



Composition of Saffron By-products (*Crocus sativus*) in Relation to Utilization as Animal Feed

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ABSTRACT

Background: In the region of Taliouine-Taznakhte, the saffron culture constitutes the pivot of the agriculture. Nevertheless, a huge amount of saffron by-products with little or no commercial value are wasted during the processing of the stigmas. To increase the overall profitability of this crop, these by-products have been investigated as a potential source of nutrition.

Methods: The different parts of *Crocus sativus* were analyzed. The leaves have high crude fibers (19, 31%), proteins (7, 24%), lipids (6, 10%), N (1, 15%), Fe (985, 59 mg/kg). The petals are the flower parts with the highest contents of crude fiber (11, 25%), ash (7, 30%), protein (6, 35%) and total carbohydrates (71, 16%). Corms have high total carbohydrates (92,41%). The fatty acids in cyclohexane extract oils from leaves were palmitic acid (21.68%) and linolenic acid (25.09%) while in the petals, palmitic acid (11.64%) and linoleic acid (22.60%).

Result: From the result obtained, it is suggested that some of the by-products of Moroccan saffron could be utilized by ruminants as feed supplement during both wet and dry seasons.

Key words: Animal feed, Bio-residues, Moroccan saffron, Valorization.

INTRODUCTION

Crocus sativus L. is an autumn-flowering geophyte extensively grown in the Mediterranean basin and near east since the Late Bronze Age (Negbi, 1999). Saffron is the most representative species of the genus *Crocus*, which consists of 88 species distributed in Central and Southern Europe, North Africa and from Southwest Asia to Western China (Petersen *et al.*, 2008). It is a triploid sterile plant species belonging to the subfamily *Crocoideae*, the largest of the four subfamilies currently recognized in the Iridaceae family (Goldblatt *et al.*, 2006). The other parts of the plant or by-products (waste) generated during the harvesting of the stigmas (saffron spice) have been much less studied (Zheng *et al.*, 2011). Nevertheless, a huge amount of saffron by-products with little or no commercial value are wasted during the processing of the stigmas. Thus, around 350 kg of tepals, 1500 kg of leaves and hundreds of corms which due to their low size cannot be reground or commercialized are generated to obtain just 1 kg of this so-called "red gold" (Jadouali *et al.*, 2019). This renders the crop very little profitable in terms of biomass and makes desirable for search of new uses of such by-products for a more sustainable management of the crop (Sánchez-Vioque *et al.*, 2012).

The present study is a continuation of previous investigations in which extracts of saffron corm, petal and leaf, showed remarkable content polyphenols (Sánchez-Vioque *et al.*, 2012). Stamens are the flower parts with the highest contents of ash, protein, lipids, total and insoluble. Flowers and saffron bio-residues show high mineral contribution, especially in iron (Jessica Serrano-Díaz *et al.*, 2013). However, the phytochemical content of sepals

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(Vignolini *et al.*, 2008), petals (Montoro *et al.*, 2008) and flowers (Nørbæk *et al.*, 2002) of *C. sativus* showed their wealth in flavonoids and anthocyanins. Although saffron leaves have not received much attention as a source of bioactive components, the presence of a number of phenolic compounds that could be used as natural antioxidants has been described (Sánchez-Vioque *et al.*, 2012; Jadouali *et al.*, 2018). The young bulbs are eaten today in Tibet and

Kashmir, as radishes. The bulbs were consumed by the animals, but also by the men during periods of famine. They were crushed and added to the bread in the region of Caussade (Bergoin 2005).

The purpose of this study was to characterize by-products of Moroccan saffron regarding ash, proteins, lipids, crude fibers, total carbohydrates, minerals and fatty acids, in order to enhance their value, by emphasizing their use in the feeding of domestic animals and the good management of saffron bio-residues.

MATERIALS AND METHODS

Our study focuses only on the bios residues of saffron namely corms, leaves, petals, stamens, styles and pruned flowers (without stigma) (Fig 1).

The Taliouine Taznakhte region includes almost all of the saffron production in Morocco. The different parts of *Crocus sativus* were obtained from "DAR ZAAFARANE" at the 2019 production. The different analyzes were carried out in the national institute of agronomic research in Agadir-Morocco.

