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Effect of Drying Temperature and Air Flow on the Production and Retention of Secondary Metabolites in Saffron

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Safranal is the compound most responsible for the aroma of saffron spice and is, together with the suite of crocin pigments, the major determinant of the product quality. The content of safranal and pigments in saffron is determined by the method of postharvest treatment of the *Crocus* stigmas. A range of drying treatments involving different temperatures, with or without air flow, was applied to stigmas from three harvest dates. Dual solvent extractions combined with quantitative measurement using GC and HPLC-UV−vis techniques were used to analyze the secondary metabolite contents of the products. It was demonstrated that these methods overcame the previously reported problems in measuring the concentration of both pigments and safranal in saffron caused by the very different polarities and thus solubilities of these compounds. The results showed that a brief (20 min) initial period at a relatively high temperature (between 80 and 92 °C) followed by continued drying at a lower temperature (43 °C) produced saffron with a safranal content up to 25 times that of saffron dried only at lower temperatures. Evidence was provided suggesting that drying with significant air flow reduced the safranal concentration. The results, moreover, indicated that high-temperature treatment had allowed greater retention of crocin pigments than in saffron dried at intermediate temperatures (46−58 °C). The biochemical implications of the various treatments are discussed in relation to the potential for optimizing color and fragrance quality in the product.

KEYWORDS: Saffron; *Crocus sativus* L.; safranal; aroma quality; dehydration conversion; crocin pigments; carotenoid secondary metabolites; postharvest treatment; solvent extraction; GC-MS and HPLC-UV−vis analysis

INTRODUCTION

Saffron, obtained from drying the stigmas of *Crocus sativus* L., is highly valued as a culinary spice for its flavoring and coloring properties (*1*) and for its medicinal use in both traditional treatments (*2*) and potentially as a clinical antitumor (*3*), anti-inflammatory (*4*), and pro-memory (*5*) agent. The postharvest treatment of the stigmas, particularly the drying process, is critical to the quality of saffron as measured by the levels of secondary metabolites crocins (color and anti-tumor properties), picrocrocin (taste), and safranal (aroma). It is during this drying that the water-soluble picrocrocin (a biodegradation product of the carotenoid zeaxanthin) is converted to the volatile and largely water-insoluble safranal either by a two-step enzymatic/dehydration process involving the intermediate 4*R*-hydroxy-β-cyclocitral (HCC) or directly by dehydration at high temperatures or extreme pH (*6*−*10*) (**Scheme 1**).

The quantitative measurement of the composition of saffron has involved some uncertainty in that the ISO-3632 (2003) (*11*) standard spectrophotometric analysis method is subject to variation due to the lack of solubility of safranal in water, interfering absorbance at 330 nm due to *cis*-crocins, and postextraction degradation of picrocrocin (*12*, *13*). Various other means have been used for measuring the components of saffron including thermal desorption GC (*12*, *14*), HPLC methods with a polarity range capable of measuring the polar crocins and picrocrocin as well as the nonpolar safranal in the one polar extract (*15*), and GC and HPLC analyses of supercritical CO_2 extracts (*16*). Not all of these methods are readily available to industry and doubt remains as to whether a single extraction can provide an accurate measure of the true relative levels of all these components.

The literature includes a wide variety of reported safranal concentrations measured by different extraction and analysis methods in saffron of different origins including ∼800 ppm in Indian saffron extracted with 80% ethanol (*17*), ∼1500 ppm in Spanish saffron obtained by supercritical CO_2 extraction (*16*), 1070−3970 ppm in Spanish saffron analyzed by thermal desorption−GC (*12*), up to ∼4000 ppm in Indian saffron obtained by simultaneous hydrodistillation/extraction (SHDE) (*8*), and up to 1200 ppm in Greek saffron cold extracted with diethyl ether or up to 6400 ppm in the same material by SHDE

