms, in some Italian traditional good practices for saffron making. Flowers, in fact, are collected as much as possible early in the morning, they are then cut and put in thin layer in baskets, afterwards stigmas are cleaned with the previously explained procedure ("*mondatura*") (Fig. 2). Finally, the clean filaments are dried at temperature no higher than 45-50 °C, usually in ventilated oven or electric drier. This last process is the most used in Italy and it is considered the best practice for saffron drying (Giupponi et al., 2019; Raina et al., 1996), together with the traditional drying method near hot charcoal for PDO (Protected Designation of Origin) saffron of Navelli and Sardinia. In Sardina, moreover, saffron is handled with hands wetted with olive oil. These practices are very different from the ones of other countries, for example in Spain a thicker layer is kept and saffron is dried at 70 °C for one hour. Instead, in India saffron is kept from 27 to 50 hours in the sun. After drying, Italian saffron is usually preserved in a dark and dry place, although without a precise monitoring of temperature nor humidity.

With the increasing popularity of this product, a careful monitoring of the quality is important. Until now, however, no scientific studies were done on how much the compliance of these meticulous and time spending good practices affect the quality of saffron. Very often in Italy, where saffron growing is a family-based activity, it is very difficult to collect saffron before the exposure to sunlight and frequently farmers discard saffron from the first category on voluntary bases, without performing formal analysis. The quality of the spice is also given by the care with which the stigmas are separated from the flower, leaving only the most precious part of bright red colour and the presence of the yellow part of the pistil is considered an index of poor quality. Finally, they often consider saffron of the previous year of worse quality, and they discard it from first category without a formal analysis.

The aim of this study was to verify how the correct pursuing of good practices affects saffron quality, in particular considering: 1) collecting the flowers when they are still in bud early in the morning; 2) the correct process of cleaning ("*mondatura*"); 3) the correct conservation in the dark

Materials and methods

Samples collection

Some saffron farmers were chosen on voluntary bases through a "call for participation". Thirty-five samples of saffron were collected from 16 farms located all along the Italian Peninsula (Fig. 3).

Six farmers performed the test: harvest of saffron when the flower is still in bud early in the morning *versus* harvest of blossomed saffron in the afternoon after some hours of light exposure (Fig. 1). Each

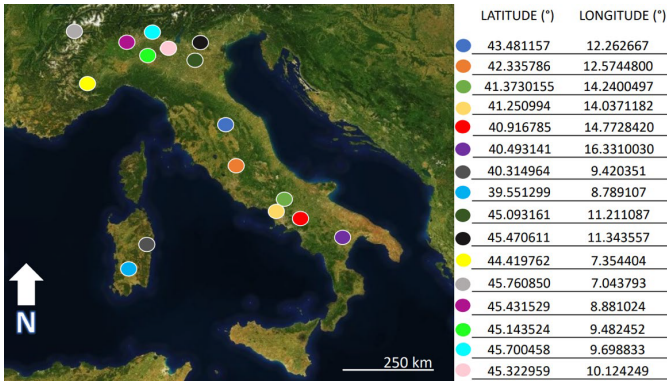

Fig. 3: Distribution of saffron producers participating in this experiment. Some farms participated to more than one experimental trial.

farmer picked 200 flowers in the first two hours after sunrise and let a part of the field unharvested; 200 flowers were collected after the sun zenith but before the sundown, approximately 3 hours after the sun zenith. The test was performed only in sunny days, avoiding cloudy or rainy days. The two samples were dried in the same conditions and kept separated. The results were obtained by six biological replications of the experiment.

Nine farmers dried stigmas from 200 flowers without performing the process of "*mondatura*" and stigmas from 200 flowers cleaned following correctly the process of "*mondatura*" (Fig. 2). The two lots were kept separated and distinguishable. The results were obtained by nine biological replications of the experiment.

Approximately 25 g of four saffron samples were collected to repeat the ISO analysis for two years, to test the effect of conservation in the light *versus* in the dark on the quality of saffron. One sample was kept exclusively for further analysis through High Performance Liquid Chromatography (HPLC) and Nuclear Magnetic Resonance (NMR). The amount of each chosen sample was divided in half, kept in the dark and in the light at 20 ± 5 °C at a relative humidity of 40-50%. Samples were kept in sterile hermetically sealed container in a light chamber (LED Growing Light T5INTEG15, Red:Blue=5:1) and concurrently in a dark chamber for two years.

Quality analysis

The samples collected were pulverized using MM400 vibrational mill (frequency: 30 Hz; time: 1 minute), then moisture content and flavour, aroma and colouring strength were measured according to procedures established by ISO 3632 1,2:2010-2011. 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:

$$wMV = m0 - m \times 100 m0\%$$

where m0 is the mass, in grams, of the test portion before incubation and m1 is the mass, in grams, of the dry residue after incubation.

