



Assessment of Commercially Safflower Oils (Carthami Oleum Raffinatum) in Terms of European Pharmacopoeia Criteria and Their Weight Control Potentials

Didem DELİORMAN ORHAN^{1*}, Sultan PEKACAR¹, Onur Kenan ULUTAŞ², Burçin ÖZÜPEK¹, Demet SÜMMEOĞLU¹, Aysel BERKKAN³

¹Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Türkiye

²Gazi University, Faculty of Pharmacy, Department of Toxicology, Ankara, Türkiye

³Gazi University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Türkiye

ABSTRACT

Objectives: Safflower oils, which are sold commercially, are in demand with food, cosmetics, and health-promoting claims. In this study, safflower oil samples belonging to 11 different brands were evaluated in terms of European Pharmacopoeia Criteria 7.0. Additionally, *in vitro* weight control potential of all samples was investigated.

Materials and Methods: Samples to be analyzed were purchased from pharmacies, herbal, online, and cosmetics stores. Acid and peroxide values of 11 safflower samples and analysis of their fatty acids by gas chromatography-mass spectrometry (GC-MS) were carried out according to the "Carthami oleum raffinatum" monograph registered in the European Pharmacopoeia 7.0. To test the effects of all samples on weight control, their inhibitory effects on carbohydrate-digesting enzymes (α -glucosidase and α -amylase) were evaluated using spectrophotometric methods.

Results: Out of 11 oil samples, only two of them had acid and peroxide values below the reference value. According to GC analysis, safflower oil samples are rich in monounsaturated fatty acids (oleic acid) and polyunsaturated fatty acids (linoleic acid) (67.10-99.53%) of total fatty acids in its content are oleic, linoleic, palmitic, and stearic acids. Saturated fatty acids were 0.58 to 12.18% of the total fatty acid methyl esters in oils. When evaluated in terms of the inhibition of α -amylase and α -glucosidase enzymes that hydrolyze carbohydrates, the results showed that safflower oil samples had no inhibitory activity on these enzymes.

Conclusion: It has been determined in this report that many safflower oil samples on the market do not meet the quality criteria recommended in European Pharmacopoeia 7.0. It was observed that safflower oil did not show any inhibitory effect on these two enzymes, which is considered a rational approach for weight control.

Key words: Fatty acids, gas chromatography-mass spectrometry, quality control, safflower oil

INTRODUCTION

Due to oil, protein, carbohydrate, mineral, and vitamins they contain, some plant seeds have a critical place in human and animal nutrition and biodiesel production. Most of the oils needed in human nutrition are obtained from vegetable oils. 92% of the world's oil production is obtained from vegetable sources and 8% from animal sources.¹ Seeds are an important

source of various vegetable oils. Fatty acid content and biological activity of seeds are the factors that determine the use of a vegetable oil in nutrition, pharmaceutical or industrial areas.^{1,2} Vegetable oil production in the world is mainly met with palm oil, soybean, rapeseed, olive, safflower, corn, sunflower, peanut, sesame, castor oil, poppy, flax, hemp, and jojoba. Most of the vegetable oil production in Türkiye is based mainly on sunflower; sunflowers constitute 69% of vegetable oil

*Correspondence: didemdeliorman@gmail.com; didem@gazi.edu.tr, Phone: +90 533 745 96 68, ORCID-ID: orcid.org/0000-0003-3916-4048

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production, 32% of total oil consumption. Therefore, safflower, whose production is low, may be a hope of closing vegetable oil gap in the market. 80% of world safflower production is carried out by Kazakhstan (24.6%), Russia (19.2%), Mexico (16.1%), India (11.9%), and Türkiye (8.2%).¹

Carthamus tinctorius L. is a bushy, herbaceous plant from Asteraceae family and grown in arid or semi-arid regions or where moderately salty water is used. Safflower oil (*Carthami oleum raffinatum*) is a fixed oil obtained by squeezing or extracting the seeds of *C. tinctorius* and then refining.³⁻⁵

Except using these oils for dietary purposes, many vegetable oils are sold with health-promoting claims or statements that they are beneficial against diseases, while safflower oils are one of them, as they are sold in “natural”, “organic products”, “local products” shops, cosmetics store chains, and pharmacies. Safflower oil, which is marketed for health benefits and cosmetics, must meet European Pharmacopoeia criteria. Currently, these safflower oils are marketed as fixed oils with the producer’s own marketing and quality criteria, while a pharmacopoeia’s core mission is to protect public health by creating and making available public standards to help ensure the quality of products while the user or procurer can make an independent judgment regarding quality, thus safeguarding the health of the public.

