Asian Food Science Journal

Volume 22, Issue 1, Page 33-42, 2023; Article no.AFSJ.96250
ISSN: 2581-7752

Quality of Saffron in Oman Markets

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SAB, AAA, AAH and AAl-Alawi managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2023/v22i1615

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/96250

Short Research Article

Received: 25/11/2022
Accepted: 06/02/2023
Published: 10/02/2023

ABSTRACT

Saffron is the most expensive spice in the world, it is highly valued as a culinary spice for its flavor and color properties. Since saffron is an expensive product, it is cheated by the dealers, therefore, quality control of saffron is important for the spice industries and also the consumers. This study aims to evaluate the saffron quality in Omani markets according to the ISO standard. A total of 21 saffron samples were examined; 2 in powder form and 19 in filaments. The samples of saffron were purchased from different hyper-markets and local markets in Muscat, Oman. Saffron's commercial value is determined according to the ISO standard which include moisture, ash, acid insoluble ash, extract %, sulfuric test, artificial colors, spectrum, Flavor, aroma and color. The saffron samples were in line with standard in terms of moisture, ash, acid insoluble ash and extract %. The two powder saffron samples failed in sulfuric, spectrum and flavor tests. From the 19 samples of saffron filament, three samples were failed to achieve the flavor and color limits required by the standard. This failure to standard quality could be due to poor processing condition such as drying and packaging or due to adulteration. The standard parameters could not distinguish between low quality and adulteration due to its limited and unspecific parameters. Therefore, we recommend improving the saffron standard with parameters targeting adulteration.

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Keywords: Crocus sativus; saffron; UV-vis spectroscopy; flavor; aroma; color; food quality.

1. INTRODUCTION

"Saffron, the dried stigmas of *Crocus Sativus* L., is one of the most precious agricultural products and also is the most expensive spice among 85 known spices in the world and thus, is called red gold. It is highly valued as a culinary spice for its flavour and colour properties and it is obtained by drying the stigma of saffron flowers" [1]. "Each saffron flower has only three stigmas which is used as a food additive due to its aroma, color, and bitter taste. Currently, saffron is produced in Iran, Spain, Greece, Italy, Turkey, Morocco, and India. The importation of saffron to Oman increased significantly during the last years, from 22.6 tons in the year 2017 to 61.8 tons in the year 2021, which valued around 25 million Omani Riyal" [2].

"Saffron possesses powerful coloring and flavoring properties due to its glycosidic constituents" [1,3]. "Its contains over 150 volatile and non-volatile compounds including proteins, carbohydrates, vitamins, amino acids, minerals, gums, and other compounds" [4,5]. "However, the crocin, picrocrocin, and safranal are responsible for saffron's sensorial attributes and are the major bioactive compounds used as markers for its quality. Furthermore, the quality and, consequently, the commercial value of saffron are based on the estimation of its coloring power, bitter taste, and aroma" [6]. "The amounts of these main compounds are used to express the quality of saffron" [7]. "These secondary metabolites are found in abundance in the final edible product, i.e., they account for 50% w/w of the dried stigmas. The bitter taste of saffron is derived from glycoside picrocrocin, which is a colorless monoterpene aldehyde" [3]. "Due to the binding of one molecule of glucose to hydroxy trimethyl cyclohexene carboxaldehyde, picrocrocin is produced and over the process of saffron drying stages, picrocrocin is hydrolyzed and changed into volatile Safranal, which is the factor for the aroma of saffron and consisting of about 30 to 72% of the aroma compounds of the stigma" [8].

Aside from its main use as a spice, saffron exhibits significant biological activity. Several scientific studies have reported that saffron and its chemical components are potential anti-ulcer agents [9], they improve digestion [10], they play a role as anti-cancerogenic [11], they reduce atherosclerosis [12,13,14], and they used as anti-depressant in the traditional medicine [15]. "Additionally, saffron presents interesting results in the prevention and maintenance of cancer due to its antioxidant properties" [16].

"The quality of saffron is a function of different factors such as climate conditions, harvesting conditions, storing conditions, method of drying, and packaging" [3]. "Reductions in saffron's commercial quality can be attributed to inappropriate harvesting methods, insufficient dehydration processing, exposure to direct sunlight, improper storage, and adulteration" [17,18]. "Saffron usually tolerates high temperatures, but it is sensitive to light and oxygen, therefore, it is necessary to place it in suitable packages like polyethylene with cellophane covering" [19].

