The colour, bitterness and aroma of l'Aquila DOP saffron

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Riassunto

Il colore, il potere amaricante e l'aroma dello zafferano dell'Aquila DOP sono stati analizzati con Spettrofotometria UV-VIS determinando i livelli di crocina, picrocrocin e safranale. Questi parametri chimici sono stati combinati tra loro in una variabile Z che definisce le caratteristiche di tipicità dello zafferano DOP dell'Aquila. I dati mostrano come non ci sono differenze significative della variabile Z tra le produzioni 2005, 2007 e 2009 e tra le produzioni 2006 e 2008. Si registra pertanto una differenza significativa, ad anni alterni, nella variabile Z, riconducibile principalmente alle caratteristiche pedoclimatiche del territorio e alle tecniche di coltivazione dello zafferano adottate dalla maggior parte dei produttori. I dati ottenuti mostrano una tendenza significativa nel corso degli anni ad ottenere prodotti con le principali caratteristiche chimiche che si alternano. Ulteriori approfondimenti, nelle produzioni successive, saranno necessari, anche al fine di monitorare e mantenere l'equilibrio della variabile Z entro i valori caratteristici che hanno reso lo zafferano dell'Aquila DOP tanto apprezzato in tutto il mondo.

Abstract

The colour, bitterness and aroma of L'Aquila DOP saffron were investigated according to crocin, picrocrocin and safranal levels, using UV-VIS spectroscopy. These chemical components were combined to define the Z variable for the specific characteristics typical of this L'Aquila DOP saffron. These data showed no significant differences for the Z variable for L'Aquila DOP saffron production between 2005, 2007 and 2009, and between 2006 and 2008. The significant differences in the Z variable thus occurred over alternating years, which largely arises from the characteristic pedoclimatic of the local area and the particular cultivation techniques of saffron used by the majority of growers. These data show significant trends over alternating years for the main characteristic chemical components, indicating the need for further analysis and monitoring of the Z variable to maintain the equilibrium that has made this L'Aquila DOP saffron so appreciated throughout the World.

Parole chiave: picrocrocina, crocina, safranale, *Crocus sativus* L.
Key words: picrocrocin, crocin, safranal, *Crocus sativus* L.

Practical applications

It is important for producers and users of L'Aquila DOP saffron that the equilibrium between colour, bitterness and aroma that has made this saffron so appreciated throughout the world is maintained. With the ancient traditions for its production passed down from father to son, the saffron growers need to continue to follow these processing technologies. To be sure of this, there is the need to define and monitor the specific characteristics that are typical of the L'Aquila DOP saffron. The present study provides such a monitoring process. Here, the combination of the colour, bitterness and aroma characteristics (the Z variable) of L'Aquila DOP saffron over the five-year period from 2005 to 2009 are shown to vary according to climate and growing conditions, indicating that the ancient processing technologies of this L'Aquila DOP saffron are indeed being respected. Similar future monitoring are needed to confirm that this situation is maintained.

Introduction

The saffron from L'Aquila (Abruzzo, Italy) is recognised as a protected designation of origin (DOP) according to EU Regulation N° 2081 of 2001 (Government Decree, 2003). The zone that comprises the thirteen boroughs of the L'Aquila province has been officially recognised as the protected production area. This zone is located at an altitude of between 350 m and 1,000 m above sea level, and it extends over two plateaus: Navelli and Prada d´Ansidonia.

The saffron produced in this area of L'Aquila is obtained from the dried stigma of the *Crocus sativus* L. plant, a cultivated species of crocus that belongs to the Iridaceae family. The *C. sativus* from which this saffron DOP is obtained is cultivated as follows: the cultivated soil (rotated every 5 years) is prepared by ploughing to a depth of 30 cm and applying organic fertilisers; the flower-beds are laid in furrows that are 2 cm to 4 cm deep and set from 20 cm to 25 cm apart; the planted bulbs are picked in mid-August, with the collection of the flowers and the successive processes carried out by hand; preservation of the finished product is obtained by drying over almond, olive or oak charcoal using a sieve, for a time that varies according to the depth of the layer of saffron. The drying phase during which the fragrant element of the saffron, safranal, develops is essential (Tammaro and Di Francesco, 1978; Massimini, 1980; Piccioli, 1932).