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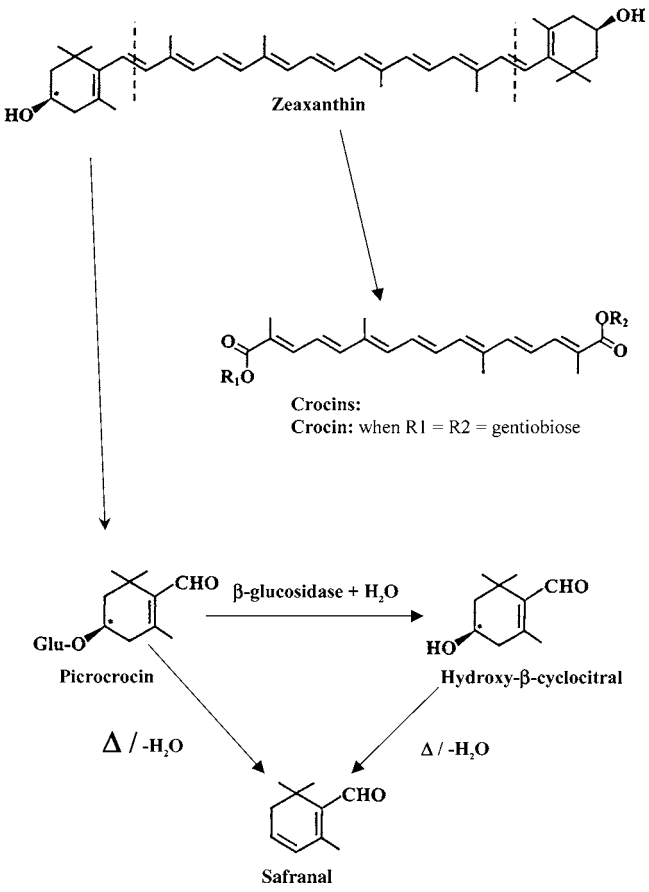
Scheme 1. Proposed Pathway for Zeaxanthin Biodegradation to Produce Principle Secondary Metabolites Responsible for Color (crocins), Flavor (picrocrocin) and Aroma (safranal) in Saffron (6, 16)



(*13*), a process by which significant extra safranal may be generated due to the heating involved. Total crocins contents of commercial samples of saffron have typically been reported from 5% of dry weight for sun-dried stigmas (*17*) to 17% of dry weight for vacuum oven dried samples having poor aroma development (*8*), although for a quality product with good aroma development values from 6 to 16% of dry weight (*8*, *17*−*19*) have been reported.

From the evidence available there is also much uncertainty about the ideal conditions for the drying of saffron. Stigmas have traditionally been dried by methods such as sun-drying (India and Iran), toasting over hot ashes (Spain), and drying slowly in a darkened room at 30−35 °C (Greece) (*1*, *6*, *8*, *13*). Of these, the Spanish methods have been regarded as producing the best quality of saffron (*3*, *6*, *8*, *14*, *19*), and although recent work has provided details of the actual conditions involved (*20*), no precise determination of optimal drying conditions has been provided.

The range of recent scientific studies and reviews available provides contradictory information as to the best drying conditions. In reviewing the topic, Cadwallader (*6*) summarized the work of Riana et al. (*8*) and others, stating that a temperature range of 35−45 °C without air flow or freeze-drying was optimum for good conversion of picrocrocin to safranal without excessive loss of color. He emphasized that temperatures below this required too long a drying period, resulting in excessive enzymatic degradation of crocins, whereas excessive temperature resulted in thermal degradation of these pigments. Other studies have, however, given evidence for quality saffron with aroma from drying conditions such as 80 °C for 30 min in an oven (*21*), presumably without strong air flow, 110 °C for 2 min (*22*, *23*), and 70 °C for 30 min with a strong air flow (*22*). The traditional Spanish methods involve maximum temperatures ranging from 75 to 121 °C for periods of 28−55 min (*20*).

In Tasmania, Australia, stigmas are generally dried using commercial food dryers employing significant air flow at temperatures ranging between 40 and 55 °C, and although this normally produces saffron of ISO category 1 (*11*), the safranal content is usually at the low end of the acceptable range. Significant problems with color degradation have also occasionally occurred, apparently associated with relatively small differences in drying temperatures or times.