Flavour, aroma and colouring strength were determined by UV-Vis spectrophotometric analyses. Powdered saffron (500 mg) 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 and analysed by spectrophotometer (Varian Cary 50 UV-Vis) to determine flavour strength, aroma strength and colouring strength expressed as the absorbance of a 1% aqueous solution of dried saffron at 257, 330 and 440 nm respectively. Flavour, aroma, and colouring strength determination [A1%1cm (λ max)] of each sample was calculated using the following formula:

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

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 triplicate. Flavour, aroma and colouring strength were used to evaluate the quality category of saffron samples according to the quality category limits established by ISO 3632 1,2:2010/2011 (Tab. 1).

HPLC and NMR analysis

All the reagents and solvents were purchased from Sigma Aldrich. Merck Silica gel 60 F254 (aluminium foil) plates were used for analytical and preparative Thin Layer Chromatography (TLC).

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

Characteristics	Specifications Categories		
	I	II	III
Moisture content (%)	≤12	≤12	≤12
Flavour strength $A_1^{1\%}$cm 257 nm	≥70	≥55	≥40
Aroma strength $A_1^{1\%}$cm 330 nm	20-50	20-50	20-50
Colouring strength $A_1^{1\%}$cm 440 nm	≥200	≥170	≥120

Picrocrocin was extracted and purified from saffron residues following as previously reported (Catinella et. al., 2022). *Trans*-crocin 4 (TC4) was obtained by purification of commercial crocin (Aldrich, Italy) by preparative TLC. Briefly, 20 mg of commercial crocin were purified using a mixture of ethyl acetate/methanol/water/acetic acid (7:2:1:0.2) as eluent. TC4 (2.8 mg) was obtained in 14% of yield with a purity of 86%. The chemical identity of the purified TC4 was checked by comparison with NMR data reported in literature (Assimiadis et al., 1998).

HPLC analysis were performed with a Liquid Chromatograph Varian ProStar. The instrument was equipped with a ternary pump with a UV-Vis detector Varian Model 345 (both from Varian, Milan, Italy). The analysis was carried out with RP 18 (Hypersil ODS, Thermo, 5 µm 300 × 4 mm i.d.). Runs were carried out using the following gradient: 90/10 (v/v) milliQ water with 15% in volume of acetonitrile until 100% of acetonitrile for 50 min in a flow rate: 1 mL/min. Picrocrocin was detected at 250 nm and crocins at 440 nm. The samples were prepared by extraction of 1.0 mg of saffron powder with 1 mL of 50% aqueous methanol. The extraction was performed in the dark with an ultrasonic bath for one hour at 25 °C. The resulting extracts were filtered through a 0.22 µm nylon filter prior the injection. All the analysis were performed in triplicate. Pure picrocrocin and TC4 were used as standard for quantification by HPLC analyses of saffron extracts. A linear correlation between picrocrocin and TC4 concentration and area of peaks were obtained for the covered concentration ranges from 0.05-2.00 mg/ml for picrocrocin and 10-1000 ppm for TC4 with regression coefficient R^2 of 0.9909 and 0.9953 respectively. As suggest by some authors (Suchareau et al., 2021; Lozzano et al., 1999) the quantitative determinations were made taking into account the molecular coefficient absorbance and were reported in milligrams per gram of saffron power.

NMR spectra were recorded on a Brucker Avance 600 MHz spectrometer using the residual signal of the deuterated solvent as internal standard. Chemical shifts (δ) are expressed in ppm and coupling constants (*J*) in Hertz (Hz). The samples were prepared extracting 10 mg of saffron powder with 1 mL of dimethylsulfoxide-d6 according to the reported procedure (Cagliani et al., 2015). The clear supernatant was collected by centrifugation and used for the analysis without any further dilution.

Statistical analysis

Data were analysed using unpaired t-test and considering degree of freedom and significancy based on P values.

