Fatty Acid Profiling of Safflower (*Carthamus tinctorius*) Genotypes Using HPLC: A Comparative Analysis

ABSTRACT

Fatty acid profiling is a process of analyzing the composition and quantity of different fatty acids. Fatty acids are organic molecules that are essential for a variety of biological processes in the body, including energy storage and membrane function. The analysis of fatty acid profiles is important for understanding the nutritional quality of food products and evaluating their health benefits or risks.According to hull types of seeds, the seed oil content ranges from 20 % to 45 %. Fatty acid profiling of safflower involves analyzing the types and quantities of fatty acids present in safflower oil. This can provide insights into its nutritional value, stability, and potential health benefits. This research is conducted to know the content of saturated as well as unsaturated fatty acid in safflower oil. The fatty acid composition of safflower genotypes reveals palmitic acid content ranges from 5.09% (GMU-2969) to 6.93% (GMU-3929). The stearic acid content ranged between 1.69% (GMU-2969) to 3.55% (GMU-3780). The oleic acid content ranged from 12.88% (GMU-2749) to 20.69% (GMI-3177) and the percent linoleic acid content ranged from 71.31 (GMU-3177) to 78.44% (GMU-2749).

Keywords: [Fatty acid, Oleic acid, Safflower, Hexane,Profiling]

1. INTRODUCTION

Safflower (*Carthamus tinctorius* L.) the oilseed crop is a member of the family Compositae or Asteraceae, is an ancient crop believed to have originated in the Near East and parts of the Mediterranean. Maniet al. [17]. Over time, safflower cultivation spread to various parts of the world, including India, Egypt, and parts of Europe, where it also began to be used for its oil-rich seeds. It is a diploid plant with 2n = 24 and it is self-pollinated. It is an annual herbaceous crop that thrives in hot and dry climatic conditions and it is particularly well-suited to arid areas due to its ability to withstand drought conditions effectively. Currently, it is commercially cultivated in India, Mexico, the United States, Kazakhstan, Australia, China, Uzbekistan, Ethiopia, Spain, Turkey, Iran, Canada, the Russian Federation and Pakistan. The cultivation of safflower, since time immemorial under diverse climatic conditions in different countries, suggests the sustainability of the crop is very high. Singh &Nimbka [19].

Safflower has spiny and spineless varieties. Spiny varieties have spines on the leaves and modified leaves associated with flower heads. Generally, varieties with reduced or absent spines have been lower in oil content than spiny types. Safflower oil quality is high due to its fatty acids' composition.

Cosge et al. [4]. The oil is high in linoleic acid, an unsaturated fatty acid that aids in lowering the cholesterol level in the blood. In addition, this oil is used in soft margarines and as salad oil besides being used raw for edible purposes.
It was initially grown for its flowers, which were used for dyeing fabrics and medicinal purposes. Safflower, a diversified crop has been extensively grown in India, mostly for its quality oil rich in polyunsaturated fatty acids (linoleic acid) and for the carthamin (orange-red dye), extracted from the brilliantly coloured flowers. Safflower flowers are commercially exploited for herbal preparations in China and have medicinal and culinary properties.Gomashe et al. [8]. It is the most drought-tolerant oilseed crop and can produce good seed yield in semi-arid regions, while its salt tolerance is a valuable asset as the area affected by some degree of salinity increases worldwide.Emongor and Oagile. [5].

Safflower oil has shown many beneficial health effects in various studies performed recently. A balanced fatty acid profile found in safflower oil has been shown to decrease fat accumulation in rats when compared to the beef tallow diet. Khalidet al. [14]. It is an oil seed crop, that contains about 80% oleic and linoleic acid, iodine value and saponification value, cultivated mostly for its high-quality oil, cut flowers, vegetables and medicinal properties. Safflower oils were used as a source of oil in the paint industry and edible oil for cooking, margarine production, and salad oil.Surmi et al. [21].

The average contents of total fatty acid were 22.16-27.23 mg/100 g, and the average contents of linoleic acid ranged between 78.54% and 82.45%. The correlation analysis showed that the total fatty acids were positively correlated with linoleic acid (C18:2) and palmitic acid (C16:0), and negatively correlated with oleic acid (C18:1n12 and C18:1n9).Lianget al. [15]. 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% corresponds 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. Liu et al. [16]. Different types of fatty acids have different effects on the body, so understanding the fatty acid profile of a food can provide insight into its potential impact on health content in safflower.

