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Evaluation of Crocus sativus L. Quality Using FTIR Spectroscopy

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Abstract: *Crocus sativus L.* is one the important spices that gives food a distinctive color and taste as well as its medicinal value. Its quality can be estimated using different analysis methods. In This study we used FTIR spectroscopy to Evaluate *Crocus sativus L.* quality at MEDICO Lab 2023. FTIR Spectroscopy is a fast and reliable analytical method for quality saffron evaluation. The results of FTIR spectra indicated functional groups related to O-H, C=C, C=O, C-O, C(O)–O, C-H, C-H (cis-) and C-H (trans-). Also, our samples lacked the distinctive bands of the active substances of crocetin. Furthermore, the flowers in our samples contained different numbers of stigmas, evidence of the genetic mixing of the bulbs used.

Keywords: *Crocus sativus L.*, Evaluation, FTIR Spectroscopy, Quality

1. Introduction:

Stigma flowers of Crocus sativus L. are considered the most expensive spices in the world. It is also characterized by its pharmacological and medicinal properties. It has many benefits in food as coloring and aromatic agents are caused by its rich composition of proteins (10-14%), carbohydrates (12-15%), total oils (5-9%), ash (4%), fiber (4-5%) as well as volatiles (0.3-0.8%) [1]. Stigma flowers of Crocus sativus L. contain crocetin (C20H24O4, 8,8' Diapocarotenedioic acid), picrocrocin (C16H26O7, 4-(β-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carbox-aldehyde) and safranal (C10H14O,2,6,6-trimethyl-1, 3-cyclohexadiene-1-carboxaldehyde) [2]. These are components responsible for the unique characteristics of saffron such as the bright yellow-red dye and bitter taste [3].

A number of alternative methods exist to evaluate the quality of agricultural products, including saffron, apart from the conventional approaches. The conventional methods include the use of chemical reagents, involve the completion of time-consuming and financially burdensome procedures, and call for a considerable amount of effort [4]. Hence, it is imperative to provide significant attention to emerging technologies like nanotechnology, as it has shown exceptional results in agricultural applications, particularly in improving agricultural production and assessing its quality [5, 6]. Moreover, it is important for researchers to take into consideration new investigation technologies for quality testing that may provide an accurate and prompt evaluation of the quality of agricultural products. One of these methods is infrared spectroscopy. FTIR spectroscopy is a very effective method for identifying organic compounds. It utilizes infrared light in the range of 4000 cm-1 to 400 cm-1 [7].

Also, it is characterized by speed, sensitivity and versatility [8]. The FTIR spectroscopy is also used to identify functional compounds of samples with an easy preparation protocol without any hazardous chemicals [9].

FTIR spectroscopy was used to characterize functional groups and to define the quality and authenticity of saffron [10], as well as to determine geographical origin [11]. Furthermore, it could character the changes in effective materials as a result of storage [12]. In addition, to distinguish authentic saffron, commercial saffron and colorant-added saffron as adulterated samples were mixed with other plant colorants [13].

As a result of the medical and economic importance of saffron, its cultivation has spread in recent times in several regions in Syria, where the bulbs were imported from different areas. Therefore, it is vital to verify the quality of saffron samples due to their efficiency being strongly dependent on plant components.

The present study aimed at studying the functional groups of some samples using FTIR spectroscopy and identifying the differences among these samples collected from local fields.

2. Materials and methods:

2.1. Plant materials:

Six stigma saffron samples were collected from different fields in Homs city. Moreover, one sample of *Crocus sativus*

L. was brought from Iran as a control sample. It was noticed that the flowers that were collected as samples for analysis contained different numbers of stigmas, starting from 2 to seven stigmas, so the samples were arranged as follows: **2.**

2.1. Plant materials:

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Table 1. The samples for analysis

The sample	Characterization flowers
1	control
2	The flower contains two stigmas
3	The flower contains three stigmas
4	The flower contains four stigmas
5	The flower contains five stigmas
6	The flower contains six stigmas
7	The flower contains seven stigmas