In European Pharmacopoeia 7.0, “safflower oil, refined; *Carthami oleum raffinatum*” is registered under the name “refined safflower oil”, which is defined as oil obtained from the seeds of *C. tinctorius* (type I) or hybrid *C. tinctorius* seeds (type II) from extraction and/or extraction followed by refining.⁶ Type II oil is rich in oleic acid and contains antioxidant.⁷ Fatty acid content is one of the main factors that determine the use of a vegetable oil in nutrition, pharmaceutical or industrial areas.²

Safflower seeds contain high levels of polyunsaturated fatty acids and are used for dietetic, medical, and industrial purposes.^{8,9} 96-99% of total fatty acids in its content are oleic, linoleic, palmitic, and stearic acids, 9.7-10.8% of saturated fatty acids.² Fatty acid composition in safflower seeds usually consists of 71 to 75% linoleic acid (C 18:2), 16 to 20% oleic acid (C18:1), 6 to 8% palmitic acid (C16:0), and 2 to 3% stearic acid (C18:0).^{2,10-12}

There is much news about the use of safflower oil in weight control in the media and on the internet. Pharmacists also state that especially women often demand safflower oil for this purpose. Literature surveys have shown us that there is no scientific study on the effect of this oil on weight control. Therefore, in this study, we tested the effects of safflower oil on enzymes that digest carbohydrates, which is a rational approach to weight control. On the other hand, quality control evaluations of 11 safflower oil samples obtained from pharmacies, akhtars, and cosmetics shops were made in terms of the criteria in the relevant monograph in the European Pharmacopoeia 7.0. For this purpose, acid and peroxide values of the oil samples by volumetric method and fatty acid analysis were performed using gas chromatography-mass spectrometry (GC-MS) technique.

MATERIALS AND METHODS

Chemicals and instruments

All chemicals used were of analytical reagent grade. Heptane (99%), potassium hydroxide (KOH), methylene chloride (CH_2Cl_2) for GC-MS Supra Solv[®], sodium chloride (NaCl), sodium sulfate (Na_2SO_4), Supelco 37-component fatty acid methyl ester (FAME) mix (FAME37, C4-24), chloroform (CHCl_3), glacial acetic acid, acetone, phosphomolybdic acid, ether, phenolphthalein, 3,5-dinitrosalicylic acid, sodium potassium tartrate were purchased from Sigma Aldrich Co. and Riedel-de Haën (Seelze, Germany). Methanol (MeOH) containing not more than 0.5% (w/w) water, starch, potassium iodide (KI), sodium thiosulfate, and peroxide-free ether were purchased from Merck, Germany. Oksan Co., Ltd. (Ankara, Türkiye) provided helium, hydrogen, and dried air gas for GC with 99.99% purity. Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

GC (7890A GC System, Agilent Technologies Inc, US), a capillary column Rt-2560 (100 m, 0.25 mm ID, 0.2 μm) (Restek Corporation Bellefonte, US), vial insert, 250 μL , glass with polymer feet, vial, screw top, 2 mL, amber and cap, screw, blue, polytetrafluoroethylene/red silicone septa (Agilent Technologies Inc., US), and ELISA (Versamax Tunable Microplate Reader) were used in GC analysis and enzyme activity studies, respectively.

Safflower oil samples

Eleven different brands of safflower oils were purchased from pharmacies, akhtars, online, and cosmetics stores in Ankara/Türkiye in 2019. The samples were stored at 4°C until used in studies.

Acid value (V_A)

About 10 g of the oil (m) was dissolved in 50 mL of 96% MeOH and ether mixture (1:1, v/v). 0.1 M KOH was used as a titrant in the presence of phenolphthalein indicator until the pink remained stable for at least 15 s (n mL of 0.1 M KOH). Acid values of samples were calculated from the equation of $V_A = (5.610 \times n)/m$ and compared with value of maximum of 2.0 in a 5.0 g oil sample.¹³

Peroxide value (V_p)

About 5 g oil (m) was placed in a 250 mL conical flask fitted with a ground-glass stopper. 30 mL of a mixture of CHCl_3 and glacial acetic acid (2:3, v/v) was added. After the oil dissolved, 0.5 mL of saturated KI solution was added and shaken exactly 1 min, then 30 mL water was added. It was titrated with 0.01 M sodium thiosulfate until yellow color was almost discharged. 5 mL of starch solution was added and continued the titration, until the color was discharged (n_1 mL of 0.01 M sodium thiosulfate). We carried out a blank test under the same conditions (n_2 mL of 0.01 M sodium thiosulfate). Volume of 0.01 M sodium thiosulfate used in the blank titration did not exceed 0.1 mL. Peroxide value was calculated from the equation of $V_p = 10 (n_1 - n_2)/m$, and compared value of max 15.0 in 5 g oil.^{14,15}