Preparation of the saffron by-products extract

Twenty grams of dried and ground corms, leaves, petals and whole flowers were transferred into a flask containing 150 mL of the solvents (hexane and cyclohexane) used for the extraction. Extraction was performed with a soxhlet for 8 h. Then it was removed under vacuum using a rotary evaporator. The samples were kept at a temperature of 40°C until the total elimination of hexane traces and then the resulting oil was stored at 4°C until it was analyzed. all the analyzes were carried out in Natural Resources and Ground Products Research Unit, Agrophysiology Laboratory, B.P. 124, Inezgane, Morocco.

Proximate composition

The moisture of freeze-dried samples and their ash content by incineration at 550°C were calculated according to ISO 3632 (2011). Protein content was obtained from the total N

content by multiplying by 6.25, as estimated by the macro-Kjeldahl method according to AOAC (1995). Lipid content, available carbohydrates and crude fiber, together with energy were also determined using the methods reported by AOAC (1995). The total sugar content was determined by the phenol-sulfuric acid method described by Dubois *et al.* (1956). The extraction of carbohydrate was made by the 80% ethanol according to the following protocol: For each sample, 200mg of sample from each concentration were homogenized in 5 ml of 80% ethanol.. Centrifuge the mixture at 5000 rpm for 10 min, Put 0, 1 ml of the supernatant in the test tube, add 1ml of 5% phenol quickly and add 5ml of concentrated H₂SO₄. Place the tubes immediately to a boiling water bath for 20 min, cool in the dark for 20 min and measure the optical density at 495 nm using UV-Visible spectrophotometer.

Caloric content

Estimates of total calories were calculated on the basis of 100 g portion using the Atwater factors for protein (4.0 kcal/g), fat (9.0 kcal/g) and carbohydrates (4.0 kcal/ g).

Mineral composition

The dried and ground petals, stamens, styles and whole flowers were ground separately in a mill to pass through a 20-mesh screen, then 0.5 g of the dried plant tissues were analyzed. Nitrogen concentration in the plant tissues was determined after mineralization with sulfuric acid by "Kjeldahl method" (Bremner, 1965), K, Ca and Na concentrations were determined by dry ashing at 400°C for 24 h, dissolving the ash in 1:25 HCl and assaying the solution obtained using a flame photometer BWB-XP. The elements, including iron and zinc, were analysed by atomic absorption spectrometry in a flame air-acetylene. The measured absorption was done at a specific wavelength of 248.3 nm.

Fatty acid analysis

Fatty acid composition was determined using the International Standard Organisation method (ISO 5508, 1990). Fatty acids were converted to fatty acid methyl esters before analysis by shaking a solution of 60 mg oil and 3 ml of hexane with 0.3 ml of 2 N methanolic potassium hydroxide. They were analyzed by gas chromatograph (Varian CP-3800, Varian Inc.) equipped with an FID. The column used was a CP- Wax 52CB column (30 m×0.25 mm i.d.; Varian Inc., Middelburg, The Netherlands). The carrier gas was helium and the total gas flow rate was 1 ml/min. Steps of 4°C/min increased the initial column temperature was 170°C, the final temperature 230°C and the temperature. The injector and detector temperature were 230°C. Data were processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA). The results were expressed as the relative percentage of each individual fatty acid (FA) present in the sample.

Statistical analysis

Values reported in tables and figures are the means ± SE of two replications. The significance level was set at P≤0.05.

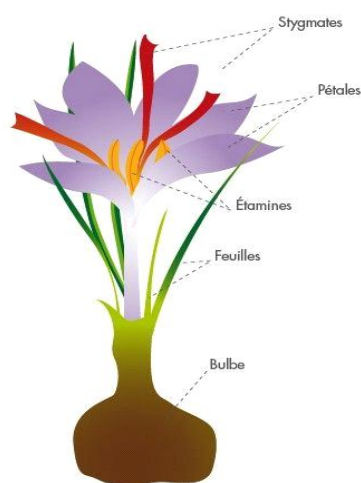


Fig 1: Morphological characteristics of saffron.

(<https://www.safrandemurols.com/le-safran-the-saffron-p184082.html>).

Separation of means was performed by Tukey's test at the 0.05 significance level using Minitab 17 software.