The main components of saffron are crocin, which is responsible for the characteristic colour, the glucoside picrocrocin, and safranal. Crocin is the digentiobiose ester of the carotenoid crocetin: the trans-crocetin di-(β-D-gentiobiosyl) ester. Crocetin itself is a conjugated polyene dicarboxylic acid that is hydrophobic, and thus oil soluble. Picrocrocin is responsible for the saffron aroma, and from this the safranal component is derived. Safranal is the principal component of the essential oil, with its characteristic scent, and thus represents the odour aspect. Safranal is only formed from the picrocrocin at a late stage during the toasting and storage of the finished product. The heat, combined with enzymatic action, splits picrocrocin to yield D-glucose and a free safranal molecule (Amelotti and Mannino, 1977; Corradi and Micheli, 1979a, b; ISO3632 (1), 2003; ISO3632 (2), 2003).

With the aim of characterising the L'Aquila DOP saffron, we carried out an investigation into these chemical components. This will also allow better differentiate of this L'Aquila DOP saffron from saffron produced in other areas across Italy and abroad, with particular regard to its aroma, as its most distinctive and recognised characteristic.

Materials and Methods

Samples

Two hundred and thirteen saffron samples from the Navelli and Prata D'Ansidonia plateaus were obtained in the period from 2005-2009. The analyses for the chemicals crocin, picrocrocin and safranal was carried out according to Corradi *et al*. 1979b, and performed according to the schemes suggested by UNI CEI EN ISO/IEC 17025:2005.

Colour and bitterness

The analyses of the crocin and picrocrocin that are responsible for the colour and bitter taste of saffron were performed using spectroscopy absorbance measurements of aqueous extracted samples, each prepared from the saffron samples as follows: following crushing and sieving, 0.10 g powered saffron was weighed and transferred into a 250 mL conical flask with an emery stopper. To this was added 200 mL distilled water, and the sample was left in the dark at room temperature for 3 h, with occasional mixing. The sample was then filtered through a cellulose acetate membrane (0.45 μm), and 80 mL distilled water was added to 20 mL of this filtered solution. The UV-VIS scans (210-550 nm) of these final samples were carried out in a 1-cm path-length UV cell, using distilled water as the blank. Absorbance readings were at the wavelengths of 257 nm, 297 nm and 440 nm. The data were expressed as arbitrary units (a.u.), calculated according to the following:

$E_{440}^{10/000}$: extinction of the water extract 1-10,000.
Crocin levels: $E_{440}^{10/000} \times 7.305$.
ΔE_{pic}: $E_{257}^{10/000} - E_{297}^{10/000}$.

Aroma

Safranal is responsible for the fragrance of saffron, and it was determined by spectroscopic absorbance of sample distillates, each prepared as follows: following crushing and sieving, 0.10 g powered saffron was weighed into a glass pot, and 20 mL 3% (w/v) barium hydroxide in distilled water was added. The sample was distilled by steam stripping, until 200 mL distillate was collected. UV analysis was carried out with the extinction reading at 318 nm (E_{318}). As for crocin, the safranal levels were determined as a.u., according to: $E_{318} \times 4$.

The Z variable

The Z variable was calculated using the relationship between the colour element, as crocin (extinction reading at 440 nm; E_{440}; a.u.) and the sum of the aromatic and bitterness components, as picrocrocin (calculated as E_{257}-E_{297}; ΔE_{pic}; a.u.) and safranal (extinction reading at 318 nm; E_{318}; a.u.), i.e. Z (a.u.) = $E_{440}/(\Delta E_{pic}+E_{318})$.

Statistical analysis

The results obtained were analysed using the SPSS programme for Windows software (version 15.0, 2006).

