Analytical Methods

An image analysis of TLC patterns for quality control of saffron based on soil salinity effect: A strategy for data (pre)-processing

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Quality of saffron, a valuable food additive, could considerably affect the consumers' health. In this work, a novel preprocessing strategy for image analysis of saffron thin layer chromatographic (TLC) patterns was introduced. This includes performing a series of image pre-processing techniques on TLC images such as compression, inversion, elimination of general baseline (using asymmetric least squares (AsLS)), removing spots shift and concavity (by correlation optimization warping (COW)), and finally conversion to RGB chromatograms. Subsequently, an unsupervised multivariate data analysis including principal component analysis (PCA) and k-means clustering was utilized to investigate the soil salinity effect, as a cultivation parameter, on saffron TLC patterns. This method was used as a rapid and simple technique to obtain the chemical fingerprints of saffron TLC images. Finally, the separated TLC spots were chemically identified using high-performance liquid chromatography-diode array detection (HPLC-DAD).

1. Introduction

Crocus sativus L., a member of the Iridaceae family, is known as saffron and widely cultivated for its valuable stigma. Saffron is a precious spice with a wide range of applications in the food and cosmetic industries as a flavoring and coloring agent (Montalvo-Hernández, Rito-Palomares, & Benavides, 2012). Moreover, saffron is widely used in modern and traditional medicine to prevent and treat different types of disease (Amin & Hosseinzadeh, 2012). Iran, is the main producer country of saffron, with >90% of world annual production (Anastakasi et al., 2009). Owing to large-scale consumption of saffron and increasing concerns over consumer health, rigorous quality control of this expensive spice is necessary. Saffron quality is determined by its three main chemical components: safranal (the main part of saffron essential oil which is responsible for saffron aroma), picrocrocin (the main cause for saffron’s bitter taste) and crocins (a family of carotenoid derivatives responsible for saffron’s color) (Sujata, Ravishankar, & Venkataraman, 1992). Several parameters such as geographical origin, cultivation conditions (e.g. soil and climate parameters), harvesting time, processing conditions (e.g. dehydration temperature), and storage time and conditions affect the concentration level of these components in saffron (Masi et al., 2016; Yuan, Liang, Yi, Xu, & Kvalheim, 2008).

Soil salinity is a general term that refers to the salt content of soils namely: (a) high salt concentration (saline soils), (b) high level of sodium cation (Na⁺) (sodic soils), and (c) high pH values (alkaline soils) (Beek & Toth, 2012). It has been proven that soil salinization can change dramatically a plant’s biochemical activity. Experiencing salinity stress, important processes such as photosynthesis, lipid metabolism and protein synthesis could be affected. Moreover, at high salt concentration, sodium ions compete with other nutritional elements, especially potassium (K) and lead (Pb), and result in the mineral nutritional imbalance in the plant matrix (Kumar & Bandhu, 2005). Although saffron can grow in a wide range of soils (Gresta, Lombardo, Siracusa, & Ruberto, 2008), it is very sensitive to soil and water salinity (Yarami & Sepaskhah, 2016). Therefore, the study of soil and water salinity effects on the saffron metabolome and quality has received much attention in recent years (Yarami & Sepaskhah, 2016).

In international trade, saffron quality is evaluated mainly by its coloring strength according to the ISO-3632 method suggested by the International Organization for Standardization (ISO) (ISO, 2011). The method is based on using ultraviolet-visible (UV-visible) spectrophotometry which is a simple and low cost technique. However, it suffers from some fundamental problems such as insensitivity and inaccuracy in measuring safranal concentration (Masi et al., 2016). Therefore, different instrumental devices have been proposed and employed to improve the saffron quality control procedure. Spectroscopic methods such as infrared (IR)
spectrometry (Ondoudi, Mozos, & Tsimidou, 2015) and nuclear magnetic resonance (NMR) spectrometry (Ondoudi et al., 2015; Petrakis, Caglioni, Polissiou, & Consomni, 2014), and chromatographic techniques such as gas chromatography (GC) (Anastakasi et al., 2009; Sereshti, Heidari, & Samadi, 2014), high performance liquid chromatography (HPLC) (Carmona, Zalacain, Sánchez, Novella, & Alonso, 2006; D’Archivio, Giannitto, Maggi, & Ruggieri, 2016; Masi et al., 2016; Tarantilos, Tsoupras, & Polissiou, 1995), and thin layer chromatography (TLC) have been widely used to obtain a comprehensive view of the saffron chemical profile (Sujata et al., 1992).