RESULTS AND DISCUSSION

Proximate composition and caloric value

Proximate compositions of different parts of Moroccan *Crocus sativus* L. are reported in Table 1. The lower moisture content was observed in leaves, the extract of leaves of *Crocus sativus* had a higher concentration of crude fiber content than the other part of *Crocus sativus*. Fiber has received a great deal of research attention among animal scientists because of its importance to the ruminant. In the ruminant, it represents the plant cell wall that is utilized as an energy source by the rumen microflora and is extensively degraded. Crude fiber helps in the maintenance of normal peristaltic movement of the intestinal tract hence diets containing low fiber could cause constipation and eventually lead to colon diseases (piles, cancer and appendicitis) (Fahim *et al.*, 2012). Fiber also plays an important role in ruminant digestion by increasing bacterial populations in the rumen. The value of crude fibre in this study is higher compared to those obtained by Zanin (2009) and Bertin (2008) for alfalfa leaves and lower than jack bean leaves fresh 34,3% (Sharazia 2017) and bamboo leaves (Jafari *et al.*, 2017). According to Mateos-Aparicio *et al.* (2012) the broad bean pod and peas pod contains total fiber content of the order of 3.37% and 4.72% of dry matter respectively. Years of investigation and cuts had a high statistical influence on the crude protein content in leaves but did not influence crude fiber content in leaves (Popovic *et al.* 2001). The crude fiber content was higher than those reported by Khoshbakht (2012) in the petals

C. sativus. Ash represents the mineral level in a feed (Verma, 2006). The ash content in the stamens and the whole flower is higher with 13.45% and 10.95 % respectively. The value obtained for the stamens, styles and whole flowers by Jessica Serrano-Diaz *et al.* (2013) are respectively 11.43; 8.33 and 7.39 g per 100 g on a dry weight. In this study the leaves have 5.63%, on the other hand, Bergoin (2005) has shown that saffron leaves have an ash content of 10,2%. Ash content of saffron petals reported by Khoshbakht (2012) was 7%. The protein content is higher in the leaves and petals with 7, 24% and 6.35% respectively. Protein also plays a part in the organoleptic properties of foods in addition to being a source of amino acid. Petals of *Rosa micrantha* used to make jam or tea also contained lower content (Guimaraes *et al.* 2010) than the petals of the flowers of saffron. Bergoin (2005) showed that the June bulb of quercy contains a protein content of 3.57%. Protein is considered as basic feed component and knowledge of the kinetics of ruminal degradation of feed proteins is fundamental to formulating diets for adequate amounts of rumen degradable protein for rumen microorganisms and for the host animal (Koukolová *et al.*, 2017). Leaves followed by whole flowers showed significantly higher lipid contents than the other parts of *Crocus sativus*. while petals, stamens and whole flowers had the lowest levels of lipids and total carbohydrates.

The saffron corms contain a higher Zn, Na and Ca content than that determined by Egbebi (2016) in white fonio flour which are 1,6 mg/100g; 1,65mg/100g and 0,83 mg/100g.

Determination of mineral elements

The mean values of the mineral contents of by-products of saffron are presented in Table 2. We observed significant

Table 1: Proximate compositions of different parts of Moroccan *crocus sativus* L. Values (%) are reported on dry matter basis.

	Petals	Stamens	Styles	Wholes flowers	corms	Leaves
Moisture %	92 ^a ±1.11	89.5 ^a ±1.5	91 ^a ±1.3	87.3 ^a ±1.02	67 ^b ±1,9	66 ^b ±0,9
Crude Fiber %	11,2 ^c ±0,97	13,3 ^b ±0,7	7,93 ^d ±0,54	10,4 ^c ±0,66	4,2 ^e ±0,31	19,3 ^a ±1,19
Ash %	7.30 ^b ±0.3	13.4 ^a ±1.06	7.86 ^b ±0.5	10.9 ^a ±0.95	5,28 ^e ±0,07	5,63 ^c ±0,27
Protein %	6.35 ^b ±0.07	5.97 ^c ±0.09	6.11 ^b ±0.42	4.3 ^d ±0.14	2,58 ^e ±0,3	7,24 ^a ±0,08
Lipid %	0.03 ^b ±0	0.03 ^b ±0	0.01 ^c ±0	0.035 ^b ±0	0,03 ^b ±0	6,10 ^a ±0
T. carb ^z %	71.1±1.4	62±0.07	69.7±0.7	67±1	92,4±0,1	80,1±0,1
Caloric value kcal/g	310±1.6	272±2.1	303±1	285±1.54	377±3.1	404±4.05

All values are mean ± standard deviation of three replicates. Different letters within a row indicate significant statistical differences (p < 0.05).^z Total carbohydrates.