Results and Discussion

The aim of the present study was to determine the chemical characteristics of a series of L'Aquila DOP saffron samples collected from 2005-2009, in terms of the principal components of crocin, picrocrocin and safranal.

Here we used the Z variable to express the equilibrium that includes all of these chemical components that together define the specific characteristics of the colour, bitterness and aroma, respectively, that are typical of L'Aquila DOP saffron. The data obtained from the descriptive analysis of these saffron samples from this five-year period are reported in Table 1. These data highlight how the index of symmetry and kurtosis are not particularly reliable because of the variability associated with the number of samples analysed (ca. 50 samples within each year of production).

For each variable and for each year of production, the distribution and the Q-Q plots for normality were constructed. Figure 1 illustrates an example of the data, as the frequency distribution (Fig. 1A) and Q-Q plot (Fig. 1B) corresponding to the safranal levels of the L'Aquila DOP saffron produced in the year 2007. Figure 2 shows the Tukey box and whiskers plots for the main components across the five years of these L'Aquila DOP saffron samples. For the Q-Q plot, the normal distribution was evaluated by the Shapiro–Wilk test (Table 2).

For commercial purposes, the market requires that the colour, bitterness and aroma properties are very homogeneous between samples produced during the same year and amongst samples produced across different years of production.

Table 1. Descriptive statistics of the samples of L'Aquila DOP Saffron from 2005 to 2009

Production year (n)	Statistical parameter	Safranal (a.u.)[1]	Standard error	Crocin (a.u.)[1]	Standard error	ΔE_{pic} (a.u.)[1]	Standard error	Z variable (a.u.)[1]	Standard error
2005 (49)	Mean	5.442	0.080	14.757	0.125	0.650	0.008	1.010	0.011
	Median	5.512		14.683		0.656		1.010	
	Variance	0.313		0.771		0.003		0.006	
	Standard deviation	0.560		0.878		0.055		0.077	
	Minimum	4.220		13.075		0.374		0.880	
	Maximum	6.460		16.684		0.523		1.230	
	Interquartile distance	0.756		1.133		0.081		0.100	
	Asymmetry	-0.327	0.340	-0.130	0.340	-0.130	0.236	0.787	0.340
	Kurtosis	-0.322	0.668	-0.449	0.668	-0.775	0.464	0.927	0.668
2006 (61)	Mean	5.665	0.079	12.949	0.260	0.633	0.085	0.869	0.017
	Median	5.688		13.383		0.635		0.880	
	Variance	0.379		4.122		0.004		0.018	
	Standard deviation	0.616		2.030		0.066		0.135	
	Minimum	3.976		9.168		0.462		0.590	
	Maximum	6.780		16.129		0.767		1.170	
	Interquartile distance	0.716		3.484		0.097		0.210	
	Asymmetry	-0.596	0.306	-0.204	0.306	-0.305	0.306	-0.034	0.306
	Kurtosis	0.497	0.604	-1.265	0.604	-0.098	0.604	-0.544	0.604
2007 (56)	Mean	5.720	0.103	15.174	0.108	0.680	0.008	0.996	0.017
	Median	5.886		15.271		0.682		0.955	
	Variance	0.591		0.652		0.004		0.016	
	Standard deviation	0.769		0.808		0.597		0.127	
	Minimum	4.096		13.492		0.536		0.790	
	Maximum	7.044		16.802		0.799		1.370	
	Interquartile distance	1.089		1.278		0.087		0.160	
	Asymmetry	-0.464	0.319	-0.310	0.319	-0.199	0.319	0.956	0.319
	Kurtosis	-0.571	0.628	-0.737	0.628	-0.434	0.628	0.307	0.628
2008 (47)	Mean	5.916	0.096	13.551	0.262	0.658	0.010	0.871	0.015
	Median	5.972		13.186		0.676		0.860	
	Variance	0.434		3.223		0.004		0.011	
	Standard deviation	0.659		1.795		0.066		0.103	
	Minimum	4.312		9.314		0.443		0.710	
	Maximum	7.304		8.992		0.834		1.160	
	Interquartile distance	0.716		2.856		0.066		0.140	
	Asymmetry	-0.320	0.347	0.207	0.347	-0.785	0.347	0.483	0.347
	Kurtosis	0.224	0.681	-0.114	0.681	2.060	0.681	0.112	0.681
2009 (53)	Mean	5.190	0.062	14.989	0.146	0.682	0.008	1.041	0.011
	Median	5.244		14.910		0.691		1.062	
	Variance	0.206		1.125		0.003		0.007	
	Standard deviation	0.453		1.061		0.058		0.082	
	Minimum	4.296		12.360		0.532		0.841	
	Maximum	6.412		17.306		0.792		1.198	
	Interquartile distance	0.696		1.457		0.086		0.116	
	Asymmetry	0.283	0.327	-0.305	0.327	-0.677	0.327	-0.563	0.327
	Kurtosis	-0.062	0.644	0.147	0.644	0.043	0.644	-0.428	0.644

