A Rapid Procedure for the Evaluation of Saffron Colouring Strength Using Tristimulus Colorimetry

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Abstract

Hunter colour parameters (L*, a*, b*) measured by a portable tristimulus colorimeter, were used to develop a rapid procedure to evaluate saffron colouring strength in situ. Such a procedure is expected to strengthen the quality control of Greek saffron under the conditions it is traded by the Saffron Cooperative (Kozani, Greece). Correlations were sought among colorimetric, spectrometric ($E_{440}^{1\%}$) and HPLC data (crocin content). The effect of parameters such as sample type (filament or powder) and moisture content on the method robustness is discussed.

INTRODUCTION

The commercial quality of saffron depends heavily on its colouring strength, bitterness and aroma values. According to the current ISO specifications, colouring strength is evaluated in the laboratory by spectrometry at 440 nm (ISO 3632-1-1993).
The present study was carried out to develop a rapid procedure to evaluate saffron colouring strength by measuring Hunter colour parameters (L*, a*, b*) using portable tristimulus colorimeters. The colorimetric, spectrometric ($E_{440}^{1\%}$) and HPLC data (crocin content) were correlated and the results were discussed.

MATERIALS AND METHODS

Samples

Experiments were carried out for representative sets of saffron samples kindly offered by the Cooperative of Saffron Growers (Kozani, Greece). Saffron in filaments was stored in glass bottles and analysed just after the delivery in the laboratory.

Reagents and Standards

All chemicals and solvents were of the appropriate grade.

Apparatus

Instrumental colour analyses were performed with a) a Minolta Chroma Meter CR-300, 8mm measuring area, diffuse illumination and 0° viewing angle (Tokyo, Japan) and b) a Miniscan XE Plus, model 45001 (large area view) 450º/0º (Hunterlab, Reston, Virginia, USA) both following the L*a*b* colour space system. Double beam UV-Vis spectrophotometers were used for absorbance measurements from 200 to 600 nm. For the HPLC analysis of saffron extracts a Liquid Chromatograph model 1090, series II (Hewlett-Packard) was used equipped with a Hewlett-Packard diode array detector. Spectra were recorded in the region 200 to 600 nm and stored on a Vectra 486/33U terminal.

Sample Preparation

Saffron in filaments was ground using an electric mixer and the powder was sieved through a 0.5 mm mesh sieve. The moisture content was determined for saffron in filaments (0.5 g) at 103 °C.

Colour Measurement using the Minolta Chroma Meter

Samples in the powder form were pressed flat manually. Prior to analysis, the instrument was calibrated with a white standard. Samples were measured in a Minolta CR-300 cell. Colour variables calculated by the instrument were CIE L*a*b* of which L* describes lightness, a* red-green chromaticity and b* yellow-blue chromaticity. Values of hue angle indicate sample colour. Hue angle (H^o) values were calculated as $H^o=\tan^{-1}(b^*/a^*)$. Chroma (C*) indicates colour purity and was calculated from the tristimulus co-ordinates a* and b*. Chroma values were calculated as $C^*=[(a^*)^2 + (b^*)^2]^{1/2}$ All samples were measured independently eight (8) times. For each sample and parameter, the mean and standard deviation were calculated.

Colour Measurement using the Miniscan XE Plus

The instrument was calibrated against a white and a black standard. Measurements were taken with reference to D65 illuminant and 10^o standard observer. Quantity of each sample was transferred independently eight (8) times in a glass Petri dish and each time eight (8) measurements were recorded (total=8x8). For each sample and parameter, the mean, standard deviation and standard error were calculated. Measurements were taken for stigmas and powders before and after sieving (0.5 mesh). A certain amount of each sample was stored in a) airtight glassware and b) in a desiccators in open glassware of standardised diameter at $a_W=0.43$.

Spectrometry

All the following operations took place away from direct sunlight. The saffron extracts were prepared with cold water according to ISO 3632-2-1993 and the spectra were recorded in the region 200-600 nm. Maxima were calculated at 440 nm, 330 nm, and 257 nm. Derivative spectroscopy (first and second derivative) was used to clarify the wavelength maxima of the extracts. Repeatability of measurements was examined for four replicates for each extract. The measurements were then made twice. The colouring strength of the extract at 440 nm was expressed as $E^{1\%}_{440}$ for which the mean and standard deviation were calculated.