"Harvesting of saffron flowers and stigma separation are still carried out by hand in most areas. The intensive hand labor is why saffron is widely known as the most expensive spice in the world" [20]. Unfortunately, since saffron is an expensive product, in various ways, it is cheated by the dealers. Adulteration of Saffron has a direct effect on the economy of the country and has serious health impacts [21]. "Therefore, quality control of saffron is important for the spice industries and also the consumers. Adulteration of saffron includes adding or mixing of similar materials such as beet, pomegranate fibers, red-dyed silk fibers, safflower, and marigold to red stigma of saffron are the most common types of fraud in saffron production" [22,23]. "Another way for adulteration, in order to increase the saffron mass, is immersion of saffron fibers in honey, vegetable oils, or glycerin" [22]. "The frauds in the saffron market of Kashmir (J&K) constitute 52% genuine, 30% poor grade and 17% adulterated saffron" [24]. Therefore, to prevent adulteration, it is necessary to establish a precise chemical identification protocol to protect producers' and consumers' interests [25].

"The quality control of saffron typically involves determination and quantification of certain colorants suspected as potential adulterants. Saffron's commercial value is determined according to the ISO 3632-1 standard" [26]. Also, the Gulf Cooperation Council Standardization Organization GSO 3632-1 [27] which adopted the ISO specification and based on minimum requirements of each quality. Guidelines for the analyses of the major bioactive compounds have

been established by the International Standards Organization ISO 3632-2 [28]. "They have defined procedures to determine these compounds by spectrophotometric analyses. According to ISO 3632-1, picrocrocin, safranal and crocins express the flavour or bitterness, the aroma and the coloring respectively. These values are defined as direct reading of the absorbance of a 1% aqueous solution of dried saffron at 257, 330 and 440 nm respectively using a 1 cm pathway quartz cell" [29].

Saffron is the most expensive spice in the world, it is highly valued as a culinary spice for its flavor and color properties. Since saffron is an expensive product, it is cheated by the dealers, therefore, quality control of saffron is important for the spice industries and also the consumers. From the literature survey, it is clear that no such work has been done till date on assessing the quality of saffron in Omani market. This study aims to evaluate the saffron quality in Omani markets according to the ISO standard.

2. MATERIALS AND METHODS

2.1 Samples and Sampling

A total of 21 saffron samples were examined; 2 in powder form and 19 in filaments. The samples of saffron were purchased from different hyper-markets and local markets in Muscat, Oman. To keep the brand names anonymous, the saffron samples were given letter F for filaments and letter P for powder, labeled accurately, mentioning the name, location, collection time, date, and then stored in room temperature (25 ºC) under dark conditions until the analysis completed.

2.2 Moisture Content

The moisture content include volatiles of saffron samples was determined according to the International Standards of ISO 3632-2 [28]. Using weighing dish previously dried, 2.5 g of saffron filaments was weighed to the nearest 0.001g. The dish placed in oven at 103ºC for 16 hrs., and allowed to cool in desiccator before checking the weight. The dried sample used for determining ash and acid insoluble ash.

2.3 Total Ash and Acid Insoluble Ash

Total ash of Saffron samples was analyzed according to the method ISO 928 [30], which measured the residues obtained after incineration at 550ºC. Where acid-insoluble ash was determined according to the method ISO 930 [31], which measured the part of total ash remaining after treatment with hydrochloric acid.

2.4 Extract in Cold Water

The extract was measured according to the methods of Test for Spices and condiments IS 1797 [32]. The sample extracted by distilled water, shacked for 30 min. interval for 8 hours and allowed to stand for 16 hours. The extract filtered by dry filter paper and 50ml of extract evaporated to dryness at 130ºC to constant weight.

2.5 Sulfuric Test

Sulfuric acid test was conducted according to Mohamad et al [33] to examine adulteration in saffron. The Carotenoid pigments like crocin, crocetin and picocrocin reacts with the sulfuric acid to give bluish color immediately, which finally changes to violet to red. This reaction is due to the hydrolysis of the Carotenoid esters, where the fake saffron produces yellow color only.