Table 2: The mineral content of different parts of Moroccan *Crocus sativus* L.

	Petals	Stamens	Styles	Whole flowers	Corms	Leaves
Na (ppm)	45.8 ^a ±5.44	53.3 ^a ±4.59	54.1 ^a ±2.40	45.8 ^a ±5.44	45,2 ^a ±3,74	55,4 ^a ±5,65
K (ppm)	23.7 ^a ±2.75	26.3 ^a ±1.34	23.6 ^a ±0.91	23.7 ^a ±2.75	17,9 ^a ±0,88	20,1 ^b ±0,74
Ca (ppm)	39.2 ^a ±1.06	14.8 ^b ±1.13	42.9 ^a ±2.26	39.3 ^a ±0.98	12,8 ^b ±1,06	13,8 ^b ±0,21
N %	1.01 ^b ±0.08	0.95 ^b ±0.01	0.38 ^d ±0.01	0.7 ^c ±0.02	0,41±0,01	1,15 ^a ±0,01
Fe (ppm)	149 ^a ±6,2	94,7 ^b ±4,13	-	727 ^d ±6,4	228 ^c ±8,8	985 ^e ±8
Zn (ppm)	47,2 ^b ±5,04	49,9 ^b ±4,3	-	42,6 ^b ±4,4	236 ^c ±8	20,5 ^a ±3

All values are mean ± standard deviation of three replicates. Different letters within a column indicate significant statistical differences (p < 0.05).

differences ($p < 0.05$) in the concentrations of Na, Ca, K, N, Fe and Zn in the different parts of Moroccan *Crocus sativus* L. The most abundant minerals in the leaves were Fe and Na with values from 985,8 ppm and 55,4 ppm. The flower parts with the highest Ca and Na contents were the styles. The petals have high Fe compared to those of flower parts of *Crocus sativus* L. and corms have high Zn content. Petals and leaves were the part with the highest N content. The tissue chemistry of by-products has been reported to show considerable variation in mineral composition which may be attributed to the age and the fertility of the aqueous environment (Banerjee and Matai, 1990).

The presence of a high content of Ca in the style and the whole flower is due to the role played by this mineral element in the sexed reproduction of the plants, because Ca plays a role in the germination of the pollen tube (Jessica Serrano-Díaz *et al* 2013; Grilli Caiola 1999). Ca is responsible for bone formation, Ca regulates many cellular processes and has important structural roles in living organisms (Berivan Tandoúan, 2005). The calcium content of cereal straw (3g / kg of dry matter) (National committee of French coproducts) which is very large compared to that obtained in saffron by-products. According to Sherazia (2017) pea straw and chickpea straw contain Ca content of 2.37% and 1.36% in dry matter respectively. The saffron corms contain a higher Zn, Na and Ca content than that determined by Egbebi (2016) in white fonio flour which are 1,6 mg/100g; 1,65 mg/100g and 0,83 mg/100g. The whole flowers, bulbs and leaves can be a very important source of Fe.

Fatty acid

Two polarities crude extracts of saffron by-products used for the determination of fatty acids content were presented in Fig 2 and Fig 3.

The mean values of the fatty acid contents in hexane extract of saffron by-products are presented in Fig 2. The main fatty acids found in the corms in relatively high

concentrations were C16, C18:1 and C18:2, with mean values of 16,47%; 22, 21% and 40,83%, respectively.