Thin layer chromatography is a convenient and low-cost method for separation of organic or inorganic species. This technique has a wide range of applications in the food industry such as study and identification of target compounds, adulterations, and contaminants (Zeb & Murkovic, 2010). It has also been employed for qualitative and quantitative analysis of carotenoids in both foods and non-food products (Djozan, Karimian, Jouyouban, Irmananesh, & Gorbanpour, 2014; Zeb & Murkovic, 2010). The advantages of TLC such as high sample throughput, the ability to separate many spots in parallel, low mobile phase usage, availability, little or no requirement to sample preparation, low cost, and simplicity of operation, have made it a useful separation tool in the chromatographic community (Poole, 1999; Sherma & Fried, 2003, chap. 10).

In recent years, by development of digital imaging systems, inexpensive digital cameras have been utilized for TLC qualification and quantification analysis. Thin layer chromatography coupled with image analysis (TLC-IA) is a rapid, convenient and low cost method for pattern recognition and fingerprint analysis of plants (Tang, Guo, Xu, Zhang, & Xu, 2014). However, TLC suffers from some problems such as low peak resolution, lack of reproducibility, low sensitivity, limited usage (Fazakas, Nascu-Briciu, & Sarbu, 2011), high level of baseline/noise contribution, peak shifts, and concavity in the spots which could significantly reduce the accuracy of the final results (Sharma & Fried, 2003, chap. 10). Moreover, TLC chromatograms are qualitatively assessed by the retention factor (Rf), but this parameter has low accuracy due to its inconstancy among different runs, and possible overlapping spots (Sharma & Fried, 2003, chap. 10). Such problems could significantly reduce the method applicability specially in the analysis of complex samples (Amigo, Skov, & Bro, 2010). Different aspects of TLC technique, such as stationary phase and instrumental parts, have been developed during the recent years to reduce the effects of TLC inherent problems. However, some of these problems mainly occur due to the complexity of the sample matrix, and cannot be fully eliminated by improving the chromatographic conditions.

In the present work, the effect of soil salinity on TLC pattern and saffron’s main secondary metabolites using a multivariate chemometric method for TLC image pre-processing was studied. The proposed method provides appropriate TLC fingerprints which help to evaluate the quality of Iranian saffron samples based on two main indicators of soil salinity (i.e. the pH and electro-conductivity (EC)).

2. Materials and methods

2.1. Reagents and materials

Eighty saffron samples from eight geographical locations of Iran were collected in the harvesting season (November 2015). General information of the saffron samples including their origins is listed in Table S1 (supplementary data file). A crude source of crocin and crocin CRM (certified reference material) was supplied from Sigma-Aldrich (St. Louis, MO, USA). All the chemicals (analytical grade) and TLC plates were purchased from Merck Chemicals (Darmstadt, Germany). The silica gel TLC plates with fluorescent indicator 60 F254 were cut into 5 cm × 7.5 cm pieces, and then placed in an oven at 100 °C for 1 h prior to use.