Results

Fig. 4 shows the results of the analysis of quality of the spice obtained from flowers collected early in the morning *versus* blossomed after some hours of light exposure, while Tab. 2 shows the statistical analysis of results: only colouring strength was significantly higher in the

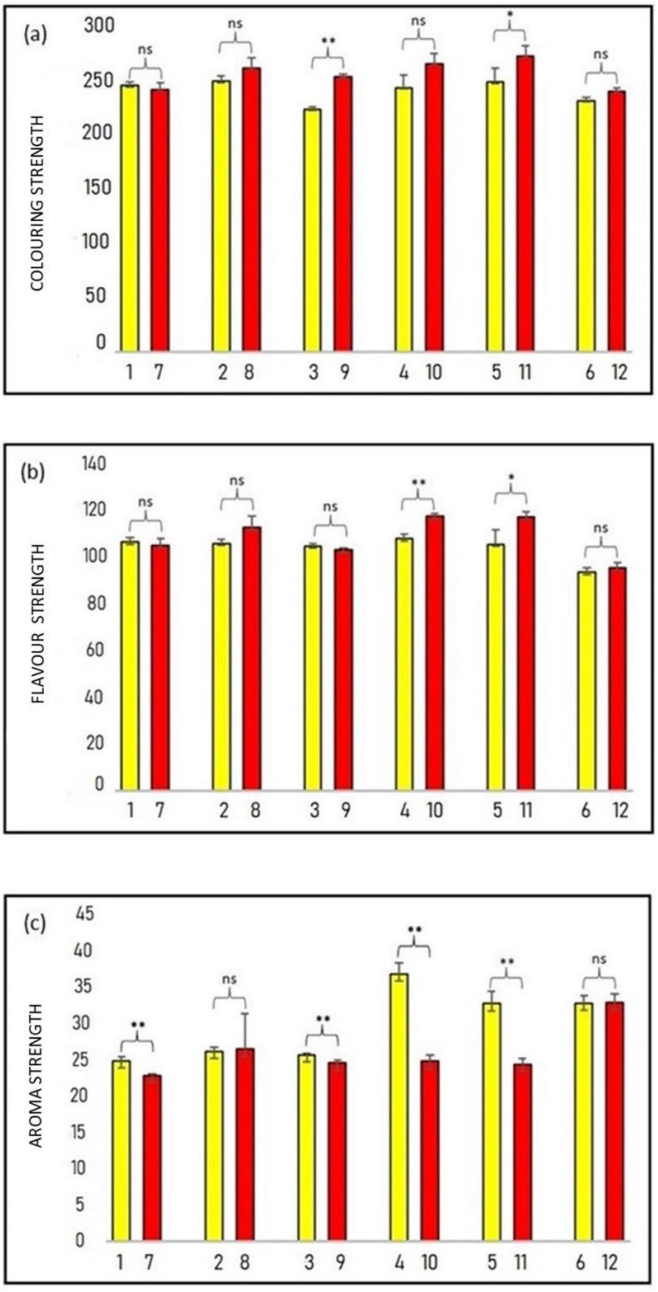

Fig. 4: Quality parameters of saffron samples: (a) colour (b) flavour (c) aroma strength of saffron collected blossomed after some hours of light exposure (yellow) and collected in bud early in the morning (red). Key: *, significantly different ($p < 0.05$); **, very significantly different ($p < 0.01$); ns, not significantly different. Numbers are the codes of samples and samples coupled are from same field (same grower).

Tab. 2: The means of the two groups (saffron collected in bud and blossomed) are compared and considered significantly: *, $p < 0.05$; ns, not significantly different.

Source of variance	t-statistic	df	p-value	
Flavour strength	1.0987	10	0.2977	ns
Aroma strength	1.5589	10	0.1501	ns
Colouring strength	2.3143	10	0.0432	*

flowers collected early in the morning (255.35 ± 11.87 *versus* 239.66 ± 10.33 for flowers collected after light exposure). On average, then, there was a decrease of -15 units in colouring strength if the flower was collected without respecting the good practice of collecting it at sunrise before the exposure to sunlight, while the decrease in flavour and aroma strength was about -4 units on average. All the saffron, nonetheless, remained of first category of quality.

Fig. 5 shows the results of the analysis of the quality of the spice correctly cleaned (following "*mondatura*") *versus* the spice uncleaned, where stigmas were whole kept and dried. In this case, also humidity was considered since it was seen to change among saffron stigmas cleaned and left entire. Tab. 3 shows in fact how humidity (p-value: 0.0463) and flavour strength (p-value: 0.0109) resulted significantly different between cleaned and uncleaned saffron while colouring strength resulted very significantly different (p-value: 0.0035).

The results of the ISO analysis of saffron along the two years of experimentation were arranged in graphics showing the linear trendline and Coefficient of Determination (R^2) to investigate the trend of degradation (Fig. 6). The trendline of the colouring strength showed a more marked slope in case of saffron preserved in the light ("B" samples). The trendline of the flavour strength, instead, did not show a great difference among saffron kept in the dark and in the light, with a decrease (intended as the average slope of the trendline), in both cases, always around -4. The aroma strength showed a positive trend, as predictable, and very similar in saffron maintained in the dark and in the light. Only in the case of colouring strength a strong statistical difference ($p < 0.01$) in the coefficients was found, with a steeper trendline for the conservation in the light regime (-13.95 ± 2.32) than the one kept in the dark chamber (-9.34 ± 0.86).

