

A Review of Fatty Acids and Genetic Characterization of Safflower (*Carthamus tinctorius* L.) Seed Oil

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Abstract

Safflower, *Carthamus tinctorius* L., is an annual oilseed crop that is cultivated on small plots all over the world. The seed oil content ranges from 20% to 45%; the oil is high in linoleic acid, an unsaturated fatty acid that aids in lowering the blood cholesterol level. Thus, safflower has long been used as medical plant in many countries, especially in China and India. However, for industrial purposes, it has long been neglected because of the low seed yield or oil content, until its physical role was revealed. In recent years, research works carried out in many countries mostly focused on improving the seed or oil yield. In this review, after illustrating the fatty acid composition of safflower seed oil as well as the genetic characteristics of safflower and their relationships with agronomic traits, a brief analysis of the current worldwide situation and future prospects of safflower utilization are presented.

Keywords: Safflower; Fatty acid; Seed development; Genetic characteristics; Agronomic traits

Introduction

Safflower (*Carthamus tinctorius* L.) is a member of the Compositae family; it has been cultivated around the world for centuries, mainly as a source of edible oil and dyes. India, the USA, Mexico, Australia, and Ethiopia are the largest producers of safflower for oil extraction, and these countries account for 85% of the world's safflower production [1]. Other important safflower-producing countries are China, Kazakhstan, Argentina, Uzbekistan, and the Russian Federation [2]. As safflower is more drought and salt tolerant than other oil seed crops, it is especially suitable for the dry and salty areas where other oil seeds are difficult to grow [3]. The fatty acid composition of a vegetable oil determines its best commercial uses [4]. Safflower has been used for centuries as a kind of ornamental plant, as medicinal plant, and as cosmetic material in China. Safflower seed oil with its high linoleic acid content was once largely used in the preparation of alkyd resins for paints and varnishes. Currently, in view of its reported role in reducing blood cholesterol levels, it is being utilized as premium edible oil [5-7]. It also may be a potential raw material for vegetable oil-based liquid fuel production in the near future [4]. Safflower oil, due to its high oleic acid content, has become a widely used frying oil because of its high stability and bland flavor [8]. In this review, after illustrating the fatty acid composition of safflower seed oil as well as the genetic characteristics of safflower and their relationships with agronomic traits, a brief analysis of the current worldwide situation and future prospects of safflower utilization are presented.

Fatty Acid Composition of Safflower Seed Oil

Oilseeds are important sources of vegetable oils. The suitability of a vegetable oil for a particular use such as in nutritional, industrial or pharmaceutical applications is determined by its fatty acid composition, which is highly variable depending on the plant species. This has encouraged researchers to look for new sources of oil or for new fatty acid compositions in different plant species. Genetic variation in the fatty acid composition is essential for the genetic improvement of oil quality and for the development of new cultivars. Safflower oil contains two main unsaturated fatty acids: oleic (18:1) and linoleic acid (18:2). They account for about 90% of the total fatty acids. The remaining 10% correspond to the saturated fatty acids, palmitic (16:0) and stearic acid (18:0). Standard safflower oil contains about 6-8% palmitic acid, 2-3% stearic acid, 16-20% oleic acid, and 71-75% linoleic acid [9]. However,