2.2. Instrumentation

A UV2601 UV–visible spectrophotometer (Beijing, China) with 1 cm optical path, and micro-cuvettes (Fisher Scientific, USA) with a sample volume of 0.1 mL were used to record the absorbance spectra of the TLC spots. A digital ultrasonic water bath (CD-4820, Korea) was used to enhance the extraction efficiency. The centrifugations were performed using a Hermle Z200A centrifuge (Hermle Labortecnik, Wehingen, Germany). The pH values of the soil samples were measured by a Metrohm 691 pH-meter (Herisau, Switzerland) equipped with a combined glass electrode. An electrical conductivity meter 712 (Metrohm, Cheshire, UK) was used to determine the electrical conductivity of the soil samples. The images of TLC plates were recorded using the in-built camera of a Samsung galaxy S6 smartphone.

2.3. The HPLC-DAD procedure

An Agilent Technologies high performance liquid chromatography (HPLC) system (1260 Infinity), equipped with a photodiode array (PDA) system, a manual injector (20 μL sample loop) and a quaternary pump VI, was used for analysis of the TLC bands. An ultra-reverse-phase C18 column (Restek Corporation, USA, 5 μm particle size 250 mm × 4.6 mm) was utilized as HPLC stationary phase system. For HPLC analysis, 20 μL of the CRM mixture along with each sample solution (the separated TLC spots, scraped off the plate and eluted in 300 μL methanol/acetonitrile) were injected into the HPLC system. The analyses were carried out using a gradient elution of methanol (10–100%) and water (containing 15% acetonitrile) as mobile phase. The flow-rate was adjusted at 1.0 mL min⁻¹ with a maximum elution time of 60 min. Chromatograms were recorded at three main wavelengths (250, 330 and 440 nm) and PAD spectra were recorded in the range of 200–700 nm.

2.4. Experimental procedure

Saffron stigmas were separated manually from the flowers and dried at room temperature in the absence of direct light. The dried stigmas were then packed in paper bags and stored in a refrigerator at 4 °C for further analysis. The procedure for extraction of saffron's main components has been optimized in our previous work (Sereshti et al., 2014). Briefly, 50 mg of soft homogenized powdered stigmas was placed in a round-bottom glass test tube and 1 mL of methanol/acetonitrile (38:62 v:v%) was added to it. Then, the mixture was exposed to ultrasound for 25 min, followed by centrifugation for 5 min at 4500 rpm to separate the particulate materials from the solution.

Using a manual micropipette, 10 μL of the supernatant was spotted onto TLC plates. Then, the plates were placed into a developing chamber which had been previously saturated with a mobile phase consisting of 1-butanol, acetic acid and distilled water (4:1:1, v/v) at room temperature (Sujata et al., 1992) for about 35 min. After removing the developed plates from the chamber, a visible image was taken under the visible light. Afterwards, each plate was placed under the UV light (256 nm) and another image was captured. Fig. S1 (supplementary data file) shows all TLC images captured under visible and UV lights. Finally, the recorded images were imported to MATLAB environment (version 8.3 R2014a) for further data analysis.
In order to measure the pH of the soil samples, 10 mL of CaCl₂ solution (1.47 g L⁻¹) was added to 5 g of each sample. After 1 h, the pH of the supernatant was measured. For determination of standard saturation electroconductivity (EC), 30 g of each soil sample was placed in an individual beaker and 30 mL distilled water was added to it. Afterward, using a Buchner funnel and a vacuum pump, a more concentrated extract was prepared (Kalra, 1997). Finally, the EC values of the concentrated soil extract were recorded by an electro-conductivity meter. The EC and pH values of the soil samples are given in Table S1 (supplementary data file). To obtain the UV spectra, each plate was placed under a UV lamp and the pH of the supernatant was measured. For determination of the UV spectra of the extraction solution and placed into UV cells. The UV spectra of the spots were recorded in the wavelength range of 200–700 nm against distilled water as a reference solution.