Tab. 4 shows the concentrations of TC4 (*trans*-crocin 4) and picrocrocin measured by HPLC analysis of three samples of saffron powder before the storage (35) and stored for two years respectively in the dark (35A) and in the light (35B). It is possible to see a time dependant-decrease of the concentration of picrocrocin. This process does not seem influenced significantly by exposure to light as both the samples 35A and 35B showed similar content of picrocrocin. Conversely, no significant changes related to time or light exposure was noticed in the content of TC4.

Fig. 7 shows the ^{1}H NMR profile (DMSO-d6, 600 MHz) of sample 35. In the picture, the expansion of the region between 6.00 and 7.80 ppm of the spectra of the samples (35A and 35B) has been included as comparison. The spectrum showed a singlet at 10.05 ppm related to the aldehydic proton of picrocrocin, a group of signals between 6.50 and 7.40 ppm related to the conjugated double bond of the crocins, signal of carbohydrate protons between 5.4 and 3.0 ppm, and signals at the higher field side of the diagram including the signals related to the methyl of picrocrocin (1.16, 1.18, and 2.08 ppm) and crocins (1.96 and 1.97 ppm). By comparison of the chemical shifts and the coupling constants of the signals between 6.50 and 7.40 ppm with the literature data, the *trans* isomers were confirmed as the major isomers of the crocins in the sample. The signal related to corresponding *cis* isomers of the crocins were not detectable in this area of the spectrum. By comparison of the same region of the spectra of sample 35A and 35B in the expansion, no significant changes induced by ageing or light exposure in the amount of the *cis* and *trans* isomers of crocins were visible.

Discussion

Both saffron collected in bud and blossomed remained in the first category of quality according to ISO 3632 1,2:2010/2011, but the colouring strength resulted sensibly reduced. Carotenoids are photolabile compounds (Lademann et al., 2011) and, in fact, the common Indian practice of sun-drying stigmas is considered the probable

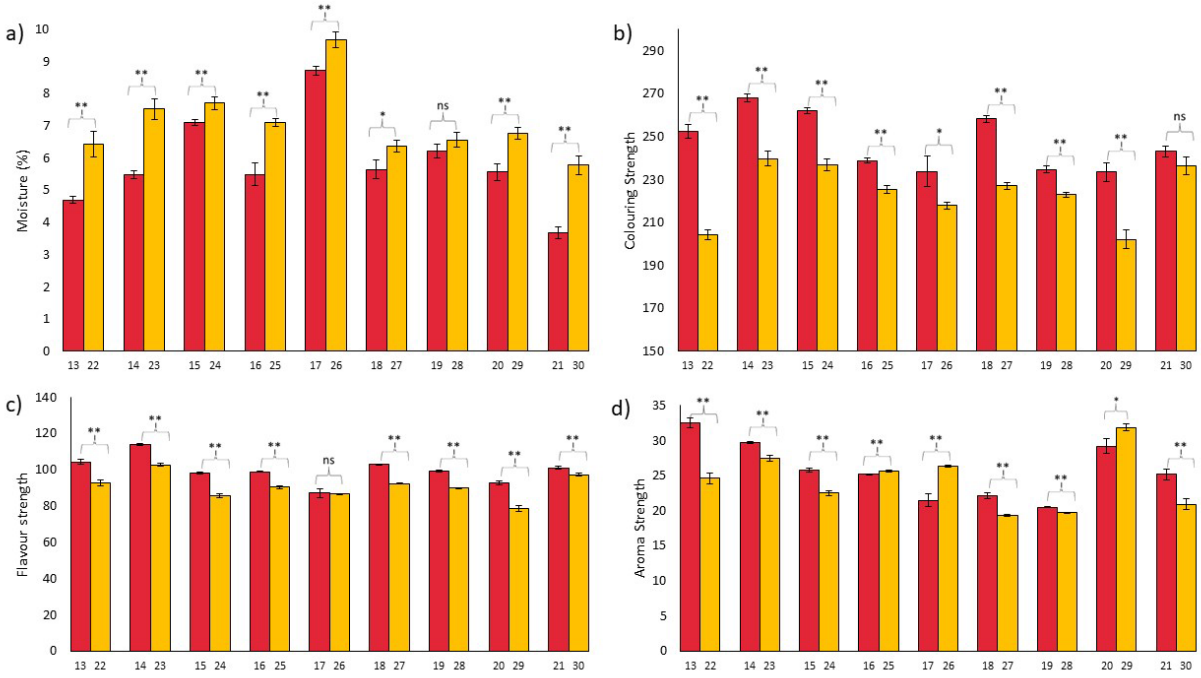

Fig. 5: Quality parameters of saffron samples: (a) moisture content (b) colouring (c) flavour (d) aroma strength of saffron correctly cleaned/"*mondato*" – red, and saffron uncleaned where stigmas are whole kept and dried - orange. Key: *, significantly different if p <0.05; **, very significantly different if p < 0.01; ns, not significantly different. Numbers are the codes of the samples and samples coupled are from same field.

