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## Saffron Crop (Golden Crop) in Modern Sustainable Agricultural Systems

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Abstract: Saffron (Crocus sativus L.) is an autumnal flowering plant valued for dried stigmas are well known for their aromatic and colouring power. It possesses a number of medicinally important activities and helpful in controlling various diseases. Saffron has a reverse biological cycle compared with the majority of cultivated plants: flowering first in October-November, then vegetative development until May, which means that the vegetative development is not directly important for the production of stigmas, but for the production of new corms. Due to its unique biological, physiological and agronomic traits, saffron is able to exploit marginal land and to be included in low-input cropping systems, representing an important and alternative viable crop for sustainable agriculture. The main biological, genetic, ecological and quality traits associated with agronomic management techniques of saffron in relation to environmental conditions have been described in this paper. The essential features on which the quality of saffron depends are colour, taste and aroma of the stigma. The quality of saffron, mainly from a chemical point of view, will be discussed.

Keywords: Saffron, chemical constituents, yields, quality, sustainability.

#### I. INTRODUCTION

The word saffron originated from the 12th century old French term Safran, which was derived from the Latin word safranum. It is also related to the Italian Zafferano and Spanish Azafran [1]. Safranum comes from the Arabic word as far which means "yellow" [2]. Saffron (Crocus sativus L.) belongs to the family of Iridaceae and to the genus Crocus, which includes about 80 species primarily distributed in the Mediterranean and south-western Asia (Fig. 1). Saffron recognised as the most expensive and most interesting and attractive species because of colouring, bitterness and aromatic power of its dried stigmas in the world [3];[4].



Fig . Saffron (Crocus sativus L.)

Saffron is a geophyte herbaceous plant, whose stigmas have been used from ancient times as a spice in food, as a dye, in perfumes and cosmetics preparation and for medicinal purposes [5]. Saffron does not propagate by seeds. The underground portion of the saffron called corms (also called bulbs), divide to produce new plants. The flowers of the saffron emerge in Autumn-November. The outstanding feature of the saffron flower is its three stigmas which are almost 25-30 mm long and droop over the petals that are what is collected as saffron. Besides this, there are also three yellow stamens, which lack the active compounds and are not collected. The quality of saffron is influenced by the size of individual stigmas and the amount of style collected. To produce 1 kg of saffron, about 70 000 or more 200 000 flowers (0.3-1 g each) are needed.

In Kashmir, saffron is grown successfully under non-irrigated conditions (1000-1500 mm per annum). The rain of spring season is considered favourable for the production of corms and the rain immediately before flowering encourages high flower yield. During flowering stage, the rain or cold weather spoils the saffron flowers and also persistent wetness and high temperatures encourage



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various dangerous diseases. Corm planting, flower harvest, stigma separation and corm lifting are the main management techniques carried out manually and this corm tribute to its high price. Commercial saffron is defined as "the stigmas of *C. sativus* L. dried, dark red in colour and trumpet-shaped, serrated or indented at the distal end. The length is between 20 mm and 40 mm. The stigmas may be isolated or joined in pairs or threes at the end of the portion of the style, which is white/yellow in colour" [51]. The three different molecular features of saffron are crocins, picrocrocins and safranal, respectively.

#### II. ORIGIN AND DISTRIBUTION

Saffron believed to have originated from Greece, Asia Minor and Persia spreading eastwards to Kashmir and China. There is an indication that this plant was grown in Palestine in the time of Solomon for saffron. Around 2400 BC, there were evidence of its use in colouring tunics in Castilla-Mancha region of Spain. Several texts speak of its use as a condiment during the reign of Hammurabi (1800-1700 BC). Saffron finds its name in the oldest text (5th century BC) of Kashmir. Its cultivation in the world extends through 0 to 90°E longitude (Spain to Kashmir) and 30 - 45°N latitude (Persia to England). The name "Saffron" is derived from the Arabic word Zafaran which means yellow [3]. The ancient Greek call it "Koricos" whereas Romans used the term "Crocum". In India, this golden spice is known as "Kum Kum" and "Keasr" in Sanskrit and "Koung" in the Kashmiri language. As far as Kashmir is concerned, the saffron cultivation started in the reign of king Laltaditya during 550 A.D. In "Tozaki-jahangari" some reference has been made regarding saffron famous Kashmiri vaids namely Vegbhatta and sushtra used saffron as an ingredient of fragrance, as dye and in herbal medicine. Ancient texts on Ayurveda have information about the herb's use as an aphrodisiac. It is a stimulant and promotes libido that probably explains the kesar milk (Saffron mixed with milk) that is a part of the wedding night ritual.

Due to the small area of cultivation and the minor importance is given to this crop, information on its diffusion throughout the world is uncertain. Total world saffron production is estimated at about 205 tons per year [4]. In the last century, saffron cultivation areas changed completely: in European countries, despite an increase in the price of saffron, traditionally cultivated areas (Spain, Italy and Greece) underwent a severe reduction. On the contrary, an enormous increase has been registered in Iran in the last 30 years. The main reason for this change is certainly due to the high requirement of manual labour, concentrated into a few days and into a few hours a day, and to the increase in labour costs. Today, the main producer countries are Iran, India and Greece (Tab. 1). Iran has the widest area cultivated with saffron: Ehsanzadeh et al. [6] report an area of 47000 ha, most of which is grown in the Khorasan province. In India, saffron is widely cultivated in Kashmir. There are also small cultivation areas in Italy, about 35 ha, for the most part concentrated in Sardinia (about 25 ha in S. Gavino, Cagliari province) and Abruzzo (about 6 ha in Al- topiano di Navelli, L'Aquila), France, Turkey, Switzerland, Israel, Pakistan, China, Egypt, United Arab Emirates, Japan and Australia.