2.5. TLC image processing

In order to extract as much information as possible from the acquired TLC images, they were subjected to different image processing steps. The image processing workflow is classified into three sections: i) image compression or size reduction, i.e. reducing memory requirements by deleting additional unwanted parts of the image; ii) image pre-processing, in which the image quality is improved by removing chromatographic artifacts; iii) image (data) analysis as the final step, in which image information is transformed into mathematical data (Juan & Ferrer, 2011). Prior to the image processing step, for simplifying the calculation, the RGB image values were converted from 8-bit unsigned integers (unit 8) to double-precision floating point using “im2double” MATLAB function.

2.5.1. Size reduction

As the first step of the data processing, the images were inverted and compressed. The inversion of image values resulted in normal positive chromatographic peaks. Since the original size of each image was too high (13,500 × 10,200 × 3), and potentially it might have slowed down the process, performing a compression step was necessary. “imresize” function implemented in MATLAB image processing toolbox was used in order to reduce the image size. In this regard, all the images were resized using bilinear interpolation method with resizing scale set to 0.05. This way, some of the pixels were replaced by a single pixel with an output image value equal to a weighted average of pixels in the nearest 2-by-2 neighborhoods. The final size of all images was equal to 675 × 510 × 3. Further data analysis were performed on each color channel (i.e. red, green and blue channels) (Juan & Ferrer, 2011).

2.5.2. Images pre-processing

As mentioned before, the obtained images contain many chromatographic problems that may significantly reduce the accuracy of the results. Therefore, image pre-processing steps were applied on the acquired digital images to enhance the quality and remove possible sources of variations (De La Mata-Espinosa, Bosque-Sendra, Bro, & Cuadros-Rodríguez, 2011).

Baseline correction was considered as the first step for image pre-processing. General baseline drift may occur because of the instrumental and experimental errors such as inaccuracy in capturing images, optical imbalance between different parts of plates, and impurities in the mobile phase. Accordingly, each compressed and inversed image was vectorized (unfolded) to the pixel level. The asymmetric least squares (AsLS) algorithm, a well-known baseline correction method in one dimensional chromatographic data sets, was used to remove the baseline deviation (Eilers, 2004; Tang et al., 2015). In this method, a second-order polynomial equation was fitted to all data points of a vector, to iteratively estimate the baseline. Then, the estimated baseline was subtracted from the original vector. The weighting (penalizing) and smoothing parameters were set to 0.001 and 1e⁻², respectively. Finally, the baseline corrected vectors were refolded to their original form (i.e. TLC images).

The Rₛ value of the separated spots in TLC chromatograms may be shifted between runs. Fluctuations in temperature, mobile phase flow rate and composition, along with matrix effects, stationary phase degradation, incomplete compacting of silica particles, lack of uniformity on TLC plates’ surface, and inconsistency in manual injections can cause shifts in Rₛ values. Moreover, for similar reasons, the shapes of spots generally fluctuate among different runs in TLC analysis (Sherma & Fried, 2003, chap. 10). Correlation optimization warping (COW), a well-known alignment algorithm in chromatography, was developed to correct random shifts and general deviation in the shape of all image spots (Berg & Andersson, 2004; Cook & Rutan, 2014). To apply COW algorithm, all the images from each channel (i.e. R, G and B) were concatenated along their non-chromatographic dimension. In the obtained image, each column (12,240 pixels) and each row (675 pixels) represented a sample and a chromatogram, respectively. The COW algorithm aimed at aligning each sample (here, each pixel row in the non-chromatographic dimension) toward a target chromatogram (vector) by dividing the vectors into a defined number of segments and then stretching or compression of these segments in a way that the correlation coefficient between the sample and the reference vectors is maximized (Berg & Andersson, 2004). The COW parameters including the segment length and slack size, were optimized to 200 and 15, respectively.

The digital TLC images were then cropped in order to remove undesirable sectors and to achieve separate chromatograms. By summing pixels in each channel of the rows at the same migration distance of the lane, a one-dimensional chromatogram was obtained (Tang et al., 2014).