The work described here was designed to establish whether significant improvement in the quality of Tasmanian saffron could be achieved by different drying methods, in particular the use of higher temperatures, and in doing so to further the understanding of the biochemistry of saffron drying. To achieve this, a reliable assay of the main saffron components was required, and thus a comparison of ISO 3632 test methods with polar and non polar solvent extraction methods was made to determine if any single method would give a reliable measure of both color and aroma components. To this end, quantitative measurement of the volatile components of the saffron was attempted using gas chromatographic (GC) analysis of polar and nonpolar extracts. For the pigment and picrocrocin contents, polar solvent extract and HPLC-UV−vis analysis were used. A brief comparison of this GC method was also made with the ISO-3632 (*11*) analysis in respect to safranal content.

MATERIALS AND METHODS

Collection of Material. The experiments were conducted on *Crocus* stigmas from the same southern Tasmanian crop at three harvest dates of April 8, 18, and 30, 2004. The flowers on each of these days were picked by hand at approximately the same time of day as part of a normal commercial harvest. It should be noted, however, that no attempt was made to test for the effect of harvest time in this study and that a variety of factors such as weather conditions and the exact time between flower picking and stigma removal would have varied between harvest dates. All conditions were kept identical between treatments on the same harvest day.

Stigmas for the experiments were separated by hand from a random selection of the picked flowers at 18 °C indoors. All stigmas were separated from the flower with the tripartite stigmas intact (connected to style) initially, although separation of some of these did occur through the treatment and extraction processes. For each treatment replicate, 20 stigmas were placed in sealed vials and transported to the laboratory in a cooled container (4 °C for 2 h). The exceptions were the samples of treatments J and K, when 100 stigmas per replicate were collected to allow enough material for ISO tests to be performed as well as the extractions.

Application of Treatments. The stigma samples were equilibrated in the vials at room temperature (20 °C) for 15 min, weighed, and then placed on Teflon trays for drying except the two frozen treatments (E and I) that were placed in vials in a −20 °C freezer and the fresh sample (A), which was extracted immediately. The drying conditions were then applied to the other treatments using either electric ovens with fans disconnected or a food dryer (Ezidri Snackmaker from Hydraflow Ltd.) set at either the medium or high temperature setting (46 and 58 °C, respectively), with the tray including the stigma samples at the second from top level of a five-level stack. The air flow over the stigmas in the dryer at both temperature settings was measured at 2.9 m/s. The temperature of the interior of both dryer and ovens was recorded with probe thermometers and found to vary within a range of ±1 °C of the set temperature. Where a treatment involved a high temperature followed by a lower temperature, the samples were transferred immediately from one oven to another at the appropriate time to avoid a temperature lag period. The frozen treatment samples

Table 1. Treatment Description Summary with Results of Component Analysis

treatment code	harvest date	drying treatment type	drying temperature and duration	air flow	safranal yield[a] (ppm) hexane extraction	safranal yield[a] (ppm) methanol extraction	HCC yield[a] (ppm) hexane extraction	picrocrocin % yield[a] methanol extraction
A	1	fresh/no drying	not dried	none	318 (c)	58 (a)	4740 (c)	16.52 (c)
B	1	food dryer	46 °C for 60 min	2.9 m/s	61 (a)	43 (a)	101 (a)	7.94 (a)
C	1	oven	43 °C for 100 min	none	93 (b)	47 (a)	170 (b)	10.22 (b)
D	1	oven	80 °C for 20 min, then 43 °C for 70 min	none	920 (d)	54 (a)	118 (a)	10.82 (b)
E	2	frozen/no drying	not dried	none	518 (g)	55 (b)	21169 (f)	9.80 (e)
F	2	food dryer	58 °C for 20 min, then 46 °C for 40 min	2.9 m/s	105 (e)	53 (b)	103 (e)	8.24 (d)
G	2	oven	87 °C for 20 min, then 43 °C for 70 min	none	1596 (h)	80 (c)	65 (d)	9.42 (e)
H	2	oven	75 °C for 20 min, then 43 °C for 70 min	none	376 (g)	74 (c)	93 (e)	10.11 (e)
I	2	frozen, thawed, and then dried	87 °C for 20 min, then 43 °C for 70 min	none	199 (f)	76 (c)	60 (d)	9.64 (e)
J	3	oven	43 °C for 100 min	none	377 (i)	73 (d)	477 (g)	10.52 (f)
K	3	oven	92 °C for 20 min, then 43 °C for 70 min	none	1106 (j)	77 (d)	70 (h)	9.78 (f)

