Appraisal of various physico-chemical characteristic and detection of synthetic color in saffron (Crocus sativus)

K.J. Patel* and V.R. Boghra

Department of Dairy Chemistry, S.M.C College of Dairy Science, Anand Agricultural University, Anand-388 001, Gujarat, India.

ABSTRACT

The saffron contained on an average 12.88 per cent moisture and volatile matter, 119.77 (E 1% \( \text{cm}^{-1} \)) coloring strength, 55.85 per cent (on dry matter basis) aqueous extract, 6.56 per cent floral waste, 2.47 per cent (on dry basis) nitrogen, 4.57 per cent total ash and 0.57 per cent acid insoluble ash, 4.63 per cent crude fibre and 4.83 per cent total oil content respectively. None of the sample of saffron exceeded the extraneous matter content of 1 per cent (maximum limit under PFA), so far analyzed. The extracted material from pure saffron in 80 per cent ethanol was subjected to thin layer chromatography resolved in 3 major distinct and 3 minor yellow orange spots. While adulterated samples gave more than 6 spots with different colors.

Key words: Characteristics of saffron, Crocus sativus, Saffron, Saffron adulteration, Synthetic colors in saffron, Thin layer chromatography.

INTRODUCTION

The word saffron is derived from the Arabic word ‘Za`faraan’. It is also known by alternate names as saffron , Crocus sativus (Scientific Name), Spanish Saffron, saffron (Amharic), kerkum (Armenian), zaffarano (Italian), keshar (Marathi), safuran (Japanese), kesari (Nepalese), acafro (Portuguese), shafran (Russian), azafrao (Spanish), zafaran (Swahili), saffran (Swedish, Norwegian), kesar / zaaffran (Hindi), (Sastri, 1953; Kirtikar et al., 1975; Kaul, 1997).

Saffron is the most expensive natural spice in the world. It is widely used for its aromatic, coloring and medicinal properties. The dried stigmas of saffron when steeped in water, lemon juice, milk and diluted ethyl alcohol, constituents responsible for coloring, flavoring and medicinal properties are dissolved imparting a delicate aroma, distinctive pleasant flavour and rich yellow tinge. Saffron is used in wide ranges of food products.

Its high value has made saffron the object of frequent adulteration and also being the object of intense chemical and biotechnological research (Francesco, 1990). The typical chemical composition and commercial characteristics of saffron are available from a sporadic and limited numbers of studies (Budhiraja, 1942; Ingram, 1969; Kaul, 1997; Alonso et al., 1998, Carmona et al., 2004). However, there is no information available on detailed physico-chemical characteristics of saffron form a large numbers of samples. Moreover, an uncertainly of production as a result of several factors has made saffron easily the costliest of non-contraband plant product (Singh et. al., 1997). Therefore, saffron also does not escape from adulteration.

*Corresponding author’s e-mail: kinpatel11@gmail.com
was then diluted with glass distilled water and volume was made to 500 ml, so as obtained a solution containing about 4 mg of saffron per 100 ml of solution. The absorbance of the final dilution was measured at 440 nm by UV-VIS spectrophotometer, in a 1 cm cell using distilled water as a reference. The specific extinction $E_{1\%}^{1cm}$ 440 nm was calculated as a function of the appropriate dilution obtained, and was reported as coloring power $E_{1\%}^{1cm}$. The total nitrogen content was determined by micro Kjeldahl method according to the procedure described by the BIS 1981. The volatile oil of saffron was measured by following the procedure as described in laboratory manual (Laboratory Manual, 2005).

In order to detect adulteration of natural and synthetic colors in saffron, three food colorants, namely Tartrazine, sunset yellow and carmoisine (Mfg. Asian food products, Ahmedabad) were procured from the local market. Annatto water soluble liquid color, norbixin was obtained from satellite Dairy (Amul cheese factory), Khatraj. All these synthetic colors are permitted food colors under PFA rules (2008) and have either red or yellow shades. The method described in ISO - 3632, 1993 was used for investigation of saffron pigments. Silica gel-G (Merck) plates were prepared by the method of Lees and De Muria (1962). The glass plates (21.5 x 21.5 cm) were thoroughly cleaned with chromic acid solution and dried. Slurry was prepared by mixing 8 g silica gel-G with 22 ml of water and shaking for about 2 min and used promptly. A plate was placed under the trough of an applicator with leading edge protruding about one inch beyond the gate. The slurry was poured into the trough to prepare single dimensional plate. Another glass plate was immediately pushed under gate of trough in a continuous movement. The coated plates were immediately place on a uniform surface for superficial drying. After about 30 min, the plates were transferred to an oven maintained at 5 °C, where the plates were dried overnight. Before use, the plates were activated at 110°C for 1 h.