Tab. 3: The means of the two groups (saffron cleaned ("*mondatura"*) and uncleaned) are compared and considered significantly: *, p <0.05; **, p <0.01; ns, not significantly different.

Source of variance	t-statistic	df	p-value	
Flavour strength	2.8809	16	0.0109	*
Aroma strength	0.8832	16	0.3902	ns
Colouring strength	3.4195	16	0.0035	**
Moisture	2.1596	16	0.0463	*

major cause of poor quality of Indian saffron on the global market (Raina et al., 1996). It must be considered that Italy is comprised in the northern temperate zone, and harvest of saffron happens in full autumn, from October to November. In this yearly cycle phase daylight is already decreasing, and incident solar radiation is weaker. For this reason, some hours of exposure to sunlight caused a significant effect on colouring strength without although the prompt fall into second category of the product. However, the saffron used for the research was all broadly in first category, which limit is ≥ 200 for colouring strength, this meaning that the exposure to light could cause the fall into second category of a saffron of lower quality. In the work by Raina et al., 1996, instead, a higher concentration of pigment was found in stigmas of flowers at full bloom (170±7 g/kg) than in flowers in bud (165±4 g/kg), attributing this result to the increased weight of stigmas at full bloom. It is to consider, however, that the research by Raina et al., 1996 did not perform a statistical comparison test of groups. It is possible to see how in the present research, in some cases, there was not a significant difference in the singular biological repetition (Fig. 4). We can conclude, hence, that our results support the good practice of collecting the flower avoiding light exposure as much as possible, although the effect is not dramatic on the quality of the product.

From the results of this research, it is possible to evidence how the good practice of "cleaning" following the Italian traditional process "*mondatura*", significantly affected the flavour strength and the humidity, and very significantly the colouring strength (Tab. 3). For what concerns humidity, this is a very important aspect to consider: although not specifically demonstrated in this research, the yellow parts of the flower, besides containing less or no pigments (Raina et al., 1996), are more aquifer tissues. To an increasing of weight corresponds a higher length/temperature of the drying process to remain in the limit of the ISO 3632 1,2:2010/2011 first category, and the correct method of drying is fundamental in influencing the quality parameters of the spice. Dehydration is not only important to keep saffron quality, but it is also a factor of the release of safranal from picrocrocin via enzymatic activity, producing D-glucose and safranal (Heydari and Haghayegh, 2014). A pushed heat treatment corresponds to a strong accentuation of pigment degradation with an increasing water activity (Tsimidou and Biliaderis, 1997). So, the incorrect cleaning, besides significantly reducing the colouring strength, determines the necessity of a more pushed heat treatment. Considering that moisture content and colouring strength are the factors that influence the quality of the spice most (Giupponi et al., 2019), this aspect become crucial to produce a top-quality spice.

Saffron preserved in the dark remained in the first category of quality for all the period considered, while all the samples from the light chamber dropped in the second category of quality in 24 months (Fig. 6). According to the study of Tsimidou and Biliaders, 1997, saffron carotenoid (mainly crocins) degradation follows first-order-like reaction kinetics but the research highlighted as well how the nature of the reaction medium has a very strong influence on pigment degradation: in anhydrous media decolouring of carotenoids seems to follow zeroth-order kinetics, whereas in an aqueous medium, the reaction is of first-order. Our samples had a moisture varying from 2.5 to 6%, so they are closer comparable to an anhydrous media. As a matter of fact, in the research by Raina et al., 1996, it was demonstrated that a proper packaging and storage with 5% moisture reduced the deterioration of saffron, thereby increasing the shelf-life of the product. Moisture content taken as a key factor of dehydration has been already identified below permitted standard level of ISO 3632 1,2:2010/2011 (Feili et al., 2012): under normal storage conditions,