#### **III.DESCRIPTION**

#### A. Structure of the plant

Saffron crocus (C. sativus L.) is a perennial herbaceous plant of the family Iridaceae. The Crocus genus to which it belongs includes about 90 autumn, winter or early flowering species widespread in the old world, and a number of these are garden plants. Saffron crocus is distinguished for its luxuriant habitus, the showy and brilliant flower with the long scarlet stigmas excels in fine appearance even the crocus flowers that are grown for decorative purpose. The stem consists of a subterranean globose corm of 3-4 cm in diameter enveloped by fibrous tunics. The corms, a tuberous-bulb formation, are squashed, flattened at the base, to about 4.5– 5.5 cm diameter, and covered by several reticulated fibrous tunics (Fig. 2). Corms have one or two main buds in the apex position and about (depending on the dimension) 4-5 or more secondary buds, arranged irregularly in spiral form. Corms derived from secondary buds are smaller than corms produced by apical buds. Each mother corm produces 1-3 medium-big daughter corms from apical buds and several small corms from lateral buds, depending on the size of the mother corm. It is a typical reserve organ rich in starch and provided with subterranean buds, both apical and lateral, to form roots, leaves, flowers, and the daughter corms. Roots are two types: fibrous, thin roots at the base of the mother corm, and contractile roots formed at the base of lateral buds [7], thicker than the former with a tuber organ aspect that give the corm the ability to maintain depth in the soil [8]. Leaves (from 6 to 9) are erect, narrow, grass-like and dark green coloured. After sprouting, the flower, usually one or several, but e ven as many as 12, is composed of a perianth of 6 violet tepals (perigon) connate at the base in a long and narrow tube. The pistil is composed of an inferior ovary from which a slender style, 9–10 cm long, arises. The style is divided into 3 dark red branches, each one up to 30–40 mm long, named stigmas, which droop over the perianth segments. Three stamens with two-lobed anthers each are also present. Some variants of saffron with a higher number of stigmas have been reported by [9]. However, they do not reappear the following year and so should be considered somaclonal variations that do not pass on to the next generations. The flowering is during October to the middle of November and is not followed by fruit and seed development.



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Fig. 2 The corms flattened at the base, to about 4.5–5.5 cm diameter and covered by several reticulated fibrous.

#### *B. Biology and physiology*

Saffron is an autumnal flowering geophyte characterised by a long summer rest in which the plant survives periods of drought by means of corms. Its biological cycle starts with its above-ground vegetative growth at the first autumn rains with the emission of leaves and flowers almost immediately and ends with the production of replacement corms in about 220 days. Unlike many other species of the Crocus genus that flower in the winter-spring period, in saffron flowering can occur from mid-October to the end of November, essentially depending on the climatic conditions. It generally starts from 60 to 90 days after planting, mainly depending on sowing time, but snowy and cold periods may retard flowering. Flower induction is a very complicated mechanism in saffron. Little information is available on flower induction in saffron; nevertheless, flowering seems to be mainly influenced by an environmental factor such as Molina et al. [10] described the ability to influence the beginning and duration of flowering to temperature, while an inferior or negligible role is ascribed to soil water content. Certainly, as in most geophyte plants, both seasonal and daily thermoperiodism is involved as the main environmental factors inducing flowering [11].

Saffron is considered a subhysteranthous plant [12]. Indeed, the phenological sequence of the different phases is not predetermined: flowers can appear before, at the same time or after leaf appearance. Hysteranthyis astrange phenomenon present in many geophytes such as Scilla autumnalis, Urginea maritima, Amaryllis belladonna, Pancratium sickenbergeri, Colchicum alpinum, Sternbergia colchiciflora, Ornithogalum pyrenaicum, etc., that flower before leaf emergence, supporting flowers only with the storage nutrients concentrated in the corm [13]. In Mediterranean geophytes, this response can be explained as a strategic adaptation to the temporal unpredictability of the onset of rain after the summer drought [14]. Hysteranthy may be of great importance in saffron with respect to mechanisation of the flower harvest. Flower-cutting machines could be used to harvest flowers and avoid damaging the leaves. Hysteranthy can be induced by controlled temperature during corm storage: a dry storage of corms at 15 °C for 35 days resulted in flowering prior to leaf appearance (hysteranthy) and in a more synchronous flowering [15]. Flower and corm production was also improved by the 15 °C pretreatment. A short growth phase of the vegetative part is also detectable in early spring, in which the photosynthetic activity of leaves allows the formation of replacement corms. Afterwards, when the temperature rises, leaves wither, and the plant remains only below-ground by means of the corm. Root growth occurs from autumn to the spring period, in which the mother corm is completely empty and daughter corms are produced to propagate the plant. The average size of replacement corms is inversely related to their number [16].