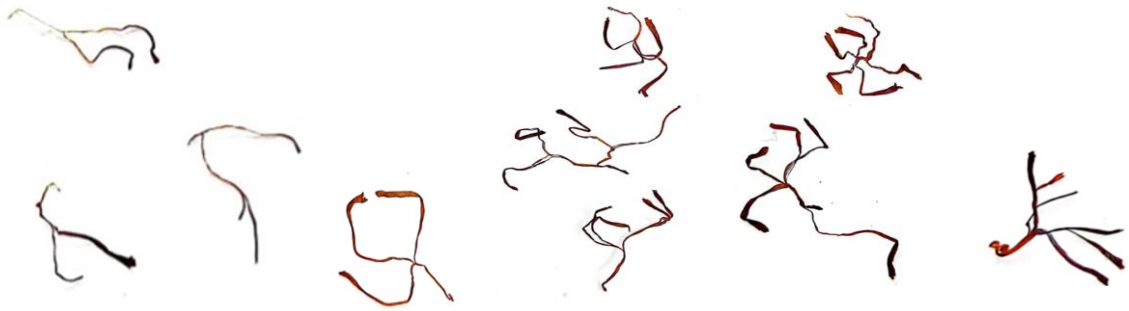

Figure 1. Numbers of stigmas in the different flowers

2.2. Sample preparation:

All samples of stigmas were finely ground using an agate pestle and mortar. After that, each sample was mixed with KBr at a 1/140 ratio (w/w) and a KBr disc was prepared.

2.3. FTIR Spectroscopy:

FTIR spectrum was performed using a (IRAffinity-1S-4100 SHIMADZU) from 4000 cm−1 to 400 cm−1 with a 4 cm−1 instrumental resolution.

3. Results and Discussion:

3.1. FTIR Spectrum

The spectra image of FTIR and the functional groups for all samples in the present study was shown in Table 2. There were three patterns of spectra images. The first one was for sample 2, Figure 2. This image included various peaks of 3394, 2935, 2885, 1649, 1456, 1384, 1228, 1045, 669, 792, 856 and 923 cm-1. The second pattern was for samples 3 to 7 due to the similarity of their spectra images in Figure 3, where it contained the peaks of (3396-3410), (2920-2924), 2852, 1651, 1456, 1384, 1228, 1047, 1051, 831, 921 cm-1. The third pattern was for the control sample which showed peaks of 3410, 2920, 2852, 1697, 1653, 1624, 1575, 1556, 1543, 1456, 1384, 1228 and 1072 cm-1 Figure 4.