Because of its reported role in reducing blood cholesterol levels, it is being utilized as a premium edible oil. It also may be a potential raw material for vegetable oil-based liquid fuel production. Safflower oil, due to its high oleic acid content, has become widely used as a frying oil because of its high stability and bland flavour. Recently, attention has been increasingly attracted to safflower seed oil as an excellent healthcare product, because it is effective for the treatment of hyperlipaemia, arteriosclerosis and coronary heart disease and it can enhance microcirculation. Han et al. [9]. Safflower oil is also known to protect body tissues and protect interior organs. Katkadeet al. [13].
Fatty acids are essential components of cell membranes and are an integral component of the intercellular barrier in the stratum corneum. This barrier is formed by the extrusion of lamellar granules containing phospholipids, glycosphingolipids and free sterols that are produced by keratinocytes.

Essential fatty acids cannot be synthesized and therefore, must be supplied in the diet. Animals are unable to change one series of fatty acids to another, eg, omega-3 to omega-6.Arslan and Kucuk. [1].

A health-conscious population has significantly increased the demand for safflower oil, which is now widely used as a salad oil, margarine and cooking oil for frying items like french fries, chips and other snacks. This versatility makes safflower oil popular for both personal and commercial uses. However, there is a lack of information about the different safflower cultivars available in the local market and their oil characteristics.

Aim of the present investigation was aimed to study fatty acid profiling of oil extracted from eighteen different safflower genotypes by HPLC.

2. Material and methods

Eighteen safflower seed samples were procured from National Agriculture Research Project, Solapur, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.S.) and were used for oil content and fatty acid composition (palmitic, stearic, oleic, linoleic) analysis.

2.1 Fatty Acid Profiling

To analyze the major fatty acidspalmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2)in seed oil of the selected material, 100–150 mg of oil was used. The oil was treated with 2 mL of 13% methanolic KOH for 40 minutes at 55°C. Hexane was added to extract the organic phase, and the upper organic layer was washed with water until it reached neutral pH. The hexane layer was dried over anhydrous sodium sulfate and concentrated under nitrogen to obtain fatty acid methyl esters (FAMEs). The fatty acid composition was then determined using an Agilent 7890B gas chromatograph equipped with a flame ionization detector (FID). A DB-225 fused silica capillary column (250 μm diameter, 30 m length, and 0.25 μm film thickness) was employed for peak separation. Samples (0.1 μL) were injected in split mode with a split ratio of 1:50. The initial oven temperature was set at 160°C for 2 minutes, then increased to 220°C at a rate of 5°C per minute, and held at 220°C for 10 minutes. The inlet and detector temperatures were set at 200°C and 230°C, respectively. Nitrogen was used as the carrier gas at a constant flow rate of 1 mL/min. Peak identification was achieved by comparing the relative retention times with a commercial standard FAME mixture (Supelco 37 Component FAME Mix), and the fatty acid composition was determined by calculating the percentage of peak areas using EZChrom Elite Compact software.Kammili and Yadav. [12].

2.2 Data analysis

The data were analyzed using INDOSTAT statistical software from Indostat Services, Hyderabad, India. Pearson correlation coefficients were used to establish the associations between different fatty acids and their relationships with oil content and seed yield. Two-tailed P-values were calculated with confidence intervals of 95% (0.05) and 99% (0.01).

3. RESULTS AND DISCUSSION

Fatty acids play a crucial role in human health. Unsaturated fatty acids, particularly omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), are essential for various bodily functions, including cell membrane integrity, inflammation regulation, and brain health. For instance, the high levels of linoleic acid (omega-6) in safflower oil can support skin health and reduce the risk of chronic diseases when consumed in appropriate amounts. Profiling allows nutritionists to recommend specific oils based on their fatty acid composition, ensuring balanced dietary intake. Calder.[3]. In the present investigation, eighteen safflower genotypes were used for the estimation of oil content and fatty acid profiling viz., palmitic, stearic, oleic and linoleic acid at the Indian Institute of Oil Seed Research (IIOR), Rajendranagar, Hyderabad.