#### IV.ADAPTATION

#### A. Climate

Saffron is native to the Mediterranean environment, characterised by cool to cold winters, with autumn-winter- spring rainfall, and warm dry summers with very little rainfall. It can withstand substantial frosts (-10°C) and can tolerate occasional snow in the winter. Significant snow damage in saffron was ascertained only during flowering because of flower freezing and decomposing [17]. While minor problems were observed in leaves even with short-lasting snow [18]. Mollafilabi [19] reports that saffron is able to tolerate substantial cold temperatures of -18°C, and occasional winter snow, even if with reduced productivity. Autumn temperatures in Azerbaijan saffron-cultivated areas fall to -5.9°C [20]. During the summer rest period, maximum temperatures of 30-4°C can be tolerated as well. Saffron prefers direct sun exposure, even though in India it is cultivated together with almond trees. According to Fernandez [4] and Mollafilabi [19], the best climatic conditions for high yields are rainfall in the autumn, warm summers and mild winters.



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Saffron is grown successfully under non-irrigated conditions (1000-1500 mm per annum) in Kashmir, India. Spring rain is considered favourable for corm production, while rain immediately before flowering encourages high flower yield. However, rain or cold weather during flowering spoils the saffron and persistent wetness and high temperatures encourage disease.

#### B. Soil

Saffron grows on a wide range of soils but thrives best in deep, well-drained clay-calcareous soils with a loose texture that permits easy root penetration. Fernandez [4] suggests that clay is a good soil for saffron, while Sampathu et al. [21] reported that saffron requires a well-ploughed sandy-loamy soil or a well-drained clay soil. Tammaro [17] suggests that the humus-clay soil of guarantees good water storage for saffron. Saffron grows well in salty soil, while a limiting factor could be calcium carbonate deficiency. Good soil pH ranges from neutral to slightly alkaline. Conflicting information is reported on nutrient needs among different authors [22].

#### V. AGRICULTURAL PRACTICES

#### A. General Description

Most crop management techniques, above all planting, weeding, flower picking and separating, are performed by hand all over the world (Bali and Sagwal, 1987) [23]. For this reason, saffron cultivation is painstaking and expensive. Saffron cultivation is generally carried out as a perennial cycle, but an annual crop system is adopted in Navelli, Italy. Perennial crop techniques have highly variable durations from place to place: from 3–4 years in Spain, 6–8 years in India and Greece and up to 12 in Morocco [18]. With the ageing of the saffron field, generally after 4–5 years, spice production declines because of increasing competition for water and nutrients, fungal infection due to overcrowding (Sampathu et al.[21] and the reduced size and reproduction capability of corms. In a ten-year experiment, Grilli Caiola [24] observed that corms left in the soil without management techniques continue producing daughter corms for up to 3–5 years and afterwards they degenerate and are no longer able to reproduce vegetatively. Every year in perennial crop techniques, daughter corms creep upwards by about 2 cm from the mother corm, and when they reach the soil surface they must be lifted and replanted.

#### B. Planting and harvesting of corms

Planting cycle, planting time, planting method and seed rate are the critical factors for saffron productivity. Mother corms once planted are retained in the field for many years, along these to produce daughter corms which continue the production cycle without interruption, though at the cost of declining productivity. The planting cycles are generally of 10-12 years duration.

Prior to the plantation of saffron corms, deep ploughing is done using bullock drawn-plough. Every month from January–September plough is carried out to keep the field clean. After the field is ready, corms of different grades are planted in September by hand dropping of saffron corms behind bullock drawn the plough. The field is laid out into  $2m \times 2m$  beds with deep drainage channels on both the sides. Sowing depth and spacing differ in annual and perennial crops. Sowing in perennial crops is deeper (10–20 cm) and wider spaced (10–15 cm between corms and 20–25 between rows) compared with annual crops (8–10cm depth, 3–8cm between corms and about 15 between rows), but shorter spacing is also used in intensive crop systems. To avoid fungal infection, corms can be disinfected before planting by immersion for around 5 minutes in a benomyl, captan or copper-based solution.

#### C. Irrigation

Irrigation is not a necessary practice. Water requirements of saffron are low and can be satisfied by the scarce rainfall when cultivated in semi-arid conditions. Saffron in Kashmir is grown under rainfed conditions as no water source is presently available in saffron karewas. Farmers are dependent on September rains for a good flush of flowers and delayed rainfall (late October) is detrimental to the crop as it is accompanied with low minimum and maximum temperature leading to flower abortion.

The region used to receive annual rainfall between 600 to 1000 mm, part of which would occur during August to mid-October and then in November, the two critical stages for normal flowering and good crop for the next year. However, since the last several years, the weather has become quite erratic. Rains are either scanty or irregular, thus adversely affecting flowering and subsequent plant stand. Experiments carried out in Greece (Skrubis [29], demonstrate that irrigation at the beginning of September resulted in an earlier onset of flowering, while irrigation at the end of September and during October determined an increase in production. Late irrigation could result in a worsening of the quality traits of saffron, especially if watered just before flowering. Certainly, the most crucial moment for irrigation is after summer to awaken the corms, but this coincides with autumn rains so, excepting a severe drought season, this may be considered unnecessary.



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For the accelerated growth of roots and floral primordial and post-flowering, vegetative and corm multiplication, the saffron crop should be sprinkler irrigated @ 700 m3/ha (7 lac litres/ha) to be distributed over 10 irrigations at weekly interval. First seven irrigations are most crucial for the accelerated growth and facilitation of flowering. The timing of this phase of irrigation (preflowering or pre-sprouting) is very important and should be started from the last week of August till 15th October based on climate conditions, otherwise flowering and vegetative growth may coincide which may interfere with the picking of flowers. For first seven irrigations, about 490 m3 (4.90 lac litres/ha) of water is required. To boost vegetative phase for enhanced corm production 3 irrigations with a total water requirement of 210 m3 (2.10 lac litres/ ha) should be applied starting from 8th November.