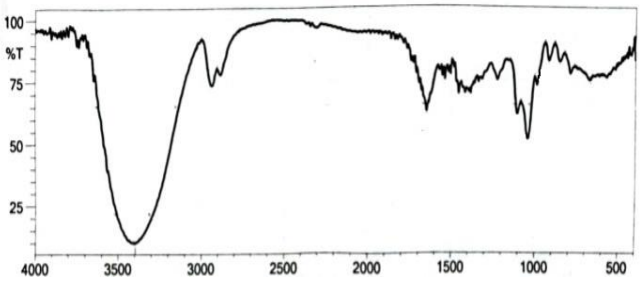

Figure 4. FTIR for control sample

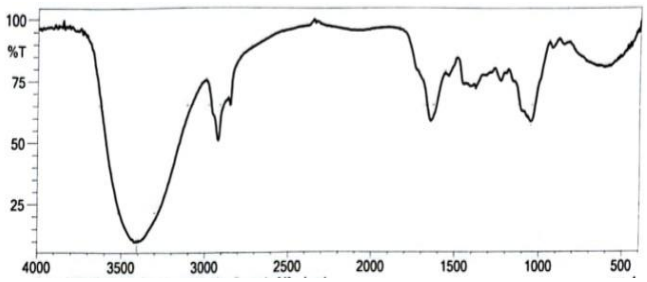

Figure 2. FTIR for sample 2

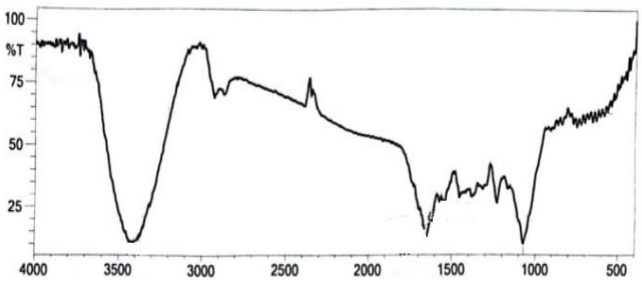

Figure 3. FTIR for sample 4

Table 2. FTIR spectrum for *Crocus sativus L.* stigmas

sample 2	Functional group	samples 3 to 7	Functional group	control	Functional group
3394	hydroxyl (-OH) group of sugars	3396-3410	hydroxyl (-OH) group of sugars	3410	hydroxyl (-OH) group of sugars
2935 2885	C-H asymmetric and symmetric stretching vibrations	2920-2924 2852	C-H asymmetric and symmetric stretching vibrations	2920 2852	C-H asymmetric and symmetric stretching vibrations
-	-	-	-	**1697**	C=O stretching in **crocetin** esters, aliphatic esters, and free carboxylic groups of **crocetin** and amino acids
1649	C=C stretching vibrations of carotenoids and apocarotenoids, the amide I band and the O–H bending vibrations of water	1651	C=C stretching vibrations of carotenoids and apocarotenoids, the amide I band and the O–H bending vibrations of water	1653	C=C stretching vibrations of carotenoids and apocarotenoids, the amide I band and the O–H bending vibrations of water
-	-	-	-	**1624-1575-1556-1543**	C–O vibrations in esters, the amide II and aromatic C=C stretching vibrations
1456-1384	alkyl and alkenyl groups, aromatic C=C bonds, O-H bending phenol	1456-1384	alkyl and alkenyl groups, aromatic C=C bonds, O-H bending phenol	1456-1384	alkyl and alkenyl groups, aromatic C=C bonds, O-H bending phenol
1228	C(O)–O stretching vibrations and –OH in plane vibrations/amide III	1228	C(O)–O stretching vibrations and –OH in plane vibrations/amide III	1228	C(O)–O stretching vibrations and –OH in plane vibrations/amide III
-	-	-	-	**1072**	C-O vibration of sugars
1045	C-O-C glycosidic linkages of oligosaccharides, bending vibration in sugars	1047-1051	C-O-C glycosidic linkages of oligosaccharides, bending vibration in sugars	-	-
669-792-856-923	C-H (*cis*-) and C-H (*trans*-) out-of-plane vibrations	831-921	C-H (*cis*-) and C-H (*trans*-) out-of-plane vibrations	-	-

Our results of FTIR indicated the presence of similar functional groups in most spectral regions in all samples except the band in 1697 cm-1 in a control sample. This band is characterized by the carbonyl (C=O) group which contains functional groups of esters, ketones and aldehydes related to the crocin content in the saffron. This agrees with Foschi et al. [4] Nakanishi and Solomon [14]. Furthermore, the presence of bands 1624, 1575, 1556 and 1543 cm-1 in control samples indicated its protein content. According to Ordoudi et al. [8], Petrakis and Polissiou [15], these bands correlated with the presence of protein. In contrast, all samples distinguish with the bands in the region 1200-700 cm-1 correlated with the presence of polysaccharides and sugars compared with the control sample. A similar study indicated that this region is associated with the presence of sugar [16].

Most research studies indicated that the bands characterizing in 1800–1500 cm-1 region provide vital information on the presence of secondary metabolites especially crocin, picrocrocin and safranal [1-4,10-17]. Therefore, FTIR spectrum results show that our samples were not of good quality. In addition, the presence of different numbers of stigmas in the flowers, evidence of impurity of the cultivated bulbs as well as the occurrence of genetic mixing.

4. Conclusion:

FTIR analysis of stigmas saffron affirmed the presence of similar functional groups for samples. Also, there weren't any bands indicating crocin compound compared with the control. Our results showed low-quality samples and impure genetic bulbs.

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تقييم جودة الزعفران *Crocus sativus L.* باستخدام التحليل الطيفي FTIR

لينا ممدوح النداف [1،*]، محمد نبيل القصير [2]، سالم فرج بامسعود [3]

الملخص: يعد الزعفران *Crocus sativus L.* من التوابل المهمة تضفي للطعام لونًا ومذاقًا مميزًا بالإضافة إلى قيمته الطبية. ويمكن تقدير جودته باستخدام طرائق تحليل مختلفة. استخدمنا في هذه الدراسة التحليل الطيفي FTIR لتقييم جودة الزعفران *Crocus sativus L.* في مخابر ميديكو لعام 2023. يعد التحليل الطيفي FTIRطريقة تحليلية سريعة وموثوقة لتقييم جودة الزعفران. أشارت نتائج أطياف FTIR إلى مجموعات وظيفية مرتبطة بـ O–H وC = C و C = O و C–O و O– (O) C و C–H و (cis–) C–H و (trans-) C–H. تفتقر العينات الى الحزم الطيفية المميزة للمادة الفعالة الكروسيتين. كذلك، احتوت الأزهار في هذه العينات على أعداد مختلفة من المياسم دلالة على الاختلاط الجيني للبصيلات المستخدمة.

الكلمات المفتاحية: الزعفران *Crocus sativus L*. النوعية، تقييم، FTIR Spectroscopy