The observation data of different parameters were subjected to statistical analysis by employing suitable statistical methods (Snedecor and Cochran, 1980).

**RESULTS AND DISCUSSION**

The average values and range for various physico-chemical characteristics of commercial samples of saffron are given in Table 1. The frequency distribution of Moisture and Volatile oil of saffrons are given in Table 2. Higher moisture content due to improper drying, spray of water on dried samples, substance (Syrups, honey, glucose, glycerin etc) added to increase the moisture content.

Coloring strength of saffron refers picrocrocine, safranal and crocin expressed as direct reading of the absorbance at specific wavelength for 1 per cent solution and with 1 cm cell.

The frequency distribution of coloring strength is shown in Table 3. Majority of the samples conformed the maximum limit of 80 set by PFA (2008). Carmona *et al.* (2004) reported a coloring power for saffron collected from different origin in Spain in the range of 25.36 to 258.23. Li *et al.* (1999) reported 29 mg/g of crocin in saffron where as Ortega *et al.* (2004) shown 45.99 mg/g of crocin from Iranian saffron. Sujata *et al.* (1992) analyzed Indian samples of saffron and isolated 67.3 mg/g of crocin. According to Sujata *et al.*(1992) there was a good correlation between the crocin results obtained from the two different methods. The extraction method of crocin according to ISO – 3632 (1993)

### Table 1: physico -chemical characteristic of saffron.

| Characteristics                        | Average | Range          |
|----------------------------------------|---------|----------------|
| Moisture and volatile matter per cent(m/m) | 12.88   | 7.63-17.74     |
| Coloring strength                      | 119.77  | 19.11-231.77   |
| Aqueous extract, per cent by wt        | 55.84   | 30.18-69.38    |
| Floral waste, per cent by wt           | 6.56    | 1.23-23.14     |
| Extraneous matter, per cent by wt      | >1      | ------         |
| Nitrogen, per cent by wt on DMB        | 2.47    | 1.64-3.43      |
| Total ash, per cent by wt              | 4.57    | 2.16-7.7       |
| Acid insoluble ash, per cent by wt     | 0.57    | 0.02-0.86      |
| Crude fibre                            | 4.63    | 2.19 – 8.00    |
| Total oil                              | 4.83    | 1.36 – 11.20   |
| Volatile oil                           | 0.42    | 0.26 – 0.73    |

### Table 2: Frequency distribution of moisture and volatile matter.

| Class Limit | Frequency | Per cent | Cumulative Frequency | Cumulative Percent |
|-------------|-----------|----------|----------------------|--------------------|
| 7.50-8.71   | 6         | 3.75     | 6                    | 3.75               |
| 8.71-9.92   | 19        | 11.88    | 25                   | 15.63              |
| 9.92-11.13  | 16        | 10.00    | 41                   | 25.63              |
| 11.13-12.34 | 22        | 13.75    | 63                   | 39.38              |
| 12.34-13.55 | 25        | 15.63    | 88                   | 55.00              |
| 13.55-14.76 | 28        | 17.50    | 116                  | 72.50              |
| 14.76-15.97 | 33        | 20.63    | 149                  | 93.13              |
| 15.97-17.18 | 9         | 5.63     | 158                  | 98.75              |
| 17.18-18.39 | 2         | 1.25     | 160                  | 100.0              |
was modified in order to improve the extraction procedure. In this modification instead of pure distilled water for extraction, mixture of distilled water and ethanol (50:50) was used. Crocin content of one of the saffron samples extracted using two different methods the value obtained 32.3 and 36.3 mg/g respectively when measured by UV-Visible Spectrophotometer.

The frequency distribution of saffron samples for aqueous extract is shown in Table 4. In this table many of the samples did not conform to the minimum limit to 55.00 per cent specified under PFA rules.

In Table 5 of frequency distribution of floral waste is shown most of the samples come under the grade 3 and grade 1 as per grading system of BIS and ISO (BIS., 1996; ISO., 1993) respectively.

Extraneous matter of saffron was not based on grade system as specified by BIS (1996) and ISO (1993). BIS grade system is stricter than ISO grade system. The limit for extraneous matter for saffron has not been specified under PFA rules (PFA, 2008). Carmona et al. (2004) reported a range from 0.00 to 1.31 for nine samples of saffron collected from different origin in Spain.