The obtained chromatograms were then normalized against the total peak area to eliminate any uncontrolled variation which occurred during sample preparation and injection. Finally, the chromatograms were mean-centered and Pareto-scaled prior to data analysis to eliminate the mean of the variables and to increase the importance of the small variables, respectively (Yi et al., 2016).

2.6. Data analysis

In the present study, principal component analysis (PCA) and k-means partitional clustering methods were used as exploratory overview of the data general structure and to evaluate the performance of the proposed pre-processing steps. As the first step of data analysis, the obtained data matrix was analyzed using PCA. This technique transforms the original variables of the data into a set of a few orthogonal principal components (PCs) that accounts for most of the data variance. Plotting the scores and loadings in the space of the significant PCs (Brereton, 2015a; Brereton, 2015b; Brown, Tauler, & Walczak, 2009), it is possible to assess the similarities and dissimilarities between the pre-processed images, and evaluate the effects of original variables on the sample properties (Brereton, 2009).

Analyzing the unscaled data matrix by k-means partitional clustering with PCA preprocessing (the first two PCs explained 98% of the variance) and Mahalanobis distance (Brereton, 2015a; Brereton, 2015b), the best discrimination between samples was obtained. The main aim of this iterative method was to cluster the samples in a way that the within-cluster sum of square euclidian (SSE) distances is minimized (Liland, 2011; Sun, Xu, Liang, Xie, & Yu, 1994). The general workflow of experimental and image analysis steps are illustrated in Fig. 1.
3. Results and discussion

3.1. Image preparation

The images of saffron TLC pattern under visible and UV lights (for one of the TLC plates) are shown in Fig. 2a and b, respectively. As described in Section 2.5.2, the images were processed through different image preparation steps including inversion, size reduction, and image pre-processing steps including baseline correction using AsLS algorithm and aligning each color channel using the COW method. Finally, the images were cropped and converted to one dimensional (1D) chromatograms. General workflow of the image preparation is shown in Fig. 2. The final preprocessed images for one TLC spot under visible and UV lights and their representative (1D) chromatograms are illustrated in Fig. 2m and n.

Seven yellow-orange bands with different intensities were observed under visible light (Fig. 2a). The \( R_f \) values assigned to these bands were 0.19, 0.29, 0.43, 0.56, 0.63, 0.80, and 0.96 respectively for the spots 1–7. Under UV light (256 nm), two additional spots were also observed. One of the spots also appeared under visible light as a very pale spot while under UV light appeared as a dark brown fluorescent spot. Therefore, under UV light, the \( R_f \) values for all 9 spots were calculated as 0.19, 0.29, 0.43, 0.56, 0.63, 0.67, 0.80, 0.85, and 0.96.

As it is clear from Fig. 2l, the chromatograms obtained from the red channel contain no information regarding the spots intensities, and thus they were not included in further analysis and in order to compare the TLC chromatograms, the information from the green and blue channels were considered. The normalized, mean-centered and Pareto-scaled chromatograms were then transformed in a matrix (size 2323 × 80) which was analyzed by PCA and k-means methods.

3.2. Identification

3.2.1. UV–visible spectrophotometry

Saffron main components have characteristic UV–visible spectra. Picrocrocin exhibits a typical broad absorption band located around 250 nm (\( \lambda_{max} \)) and the safranal maximum absorbance appears at 330 nm. Crocins, both cis- and trans-derivatives, exhibit a double peak at about 440 nm and a secondary band near 260–264 nm (Sujata et al., 1992; Tarantilis et al., 1995). On the other hand, the cis-crocins exhibit an additional adsorption peak between 320 and 340 nm. Moreover, the additional minor absorption bands in 260–264 nm permits the identification of trans-isomers of crocins (Cossignani et al., 2014; Tarantilis et al., 1995). It should be noted that with the proposed TLC solvent system (\( n \)-butanol-acetic acid-water), safranal cannot be eluted because it requires a different solvent system (Sujata et al., 1992). Fig. S1 (supplementary data file) indicates the structural formula of crocins and picrocrocin.