[a] Means within each harvest interval annotated by the same letter in parentheses are not significantly different at the $P \geq 0.05\%$ level (statistical comparison only made within each harvest time). Yield values are all calculated on a ppm/dry weight of saffron basis.

were thawed for 15 min the next day and then dried as described above or extracted immediately. Each treatment was replicated four times, and they are detailed in **Table 1**.

Extraction of Samples. For the determination of the pigments, picrocrocin and possibly safranal, a polar solvent was required, and, as for other commonly used methods (*10*, *15*, *19*, *20*, *24*), methanol was chosen. For comparison of safranal (and HCC) extraction a relatively nonpolar solvent was required (*13*, *21*), and hexane was used in this case.

After treatment, samples were reweighed and then divided with a four-stigma subsample of each replicate taken, combined with those from the same treatment, weighed, dried at 104 °C for 24 h, and then reweighed for mean moisture determination (as per ISO test procedure), which was used to calculate concentrations on a dry weight basis for all treatments. The remaining stigmas of each replicate were split into two subsamples of approximately eight stigmas, which were each weighed (to four decimal places at 20 °C) into separate sealed vials. To each of these treatment replicates were added 5 mL of HPLC grade hexane and 0.492 mg of β-cyclocitral (as an internal standard), and the vials were sealed. To the remaining subsample from each replicate was added 5 mL of HPLC grade methanol, and the vial was sealed.

The extraction method used was adapted from the ultrasound-assisted method used by Kanakis et al. (*13*) as this did not employ heat, which could cause compositional changes. Extraction samples were subjected to ultrasonification for 1 h in the dark before being left on a shaker at 100 rpm for 20 h in the dark at 15 °C. The samples were then allowed to settle out in the dark for 1 h before an aliquot of 1 mL of each of the extracts was transferred to 2 mL GC vials and sealed. These samples were then stored in a freezer at −10 °C before analysis.

Gas Chromatographic Analysis. A Hewlett-Packard 5890 series II gas chromatograph equipped with a flame ionization detector (FID), a split injection system, and an HP-1 cross-linked methyl silicon gum column (30 m × 0.32 mm i.d., 0.33 μm film thickness) was used. Injections of 2 μL were made with an injector temperature of 250 °C in splitless mode with purging resuming after 2 min. Carrier gas was N_2 at a column flow of 1.8 mL/min, a head pressure of 8 psi, and a split ratio of 1:50. The oven temperature program was 50 °C for 2 min, then rising at 9 °C/min to 290 °C, and held for 11.3 min. The detector temperature was 295 °C. To accurately quantify the response of safranal by GC-FID, calibrations involving determination of the standard curves for the response of eight concentrations of safranal (Fluka, 75%, catalog no. 17306) between 26 and 500 μg/mL in both hexane and methanol were run. The determination of HCC was made relative to the response of safranal in hexane, as this compound is structurally very similar. The internal standard was used only as a check of overall response of the GC-FID.

GC-MS Analysis. Samples were analyzed by GC-MS to confirm the safranal and HCC identities using a Varian 3800 GC coupled directly to a Varian 1200L triple-quadrupole mass spectrometer. A Varian Factor-Four VF5-MS (25 m × 0.25 mm × 0.25 μm film) with helium as carrier gas at a flow rate of 1.2 mL/min was used. Two microliter aliquots were injected into a Varian 1177 injector using the split mode (15:1) at a temperature of 210 °C, and the column oven was held at 6 °C for 1 min and then ramped to 14 °C at 5 °C/min and to 140 °C, and then to 28 °C at 20 °C/min. The *m*/*z* range from 35 to 350 was scanned every 0.3 s. A reference spectrum of HCC was available from an in-house specialized terpene library.