The frequency distribution of nitrogen content in 160 saffron samples has been shown in Table 6. Only 5 samples exceeded the maximum limit of 3.0 per cent. The saffron derives the nitrogenous compounds from soil as well as some additives used as adulterants.

was modified in order to improve the extraction procedure. In this modification instead of pure distilled water for extraction, mixture of distilled water and ethanol (50:50) was used. Crocin content of one of the saffron samples extracted using two different methods the value obtained 32.3 and 36.3 mg/g respectively when measured by UV-Visible Spectrophotometer.

The frequency distribution of saffron samples for aqueous extract is shown in Table 4. In this table many of the samples did not conform to the minimum limit to 55.00 per cent specified under PFA rules.

In Table 5 of frequency distribution of floral waste is shown most of the samples come under the grade 3 and grade 1 as per grading system of BIS and ISO (BIS., 1996; ISO., 1993) respectively.

Extraneous matter of saffron was not based on grade system as specified by BIS (1996) and ISO (1993). BIS grade system is stricter than ISO grade system. The limit for extraneous matter for saffron has not been specified under PFA rules (PFA, 2008). Carmona et al. (2004) reported a range from 0.00 to 1.31 for nine samples of saffron collected from different origin in Spain.

The frequency distribution of nitrogen content in 160 saffron samples has been shown in Table 6. Only 5 samples exceeded the maximum limit of 3.0 per cent. The saffron derives the nitrogenous compounds from soil as well as some additives used as adulterants.

was modified in order to improve the extraction procedure. In this modification instead of pure distilled water for extraction, mixture of distilled water and ethanol (50:50) was used. Crocin content of one of the saffron samples extracted using two different methods the value obtained 32.3 and 36.3 mg/g respectively when measured by UV-Visible Spectrophotometer.

The frequency distribution of saffron samples for aqueous extract is shown in Table 4. In this table many of the samples did not conform to the minimum limit to 55.00 per cent specified under PFA rules.

In Table 5 of frequency distribution of floral waste is shown most of the samples come under the grade 3 and grade 1 as per grading system of BIS and ISO (BIS., 1996; ISO., 1993) respectively.

Extraneous matter of saffron was not based on grade system as specified by BIS (1996) and ISO (1993). BIS grade system is stricter than ISO grade system. The limit for extraneous matter for saffron has not been specified under PFA rules (PFA, 2008). Carmona et al. (2004) reported a range from 0.00 to 1.31 for nine samples of saffron collected from different origin in Spain.

The frequency distribution of nitrogen content in 160 saffron samples has been shown in Table 6. Only 5 samples exceeded the maximum limit of 3.0 per cent. The saffron derives the nitrogenous compounds from soil as well as some additives used as adulterants.

was modified in order to improve the extraction procedure. In this modification instead of pure distilled water for extraction, mixture of distilled water and ethanol (50:50) was used. Crocin content of one of the saffron samples extracted using two different methods the value obtained 32.3 and 36.3 mg/g respectively when measured by UV-Visible Spectrophotometer.

The frequency distribution of saffron samples for aqueous extract is shown in Table 4. In this table many of the samples did not conform to the minimum limit to 55.00 per cent specified under PFA rules.

In Table 5 of frequency distribution of floral waste is shown most of the samples come under the grade 3 and grade 1 as per grading system of BIS and ISO (BIS., 1996; ISO., 1993) respectively.

Extraneous matter of saffron was not based on grade system as specified by BIS (1996) and ISO (1993). BIS grade system is stricter than ISO grade system. The limit for extraneous matter for saffron has not been specified under PFA rules (PFA, 2008). Carmona et al. (2004) reported a range from 0.00 to 1.31 for nine samples of saffron collected from different origin in Spain.

The frequency distribution of nitrogen content in 160 saffron samples has been shown in Table 6. Only 5 samples exceeded the maximum limit of 3.0 per cent. The saffron derives the nitrogenous compounds from soil as well as some additives used as adulterants.
The ash content of saffron in percent study compared well with the range 4.0 – 13.30 per cent in commercial saffron (Budhiraja, 1942; Ingram, 1969; carmona et al., 2004. The frequency distributions of samples of saffron for total ash are shown in Table 7. The highest percent of samples were in the range of 4.78-5.45 percent. The maximum limit established by PFA and BIS is 8 per cent, a value far from the average of 4.57.