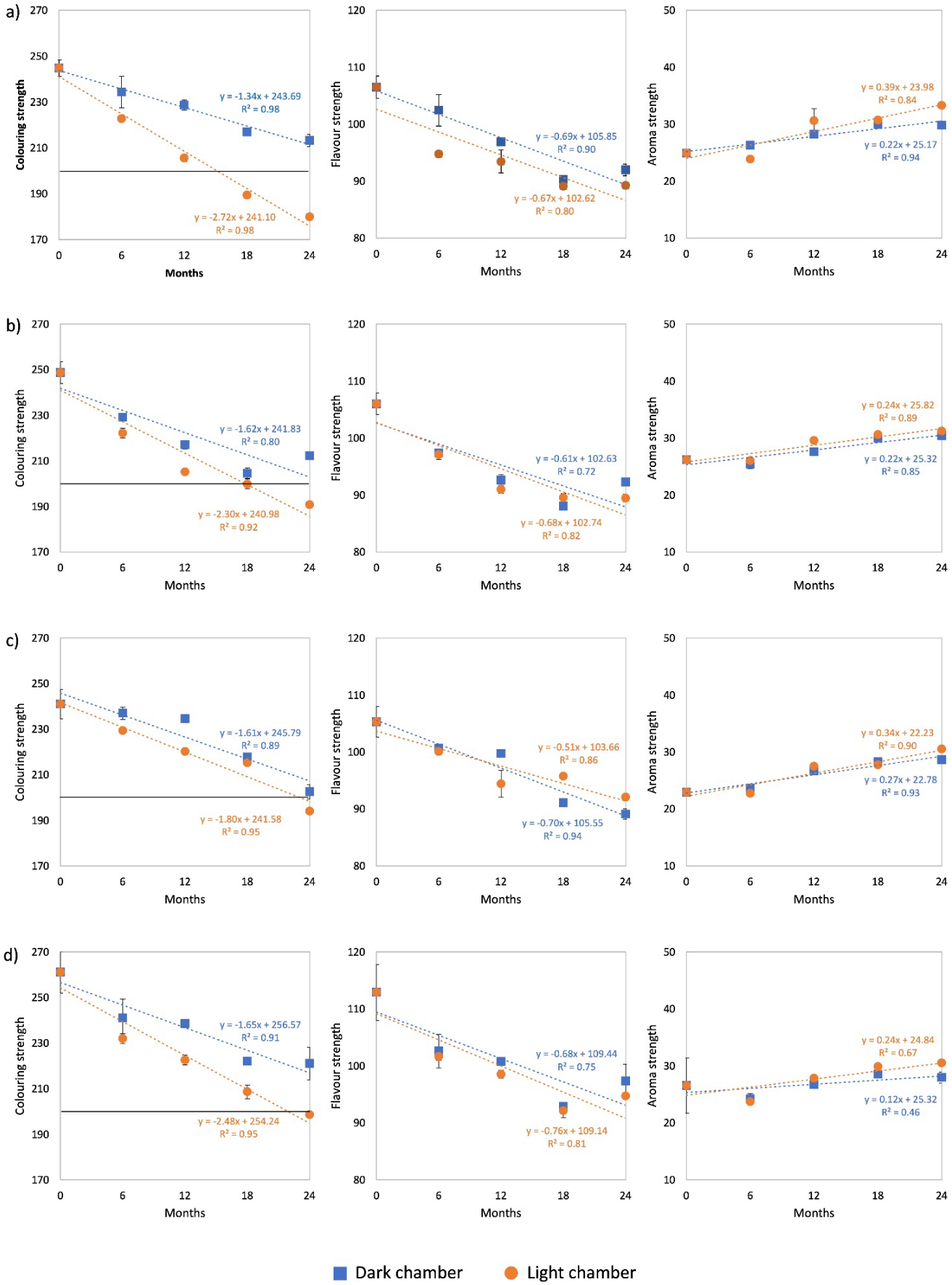

Fig. 6: Colouring, flavour, and aroma strength degradation trendline for samples 31 (a), 32 (b), 33 (c) and 34 (d) preserved in the dark chamber and in the light chamber. The black line indicates the limit of the first quality category for colouring strength.