2.6 Artificial Colors

Artificial water-soluble acidic colorants were measured according to ISO 3632-2 [28]. They are isolated and eluted by chromatography on a polyamide microcolumn, and identified by TLC. This method is directly applicable to saffron in powder form and to saffron filaments and cut filaments.

2.7 UV-Vis Spectrum

The analyses were carried out following the specifications indicated in the ISO 3632-2 [28], around 125 mg of dried saffron was transferred to a 250 mL volumetric flask and approximately 230 mL of deionized water was added. The flask was covered with aluminum foil to avoid direct contact with light sources, placed in a cold-water bath (<22ºC), and the content was stirred for 1 h. The solution was diluted up to the mark with deionized water, then 60 mL was filtered using rapid filtration filter paper. The first 40 mL was discarded and the last 20 mL was kept. Half of this volume was withdrawn and diluted up to the mark in a 100 mL volumetric flask. The absorption characteristics of this solution were measured by recording a spectrum between 200 nm and 600 nm and maximum absorbance was recorded as λmax.

2.8 Flavor, Aroma and Color Using UV-Vis Method

Flavor, aroma and color of saffron samples were determined according to ISO3632-2 [28]. This method enables the determination of picrocrocin for flavor, safranal for aroma and crocin for color. Briefly, sample of 500 mg dissolved in 1000ml distilled water using magnetic stirrer for 1 hr at dark. Then 20ml aliquot transferred to 200ml volumetric flask and filed up to the mark with distilled water then mixed and filtered at dark. The absorption was measured using UV-Vis spectrophotometer (Thermo, Evolution 201) at 257nm for picrocrocin, 330nm for safranal and 440nm for crocins determinations. The following equation was used to for calculation:

Absorbance = D x 10000/m x (100-M)

Where:

D is the specific absorbance,
m: sample mass in grams,
M: moisture of sample.

3. RESULTS AND DISCUSSION

The chemical parameters of saffron samples, moisture (include volatiles content), ash, acid insoluble ash and extract % are presented in Table 1. The ISO 3632 standard limits of these parameters are also listed in that table. The moisture content of saffron samples ranged between 2.01% to 8.35% for filament samples and between 2.35% to 2.45% for powder saffron. The moisture content of saffron samples was in line with the ISO standard which has a maximum of 12% moisture for filament saffron and 10% for powder saffron. However, there is a significant difference observed between filament and powder samples. Bergomi et al [34] reported higher moisture content ranged between 4.1% to 11.7% for saffron in filaments and 4.5% to 9.3% for powder samples. Other studies reported similar results for filament samples such as Manzo et al [35] results ranged from 6% to 9%; Saber & Guner [36] values ranged between 5.9-6.5%; Giorgi et al [37] reported moisture between 5.3-9.4% and Sharifi et al [38] reported moisture ranged between 9.7-11.9%.

“The amount of moisture and volatile matter in saffron samples is an important parameter in defining the quality of the final product, and values outside the recommended ranges are considered as signs of adulteration” [39]. “The most important factors affecting the moisture content are the method of drying, duration of drying, and storage conditions” [39]. “There are different methods of saffron drying such as drying in shade, toasting, freeze drying, microwave, and solar drying” [40]. Carmona et al. [41] have pointed out that, “unless a heat source is used, changes in the chemical composition of saffron, due to the degradation of some compounds, and their fermentation, due to the high water activity, may be observed”.

Ash content of saffron samples was ranged between 4.55% to 6.55% for filament saffron and 5.1% for powder saffron (Table 1). Ash content of saffron samples was also according to standard requirements which has a maximum of 8% ash content. Values reported in this study were similar to other studies such as Saber & Guner [36], they reported saffron ash between 6.1-6.85% and Sharifi et al [38] reported “ash between 5.38-8.0%. Ash content in saffron implies the amount of minerals in saffron, it is indicative of the presence of minerals in saffron depending on the type of soil and climatic conditions as well as the rate of the pollutants existing in air” [42].

The acid insoluble ash content in samples was between 0.04% to 0.79% in filament samples and between 0.07% to 0.11% in powder samples (Table 1). Which was in agreement with the ISO standard requirement of 1% as a maximum allowance. Saber & Guner [36] reported similar acid insoluble ash content ranged between 0.5-0.66%. The amounts of acid insoluble ash within Iranian and Turkish saffron were found to be 0.50% and 0.66% respectively. The variation of ash and acid insoluble ash contents in saffron varied linearly with the composition of soil and fertilizers [36].