It was also in consonance with the TLC pattern of the respective samples run. The ash resulted in slight grayish color with adulteration as was also evidenced by TLC pattern were there were more than six spots of pink or reddish color. Alonso et al. (1998) reported that many substances like barium sulfate, calcium carbonate, gypsum, potassium hydroxide, salt peter, Glauber’s salt, borax etc are added to increase the weight. As there substances are inorganic, they tend to increase the ash content. Parameter of acid insoluble ash are within the range of 0.14 to 1.50 as reported by Carmona et al. (2004). The frequency distribution data of acid insoluble ash (Table 8) showed that 140 out of 160 samples were in the limit of 0.42 to 0.82 per cent.

Other characteristics of saffron like crude fibre, total oil and volatile oil were also measured, but with limited number of samples (10) because of availability of sample and being costly. Analysis of crude fibre was observed that some samples also exceeded the maximum limit of 6.0 per cent as set under ISO (1993), BIS (1996) and PFA (2008). The addition of fruits, plants and grass fibers might be the probable adulterants like substances to increase the fiber content of pure saffron to some extent. The total oil content of saffron ranged between 1.36 and 11.20 per cent with an average value of 4.83 per cent which is given in Table 1. Mehta et al. (2002) reported in their review paper, a range of 5.0 to 9.0 per cent in commercial samples of saffron, while as high as 13.4 per cent was reported by Pruthi, (1976). The parameter of volatile oil analysis of saffron is boiled under reflux conditions with water where the oil separates on top of water and can be read off in volume proportional to the mass of the product under test. Safranal is the main component of the essential oil and is responsible for the characteristic saffron aroma. The concentration of safranal in saffron depends strongly on both the drying and storage conditions. The average value and range of volatile oil was given in Table 1. The values of essential in the present study were in accordance with the values (0.3 – 0.8 per cent) as reported by Mehta et al. (2002). However Pruthi (1976) has reported the yield of essential oil as high as 1.37 per cent.

Saffron is a very expensive spice used mainly as a herbal medicine and as a food coloring in various parts in the world because of its high price limited production and increased consumption, saffron is fiercely the subject of adulteration usually by adding other plant materials dyed with artificial colorants to increase its weight and yellow red colors. More recently, these has been adulteration with florets of an Onopordon species that is artificially dyed with tartrazine yellow, erythrosine, sunset yellow, carmoisine, ponceau 4R (the commercially avoidable synthetic colors) and weighted with borax, glycine and salt peter with the aim of misleading the uses for improving the appearance of the dried stigma or even other extraneous materials to give rise to coloring strength of the aqueous extract Carmona et al. (2004).

It was observed that most of the samples received from different sources were admixed with both, the synthetic color and weight enhancers, mainly mineral compound. The most preferable and admissible synthetic colors were tartrazine yellow, erythrosine, sunset yellow, carmoisine, ponceau 4R and sometimes annatto water soluble liquid, norbixin. The details of pattern of thin layer chromatogram developed by following procedure was given in ISO – 3632, 1993.

The extracted material from saffron as said above method in 80 per cent ethanol was subjected to thin layer chromatography on silica gel G plate. The chromatogram of pure saffron was developed in butanol: acetic acid: water (4:1:1, V/V) showed the presence of six yellow orange spots which shown in Plate 1. There were three major spots of much darker color while three were of very faint yellow orange shade. The first spot was significant spot among three principal spots. There was no presence of other spot(s) of different color not corresponding to saffron pigment. The RF values (distance travelled by solute/ distance travelled by solvent) of these spots were not exactly same but more or less of same value as given in TLC pattern for saffron by given ISO method. The RI values of different spots of pure

| Name of colorants | No. of spots | Color of spots | RF values |
|-------------------|-------------|----------------|-----------|
| Tartrazine        | 1           | Yellow         | 0.24 – 0.28|
|                   | 2           | Orange - yellow| 0.47 - 0.50|
| Sunset yellow     | 1           | Yellow         | 0.24 – 0.28|
|                   | 2           | Orange-yellow  | 0.20 – 0.24|
| Carmoisine        | 1           | Orange - yellow| 0.49 – 0.52|
|                   | 3           | Pink           | 0.59 – 0.63|
| Norbixin          | 1           | Orange - yellow| 0.96       |

Table 9: Thin layer chromatographic pattern of pure saffron.
saffron appeared on plate was of the following order as given in a Table 9.