#### D. Crop rotation

Saffron cultivation needs to be included in a crop rotation. No definitive information is available on the effect of the previous crop on saffron yield and quality, but traditionally between 3 and 8 years should pass before it is cultivated on the same soil. Tammaro [17] reports that a decrease in stigma production and an increase in weed number have been observed when saffron was cultivated on the same soil. In central Italy, saffron is profitably rotated with legumes and wheat [17].

#### E. Fertilising

The application of about 20–30 tons per ha of organic manure is the most common fertilisation practice all over the world [25]. In traditional saffron culture, large amounts of farmyard manure were applied to the saffron fields before planting, and typically 20-30 tons per hectare are incorporated during cultivation. This material supplies nutrients, but its other major role is to improve soil moisture holding capacity and structure under non-irrigated conditions. Under traditional growing systems, no further fertiliser was applied after corm planting.

Behzad et al. [26] found that 25 t/ha of cow manure significantly increased the dried stigma production in a soil with low organic content (0.3% in Organic Carbon), but had no effect in a soil with 1.0% organic content. They also observed that annual distribution of 50 kg/ha of nitrogen increased saffron yields and that phosphorus and potassium seems unnecessary. Sadeghi [27] reports promising results of applying chemical fertiliser, while, in a three-year experiment in two sites in Iran, Behnia et al. [28] found contrasting results on nitrogen fertiliser, and no effect was shown by the application of phosphorus. Urea foliar fertilisation applied on saffron in winter (from January to March) resulted in a significant increase in flower number in a two-year experiment carried out in Iran [30].

#### F. Weed and Rodent management

Weeds are probably the main problem for saffron since it is unable to compete, above all because of its very low height. Nevertheless, due to the short time between the corm planting and flowering, severe weed problems start the following spring in perennial cultivation. From flowering, weeds can be left to grow until May and then cut without damaging the crop when the saffron leaves wither.

Weeds are managed by hand in annual crops in Italy, while in perennial crops a good chemical control is generally achieved with 10 kg/ha of Simazine (Gesatop 50%) or Atrazine (Gesaprim 50%) [22]. In Iran, broad leaves are controlled with pre-emergence and post-emergence treatments of Sencor (Metribuzin) and narrow leaves with Gallant (Haloxy fopetoxy-ethyl) treatments after flower harvest. During the summer rest, general herbicides such as Roundup (Glyphosate) or Buster (2,4-D, 2,4-DP) are applied. Little research has been carried out on indirect weed control methods. Interesting results in reducing weeds with agronomic methods have been obtained with wood chips and sawdust mulch [31].

Rodents cause considerable annual loss to the saffron crop by damaging the saffron corms. Management of rodents using rodenticides is sporadic and not taken as a campaign and with missionary zeal. Saffron rodents (*Pitymys lucuru*) can be managed through six-day management schedule which is given below:-

- 1) Day 1: Plugging of burrows.
- 2) Day 2: Identification of live burrows/pre-baiting with 10-15 g bait/burrow (containing 48 g broken rice, 48 g crushed wheat, 2ml mustard and 2 g sugar).
- 3) Day 3: Identification of live burrows/zinc phosphide baiting with 5-10 g bait/burrow (containing 48 g broken rice, 48 g crushed wheat, 2 g zinc phosphide, 2 ml mustard oil and a pinch of sugar).
- 4) Day 4: Collection and burying of dead rodent. Close all burrows.
- 5) Day 5: Identification of live burrows.



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6) Day 6: Fumigate live reopened burrows with aluminium phosphide pellets @ 2 pellets/ burrow or 5-10 g aluminium phosphide pouch (56% poison)/ burrow and cover with wet mud.

#### G. Pests and disease

Rabbits, rats and birds can cause problems in saffron fields by eating or lifting the corms. Fungal attacks are mostly promoted by humid conditions. High moisture percentage together with high temperatures create ideal conditions for the rapid development and spread of nematodes and fungi (Fusarium, Penicillium, Rhizoctonia, etc.) and consequently corm rot. These conditions generally occur in the hot and rainy spring. Tammaro [17] indicates that temperatures above 10-12 °C with rainy weather are a favourable climatic combination for the establishment of fungal disease on saffron. On the contrary, the hot and dry Mediterranean summer inhibits the spread of parasites. To avoid fungal infection, the best practices are crop rotation, the removal and burning of infected plants and corm treatments with anti-fungal products before planting, such as benomyl or copper-based solution.

#### H. Flowering and Harvesting

Saffron flowers in the autumn, about 40 days after planting, and continues for 30-40 days, depending on the weather. The flowering period of each plant may last up to 15 days. Rain 10-15 days before flower picking results in excellent flowering and high production, whereas under drought conditions, small flowers with small stigmas can be expected. Gathering saffron flowers requires care and intensive manual labour: the flowers only grow a few centimetres above- ground and, depending on vegetative activity, might be surrounded by several leaves which must not be damaged otherwise daughter corms will not be produced. Corm size has a large effect on the production of flowers per corm. Large corms (>45 g) can produce up to 12 flowers per corm, while more average sized corms (20- 30 g) produce six flowers per corm.