The extract % values of saffron samples in Table 1 ranged between 35.2% to 95.9% for filament samples and between 37.7% to 64.3% for powder samples. All samples were in compliance with standard limit (65%), except sample F019 which has extract of 95.9%. This higher value of extract %, although it’s above the standard limit, it doesn’t regard as a sign of adulteration.

Table 2 presented the results of sulfuric test, artificial color test and spectrum of saffron samples. All filament samples passed the sulfuric test as all samples gave the bluish color immediately, which changes to violet to red. The powder samples failed in this test, as it gave a yellow color after treating with sulfuric acid. “The

Carotenoid pigments in saffron like Crocin, Crocetin and Picocrocin reacts with the sulphuric acid to give bluish color immediately, which finally changes to violet to red, the reaction is due to the hydrolysis of Carotenoid esters. In contrast, the fake saffron fails the y test and yield yellow color instead of blue when hydrolyzed by sulphuric acid. The sulphuric acid chemical test is reliable, rapid and sensitive method to find originality of the saffron in quick time" [43].

The detection of artificial colors in saffron sample were absent in all samples as shown in Table 2. This Chromatography method is one of the most common techniques used to detect adulteration induced by acid-soluble dyes in water [44]. "Since saffron mainly adulterated by addition of synthetic dyes, chromatographic methods can easily detect them" [45]. The most common chromatographic techniques that used for assessing of Safron adulteration include Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC).

The UV-Vis Spectrum of saffron samples was measured to identify the highest λmax for each sample as source of identity. The samples λmax ranged between 420 nm to 443 nm, most of the samples were in line with the standard λmax 441 nm, except P001 which has the highest λmax at 420nm. This value could be a sign of adulteration and can be confirmed by advance Chromatography analysis such as HPLC or GC.

"There are various chemical and biochemical techniques for detection saffron adulteration including those based on chromatography, spectroscopy, immunology and electrophoresis methods. Despite the fact that these analytical methods are more accurate, sensitive and specific than physical methods, sample analysis is expensive and needs specialized expertise" [46,47].

Fig. 1 presented the absorbance of the main characteristics of saffron picrocrocin, safranal and crocin which are the source of flavor, aroma and color respectively. The horizontal lines represent the ISO standard limits for each characteristic. A quality category has been also attributed to these limits, defined by ISO as: I, II or III. In order to be classified as category I, II or III, the sample needs to satisfy the requirements of all three characteristics and have a moisture content below 12% for saffron filaments or below 10% for saffron powders [26]. Based on the results of Fig. 1, the flavor absorbance of most saffron samples was in the range of ISO limits (40-60), except P001, F018 and F019 which were below the limit. The aroma absorbance of saffron samples was all within the ISO limits (20-50) and the color absorbance was all above the lowest limit (120) except sample F004. Therefore, the samples P001, F018, F019 and F004 are not complying with the standard requirement, this could be due to processing conditions such as drying and packaging or due to adulteration. Several studies reported similar values for flavor, aroma and color in saffron, Saber & Guner [36] reported 61-71, 34-43 and 162-197 respectively. Giorgi et al [37] reported flavor between 73-112, aroma between 20-49 and color between 163-277. Sharifi et al. [38] reported flavor between 71-84, aroma between 21-38 and color between 141-236.

Most of the samples studied showed little to no signs of adulteration or contamination despite 4 samples being denied classification in one of the three commercial categories. all of the 4 samples were denied classification solely due to the flavor and color being below the standard limit. This was especially the case for saffron filament samples, which all in agreement with ISO limits in terms of moisture, ash, acid insoluble ash and extraction % and in line with literature values. However, the P001sample has failed in sulfuric, spectrum and flavor tests, which is an indicator of low-quality product or adulteration case.