RP-HPLC Analysis

The separation of carotenoids of saffron extracts (10ml) was achieved on a Superspher 100 RP-18, 4mm column (250x4mm i.d.) (Merck, Darmstadt, Germany) using a linear gradient from 20 to 100 % acetonitrile in water in 30 min. The solvent flow rate was 1ml/min. Detection was performed at 442, 330, and 257 nm. Fluorescein sodium salt was used as an external standard within the range from 0.5 mg/10ml injection to 2.5mg/10ml injection.

Statistical Analysis

Univariate and multivariate regression analyses for the search of correlation among analytical data, ANOVA, Tukeys and Duncan tests were carried out using suitable statistical packages.

RESULTS AND DISCUSSION

Regression between the colorimetric and spectrometric or HPLC data of Table 1 indicated some promising relationships shown in Table 2. Better correlation was found between the crocin content and the various chromaticity parameters. Multivariate regression improved slightly the correlation unless more complex models were employed.

The derived parameters H and C were included in all those models. However, in practice, parameter a* seemed to be appropriate for examining the colouring potential of any saffron quantity that reaches the check in point in the entrance of the Cooperative installation. Thus, application to a new set of data for thirteen (13) samples from the Cooperative indicated a similar type of ranking using either the a* measurement or colouring power values (Table 3).

Though colorimetry is often applied to the food industry, there are some restrictions concerning the characteristics of the instruments that are used. Moreover, standardization of the sample characteristics and status also seems important considering the fact that saffron is traded either in filaments or in powder form. Therefore, measurements on saffron samples using a Hunterlab colorimeter revealed differences in the size of values recorded in comparison to that observed using the Minolta meter. It can be said that it is difficult to standardize a procedure for universal use but within a specific installation for quality control reasons any instrument can give meaningful information about the colouring potential of an authentic saffron sample. Electronic storage of measurements for further treatment seems essential, thus, this facility is a prerequisite for the choice of a portable colorimeter.

In order to further simplify the application of colorimetry to the quality control of saffron it was considered useful to examine whether the sample form (filament or powder) or particle uniformity (powder before and after sieving) as well as moisture content could influence the values of L*a*b*. The type of data collected and the treatment for each of the 20 samples examined are illustrated in Table 4.The analysis of 20 saffron samples in all various forms indicated that the moisture level did not influence significantly the measurements in particular when the samples were in powder form. Sieving did not change the size of the recorded measurement. This finding is important for a fast application such as the proposed procedure. Moisture content affected the a* values (75% of the sample pairs differed significantly) whereas the parameter b* was affected less (30% of the pairs differed significantly). Difference in the size of measurements was found between filaments and powders: filaments:[L* (15.817-17.146), a* (10.551-12.577), b* (5.700-7.294)]; powder without sieving: [L* (21.547-27.056), a* (16.976-20.818), b* (9.942-13.480)]; powder after sieving: [L* (22.352-27.769), a* (17.510-21.473), b* (10.800-14.314)]. Regression analysis for L*, a*, b*, C* and H^o values and $E_{440}^{1\%}$ for the 20 samples in powder form (sieving, a_W=0.43) indicated once again a good correlation between a* values and colouring strength (r=0.414). Multiple regression analysis improved slightly the value of the coefficient. The best equation found was E=96.02+6.29L*+5.80a*-12.36b* (r=0.595).

Tristimulus colorimetry is a procedure applicable to food quality control. Though the correlations found were not very high, the procedure can be suggested as a tool for monitoring the product at the check in point of the Cooperative, during storage and before shipment. It is stressed that such a procedure due to instrumental limitations should be optimised for every installation that wants to adopt it.