Nine saffron spots on one of the TLC plates were analyzed using UV–visible spectrophotometry (Fig. 3). The UV–visible spectra in Fig. 3a–e, g and f confirm the presence of crocins. It is important to note that each spot may contain more than one compound. The spectra in Fig. 3f and h with a sharp peak at about 240 nm could be assigned to picrocrocin and its derivatives. Moreover, the fact that picrocrocin and its derivatives are colorless under
visible light and appear as a dark brown fluorescent spot under UV light, is further evidence for the presence of picrocrocin (Sujata et al., 1992). Using all the mentioned information, the TLC separated compounds were identified and given in Table 1.

3.2.2. HPLC-DAD analysis

Identification of the separated compounds was carried out by comparing the data obtained from HPLC-DAD chromatograms of the CRM and those obtained from the literature under the same conditions.
operating conditions (Caballero-Ortega, Pereda-Miranda, & Abdullaev, 2007). Fig. 4 shows the three representative chromatograms of the CRM at three main wavelengths, 250, 330 and 440 nm. Accordingly, the following information was obtained: picrocrocin \((t_R = 8\, \text{min})\), HTTC \((t_R = 16.18\, \text{min})\), 3-gentiobiosyl-kaempferol \((t_R = 17.4\, \text{min})\), trans-crocin 4 \((t_R = 25.9\, \text{min})\), trans-crocin 3 \((t_R = 28.6\, \text{min})\), trans-crocin 2’ \((t_R = 32\, \text{min})\), cis-crocin 4 \((t_R = 35.4\, \text{min})\), trans-crocin 2 \((t_R = 37.9\, \text{min})\) and cis-crocin 2 \((t_R = 44.1\, \text{min})\) (Caballero-Ortega et al., 2007). In addition, each TLC spot was carefully scraped off the plate and eluted in 300 µL methanol/acetonitrile, and then analyzed by HPLC-DAD. The second TLC spot which corresponds to a HPLC-DAD peak at \(t_R = 25.4\) (Fig. 4c) is related to trans-crocin 4, the most abundant crocin in saffron with the sharpest peak at the range of 400–500 nm in UV–visible spectrophotometry (Caballero-Ortega et al., 2007).

The analysis of the 3rd and 4th TLC spots revealed that both spots are the mixture of two components appeared on the HPLC chromatogram at \(t_R = 25.6\) and 29.1 min. These peaks are ascribed by the authors to trans-crocin 3 and trans-crocin 4, respectively.

Table 1
Identification of crocins and picrocrocin derivatives of the separated TLC spots.

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Compound name</th>
<th>(R_f^a)</th>
<th>(t_R^b)</th>
<th>(\lambda_{\text{max}}) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crocin derivatives</td>
<td>0.19</td>
<td>–</td>
<td>260, 289, 436, 461</td>
</tr>
<tr>
<td>2</td>
<td>trans-Crocin 4</td>
<td>0.29</td>
<td>25.43</td>
<td>260, 436, 462</td>
</tr>
<tr>
<td>4 &amp; 5</td>
<td>trans-Crocin 3/ trans-Crocin 4</td>
<td>0.43/0.56</td>
<td>25.6/29.1</td>
<td>265, 436, 452</td>
</tr>
<tr>
<td>5</td>
<td>cis-Crocin 4/trans-Crocin 2’</td>
<td>0.63</td>
<td>32/35.2</td>
<td>262, 322, 433, 458</td>
</tr>
<tr>
<td>6</td>
<td>Picrocrocin</td>
<td>0.67</td>
<td>8.1</td>
<td>243</td>
</tr>
<tr>
<td>6’</td>
<td>cis-Crocin 4/trans-Crocin 2</td>
<td>0.8</td>
<td>35/38.5</td>
<td>265, 288, 320, 435, 460</td>
</tr>
<tr>
<td>6’’</td>
<td>HTTC/3-Gentiobiosyl-kaempferol</td>
<td>0.85</td>
<td>16.1/17.4</td>
<td>236, 283</td>
</tr>
<tr>
<td>7</td>
<td>cis-Crocin 2</td>
<td>0.96</td>
<td>44.1</td>
<td>260, 281, 430, 456</td>
</tr>
</tbody>
</table>

\(R_f^a\): Retention factor.