"Saffron's quality depends on its chemical profile, which provides the bitter taste, desirable aroma, and attractive yellowish-red color of this spice" [48,49]. Several studies related saffron stability to temperature, humidity, pH, light, oxygen [50], geographical growth location, and drying and storage conditions [51]. "Despite the fact that the ISO 3632 grading remains the main frame of reference for saffron quality, several studies suggest that the spectrophotometric method is not the best way to assess the overall quality of this spice" [52]. One of the main limitations is the inability to correctly quantify the amount of safranal in the sample [52,53] and consequently the failure to assign saffron to the proper commercial category. Despite attempts made to quantify the three molecules responsible for saffron quality through the use of an alternative HPLC-DAD methodology [54], other studies show that the problems of the ISO method do not lie solely in the detection of safranal [55,56,57]. For example, the method is incapable, in certain cases, of revealing adulterations with other types

of plants, and the use of diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) needs to be employed as an alternative [58]. Moreover, the ISO method is also unable to distinguish between synthetic components and natural ingredients.

Table 1. Chemical parameters of saffron samples

Samples	Moisture (%)	Ash (%)	Acid ins. ash (%)	Extract (%)
ISO 3632	*Max. 10-12*	*Max. 8.0*	*Max. 1.0*	*Max. 65*
F001	4.88±0.10	4.97±0.12	0.51±0.06	35.2±0.1
F002	4.15±0.07	5.17±0.31	0.11±0.05	43.4±1.4
F003	5.67±0.12	5.26±0.26	0.24±0.00	41.6±0.1
F004	7.20±0.10	5.07±0.06	0.04±0.00	40.7±0.7
F005	5.20±0.14	4.93±0.15	0.79±0.00	44.8±0.3
F006	7.00±0.14	4.55±0.07	0.24±0.00	48.1±1.9
F007	6.96±0.22	4.96±0.08	0.05±0.00	49.6±0.8
F008	7.70±0.14	5.10±0.28	0.56±0.04	46.6±0.4
F009	7.45±0.35	5.55±0.07	0.46±0.03	47.2±0.9
F010	4.75±0.07	6.55±0.07	0.59±0.01	49.8±1.7
F011	5.86±0.06	5.05±0.07	0.05±0.00	49.4±1.2
F012	8.35±0.07	5.30±0.28	0.04±0.01	46.4±1.4
F013	6.20±0.42	4.60±0.00	0.49±0.00	45.9±1.6
F014	6.11±0.13	5.70±0.14	0.45±0.00	39.1±0.3
F015	6.85±0.07	4.95±0.21	0.61±0.03	48.8±2.8
F016	7.30±0.28	5.55±0.49	0.39±0.00	43.4±1.8
F017	3.63±0.63	4.62±0.25	0.24±0.05	52.5±1.1
F018	2.01±0.04	5.63±0.40	0.27±0.08	52.9±0.7
F019	3.10±0.14	5.17±0.26	0.51±0.06	95.9±0.9
P001	2.35±0.18	5.10±0.25	0.11±0.03	37.7±0.2
P002	2.45±0.12	5.10±0.20	0.07±0.03	64.3±0.8

F and P stand for Filament and powder saffron respectively

Table 2. Sulfuric test, artificial color and spectrum of saffron samples

Samples	Sulfuric test	Artificial color	Spectrum (λ max)
ISO 3632	*Pass*	*Absent*	*441*
F001	Pass	Absent	441
F002	Pass	Absent	443
F003	Pass	Absent	441
F004	Pass	Absent	441
F005	Pass	Absent	441
F006	Pass	Absent	441
F007	Pass	Absent	443
F008	Pass	Absent	442
F009	Pass	Absent	442
F010	Pass	Absent	442
F011	Pass	Absent	441
F012	Pass	Absent	443
F013	Pass	Absent	442
F014	Pass	Absent	442
F015	Pass	Absent	441
F016	Pass	Absent	442
F017	Pass	Absent	442
F018	Pass	Absent	440
F019	Pass	Absent	441
P001	Fail	Absent	420
P002	Fail	Absent	439