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Tables

Table 1. Estimation of saffron colouring strength using colorimetry, spectroscopy and RP-HPLC

Sample no.	Analytical data: Colorimetric L*	a*	b*	H	C	spectrometric $E^{1\%}_{1cm,440nm}$	HPLC Crocin content, µg fluorescein /10µl injection
1.1	39.03	34.32	40.60	49.80	53.16	227	1.50
1.2	44.06	35.14	47.75	53.65	59.28	213	1.40
1.3	43.93	37.00	47.80	52.26	60.45	193	1.44
1.4	43.17	34.97	45.98	52.75	57.77	236	1.17
1.5	44.35	35.50	48.58	53.85	60.17	251	1.18
1.6	43.66	38.09	47.61	51.34	60.97	258	1.18
1.7	44.96	36.62	48.73	53.08	60.96	224	0.90
1.8	38.63	33.65	39.06	49.26	51.56	226	0.87
1.9	37.64	34.41	39.37	48.85	52.29	218	0.87
2.1	41.13	34.26	43.32	51.66	55.23	230	0.85
2.2	32.59	33.24	31.91	43.83	46.08	186	0.98
2.3	35.85	32.05	35.99	48.31	48.19	220	0.92
2.4	42.58	37.45	45.49	50.53	58.93	259	1.34
2.5	41.37	37.97	44.94	49.80	58.83	254	1.73
2.6	43.55	38.25	48.90	51.97	62.08	259	1.68
2.7	43.62	36.80	47.39	52.17	60.00	211	1.32
2.8	47.63	37.66	53.55	54.88	65.47	229	1.48
2.9	41.98	38.43	46.19	50.24	60.09	269	1.16

Standard deviation for L*:0.01-.04; a*0.01-0.03; b*:0.01-0.03; E1%:0.2-1

Table 2. Regression data between colouring strength values (L*a*b*) and absorbance readings ($\mathbf{E^{1\%}_{1cm}}$) or HPLC data (crocin content [C])

Regression equation	Correlation coefficient (r)	Regression equation	Correlation coefficient (r)
$\mathbf{E^{1\%}_{1cm}}$ = 6.85a* - 14.61	0.574	[C]= 0.09a* - 2.00	0.620
$\mathbf{E^{1\%}_{1cm}}$ = 1.82b* + 150.35	0.172	[C]= 0.03b* + 0.01	0.511
$\mathbf{E^{1\%}_{1cm}}$ = 2.40L* + 131.45	0.144	[C]= 0.04L* -0.24	0.465
$\mathbf{E^{1\%}_{1cm}}$ = 2.54H + 101.88	0.078	[C]= 0.0037H+-0.01	0.334
$\mathbf{E^{1\%}_{1cm}}$ = 2.11C + 110.08	0.216	[C]= 0.031C-0.54	0.558

Table 3 Saffron sample ranking in descending order on the basis of a* values

Ranking	1	2	3	4	5	6	7	8	9	10	11	12	13
a* value	36	24	32	31	30	29	28	27	26	26	25	24	21
$\mathbf{E^{1\%}_{1cm}}$ value	129	124	171	183	166	175	102	106	106	67	129	68	105

Table 4. ANOVA treatment of values of L*, a* and b*

Measurements	Mean	p=0.05		p=0.01	
		Tukey HSD	Duncan	Tukey HSD	Duncan
Sample O1					
L*					
L* (a_w=0.43) filaments	15.817	A[1]	A	A	A
L* (a_w uncontrolled) filaments	16.393	A	B	A	B
L* (a_w=0.43) powder, no sieving	25.317	B	C	B	C
L* (a_w uncontrolled)) powder, no sieving	25.317	B	C	B	C
L* (a_w=0.43) powder, after sieving	26.184	C	D	C	C
L* (a_w uncontrolled) powder after sieving	25.592	B,C	C	B,C	D
a*					
a* (a_w=0.43) filaments	12.153	A	A	A	A
a* (a_w uncontrolled) filaments	12.577	B	B	B	B
a* (a_w=0.43) powder, no sieving	20.818	E	E	E	E
a* (a_w uncontrolled) powder, no sieving	19.746	C	C	C	C
a* (a_w=0.43) powder, after sieving	21.110	F	F	F	F
a* (a_w uncontrolled) powder, after sieving	20.341	D	D	D	D
b					
b* (a_w=0.43) filaments	6.717	A	A	A	A
b* (a_w uncontrolled) filaments	6.911	A	A	A	A
b* (a_w=0.43) powder, no sieving	1.907	B	B	B	B
b* (a_w uncontrolled) powder, no sieving	1.720	B	B	B	B
b* (a_w=0.43) powder, after sieving	13.633	C	C	C	C
b* (a_w uncontrolled) powder, after sieving	13.006	B	B	B	B

1 Measurements having the same letter did not differ significantly at the selected probability level