HPLC-UV−Vis Analysis: HPLC Coupled to a Waters 996 Photodiode Array Detector. *trans*-4-Crocin, *trans*-3-crocin, *cis*-4-crocin, *trans*-2-crocin, *cis*-3-crocin, and *trans*-2-crocin were measured at 440 nm, and picrocrocin was measured at 250 nm. Separation and identification of these compounds was made with reference to the methods and results reported previously by Castellar et al. (*24*) and Lozano et al. (*15*). A Waters Nova-Pak C18 column (3.9 mm × 150 mm) and an Alltech Econosphere 5 μm C18 guard cartridge at a flow rate of 1 mL/min were used. Solvent A was methanol, and solvent B was 1% acetic acid in Milli-Q water. The gradient was 20% A to 80% A at 45 min, then to 100% A at 45.01 min, and this was held to 52 min. Re-equilibration between runs was 10 min. The known molar absorptivities of crocins and picrocrocin (in water and alcohol) were used to calculate concentrations of these compounds in the extracts, using the known conversion factor between molar absorptivity and raw PDA peak area at this flow rate based on a β-carotene standard. The molar absorptivities were those stated by Casteller et al. (*24*) or calculated from the extinction coefficients ($E_{1\%/1cm}$) at each wavelength, given by Davies (*25*). For crocin (used for all of the crocins that possess the same chromophore) this was $\epsilon_{440} = 133\,750\ M^{-1}\ cm^{-1}$, whereas for picrocrocin, $\epsilon_{250} = 10\,100\ M^{-1}\ cm^{-1}$ and for β-carotene the $E_{1\%/1cm}$ at 440 nm was 2620 and thus $\epsilon_{440} = 140\,432\ M^{-1}\ cm^{-1}$.

Statistical Analysis of Results. Linear regression analyses of the standard curve plots of safranal GC-FID response were performed using Microsoft Excel 2000. Statistical analysis of the comparison of the treatments was carried out using the procedures of the SAS statistical package, version 6.12, 1989−1996, SAS Institute Inc., Cary, NC. This included a log transformation of the safranal and HCC data sets before analysis of variants (ANOVA) as this provided a closer fit to a normal distribution. It should be noted that ANOVA was performed independently for each harvest time.

ISO Analysis Comparison. A variety of samples were analyzed using the ISO-3632 (2003) test procedures (*11*) for comparison with hexane extraction. These included the remaining saffron from treatments J and K in addition to five industry-produced samples comprising three from the 2004 harvest, dried at the three temperature settings (measured at 46, 64, and 72 °C) in a commercially used food dryer (of the same make as that used in the laboratory), as well as two samples from the 2003 harvest representing high- and low-quality examples of local product. These samples were ground, subsampled for moisture determination, water extracted, and analyzed according to the ISO-2632 procedure. The absorbances at 330 nm were measured on a Shimadzu UV-160 spectrophotometer.

Table 2. Comparison of ISO Test Measurements with GC (Hexane Extracted) Safranal Analysis

measurement type	treatment J	treatment K	food dryer at 46 °C for 100 min	food dryer at 62 °C for 25 min	food dryer at 74 °C for 12 min	low-quality commercial sample from 2003	high-quality commercial sample from 2003
ISO 3236 (clause 11) fragrance absorbance reading $E^{1\%}$ (1 cm, 330 nm)	22	27	25	24	23	23	27
safranal content (ppm) from hexane extraction and GC-FID analysis	377	1106	503	359	81	387	916

The GC analysis of the safranal content of these commercially dried samples was conducted as for the treatment samples (although with only two replicates).