(n) number of samples.

[1]Safranal (a.u.): $E_{318} \times 4.0$; Crocin (a.u.): $E_{440}{}^{10/000} \times 7.305$; ΔE_{pic} (a.u.): (E_{257}-E_{297}); Z variable (a.u.): (E_{440}/(ΔE_{pic}+E_{318})) (see Methods).

Figura 1A

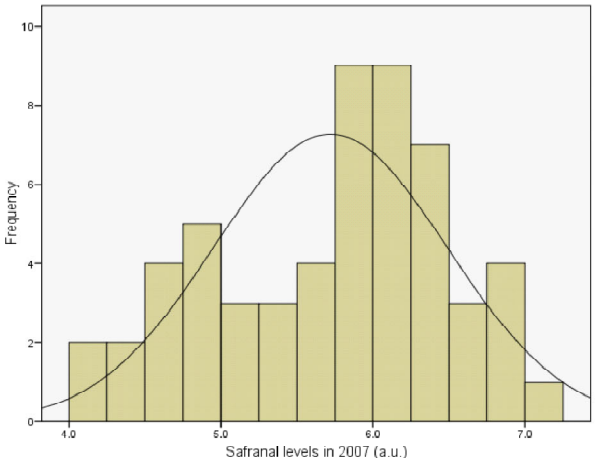

Figura 1B

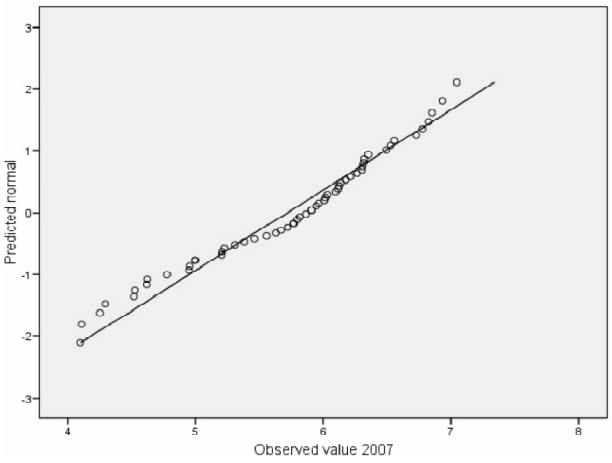

Figure 1. Safranal levels (a.u.) of the 2007 production of L'Aquila DOP saffron as frequency distribution (A) and Q-Q plot (B)