These results were similar to those reported for corms samples from France (Bergoin 2005). The most abundant fatty acids in leaves saffron were linoleic (C18:2), linolenic (C18:3) and palmitic acid (C16:0) with mean values of 20,64%; 30,72%; 20,06%, respectively. The major fatty acids in saffron petals were palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). The mean value concentrations of palmitic acid, oleic acid, linoleic acid and linolenic acid were $20,06 \pm 1,07$; $9,15 \pm 0,04$; $18,20 \pm 0,09$ and $13,88 \pm 0,49$ %, respectively. Other fatty acids in lower scale are shown in Fig 2.

These results were similar to those reported for petal samples from Iran (Faizy and Reyhani 2016). The main fatty acids found in whole flowers (flower without stigmas) were palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and gadoleic acid (C20:1), with mean values of 13, 88%; 8,08%; 15,59%; 13,05% and 5,26%, respectively. Study carried by Arapcheska (2014) investigated the fatty acid composition of saffron (*Crocus sativus* L.) from different origins. In their studies three different saffrons from Hungary, Spain and Greek were studied. The results showed that the Hungarian saffron sample contained palmitic acid (C16:0) 37.101%; stearic acid (C18:0) 9.939%; linoleic acid (C18:2) 24.966% and linolenic acid (C18:3) 14.209%. Fatty acids composition of the Spanish saffron samples was pentadecanoic acid (C15:0) 6.094%; palmitic acid (C16:0) 21.434%; oleic acid (C18:1) 10.135%; linoleic acid (C18:2) 52.684% and linolenic acid (C18:3) 7.971%. The most abundant fatty acids in the Greek saffron samples was linoleic acid (C18:2) 40.104%; palmitic acid (C16:0) 33.910%, oleic acid (C18:1) 10.397%; linolenic acid (C18:3) 10.206% and lauric acid (C12:0) 4.229%. If we compare this result with the fatty acid composition of some forage like fresh alfalfa that contains a palmitic acid 23.2%; linoleic acid 19.9% and linolenic acid 41.9%. The main fatty acids found in White clover, fresh

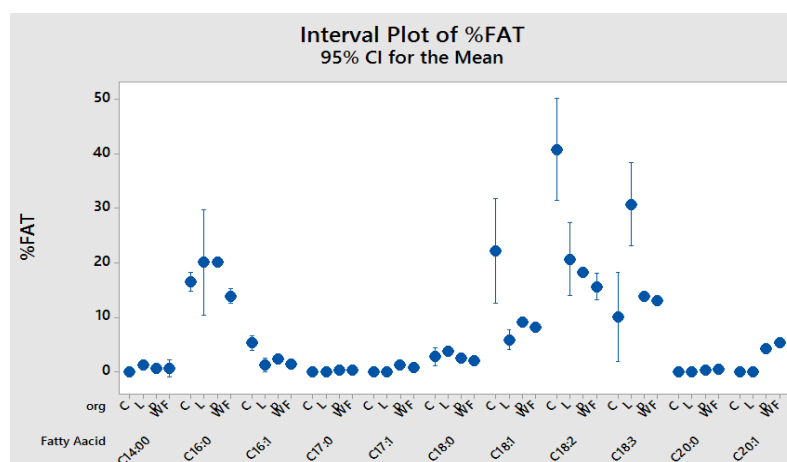


Fig 2: Fatty acid of the hexanic extract for different saffron by-products.

C: Corm; L: Leaf; P: Petals; WF: Whole flower.

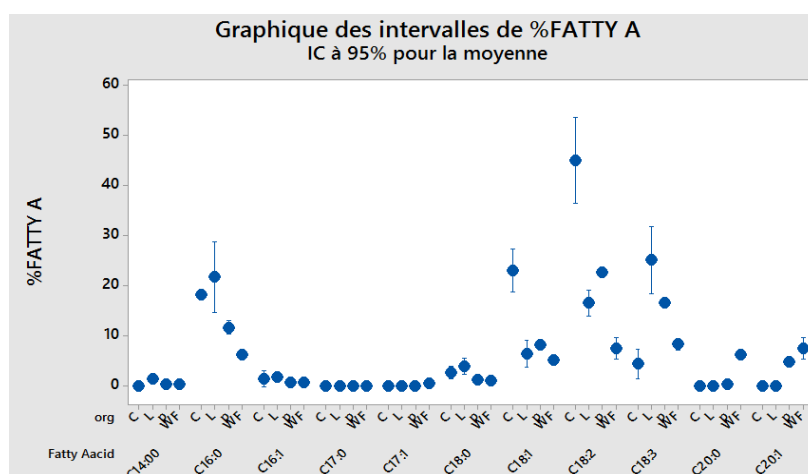


Fig 3: Fatty acid of the cyclohexanic extract for different saffron by-products.