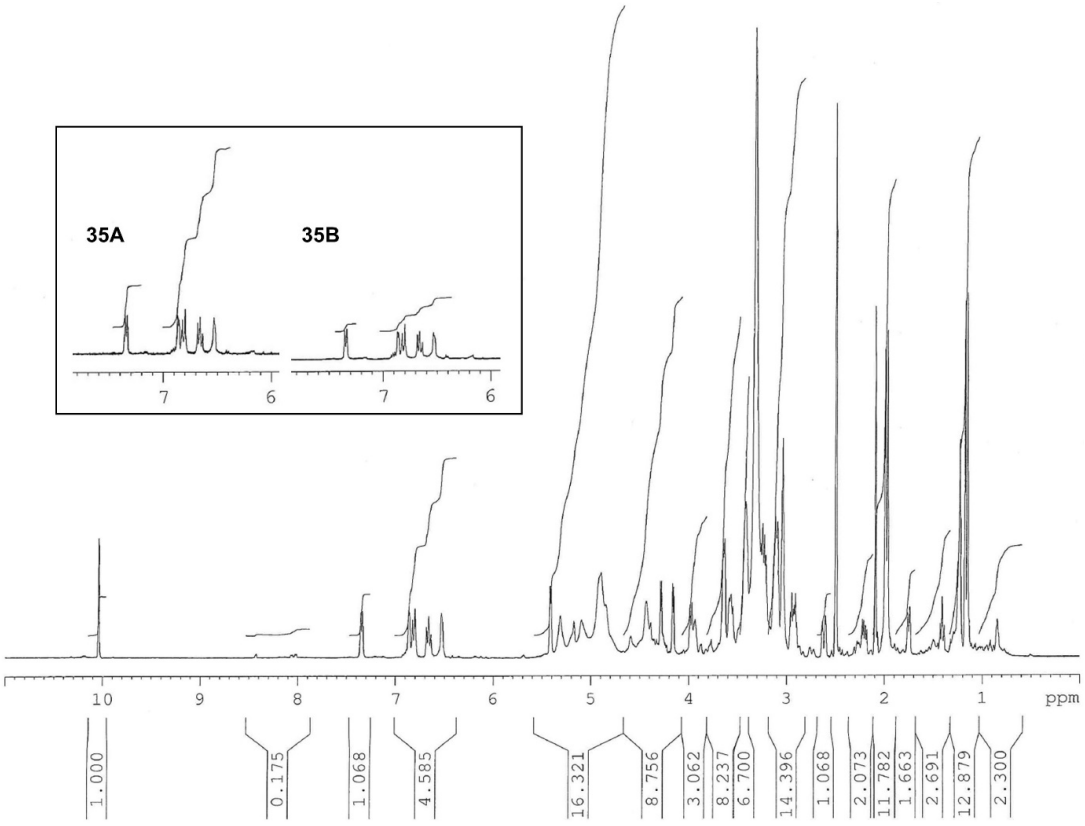

Fig. 7: ^{1}H NMR (DMSO-d6, 600 MHz) profile of sample 35 and after two years kept in the dark (35A) and in the light (35B).

Tab. 4: Concentration of picrocrocin and TC4 of saffron powder before the storage (sample code 35) and stored one year in the dark (35A) and in the light (35B).

Sample	Picrocrocin (mg/g)	TC4 (mg/g)
35	0.72 ± 0.01	64.46 ± 0.02
35 A	0.52 ± 0.01	64.50 ± 0.03
35 B	0.53 ± 0.02	63.16 ± 0.01

12% of moisture and volatile matter content, limits established by ISO standard appeared to be sufficient to hydrolyse crocins (RAINA et al., 1996; FEILI et al., 2012). The solubility of crocins in water, against most carotenoids, promotes its contact with oxygen, and crocins and picrocrocin stability was clearly improved by a reduction in relative humidity rather than other conservation practices, as for example a decrease in temperature (ALONSO et al., 1998). Saffron pigments are among the few soluble carotenoids, and this make their behaviour quite different from the other compounds of this category. While the protective effect of water on the oxidative losses of the most of carotenoids is similar to what observed for oxidative deterioration of lipids, in the case of saffron pigments, it is more similar to the kinetic responses of water-soluble pigment degradation and non-enzymatic browning reactions (RAINA et al., 1996).

The overall loss of bitterness, instead, was best described in the past by a second-order reaction model (ALONSO et al., 1990). Analysis of flavour strength during the two years of storage, did show anyway reasonably good fits to a linear trend (R^2 always > 0.7, and on average > 0.8) (Fig. 6), presumably due to the very slow kinetic responses for this compound (RAINA et al., 1996). The fact that there was not a statistically significant difference in the slope of the trendline of saffron maintained in the dark *vs* in the light can be attributed to the nature of the reaction: one of the main components of saffron volatile organic compounds profile responsible for its aroma is safranal, that is a monoterpene aldehyde, formed in saffron during drying and storage by hydrolysis from picrocrocin (TARANTILIS et al., 1994). β-Glucosidase action on picrocrocin liberates in fact the aglycone: 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, which is then transformed to safranal during the drying process (HIMENO and SANO, 1987). Considering that picrocrocin is degraded into safranal (PFANDER and SCHURTENBERGER, 1982), one of the main compounds of saffron responsible for its aroma, conditions that favour picrocrocin stability have an impact on flavour generation. Our research confirms previous knowledge about the aroma development in saffron: in two years of conservation aroma strength increased, without significant differences between saffron maintained in the dark and in the light, and the loss in flavour strength was accompanied by an increase in aroma strength, although with a less pronounced gradient (average slope for Flavour strength around -4, while for aroma strength between +2 and +3).