The flowers are harvested manually, generally by family members, by cutting the base of the flower stem with the fingernail. About 350–450 man-hours are needed to harvest 1 kg of the spice, corresponding to between 200000 and 400000 stigmas, depending on the unitary weight. The saffron flower is highly ephemeral; given its very short life, it should be picked the same day of flowering and placed in baskets. The best practice is to pick the flower early in the morning each day, when the corolla is still closed, thereby preventing the stigmas from losing colour and quality, avoiding any sudden deterioration by wind or rain [32] and allowing a ready separation into their constituent parts. After harvest, stigmas must be separated from the tepals and stamen as soon as possible by opening the corolla and cutting the stigmas with the fingers below the branching where the style changes colour (from red to yellow).

#### I. Mechanisation

Tentative mechanisation procedures of some crop techniques in saffron have been carried out but it is a rather difficult crop. Lack of mechanisation in saffron is certainly due to the delicacy of corms and flowers, which require handling with care, but also to the considerable variation in the size of corms. Other reasons are the cultivation of saffron in countries with very low manual labour costs and, on the contrary, the limited areas of land to which this crop is devoted in high labour-cost countries. Planting requires regular and correctly oriented placement of the corms. A modified onion planter has been used to plant saffron, but the impossibility of placing the corms with the apex in the upward direction led to a delay in the emergence and a decrease in production.

In fact, the corm reduces emergence when the apex is not pointing upward. A potato planter was also tested, enabling more control over corm orientation, but resulting in lower production compared with the onion planter. A normal hoeing machine can be used to mechanise weed control, by adapting row distance, especially in the first year. Lower efficacy was obtained the following year with the increase in weeds and the rising of the daughter corms. During the rest period of the crop, flaming was also used with good results against young weeds [33]. No suitable results have been obtained for flower separation, while adapted bulb and tuber-picking (such as a potato digger) can replace human labour successfully for corn lifting.

#### J. Drying and storage of stigmas

Following the separation of the stigmas from the flowers, it is essential to dry the flower heads immediately. Drying experiments show that drying at temperatures up to 1100C can be used. Drying and storage methods are very important because a poor undertaking of this procedure can completely compromise qualitative features of saffron [34]. Stigma should be separated within 10-20 hours of flower picking to achieve maximum pistil recovery. Delay in separation by 36-72 hours results in loss of recovery from 37 g/kg of fresh saffron flowers achieved under quick separation and controlled drying to 7 g/kg of fresh saffron flowers. According to the ISO norm, the moisture content may range between 10 and 12% [51]. The critical issue is the length of drying time (e.g. at 1100C for 2 minutes). Recent Spanish research shows drying in a hot air flow at 700C for 6 minutes will give quality saffron. Concerning Italian production, the stigmas are normally spread over a large area and dried at room temperature in the sunlight or



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with forced air. In Navelli, dehydration is traditionally carried out by placing the stigmas on a sieve 20 cm above a charcoal fire [17], while in Sardinia it is performed by drying stigmas in the sun or at room temperature (for several days) or in the oven at low temperature (35–40°C) in less time until moisture is reduced to 5–15%. The brightness of colour is aided by quick high-temperature drying. Slow drying gives a poor quality product. Another method is to use a dehydrator at 480C for 3 hours. Saffron is sun-dried in India and Iran and toasted over hot ashes in Spain, while it is dried slowly at 30–35 0C in dark rooms in Greece; therefore, many procedures are applied and, as is usual in such cases, there are still substantial disagreements over the best drying conditions [34]; [35]. Irrespective of the drying method, it is important not to over dry. A final dry matter close to 10% moisture is adequate for long-term storage. Storage of saffron must be done in the dark and possibly in a modified atmosphere, since saffron pigments are light-, oxygen- and temperature-sensitive. The best way to store saffron spice is to keep it hermetically closed in darkened glass containers, and possibly at low temperature (5–10 0C) [36].

#### K. Yield

A wide range of yields has been reported from various countries under different growing conditions. Yield is quite a difficult parameter to forecast in saffron: It is a function of many agronomic, biological and environmental factors able to exert a great influence on production. As far as is known, production is strictly influenced by dimension [37] and storage conditions of corms [10], climatic conditions [17], sowing time [38], cultural techniques(annual or perennial), crop management (irrigation, fertilisation and weed control) and disease. Moreover, saffron production increases from the first to the third-fourth years of cultivation [39]. Generally, one hectare of saffron may produce 10-15 kg of dried stigmas, but it can range widely, depending on the abovementioned factors, from 2 to 30 kg.

Yields of 2.5 kg/ha are reported in Kashmir, India and Morocco [23] in rain-fed conditions, while it can reach 15 kg/ha in Spain under irrigation and fertilisation [40]. In irrigated Moroccan areas, yields of about 2.5–6 kg are obtained [18]. In Iran, the average yield of saffron is around 5.4 kg/ha [41]. The average weight of fresh stigmas is 0.03 g per flower and dry weight is 0.007 g per flower. About 150 flowers are needed to obtain 1 g of dry stigmas. The size of individual stigmas and the amount of style collected influence the total yield and quality of saffron.