Table 1: Fatty acid profiling of safflower genotypes

Sr. No.	Genotypes	Palmitic	Stearic	Oleic	Linoleic
1.	GMU-6057	5.64	2.48	15.27	76.61
2.	GMU-2969	5.07#	1.69#	16.5	76.75
3.	GMU-3386	5.39	2.4	17.28	74.93
4.	GMU-330	5.17	2.01	16.21	76.61
5.	GMU-3436	5.76	2.03	16.3	75.91
6.	GMU-1626	5.17	2.24	16.43	76.17
7.	GMU-1047	5.54	2.06	17.06	75.35
8.	GMU-2749	5.6	3.07	12.88#	78.44*
9.	GMU-3780	5.69	3.55*	14.42	76.34
10.	GMU-3852	5.47	2.03	18.5	74
11.	GMU-774	5.83	2.49	16.09	75.59
12.	GMU-3177	5.71	2.3	20.69*	71.31#
13.	GMU-3929	6.93*	1.92	14.46	76.68
14.	GMU-5825	5.32	2.17	17.12	75.39
15.	GMU-4549	5.71	2.55	18.52	73.22
16.	SSF-1565	5.33	2.17	17.12	75.39
17.	SSF-1602	6.13	3.04	17.29	73.54
18.	SSF1371	5.67	2.66	15.3	76.38
	MAXIMUM	6.93	3.55	20.69	78.44
	MINIMUM	5.07	1.69	12.88	71.31

*High content of Palmitic, Stearic, Oleic, Linoleic
#Low content of Palmitic, Stearic, Oleic, Linoleic

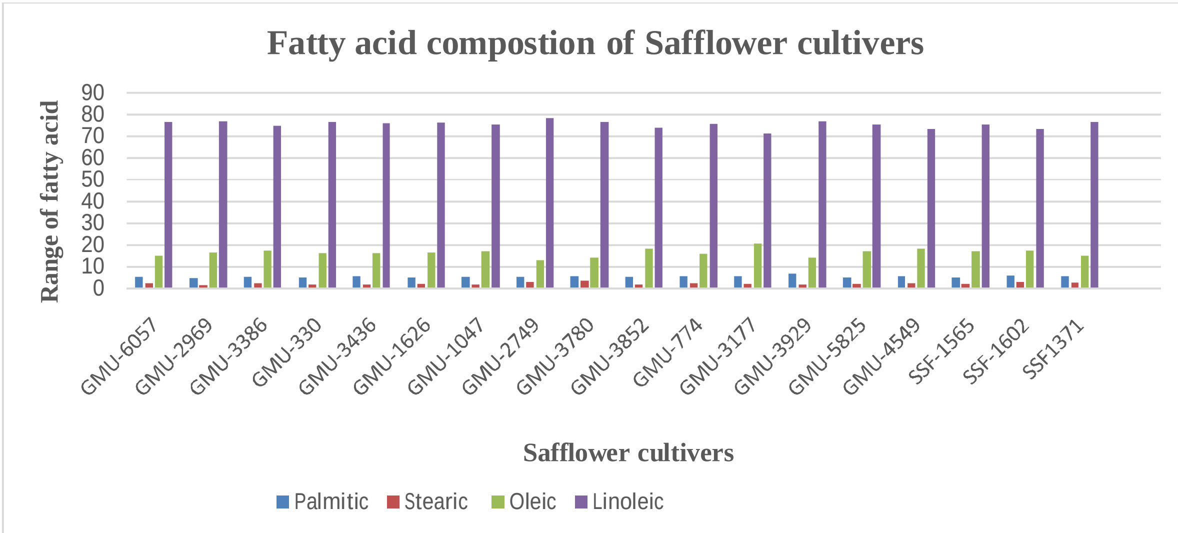

Fig1. Graphical representation of safflower fatty acid profile.

Safflower includes very high content of monounsaturated (oleic acid) or polyunsaturated (linoleic acid) fatty acids, which helps to reduce the cholesterol level in blood, to maintain the health of heart and to prevent the occurrence of cardiovascular disease e it includes very high content of monounsaturated (oleic acid) or polyunsaturated (linoleic acid) fatty acids, which helps to reduce the cholesterol level in blood, to maintain the health of heart and to prevent the occurrence of cardiovascular diseases.