RESULTS AND DISCUSSION

Analytical Methods. The calibration curves for the GC-FID response of safranal in hexane and methanol were represented by linear regression equations of $y = 3242x$ ($R^2 = 0.9979$) and $y = 31204.5x$ ($R^2 = 0.9918$), respectively. A comparison of the safranal concentrations (calculated per unit of dry weight of saffron) measured in the hexane extracts with the those measured from the methanol extracts (**Table 1**) indicates that the large differences (up to 25×) between treatments for the hexane extracts correlated very poorly with the results of the methanol extracts ($R^2 = 0.19$). Significant differences did occur between methanol extracts of some treatments at harvest time 2, but not at the other times, and these differences did not exceed 20%. This confirms the unsuitability of polar extraction for measuring saffron safranal levels indicated by Pardo et al. (*22*). This was despite the calibration curve for safranal in methanol giving a very good linear fit indicating that the poor extraction was not the result of safranal insolubility or saturation of methanol over the concentration range involved. It is suggested that this effect was a result of poor partitioning between the lipid-based membrane tissues and the relatively polar solvent. Furthermore, it should be noted that polar solvents extract picricrocin and that this compound can undergo postextraction conversion to safranal, leading to the potential for erroneous measurement of safranal content as observed by Loskutov et al. (*21*).

The comparisons of the safranal levels and ISO-3632 test results for treatments J and K and five commercially dried samples are shown in **Table 2**. These results clearly show that the large differences between samples in safranal content (as measured by hexane extraction and GC analysis) were not well reflected in the ISO-3632 UV absorbance readings at 330 nm (fragrance) as the two sets of results gave a poor correlation ($R^2 = 0.78$). This difference is likely due to a combination of poor solubility/extraction of safranal in water and interfering absorbance at 330 nm by *cis*-crocins (*12*, *13*, *22*) tending to mask any differences that exist between samples.

Both of these comparisons provide strong evidence that single-polarity extraction methods (for measuring all three main components), such as the ISO-3632 procedure and that used by Lozano et al. (*15*), are not appropriate for estimating the relative level of the aroma compound in saffron. Hexane extractions were therefore chosen as the better relative measurements of safranal content for comparing drying treatments in this study. This should not be confused with absolute concentration, however, as this assumes 100% extraction recovery, which would require multiple solvent washes as indicated by the work of Kanakis et al. (*13*).

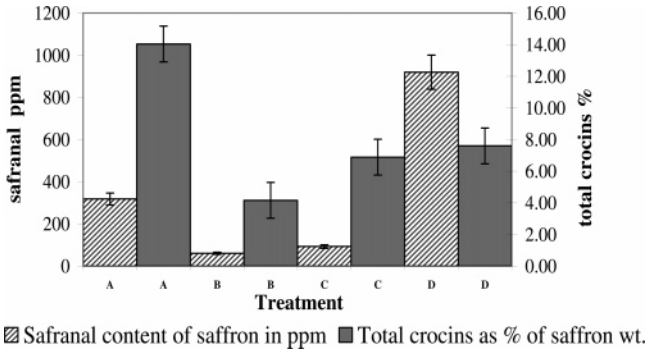


Figure 1. Comparison of safranal and crocin contents of drying treatments at harvest time 1.

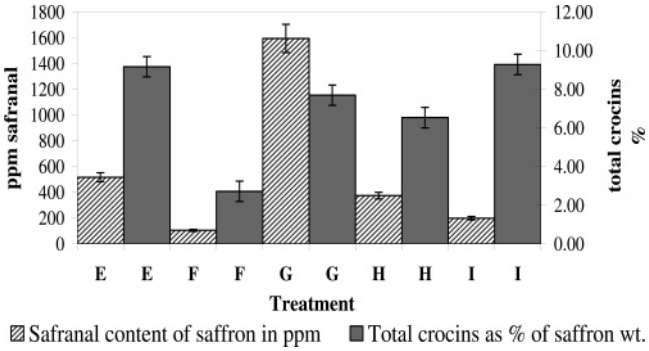


Figure 2. Comparison of safranal and crocin contents of drying treatments at harvest time 2.