#### VI. CHARACTERISTICS OF STIGMAS

#### A. Secondary metabolites

Apart from the ubiquitous primary metabolites such as carbohydrates, minerals, fats and vitamins) [21], the *Crocus sativus* L. plant contains a large number of components belonging to different classes of secondary metabolites, namely, carotenoids, monoterpenoids, flavonoids and anthocyanins. Carotenoids are the most characteristic and important components of saffron stigmas, responsible for the particular colourant features of this spice. They include both fat soluble carotenoids such as lycopene,  $\alpha$ - and  $\beta$ -carotene and zeaxanthin, and, mainly, the water-soluble C20 apocarotenoid, crocetin (8,8'-diapo-8,8-carotenedioic acid), and its ester derivatives, with one or more molecules of sugar, the trans crocetin ( $\beta$ -d-digentibiosyl) ester being the most important and abundant component of this class [42]. Amongst the other minor components belonging to this class,  $\beta$ -crocetin and  $\gamma$ -crocetin, the mono- and dimethyl ester of crocetin, respectively, and mangicrocin, an unusual xanthone-carotenoid glycosidic coniugate, have also been identified [4].

The other two typical features of saffron spice, namely the bitter taste and flavour, again derive from the carotenoid oxidation products: the bitter glucoside picrocrocin and safranal, respectively [43]. The first, picrocrocin, is a colourless glycoside and is considered the main bitter principle of saffron, even though other components, such as flavonoids (vide infra), concur to give saffron's bitterness [44]. Picrocrocin, according to the accepted biogenesis, should derive, like the members of the crocin family, from the enzymatic degradation of zeaxanthin; in turn, the transformation of picrocrocin gives the volatile safranal [21]. The latter is the main volatile component of saffron, responsible for the particular aroma of this spice. Other typical volatile components of saffron possess the same skeleton of safranal, and like this latter, are considered to derive from picrocrocin, even though the recent discovery of several new glycosides suggests that picrocrocin is not the sole glycosidic aroma precursor in saffron [45]. However, it is worth underlining that the saffron essential oil is very prone to absorbing oxygen and becoming thick and brown, and because of this high instability, this volatile oil is not commercially available.

#### B. Quality characters: aroma, bitter taste and colouring power

Saffron quality is chemically defined as the sum of the colouring power of its pigments, the bitterness of picrocrocin, and the intensity of aroma due to the composition of its volatile oil. Chemical requirements for saffron filaments or powder are given by the ISO norm No. 3632-2 (2003), and presented in Table II. This norm is based on a spectrophotometric test at three different reading



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wavelengths, namely, 440, 330 and 257 nanometers (nm), corresponding to the maximum absorbance of crocetin esters, safranal and picrocrocin, respectively.

The absorbance can be directly related to the concentration of the molecules present in solution, provided the latter is exactly the same for all samples, as must also be the other experimental conditions (temperature, path length, solvent used, physical form, etc.); the values obtained are an estimation of the quality of the sample submitted to the analysis.

Saffron quality is based on a test that is rapid, cheap and easily performed (only a spectrophotometer is needed for an accurate measurement), there are definitely several disadvantages: it is difficult to distinguish between authentic saffron and contaminants (the most recent adulterants have similar-shaped absorbance spectra to authentic natural pigments), and therefore to reliably assign a quality category on the international market [46]. The development of new analytical techniques has prompted researchers to explore new methods to evaluate saffron quality and chemical composition, mainly in order to characterise chemotaxonomic connections between, for instance, the composition and geographical origin of the sample and/or composition and storage conditions. For example, two volatile components, namely, 3,5,5-trimethyl-2- cyclohexenone (isophorone) and 2,6,6-trimethylcyclohexane- 1,4-dione, together with the flavonoidic fraction and in particular kaempferol 3-sophoroside, have been demonstrated to be useful in determining saffron's geographical origin. Furthermore, with the advent of the holistic approach and metabolomics, there is the need for reliable, reproducible high-throughput devices to analyse large amounts of samples in order to build up libraries of spectra available for comparison. Therefore, the exploitation of even more sophisticated analytical techniques with highly standardised procedures appears essential to guarantee the quality of the spice, to determine its geographical origin and to counter adulterations in order to evaluate and certify the best productions.

#### C. Adulterations

Under the prevention of Food Adulterant Act, an adulterant is any material which is employed for the purposes of adulteration, so any article of food is adulterated if: any inferior or cheaper substance has been substituted wholly or in part, any constituent of the article has been wholly or in part abstracted, the article has been prepared, packed or kept under unsanitary conditions, the article consists in part filthy, rotten, decomposed or diseased animal or vegetable or is infested with insects, the article contains any poisonous ingredient, the article hasn't prescribed coloring substance or the coloring substance is in excess of the prescribed limits, the article contains any prohibited or excessive preservatives. Adulteration of saffron dates back to the middle ages in Europe, and given its high value, the penalty for those adulterating this spice could be death (Safranshou Code). Adulteration is normally carried out with vegetable or synthetic substances, as well as with inorganic and organic matter. The most common adulteration is with different parts of the flower itself: styles, stamen, strips of the corolla; other vegetable adulterants often commonly used are safflower, calendula, poppy, arnica, onion skins, turmeric, annatto, capsicum and stigmas of maize.