Three synthetic colorants (Tartrazine, sunset yellow, carmoisine) and one natural water soluble Annatto color were selected based on wide use as adulterant in saffron. The colorants were dissolved in a little quantity of water to affect its perfect dissolution followed by ethanol maceration for 2 h before their application of thin layer chromatography. About 1µl of dye solution was applied on thin layer, in strips as well as in round spots the chromatogram was developed following the same procedure under identical conditions as for pure saffron of plate 2. Different color spots were appeared and Rf values were calculated and shown in Table 10.

In this study, the pure saffron samples were adulterated three different synthetic colorants and norbixin with different concentration like 1, 2 and 4 per cent has been shown in Plate 3, 4 and 5 respectively. It can be seen that pure saffron developed as usual six spots of yellow orange color. The adulterated samples showed additional spots either interacted (mingled) or separate spot(s). Plate 6
has been shown TLC pattern of saffron which were received from eight different dairy plants. The Plate 7 has been shownto the pure saffron sample with subjected to TLC pattern, the result indicated that it resolved into one yellow and two pink spots only. Six yellow spots as per ISO normative were totally missing though it was saffron in filaments with its all look as pure saffron.

CONCLUSION

It is difficult to judge the purity of saffron by either look or by non-specific ways and means tried in the commerce trade. The results of this study and available data on physico-chemical characteristics are concluded that the authenticity or purity could only be established by specific tests under standard condition as laid down by regulatory authorities.

REFERENCES

Alonso GL, Salinas MR, and Saez JR. (1998) Crocin as coloring in the food industry. Recent Research Developments in Agric. and Fd. Chem. 2: 141-154.

BIS 1996. IS: 5453 (Part I and II). Specification for saffron and methods of analysis.

Budhiraja KL (1942) Kashmir saffron with methods for testing its purity. J. Indian Chem. Soc. Educ. 5: 135.

Bureau of Indian Standards, New Delhi.

Carmona M, Carrion ME, Zalacain A and Alonso GL (2004) Detection of adulterated saffron through UV-Vis spectral analysis. J. Fd. Sci. Tech. 41: 451-455.

Francesco, L and Di-Francesco, L. (1990). Saffron. [Edizioni L’ Informatore AgrarioVerona, Italy]. p. 38.

Ingram JS (1996) Saffron (crocus sativus L.) Tropical sci. 2: 227-232.

ISO, 1993. ISO 3632-1, Saffron- Specifications, second revision. International Organization of Standardization. Geneva, Switzerland.

Kaul MK (1997) In: Medicinal plants of Kashmir and Ladakh Temperate and Cold Arid Himalaya. Indus Publishing Co., New Delhi. 112.

Kirtikar KR, Basu BD and ICS (1975) Crocus sativus. In: Indian Medicinal Plants, 2nd ed. MIS Bishen Singh Mahendra Pal Singh, Dehra Dun. 4: 2462-2463.

Laboratory Manual (2005). Methods of Analysis of Foods (Spices and Condiments). Directorate General of Health Services. Ministry of Health and Family Welfare. Government of India, New Delhi. pp-14.

Li N, Lin G, Kwan YW, and Min ZD (1999) Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography. J. Chromatography A. 849: 349–355.

Mehta BM, Borkhatriya VN, Boghra VR (2002) Saffron- The coloring and flavouring agent in dairy and food industry. J. Medicinal and aromatic plant Sci. 24: 1038-1049.

Ortega H, Pereda-Miranda R, Rivero’n-Negrete, L Hernández JM, Mede’cigo-Ryós M, Castillo-Villanueva A (2004) Chemical composition of saffron (Crocus sativus L.) from four countries. Acta Horticulturae. 650: 321–326.

Pruthi JS (1976) Spices and condiments. National Book trust, New Delhi, India. P.221-226.

Sastri BN (1953) In: The Wealth of India, Industrial products, part III: D-E. Council of Scientific and Industrial Research, New Delhi. p 67.

Singh R, Kumar K, and Sagwal RC (1997) Research gaps in scientific cultivation of saffron (Crocus sativus L.) in Kashmir. Agric. Sci. Digest. 17: 161-164.

Snedecor GW and Cochran WG (1980) Statistical Methods. 6th edition. Oxford and IBH Publishing Co. Pvt., New Delhi.

Sujata, V.; Ravishankar, G. A. and Venkataraman, L. V. (1992). Methods for the analysis of the saffron metabolites crocin, crocetins, picrocrocin and safranal for the determination of the quality of the spice using thin-layer chromatography, high-performance liquid chromatography and gas chromatography. J. Chromatography, 624: 497-502.