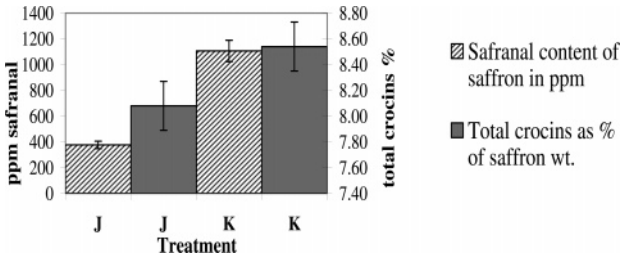


Figure 3. Comparison of safranal and crocin contents of drying treatments at harvest time 3.

Drying Experiments. The comparison of relative (hexane extracted) safranal and (methanol extracted) total crocins contents of the saffron obtained from the different drying treatments is made for each respective harvest date in **Figures 1−3**, and picrocrocin and HCC levels for these treatments are included in **Table 1**. All of the drying treatments (except A and E, which were not dried) produced saffron with a final moisture contents approximately at or below the recommended maximum (12%) required by the ISO-3632 standard.

Effect of Harvest Time. Technical constraints limited the number of flowers and thus treatments that could be performed on each harvest day, and although it was not the intention of

this study to investigate the effect of harvest timing, treatments C and J were identical other than for harvest date (**Figures 1** and **3**). The safranal and HCC contents significantly differed between these, indicating such an effect had occurred, although the factor(s) responsible for this is (are) unclear. Variables such as weather conditions on or before the day of harvest and even relatively small differences in the intervals between flower picking, stigma removal, and drying treatment could have been responsible as much as any physiological effect of harvest timing. For this reason all of the following comparisons are made only within each harvest time, for which the flowers used for the treatments were taken randomly from the total picked that day. For future studies, improvements in experimental design are required to better separate harvest-timing effects from drying treatments.

Effect of Temperature. The results (**Figures 1**−**3**) clearly show that the higher temperature oven-dried unfrozen treatments (D, G, and K) caused very significantly greater conversion to and/or retention of safranal in the saffron than all other treatments at the respective harvest times. Such highly significant increases in the relative safranal contents obtained here (up to 25 times saffron dried at lower temperatures) are most likely due to direct thermal conversion of picrocrocin (**Scheme 1**) at these high temperatures (80−92 °C), as opposed to the enzymatic conversion pathway via HCC. Enzymes, such as β-glucosidases, although variable in their temperature characteristics, are generally known in plants to undergo thermal inactivation at temperatures >60 °C and become completely denatured at 80 °C (*26*, *27*). The fact that these treatments (D, G, and K) also exhibited equal or significantly better retention of crocin pigments at each time in comparison to the lower temperature drying treatments (especially those employing the food dryer, B and F) indicates that this type of drying would be a means for producing quality saffron with increased aroma. The optimal temperature and duration of this temperature is not determined from these results, although it would appear to be between 80 and 92 °C as the highest safranal content was achieved at 87 °C, but this is not proven because of the different harvest times.

Although the safranal yields from these high-temperature treatments were less than some of those reported for quality saffron such as 3970 ppm in Spanish product (*12*), the work of Kanakis et al. (*13*) suggests that the use of further solvent washes could increase this by 2−3 times, resulting in a safranal content comparable to all but those results reported from methods involving distillation, when heating would have caused further conversion of picrocrocin to safranal (*8*, *13*). Moreover, the levels of picrocrocin (**Table 1**) detected in saffron samples in this study, being up to 40 times the molar equivalent of the highest levels of safranal detected, suggest that significant further potential for safranal conversion exists.

These results appear to contradict both the conclusions of Raina et al. (*8*) that temperatures of 35−45 °C are optimal and the results of Pardo et al. (*22*), who used air flow with high-temperature treatments and measured aroma by the ISO-3632 method. However, the latter also undertook organoleptic comparison that did support the use of higher temperature treatments, and they acknowledged the possible error inherent in the ISO method. These results are consistent with the findings of Loskutov et al. (*21*), although the safranal increases they reported by drying at 80 °C were only 3−4 times that of saffron dried at lower temperatures compared to up to 25 times in this study. The more polar extraction solvents (acetonitrile and ethanol) used in their study suggest that it is possible that their determination involved an underestimation of the safranal content due to poor extraction.