\(t_R^b\): Retention time (min).

Fig. 3. The spectra obtained from the TLC spot using UV–visible spectrophotometry. Figures (a) to (i) correspond to the TLC spots in order from lower edge to upper edge of the plate. Spectra (a) to (e), (g) and (i) are assigned to crocin compounds due to the double peak in the range of 400–500 nm. Spectra (f) and (h) are ascribed to picrocrocin and its derivatives.
mental repeatability is lower than variability within the data sample) were placed near each other. This means the experi-
mentally, two different unsupervised methods (i.e. PCA and k-
means) were exploited to remove the chromatographic artifacts such as baseline drifts and spots misalignment from the obtained TLC images. Finally, the images were converted into one dimensional chromatograms and analyzed by PCA and k-means to evaluate the pre-processing strategy performance and observe the effects of soil salinity indicators (i.e. pH and EC) on the samples properties. Only EC showed a significant effect on the saffron analysis patterns whereas the effect of soils’ pH values was negligible. The soil samples with high level of salinity have positive score values on the first PC that results in similar grouping to k-means. Therefore, saffron samples were divided into two main groups (the samples with soil EC < 2 and the samples with soil EC > 2). Both PCA score plots (Fig. 4b) and k-means dendrogram showed a slight grouping among saffron samples based on the soil EC values. The clear distinction between two groups was observed on the PCI in PCA scores plot (Fig. 4b). Generally, the samples with ECs higher than 2 have positive score values on the first PC. This is in good agree-
ment with the clustering results from the k-means method. The loading plots and TLC patterns of the samples cultivated in the slightly and moderately saline soils, revealed that the second spot (trans-crocin 4) and the fourth spot (containing a mixture of trans-crocin 3 and trans-crocin 4) have less intensities in the green-visible channel and both blue channels (blue-visible and blue-UV). Moreover, the mixture of cis-crocin 4/trans-crocin 2, the mix-
ture of HTCC/3-gentiobiosyl-kaempferol, and cis-crocin 2 corre-
sponding to the 6th, 6th and 7th spots, respectively showed less intensity in blue-visible and green-UV channels. On the other hand, the fifth spot (a mixture of cis-crocin 4/trans-crocin 2) showed high intensities in all channels of this group of samples. The intens-
ities of the spots on the TLC plates are directly proportional to the concentration of the related components in the samples matrices. Therefore, it can be concluded that saffron samples that were cul-
tivated in even slightly saline soils, generally had lower concentra-
tions of their important coloring metabolites.

4. Conclusion

In the present work, a simple and low cost method based on TLC-IA was introduced for patterns recognition (chemical fingerprinting) of saffron samples. The method showed a significant potential to discriminate between different saffron samples on the basis of their soil EC values. Moreover, an established strategy for chromatographic data pre-processing was successfully developed to remove the chromatographic artifacts such as baseline drifts and spots misalignment from the obtained TLC images. Finally, the images were converted into one dimensional chromatograms and analyzed by PCA and k-means to evaluate the pre-processing strategy performance and observe the effects of soil salinity indicators (i.e. pH and EC) on the samples properties. Only EC showed a significant effect on the saffron analysis patterns whereas the effect of soils’ pH values was negligible. The soil samples with high level of salinity have positive score values on the first PC that results in similar grouping to k-means. Therefore, according to the soil electroconductivity data obtained from the soils of saffron growing areas (that affects its main secondary metabolites content), the quality of saffron can be evaluated and classified.
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.07.012.

References