C: Corm; L: Leave; P: Petals; WF: Whole flower.

were palmitic acid 15,3%; linoleic acid 16,5; and linolenic acid 58% (Glasser *et al* 2013).

The results of this investigation showed that all parts of saffron plant have a low content of myristic acid (C14:0), palmitoleic acid (C16:1), margaric acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), arachidic acid (C20:0) and galeoic acid (C20:1). Indeed, the leaves are the part of saffron plant with the highest content of linolenic acid (30,72%). Corms had high oleic acid (22,21%) and linoleic acid (40,83%) content.

The averages and variations in fatty acid contents of the studied saffron by-products in cyclohexanic extract are presented in (Fig 3). Were the first studies on the fatty acid composition of the by-products of the saffron of Taliouine "Morocco". The results for fatty acids are expressed in percentages (%). The majority fatty acids found in the corm are palmitic acid (C16:0), oleic acid (C18:1) and linolenic acid (C18:2), with mean values of 18,21%; 23,01% and 45,01%, respectively. The same results are found in hexane extracts with extracting yield and higher in cyclohexane extract; in the leaves were palmitic acid (21,68%), linoleic acid (16,54%) and linolenic acid (25,09%). for petals were palmitic acid (11,64%), linoleic acid (22,60%) and linolenic acid (16,63%). the minimum content of fatty acids was found in whole flowers. A study is carried by Bergoin (2005) investigated fatty acid composition of corms saffron. The obtained results showed that corm contained palmitic acid (22,2%), linoleic acid (36,0%) and oleic acid (21,3%). Other fatty acid in lower scale are shown in (Fig 2).

However, fatty acid compositions may be influenced not only by the part of the plant, but also by regional, climate, degree of ripeness, harvesting and processing conditions (Dong-sun *et al* 1998). The content of palmitic acid is very high in the bulbs and leaves when compared with the results obtained by Dong-sun *et al.* (1998) in sesame, soybean and corn germ. Moreover, the corms and the sesame represent the same results in linoleic acid; more corms and sesame

represent the same results in linoleic acid and more than that in perilla, rapeseed and coconut (Zambiasi *et al* 2007; Lee *et al.*, 1998). while green forages contain 10 to 30 g/kg DM of total fatty acids (AG) composed of 35 to 70% linolenic acid (C18: 3). Many factors influence the forage content and composition of fodder (Dewhurst *et al.*, 2006 ; Khan *et al.*, 2012; Glasser *et al.*, 2013): the botanical family, the plant species, the vegetative stage, the mode of conservation and the nitrogen fertilization.

The result of fatty acid of the cyclohexanic extract for different saffron by-products is presented in the (Fig 3). whole flowers oil and petal oil was the only two that showed significant amounts of long chain unsaturated fatty acids, over 20 carbon atoms, attempting to 7,53% and 4,83%, respectively. The leaves and petals were the richest on in fatty acid linolenic with 25,09% and 16,63%. The average linolenic acid content was higher in the corms in comparison with others part of the plant. The main fatty acids found in the petals in relatively high concentrations were palmitic acid, linoleic acid and linolenic acid, with mean values of 11,64%; 22,60% and 16,63%, respectively. But the results obtained with Javad faizy and Nastaran Rarhani showed that petals contained, palmitic acid 16,21%; linoleic acid 28,48% and linolenic acid 21,06%, respectively.

CONCLUSION

The by-products of Moroccan *crocus sativus* proved to be useful as ruminant diet. The relatively high crude protein, crude fiber, total carbohydrate and mineral elements (Fe, Ca, N, Mg and Na) appeared satisfactory in animal production since they exceeded the minimum requirement for ruminants. Further study, on other aspects of feed quality such as palatability and digestibility experiments on these by-products should be initiated. Owing to the acute shortage of fodder in Taliouine. This type of work enables farmers to improve and diversify their sources of income so that the saffron market is more profitable for local households.

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