The higher levels of safranal in the two undried treatments (A and E) compared with all but the high-temperature treatments at respective harvest times are explained by continued enzymatic activity occurring in the stigmas during the extraction process as the enzymes would not have been denatured by heat and the stigma tissue would have had sufficient water content for activity to occur. The second step of this conversion pathway, the dehydration of HCC to form safranal, would not have been favored in the absence of drying, although some of this conversion would have been driven by product removal as safranal partitioned into the organic phase. This conversion would have been further promoted by the tissue disruption caused by freezing and thawing in treatment E, bringing substrates and enzymes together. The very high level of HCC (**Table 1**) in treatment E further supports this explanation. The very low level of HCC found in treatment I, despite its being frozen and thawed prior to drying, may be explained by the glucosidase being denatured at the high temperature of initial drying (87 °C) with concurrent thermal degradation of any HCC present to produce compounds other than safranal, possibly isophorone-related oxidation products (*6*, *9*). This suggests that the dehydration step from HCC to safranal in this pathway may be favored only by drying at moderate temperatures as indicated by Cadwallader (*6*), and certainly the safranal content of the saffron from this treatment was much lower than in treatment G, which was identical except for the freezing. Why there was not more safranal produced in the samples from treatment I is not fully explained, however, as there was still a significant pool of picrocrocin available for conversion to safranal via direct thermal dehydration (**Table 1**). A better understanding of the kinetics of this reaction process, possibly in relation to changes in the cellular and subcellular structure with drying (*20*), may provide this understanding.

The crocin content of treatments B and F were also significantly reduced in comparison to all other treatments. There is no obvious reason to attribute these lower crocin levels to the effect of air flow, whereas the temperatures of these treatments (46 and 58 °C) would have allowed greater enzymatic activity than the oven treatments at each respective harvest time, treatment C being below this temperature range, whereas treatments D, G, H, and I were at temperatures above that at which thermal denaturing would occur. This explanation is supported by the work of Tsimidou and Biliaderis (*28*), who demonstrated the temperature dependence of crocin degradation between 25 and 60 °C.

The loss of crocins occurring in the higher temperature treatments would be the result of nonenzymatic thermal degradation (*6*), but it would appear that by keeping the high-temperature period relatively short, this loss was minimized and no enzymatic degradation would have occurred at the subsequent lower temperature as these enzymes were denatured. In this respect the process is comparable to the way in which many food products are blanched to preserve color, with such treatments involving brief temperatures >60 °C (typically 70−90 °C), which seems to be the range in which enzymes such as polyphenol oxidases, responsible for browning or color loss, are significantly and irreversibly denatured (*29*−*33*).

Effect of Air Flow. Significant cross air flow (treatments B and F) in the drying process at temperatures up to 58 °C may be deleterious to the quality of the end product as these treatments exhibited the significantly lowest safranal and pigment levels at each harvest time (**Figures 1** and **2**). This

effect, however, is not clearly separated from other effects due to the different apparatus used (dryer vs oven) and the temperature differences between them. However, the picrocrocin levels were significantly lower in these treatments than all others at each harvest time, but without elevated HCC concentration (**Table 1**), and this suggests that considerable conversion to safranal had occurred but that the product was lost. It is suggested that this loss was the result of evaporation as the headspace was continually exchanged with air flow. This seems to be consistent with the work of Carmona et al. (*20*) in which Spanish saffron, dried at high temperatures according to the traditional method of toasting over vine shoot charcoal, had aromatic strength and color higher than saffron dried in hot air flow at similar temperatures. The interpretation that the lower crocin levels in treatments B and F (**Figures 1** and **2**) is the result of enzymatic activity being favored at these temperatures seems to be likely, although the results of Carmona et al. suggest that some direct effect of air flow is possible. They proposed that this may be a factor of dehydration rate and its effect on porosity and thus crocin extractability (*20*).

More work is required to properly determine these effects, particularly the use of different rates of air flow at each specific temperature used (i.e., with the same apparatus) and possibly also the use of headspace analysis techniques to determine if evaporation is a significant cause of safranal loss during drying.

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