in many other studies, the fatty acid composition of safflower seeds has shown great variability [10]. Fernández-Martínez et al. [11] have reported the fatty acid compositions of 200 safflower accessions originating from 37 countries; indicating that oleic and linoleic acid have a tremendous range of variation, from 3.1% to 90.60% and from 3.9% to 88.8%, respectively. Guan et al. [12] investigated the oil content and fatty acids of 21 safflower accessions from 12 countries. Their results showed that the content of oil ranged from 9.10% to 25.10%, with palmitic acid ranging from 4.04% to 7.86%, stearic acid from 1.5% to 2.75%, oleic acid from 7.9% to 32.99%, and linoleic acid from 62.7% to 83.74%. Sabzalian et al. [13] reported the fatty acid compositions of three safflower species. Their results showed that the oleic acid contents in the seed oil of *C. tinctorius*, *C. oxyacantha* ВІЕВ., and *C. lanatus* L. were in the range of 12.24-15.43%, 14.11-19.28%, and 16.70-19.77%, respectively. The corresponding ranges for linoleic acid were 71.05-76.12%, 63.90-75.43%, and 62.47-71.08%. In the past several decades, varieties with higher oil content (34-37%) and mutant types with high levels of oleic acid were developed. A safflower variety from Israel with 5-10% stearic acid, compared to 1-3% in the normal genotypes, was identified [14]. Very high levels of linoleic acid (87-89%) and very low oleic acid (3-7%) levels were found in safflower from Portugal [15]. Additionally, accessions with very high oleic acid content (>85%) have been reported [11,16]. Sabzalian et al. [13] reported that the fatty acid compositions among oils from cultivated and wild species were not very different, indicating that the seed oil of the wild safflower may be suitable for human consumption and industrial purposes. The oil content was in the range of 29.20-34.00%, 20.04-30.80%, and 15.30-20.80% in *C. tinctorius*, *C. oxyacantha* ВІЕВ., and *C. lanatus* L., respectively. The main fatty acids such as oleic, linoleic, palmitic, and

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stearic acid accounted for 96-99% of the total fatty acids in all species.

Changes in the Fatty Acids of Safflower Oil During Seed Development

Safflower showed great changes in the contents of seed oil and in the fatty acid compositions during seed development. Sims et al. [17] reported that, in developing safflower seeds, the oleic acid concentration increased slowly during the first 30 days after fertilization and then in some cases appeared to level off with approaching maturity. Initially, linoleic acid was present at almost the same amount as oleic acid, but by day 20 after fertilization its concentration was three times that of oleic acid. This ratio of linoleic to oleic acid tended to increase steadily during the latter part of seed development. Hill and Knowles [18] revealed that the actual amount of palmitic acid in their safflower varieties remained at about the same level until 10 days after flowering, and then slowly increased until about 25 days after flowering, although the palmitic acid percentage in relative amounts decreased during seed development. Ichihara and Noda [19] reported that saturated fatty acids tended to accumulate at the 1- and 3-positions of the glycerol molecule, and the more highly unsaturated acids, at the 2-position. The fatty acid compositions at the 1- and 3-positions were similar in all cases investigated, but in none of the triacylglycerols (TAG) was the distribution completely symmetrical. The positional distribution of linolenic acid in TAG prepared from the immature seeds 2 days after flowering and from the leaves was unusual. Hamrouni et al. [20] working with safflower seeds found that the oil content and total lipids were increased during seed ripening; the linoleic acid content was also significantly increased, while the palmitic acid content had decreased considerably, from 13.7% to 4.0%, during seed ripening. In addition, the ripening process of the safflower seeds was also characterized by an increase in the TAG content and a decrease in the diacylglycerol (DAG), phosphatidylcholine (PC), and phosphatidic acid (PA) contents. Geçgel et al. [21] reported that the moisture content declined in the 15 days from the flowering period to maturity, while the oil content increased. The rate of the palmitic acid formation decreased in both varieties in the 15 days from flowering to maturity, whereas the oleic and linoleic acid formation rates increased in the Montola-2001 and Centennial varieties, respectively.

Genetic Characteristics Related to the Fatty Acids

Safflower is one of the cultivated oil crops from which a great number of different types of oil can be developed through the combination of major genes controlling the levels of stearic, oleic, and linoleic acid, and by changing the environmental conditions [10]. In 1964, Knowles and Hill [22] reported that the major gene locus, *ol*, governs the proportions of oleic and linoleic acid, with the genotype *olol* leading to 72-80% oleic acid in the safflower seed oils, the genotype *OLOL* resulting in 72-80% linoleic acid, whereas the genotype *ol'ol'* or *OLol₁* has about equal amounts (45%) of each acid. In 1989, they reported that the genotypes *OLOL* and *olol* were more stable with regard to temperature changes, in contrast to the gene *ol1*. At the highest temperature, in the genotypes *ol₁ol₁* and *OLol₁*, the linoleic acid content was slightly decreased and oleic acid was increased by the corresponding amount. A new gene locus (*li*) - different from *st* (governing the levels of oleic and stearic acid) or *ol* - controlling for high levels of linoleic acid was found in the genotype from Portugal (Portugal 253568) with very high levels of linoleic acid (87-89%) and very low oleic acid content (3-7%) [15]. Hamdan et al. [23] reported on the inheritance of the very high linoleic acid content in safflower and its relationship with nuclear male sterility. The results showed a linkage of five random amplified polymorphic