Figura 2A

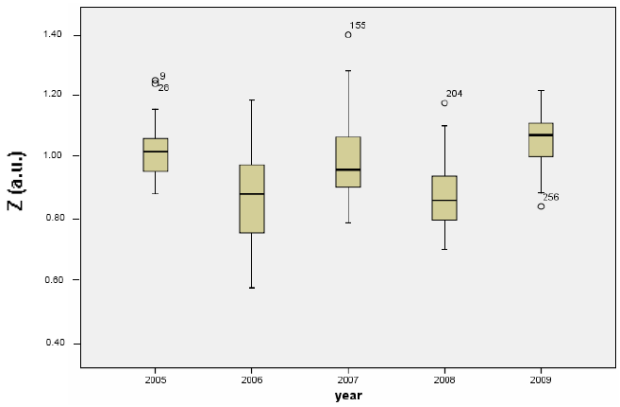

Figura 2B

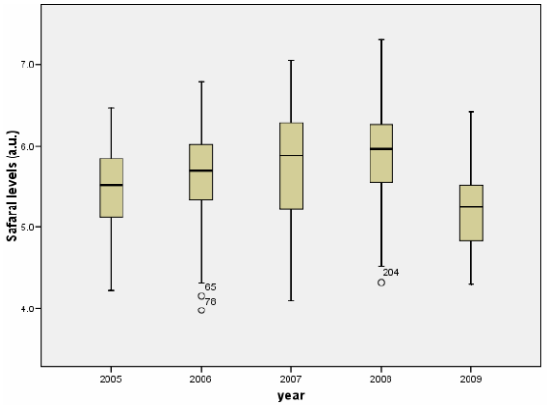

Figura 2C

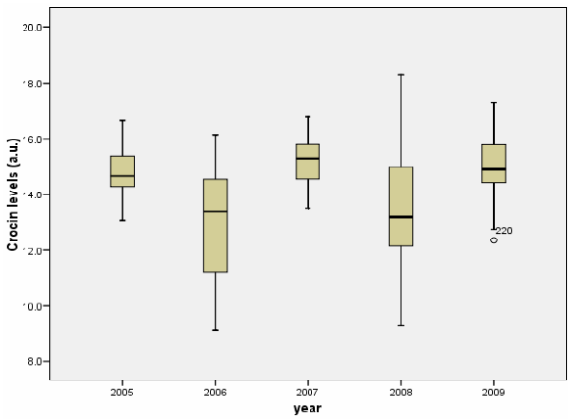

Figura 2D

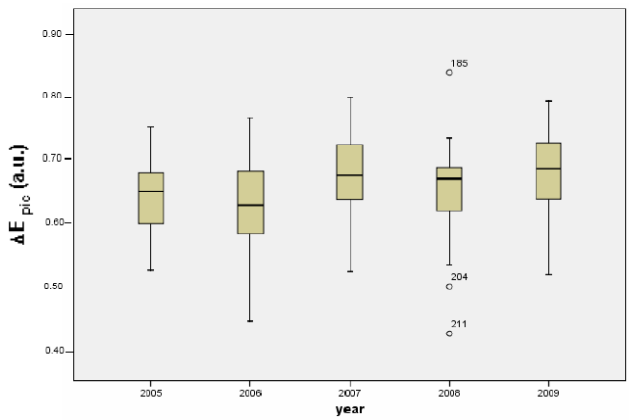

Figure 2. Tukey box and whisker plots across the five years from 2005-2009 for the L'Aquila DOP saffron for Z variable (A) and levels (a.u.) of safranal (B), crocin (C) and ΔE_{pic} (D)

Table 2. Shapiro-Wilk test of the samples of L'Aquila DOP Saffron from 2005 to 2009

Year	Number of samples	Safranal	Crocin	ΔE_{pic}	Z variable
		Significance (P)			
2005	49	0.465	0.464	0.274	0.066
2006	61	0.064	0.004	0.709	0.465
2007	56	0.055	0.151	0.792	0.001
2008	47	0.627	0.307	0.002	0.177
2009	53	0.722	0.568	0.054	0.044

(α=0.05)

Table 3. Levene test

Parameter	Safranal	Crocin	ΔE_{pic}	Z variable
Levene test	3.410	28.828	0.470	7.250
df1	4	4	4	4
df2	261	261	261	261
Significance (P)	0.010	0.000	0.757	0.000

(α=0.05)