- D. Various types of Adulteration
- 1) Traditional Manner
- a) In order to increase the saffron mass and decrease price the tasteless and odourless yellow stamens of saffron were/are mixed with the powder or saffron threads materials such as beet, pomegranate fibres, red-dyed silk fibres.
- b) Immersing saffron fibres with viscid materials such as honey, vegetable oil or glycerin were/are another ways for falsification. Sometimes the flowers of other plants, particularly Carthamus tinctorius, or safflower, Calendula officinalis, or marigold, arnica and tinted grasses are fraudulently mixed with the genuine stigmas. A specimen of this adulteration was at one time introduced into the American market, by the name of African Saffron [47].
- c) An adulteration which has been largely practised appears to consist of yellow- coloured chalk or barium sulphate, made into a thin paste, probably with honey and attached to the stigmas[47].
- d) A record Read at the Pharmaceutical Meeting November/19/1889, Botanical Medicine Monographs and Sundry, Note On Adulterated Spanish Saffron, adulterated saffron was loaded with a calcium sulphate artificially coloured and attached thereto with some saccharine substance, most likely glucose which yielded on incineration 40 % of ash [48].
- e) In various European markets, there has been offered saffron largely adulterated with borates, chlorides, carbonate, sulphate and other salts of sodium and potassium, or other various mineral substances[47].
- f) Cape saffron, which has a remarkable resemblance to genuine saffron, having a similar odour, and yielding a similar colour to water, though the flowers themselves are differently coloured. It is the flower of a small plant very abundant at the Cape, belonging to the family of Scrophulariaceae, and is said by Pappe of Cape Town, to possess medicinal virtues closely resembling those of true saffron[47].



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#### 2) Current Adulteration Manners

Powdered saffron is far more susceptible to adulteration, however. Turmeric, paprika, and other substances were and still are often combined with saffron powder. The illegal mixing of relatively lower grades (cheaper) with premium categories of saffron is more likely to be adulterated [49]. The common mislabelling of turmeric (Curcuma longa) as "Indian saffron", "American saffron" or "Mexican saffron", also borders fraud, so be wary of packets listing above because neither of them are from Crocus sativus [50]. Addition of artificial colourants is a most common way of adulteration with the aim of misleading of the consumer for improving the appearance of the dried stigmas or even other extraneous materials to give rise to the colouring strength of the aqueous extract. Sometimes saffron is used as a therapeutical plant. For this reason, adulterations make it completely useless or even harmful.

#### E. Detecting Adulteration

In spite of, available international standards, with detailing tests for determining of saffron quality, there are many other methods used to detect adulteration of saffron. One of the least costly is to throw saffron in water. It immediately expands into a characteristic form that is easily distinguishable from Crocus stamens or florets of safflower, marigold or arnica. Also genuine saffron dissolves easily in water giving the aroma of saffron. If the water extract is dried and a rod dipped in sulphuric acid is drawn across the surface, a blue colour which immediately turns purple and then reddish-brown indicates pure saffron. Other detection methods include colour reactions, microscopic study, thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

In the last two decades, the most common chromatographic method used for the qualitative and even semi-preparative separation and analysis of saffron constituents was thin-layer chromatography (TLC), with silica gel used as the stationary phase and a mixture of highly polar solvents, namely, butanol, acetic acid and water as the mobile phase [21]. More recently, reverse-phase high-performance liquid chromatography (RP-HPLC), coupled with a UV-Vis detector or, more often, a UV-Vis-DAD (Diode Array Detector) for non-volatile constituents [61], and Gas Chromatography (GC), with a mass spectrometer (MS) detector for the volatiles [52] are the methods of choice, allowing the separation on an analytical level and the identification and quantification of the metabolites of interest. Due to the peculiar characteristics of the molecules belonging to the crocetin esters (high degree of conjugation and a certain rigidity of the terpenoid scaffold) fluorescence can also be used. The use of fluorimetry offers a better selectivity and sensitivity, though more precautions must be taken in sample handling [53].

#### VII. BIOLOGICAL PROPERTIES

As a medicinal plant saffron is still used in traditional medicine in several countries (Russia, India and Iran [32], and from a toxicological point of view, it can be considered safe since its LD50 = 20 g/kg. It has traditionally been considered as an anodyne, antidepressant, respiratory decongestant, antispasmodic, aphrodisiac, diaphoretic, emmenagogue, expectorant and sedative. It was used in folk medicine as a remedy against scarlet fever, smallpox, colds, asthma, eye and heart disease, and cancer [54]. Saffron can also be used topically to help clear up sores and to reduce the discomfort of teething infants [55].

It has been reported that saffron or the compounds it contains, such as crocin and dimethylcrocin, are not mutagenic or genotoxic [56]. Premkumar et al. [57] also showed that saffron aqueous extract protects from genotoxicity as well as genotoxins-induced oxidative stress in mice. In these studies, oral pre-treatment with aqueous saffron extract (20, 40 and 80 mg/Kg) for 5 consecutive days significantly inhibited the genotoxicity of antitumor drugs (cyclophosphamide, mitomycin C and cisplatin), in vivo, as revealed by micronucleus and comet assay. It was suggested that saffron could exert its anti genotoxic and chemo preventive effects by the modulation of antioxidants and/or detoxification systems [57]. It was also reported that crocetin could significantly inhibit the genotoxic effects and neoplastic transformations of C3H1OT1/2 cells by benzo (a)- pyrene [58]. An inhibitory effect of safranal on MMS-induced genotoxicity has also been shown in multiple mice organs [59]. The protective effects of saffron and crocin observed may be related to its antioxidant and radical scavenger properties. Saffron and crocin may also decrease the MMS-induced genotoxicity by enhancing the systems involved in detoxification and mutagen/ carcinogen inactivation [60]. It shows antioxidants reducetion induced carcinogenesis and inhibited DNA lesions induced by alkylating agents such as MMS [62]. Saffron and its carotenoids scavenge free radicals and thereby may protect cells from oxidative stress [63]; [64].