DNA (RAPD) bands to the *Li* (controlling for the very high linoleic acid content) and *Ms* (controlling for nuclear male sterility, NMS) gene loci. The RAPD fragments were converted into sequence-characterized amplified region (SCAR) markers. A linkage map including the five SCAR markers and the *Li* and *Ms* gene loci was constructed. The SCAR markers flanked the two loci at minimum distances of 15.7 cM from the *Li* locus and 3.7 cM from the *Ms* locus.

Relationship of Agronomic Traits and Seed/Oil Yields

The seed yield per plant and the oil content are the primary selection criteria for safflower breeding [24]. The importance of selection for a particular trait depends on the extent of direct or indirect effects of the trait on the seed/oil yield [25]. Therefore, before initiating selection in a seed/oil yield improvement program, it is necessary to know the relative importance of each of the different traits in influencing the trait of economic importance in a desired direction [26]. Rao et al. [27] reported that the capsule number, the capsule weight, and the hull percentage were the most important components in breeding for higher yield and oil content. The Spine index showed a non-significant association with yield and oil content. The yield per plant and its major components (the number of capsules and the capsule weight) revealed a negligible relationship with the oil content. Nie et al. [28] worked with safflower and showed that the first branch height, the plant height, and the heads per plant were the principal components of safflower seed/oil yield. Therefore, an appropriate combination of these components would constitute the most desirable plant type, but the strong negative associations between these desirable traits were the major problem in the selection programs. Bagawan and Ravikumar [29] reported that the oil content and the seed yield are negatively correlated. Pahlavanim [30] stated that an improvement of the seed yield in safflower could decrease the oil content and the 100-seed weight due to negative association between these traits. Alizadeh and Carapetian [31] reported that the average number of seeds per head has a significant positive relationship with the grain yield. Thus, genetic gain may be achieved in the future by augmenting the number of seeds per head. Yang et al. [32] reported that the number of heads per plant, the number of branches, the plant height, and the first branch height are the most important characters in safflower improvement for increasing the seed yield. Our previous research indicated that photosynthetic parameters were significantly correlated with some agronomic traits such as the 100-seed weight and the head diameter [33]. After this, our research showed that most of the correlation coefficients between agronomic traits and fatty acids were insignificant, indicating that the fatty acid content was hardly influenced by the 100-seed weight, the plant height, and the number of seeds per plant [12]. Thus, the safflower oil quality and agronomic traits could be improved simultaneously through breeding of safflower for high photosynthetic efficiency. In addition, the results also showed significant correlation coefficients between some photosynthetic parameters and the percentage of fatty acids, which inferred that the photosynthesis rate can be used as an early selection marker in genetic improvement programs. Abd El-Lattief [34] evaluated 25 safflower genotypes for seed and oil yields in an arid environment in Upper Egypt. The results showed that the oil yield was significantly correlated with the plant height ($r=0.566^{**}$), the branches per plant ($r=0.591^{**}$), the capitula per plant ($r=0.625^{**}$), the seed weight per plant ($r=0.863^{**}$), the seed yield ($r=0.990^{**}$), and the seed oil content ($r=0.711^{**}$). Safflower cultivars with very high levels of linoleic or oleic acid would increase the value of the oil [10]. Usually, there was an inverse relationship between oleic acid and linoleic acid [35]. Dajue [16] reported that linoleic acid is negatively correlated with