The homogeneity of these L'Aquila DOP saffron samples within the same year of production was evaluated using a Levene test (Table 3). In particular, safranal showed a lower variance during 2009, while 2007 was characterised by the highest variance (Table 1). The crocin during 2007 showed the lowest variance, while in 2008, higher variance was seen. The ΔE_{pic} and Z variable showed lowest variance (0.006) for 2005, while in 2006 the Z variable gave the highest value (0.018). These data also indicated that the L'Aquila DOP saffron samples produced during 2005 had the lowest variability, especially for aroma and bitterness. These data were confirmed by the lower values calculated for the Z variable for 2005. Over the following years, the Z variability ranged from 0.007 (2009) to 0.018 (2006). The changing technology and the characteristic pedoclimatic of the local area of production of L'Aquila DOP saffron determine the product originality, and these factors can be identified as possible causes for the differences over the years in the variabilities of the mean levels of these determining chemicals.

Thus, the variance was not homogeneous for the safranal and crocin levels and for the Z variable, taking into account the large asymmetry and kurtosis for the ΔE variable for picrocrocin. To highlight the relevant statistical differences amongst these mean variables relating to the L'Aquila DOP saffron samples produced from 2005-2009, non-parametric ANOVA analysis was carried out using the Kruskal-Wallis test (Table 4).

Table 4. Kruskal-Wallis test

Parameter	Safranal	Crocin	ΔEpic	Z variable
Chi^2	41.11	62.3	23.75	86.11
Df	4	4	4	4
Probability	0.000	0.000	0.000	0.000

(α=0.05)

From this test it was seen that the medians of the variables analysed differed across these years. To obtain more information on the differences

between the means of these variables, we carried out an *a-priori* comparison test with different variances, as this was more suited. These results are shown in Table 5, where it can be seen that the Z variable relative to 2005 was significantly different from that of 2006, and that of 2007 was significantly different from 2008, as also seen between 2008 and 2009. No significant differences were seen here between the saffron production of 2005, 2007 and 2009, and between 2006 and 2008.

Table 5. Tests of planned comparisons

	2005 vs. 2006	2006 vs. 2007	2007 vs. 2008	2008 vs. 2009	2005 vs. 2007	2005 vs. 2009	2006 vs. 2008
	Z variable						
	0.000	0.000	0.000	0.000	0.488	0.055	0.931
Different variance	Safranal						
	0.052	0.674	0.165	0.000	0.037	0.014	0.046
	Crocin						
	0.000	0.000	0.000	0.000	0.013	0.231	0.106
	ΔE_{pic}						
	0.157	0.000	0.075	0.056	0.008	0.005	0.063

(α=0.05)

On the basis of these data for the Z variable, it is possible to see that through this 5-year period analysed there were similar chemical characteristics for this L'Aquila DOP saffron production over alternate years. This can be explained by the particular cultivation techniques of saffron that are used by the majority of these growers, which involves the seeding of the bulbs every two years in humus-rich clay soils.

For the other variables, it was seen that the safranal levels increased from 5.442 in 2005 to 5.916 in 2008 and then decreased in 2009 (5.190). The crocin levels showed an alternating behaviour: from 14.757 in 2005 to 12.949 in 2006, followed by an increase in 2007 (15.174) and a reduction in 2008 to 13.551; this increased again in 2009, to 14.989. The ΔE for picrocrocin increased from 2005 to 2007, and then remained constant over 2008 and 2009.

The Z variable describes the characteristic ratio of this L'Aquila DOP saffron for colour, bitterness and aroma. Moreover, through the statistical analyses, the Z variable allowed the grouping of the production of this saffron over these five years into two groups. Within each of these two groups, the chemical characteristics show no significant differences: the first production group in the years 2005, 2007 and 2009, and the second in the years 2006 and 2008.

As a future objective, it is hoped that the saffron growers will strictly respect these processing technologies that are the fruit of the ancient traditions that have been passed down from father to son. The aim will thus be to continue to produce a product with the particular characteristics that provide the equilibrium between the main components of crocin, safranal and picrocrocin that have made L'Aquila DOP saffron so known and appreciated throughout the World.

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