F and P stand for Filament and powder saffron respectively

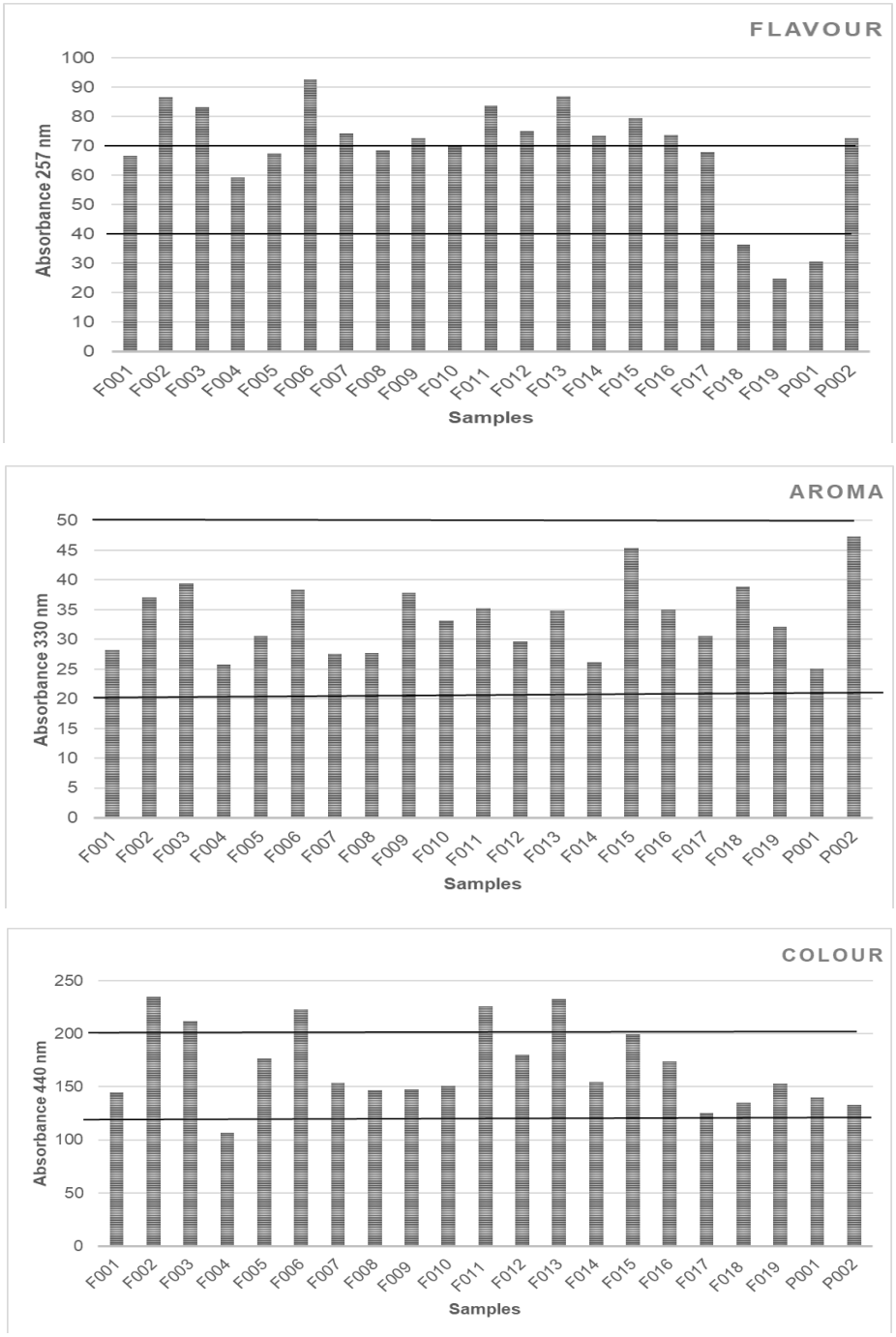

Fig. 1. The flavour, aroma and color values of saffron samples, the horizontal lines indicate the ISO standard limits, F and P stand for filament and powder saffron respectively

4. CONCLUSION

A total of 21 saffron samples purchased from different hyper-markets and local markets in Muscat, Oman, were examined to ISO quality standards. The saffron samples were in line with the standard in terms of moisture, ash, acid insoluble ash and extract % and in agreement with literature values. Two powder saffron samples P001 and P002 failed in sulfuric, spectrum and flavor tests. From the 19 samples of saffron filament, three samples F004, F018 and F019 were failed to achieve the flavor and color limits required by the standards. This failure to standard quality could be due to poor processing condition such as drying and packaging or due to adulteration. Due to the limited and unspecific parameters of the

standards, it could not distinguish between low quality and adulteration. Therefore, we recommend improving the standards with parameters targeting adulteration in saffron and regular quality monitoring of saffron products in particular the powder products.

ACKNOWLEDGEMENTS

The Central Lab, The Food Safety & Quality Center for providing the financial and material support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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https://www.sdiarticle5.com/review-history/96250