Conventional safflower oil contains 6–8% palmitic acid, 2–3% stearic acid, 16–20% oleic acid, and 71–75% linoleic acid.Gecgel et al. [7]. Great genetic variation was reported for linoleic acid and oleic acid contents in world safflower germplasm. Fernandez-Martinez et al. [6]. Major unsaturated fatty acids are linoleic and oleic acid comprising 77.9–79.5% and 9.5–11.3% of total fatty acids respectively. Mihaela et al. [18]While saturated fatty acids are present in lower proportions ranging from 9.7% to 10.8% of total fatty acids. Major saturated fatty acids are palmitic and stearic acids consisting of 7.2–8.6% and 2.0–2.4% content respectively.Ben et al. [2].The percent palmitic acid content in fatty acid composition ranged from 5.07 % (GMU-2969) to 6.93% (GMU-3929). The all-safflower genotypes have low palmitic acid content. The stearic acid content was between 1.69% (GMU-2969) to 3.55% (GMU-3780). In that GMU-2969 and GMU-3929 showed the low steric acid content and GMU-6057 (2.48%) GMU-3386 (2.4%) GMU-330 (2.01%)GMU-3346 (2.03%) GMU-1626 (2.24%) GMU-1047 (2.06%) GMU-2749 (3.07%) GMU-3780(3.55%) GMU-3852 (2.03%) GMU-774 (2.49%) GMU-3177 (2.3%) GMU-4549 (2.55%) SSF-1565 (2.17%) SSF-1602 (3.04%) SSF-1371 (2.66%) this genotype has medium stearic acid content. The range of low oleic acid is 10-30% and high oleic acid range 60-80%. The oleic acid content ranged from 12.88% (GMU-2749) to 20.69% (GMU-3177), it means all genotypes have the lowest oleic acid content. The range of low linoleic acid is 40-60%, 60-80% is medium and 80-90% is consider as high. The percent linoleic acid content ranged from 71.31% (GMU-3177) to 78.44% (GMU-2749) the all-safflower cultivar shows the medium linoleic acid content. The fatty acid profiling data is contained in (Table 1). The data pertaining to fatty acid profile of safflower oil is presented graphically in(Figure 1) Earlier scientists.Vosoughkia et al. [22] also reported the most principal fatty acids of safflower samples analysed were linoleic (75.81–77.86%), oleic (12.57–13.75%), palmitic (6.09–7.07%) and stearic (2.17–2.62%) in respecting decreasing order and these fatty acids together composed about 98.86–99.47% of the total fatty acids in all Safflower genotypes analysed. Similarly, Juhaimi et al. [11] also reported the unsaturated fatty acids, linoleic acid was the dominant acid in all oils, which varied from 48.07 to 49.62%, followed by oleic acid（40.58–42.54%）in safflower seed oils. Similar findings for fatty acid profiling are reported by Ismail and Arafat [10] showing palmitic acid ranged between 4.29% to 6.91%, stearic acid ranged between 2.90% to 5.32%, oleic acid ranged between 51.24% to 82.87% and linoleic acid between 37.57% and 5.48%. The respective genotypes with a higher range of linoleic, oleic, palmitic and stearic acids may be utilized in further improvement studies. Subasi et al. [20]studied, the primary fatty acids of the oil of the control plot plants were identified as 73% linoleic, 12% oleic, 5.8% palmitic, and 2.2% stearic acid. Surmi et al. [21] reported fatty acids composition of total lipids extracted from Ethiopian, Malawi and Giza1 safflower seeds are shown that the unsaturated fattyacids of linoleic (74.60, 78.24 and 77.90, %) and oleic (14.19, 11.22 and 11.39%) and the saturated fatty acids of palmitic (6.03,6.57and6.66%)andstearic(2.6,2.01and2.06%).

4. CONCLUSION

The present investigation into the fatty acid profiling of eighteen safflower genotypes highlights significant variation in their fatty acid composition, particularly in terms of palmitic, stearic, oleic, and linoleic acids. Across all genotypes, linoleic acid dominated the fatty acid profile, ranging from 71.31% (GMU-3177) to 78.44% (GMU-2749), aligning with previous studies that found safflower oil to be rich in polyunsaturated fatty acids. This suggests the potential of safflower oil for promoting heart health by lowering cholesterol levels and reducing cardiovascular disease risks.The genotypes showed relatively low palmitic acid content (5.07%–6.93%) and stearic acid content (1.69%–3.55%), with GMU-2969 having the lowest levels for both. Oleic acid content ranged more widely, from 12.88% (GMU-2749) to 20.69% (GMU-3177), which is below the high-oleic safflower varieties reported in some other studies.In conclusion, the safflower genotypes studied predominantly contain high levels of linoleic acid and moderate levels of oleic acid. This fatty acid profile suggests that these genotypes can be further utilized in breeding programs to enhance oil quality, focusing on traits such as higher oleic acid content for improved stability and nutritional value. The genotypes with higher levels of specific fatty acidsGMU-2749 for linoleic acid and GMU-3177 for oleic acidmay serve as candidates for developing nutritionally superior safflower oil varieties.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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