all the other components, and this negative correlation is greatest with oleic acid ($r=-0.9502$), followed by that of palmitic acid ($r=-0.3247$). Arslan [36] positive and significant relationships between oleic acid and palmitic acid ($r=0.317^{**}$), and oil stability ($r=0.920^*$), while the greatest negative and significant relationship was found between oleic acid and linoleic acid ($r=-0.999^{***}$). The results of our previous study also showed that linoleic acid is negatively correlated with oleic acid, but palmitic acid was positively correlated with linoleic acid. All the correlation coefficients between the content of oil and that of each fatty acid were insignificant, indicating that it should be possible to breed new safflower varieties with simultaneously high oil content and high linoleic or oleic acid content in the future [12].

Environment and Cultivation Techniques Affect the Safflower Agronomic Traits and the Seed/Oil Yields

Besides genetic factors, cultivation methods and environment conditions can affect the safflower growth and seed/oil yields. Arslan [37] analyzed the broad-sense heritability and variance components of the seed yield and some components of the safflower cultivars. The results showed that heritability was low for the number of primary branches per plant (76%) and the number of heads per plant (78%), indicating that these traits may be greatly affected by the environment. However, Akbar and Kamran [38] found that the safflower plant height and the number of seeds per capitulum have the highest genotypic and phenotypic variances. To identify safflower genotypes with both high and stable yield performance across different environments, Abdulahi et al. [39] evaluated 16 safflower genotypes in multi-environment trials by nonparametric methods. The nonparametric tests of $G \times E$ and a combined ANOVA across environments indicated the presence of both crossover and usual crossover interactions, and the genotypes varied significantly for the grain yield. And according to the RS and TOP measures, the genotype G16 (PI537598) was the best genotype with the highest yield and the widest range of adaptation. Mozaffari and Asadi [40] demonstrated significant differences among safflower mutants for plant height, capitulum diameter, number of seeds per capitulum, capitulum weight, days to 50% flowering, days to maturity, and yield, between irrigated and drought stress conditions. However, the differences in oil content and number of capitula in the two conditions were insignificant. In 2002, Oad et al. [41] determined that the inter- and intra-row spacing affects the growth as well as the seed and oil production of safflower. They found that crop maturity, plant height, number of branches, number of capitula, and seed and oil content varied significantly between different extents of inter- and intra-row spacing as well as their interactions. A safflower crop should be planted at 45×30 cm of row and plant distance for maximum seed and oil production. Nikabadi et al. [42] reported that the numbers of seeds per capitulum and the seed yields of two spring safflower cultivars (Isfaha14 and I.L111) were significantly decreased as the sowing dates were delayed from March 6 to June 21.

Prospects of Safflower Seed Oil

Safflower oil for both edible and industrial purposes will become increasingly important. In recent years, the results of nutritional studies with high-linoleic and high-oleic oils have been promising. Safflower oil, with high linoleic acid content, can be blended with other vegetable oils to nutritionally upgrade them. In a different system of utilization, the high percentage of linoleic acid in safflower oil may be exploited for lipochemical applications. Therefore, safflower oils and meals will provide versatile products that will find many applications in livestock nutrition, human nutrition, and in technical industries. Industrial

uses of safflower oil can be expanded to counteract environmental concerns raised by the exclusive use of fossil fuels. Bio-diesel and fuel additives, as well as applications, e.g., as chainsaw bar oil, may reduce the polluting effects of exhaust gases. Research in Montana, USA, is continuing to make high-oleic safflower oil more economical for use as a biofuel, by adding value to safflower meal through genetic breeding and improvement. Nowadays, the safflower products show great potential in the world market, with their important contributions to edible oils or industrial oils as well as the highly nutritious by-products for animal feed and the petals as coloring for foods and textiles. Moreover, development of pharmacophore point theory greatly increases the effectivity of virtual screening in the database [43-46]. It is also a valid method for the identification of a natural product in safflower later period. Therefore, technologies related to medicine, tea, food color and textile color applications need to be developed in the international research system and the crop can thus be promoted to achieve increasing market opportunities in the world.

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