As already demonstrated in the previous research by GIUPPONI et al., 2019, quality of saffron, also considering its shelf life, seems to be linked strongly to the changes in its pigments, the crocins (see also RAINA et al., 1996). In general, moisture content and colouring strength are the factors that influence the quality of the spice most (GIUPPONI et al., 2019) and for saffron old more than one year, the colouring strength is usually the parameter that cause the decline of the product from first to second category (LUCA GIUPPONI, personal communication). It is also to point out that all the sample analysed were broadly in the first category of quality, confirming the good quality of Italian saffron, as demonstrated by GIUPPONI et al., 2019 analysing a batch of 143 samples in 2018, that resulted for more than 90% of first category. We can then reasonably say that the drying method mostly used in Italy, which is drying saffron at 40-50 °C in an electric oven for a time depending on the quantity of saffron, can

be considered a good practice, although not specifically analysed in the present research. Since drying saffron on stoves or near the fire coals is a used method in Italy (Zafferano Italiano, 2022), a further field of investigation would be to explore how these different methods influence (if they do it) the sensory characteristics of saffron. It has been already hypothesised that the traditional Sardinian drying method, drying saffron near the fire coals after being gently moistened with olive oil, could reduce oxidation, and improve conservation (Ferri et al., 1997).

Sample 35A and 35B showed a very similar picrocrocin content (Tab. 4), evidencing a negligible difference in keeping saffron in the light chamber or in the dark chamber, at least considering the time-span of the experimentation. This is coherent with the trendline of the flavour strength built through the spectrophotometric analysis, that did not show a significant difference among saffron kept in the dark and in the light. What was possible to observe, was a reduction of the content of picrocrocin after two years of conservation.

All the samples showed concentrations of TC4 (*trans*-crocin 4) comparable with literature data (Caballero-Ortega et al., 2004). In contrast with the ISO analysis, there was not a detectable difference in the content of TC4 correlated to the ageing of saffron, and differences among conservation in the light or dark chamber in the time-span of the experimentation. The NMR spectrum was in good agreement with the data reported by Cagliani et al. (2015). Also considering ^{1}H NMR analysis, no significant changes induced by ageing or light exposure in the amount of the *cis* and *trans* isomers of crocins were visible. This result could appear in contrast with the result of ISO 3632 1,2:2010/2011. It has been already investigated how the effect of light can promote the *trans*-to-*cis* isomerization, while the thermal effect detaches the glycosyl moieties (Vickackaite et al., 2004). However, studies of this kind have been always conducted in condition of accelerated ageing trials (Vickackaite et al., 2004, Raina et al., 1996). It is possible that the time-span of two years is not enough to see clearly this changes. High quality Saffron, dried at the right temperatures and stored correctly, can remain fresh for five years, and changes were observed at the NMR in saffron ten years old (Gigliola Borgonovo, personal communication). Similarly, in the study by Vickackaite et al. (2004), the absorption and fluorescence spectra of a freshly prepared saffron solution stored in the dark at room temperature for 75 days, and a solution irradiated (436 nm, Hg line) for 2 hours were quite similar and few changes were detectable, and just a slight decrease in the intensity of some chromatographic peaks identified as *trans*-crocins against an increase of other identified as *cis*-crocins was detected through HPLC, but these changes were almost undetectable in the absorption and fluorescence spectra because the spectra of all the components are very similar.

If saffron is well preserved, keeping a low water activity and reducing the light exposure, it is possible to realistically affirm that it can last more than two years without changing dramatically its quality. Further, the fact that the isomers tend towards a photostationary state where the *cis* isomer makes up a much lower percentage than the *trans* isomer was confirmed by this study.

Conclusion

The present study confirmed scientifically some good practices adopted by Italian farmers in making saffron, meaning collecting flowers before light exposure, the accurate cleaning of stigmas ("*mondatura*") and the conservation in the dark, also giving an estimation on how much these good practices affect the quality of the spice. The quality of saffron, also considering its shelf life, seems to be linked strongly to the changes in its pigments, the crocins, responsible of the colouring strength, the factor that influence the quality of the spice most. Since the production process of this spice is so unique and its quality strongly depend on meticulous manual operations for its production, it is to be hoped that this Italian product from small producers will receive adequate promotion and valorisation.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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