#### VIII. CONCLUSIONS AND PROSPECTS OF SAFFRON

From an agronomic point of view, saffron is a very unusual plant for its agrological and ecophysiological characteristics. It is unable to produce seeds and multiplies by means of a subterranean stem. It does not exist in the wild state and only very recent studies have been addressed to the individuation of ancestors so that we have not yet had the possibility of acquiring information from these for improving the crop. Saffron has a reverse biological cycle compared with the majority of cultivated and spontaneous plants:



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flowering first in October-November, then vegetative development until May, which means that the vegetative development is not directly important for the production of stigmas, but for the production of new corms. The plant itself has an annual cycle, but the crop is perennial, precisely owing to its vegetative multiplication. Saffron has a low water use and a very low harvest. Above all, the parts harvested for production are the stigmas, from which a very expensive spice is obtained, probably a unique case in an agronomic context. Last but not least, saffron cultivation has been neglected for many decades by farmers, who have relegated it to adverse soil and climate conditions, and by research, which has led to a lack of innovation. All these reasons should induce revision of the most common agronomic knowledge for an effective revaluation of the crop. The synergy between the empirical knowledge of producers and scientific knowledge is able to generate new agronomic knowledge, especially in poorly-known crops, such as saffron, in which technical management represents a major hindrance to development.

Saffron production and quality can be achieved by means of plants with more flowers per plant, flowers with a higher number of stigmas, increased stigma size or stigmas with a greater amount of dye and aroma. High-quality saffron production from selected areas with appropriate and safe management techniques cannot compete in the world market with the saffron from low-cost manual labour-intensive countries but must be addressed towards a potential niche market of high-level quality. To reach it, more attention should be focused on using modern techniques and the evaluation and promotion of saffron quality. The process must be accompanied by traceability, quality marking in or to attract more consumer interest, the adoption of organic agriculture management techniques (no pesticide and chemical fertilisation) and the reduction in manual labour. In saffron, the commercial products (stigmas) are not storage structures as in most cultivated plants, so an increase in nutrients in the soil is not directly linked to an increase in stigma weight. Certainly, a fertile soil is the basis for good saffron production, but organic manure along with different biofertilizers represents the best support for saffron, especially under non-irrigated conditions, supplying nutrients, but above all, improving soil moisture and soil structure. In very nutrient-poor soil, limited chemical fertilising can be adopted. The biological and agronomical traits of saffron (autumn flowering, overcoming adverse season by corms, very low fertiliser requirements and good adaptation to poor soil) make it an alternative plant for low-input agriculture, able to offer good production in sustainable agricultural systems. It may be considered a viable alternative crop for marginal lands, especially where low water availability severely limits the cultivation of many crops. Certainly, the improvement and, above all, the diffusion of knowledge on this species will encourage farmers in low-fertility areas to increase their income with saffron cultivation.

TABLE I ESTIMATE OF SAffRON WORLD PRODUCTION (MODIfied FROM NEGBI [16]).

Country	Area (ha)	Production (kg)	
Iran	47000	160000	Ehsanzadeh et al., 2004
India	-	8000-10000	Fernandez, 2004
Greece	860	4000–6000	Fernandez, 2004
Azerbaijan	675	-	Azizbekova and Milyaeva, 1999
Morocco	500	1000	Ait-Oubahou and El-Otmani, 1999
Spain	200	300–500	Fernandez, 2004
Italy	35	120	(personal communications)
France	1	4	Girard and Navarrete, 2005
Turkey	-	10	Thiercelin, 2004
Switzerland	-	0.4	Negbi, 1999



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### TABLE II ISO NORM FOR THE QUALITY OF SAMRON.

Test method	Characteristic	Requirements	
		Filaments	Power
ISO/TS 3632-2:2003, art. 7	Moisture and volatile matter, % (m/m), max	12	10
ISO 928:1997, art. 8 &ISO/TS 3632-2:2003, art. 12	Total ash, % (m/m), max	8	8
ISO 930:1997, art. 7 & ISO/TS 3632-2:2003, art. 13	Acid-insoluble ash, % (m/m), on dry basis, max: Categories I and II	1	1
	Categories III and IV	1.5	1.5
ISO 941:1980, art. 7	Solubility in cold water, % (m/m), on dry basis, ma	65	65
ISO/TS 3632-2:2003, art. 14	Bitterness, expressed as direct reading of the		
	absorbance of picrocrocine, on dry basis,	70	70
	min: Category I	55	55
	Category I1 Category I1	40	40
	Safranal, expressed as direct reading of the		
ISO/TS 3632-2:2003, art. 14	absorbance at about 330 nm, on dry basis: min	20	20
	max	50	50
ISO/TS 3632-2:2003, art. 14	Colouring strength, expressed as direct reading of the absorbance of crocine at about 440 nm, on dry basis, min:		
	Category I	190	190
	Category I1  Category I11	150	150
		100	100
ISO/TS 3532-2:2003, art. 14	Synthetic hydrosoluble acid dyes	0	0

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