Fatty acid analysis by GC-MS

Standard mixture of 37 fatty acids methyl esters (Supelco™ 37 Component FAME Mix, FAME37, C4-24) used for the GC analyses. FAME37 (100 mg) was stored at -20°C and all standard solutions were prepared in an ice bath. To prepare 400 mg/mL FAME 37 standard solution, 250 µL of CH₂Cl₂ solution was added to 100 mg of FAME37 standard, and vortexed. 75 µL of this solution was taken into a GC vial and 925 µL CH₂Cl₂ was added, and then vortexed by closing the mouth. FAME37 standard solution (100 µL; 30 mg/mL) was placed in a 250 µL polymer-footed glass tube placed in 2 mL amber colored vial and sealed, then analyzed by GC-MS under the following chromatographic conditions (Table 1).

FAME were prepared by *trans*-esterification of the oils with MeOH, using KOH as a catalyst before GC analysis. 2 mL of safflower oil sample was placed in a flat bottom, approximately 50 mm diameter and 30 mm long container and dried in the oven at 100-105°C. It was allowed to cool in a desiccator with silica gel. 1 g of the oil was weighed into a 25 mL round-bottomed flask with a ground-glass neck fitted with a reflux condenser and a gas port into the flask. Anhydrous MeOH (10 mL) and 0.2 mL of 60 g/L KOH in MeOH were added. Then, flux condenser was attached, passed nitrogen through the mixture

Table 1. GC-MS conditions

GC conditions	
Component	Condition
Device	Agilent Technologies 7890A GC system, Agilent Technologies Inc, Santa Clara, MS detector
Column	Restek-2560, bisssano propyl polysiloxane (100-m x 0.25 µm ID x 0.20 µm)
Oven temperature	100°C (hold 4 min), increased to 24°C by 3°C min (hold 15 min)
Injection temperature	225°C
Detector temperature	250°C
Carrier gas, flow rate	He, 1.0 mL/min
Injection volume	2 µL
Split ratio	100:1
MS condition	
Component	Condition
Device	Agilent Technologies 5977E MS
Mode	Scan
Solvent Delay	11.5 min
Mass range	20-300
Step size	0.1 m/z
Scan speed	3.125 (u/s)
Frequency	9.0 scan/sec
Cycle time	110.96 ms

at a flow rate of about 50 mL/min, mixed, and heated to boiling. When the solution was clear (usually after about 10 min), it was continued heating for a further 5 min, and cooled the flask and transferred the contents were to a separating funnel. The flask was rinsed with 5 mL heptane, transferred the rinsing to the separating funnel, and stacked. NaCl solution (10 mL of a 200 g/L) was added and stacked vigorously. It was allowed to separate two phases and transferred the upper organic layer to a vial containing anhydrous Na₂SO₄, allowed to stand, then filtered, and FAME compositions were determined by GC-MS under the chromatographic conditions (Table 1).¹⁶ Results are expressed as % (w/w) with respect to all fatty acids detected.

Enzyme inhibition methods

α-Amylase enzyme inhibition

The effects of safflower oil samples in *α*-amylase enzyme were determined by modifying the method of Ali et al.¹⁷ *α*-Amylase enzyme (EC 3.2.1.1, type I-A, Sigma) was prepared in water at a concentration of 4 U/mL. Potato starch solution (0.5%) prepared in 20 mM phosphate buffer was used as the substrate. Safflower oil samples were prepared in 100% MeOH at concentrations of 1, 0.5, and 0.25 mg/mL. The samples were treated with *α*-amylase enzyme (4 U/mL) at 37°C. They were incubated at 37°C and starch solution was added. Further, 50 mL of DNS color solution (96 mM 3,5-dinitrosalicylic acid, 5.31 M sodium potassium tartrate in 2 M NaOH) was added and kept at 85°C for 15 min. Later, the mixtures were diluted with water and the tubes were allowed to cool to complete the reaction. The absorbance of the mixture was measured at 540 nm with an ELISA (Versamax Tunable Microplate Reader) plate reader. Acarbose was used as the reference substance. The change in absorbance resulting from the amount of maltose formed was calculated using equation 1.

The amount of maltose formation was measured using the maltose standard calibration curve (between 0.0 and 0.1%, w/v) maltose concentration *versus* net absorbance value, $y = 0.4428x + 0.0264$, $r^2 = 0.9926$. Percentages of inhibition were calculated using equation 1.

$$[A (\text{control solution}) / A (\text{oil sample})] = [A (\text{oil sample}) / A (\text{blank})] \quad (\text{equation 1})$$

A: Absorbance

α-Glucosidase enzyme inhibition

Inhibitory effects of the safflower oil samples on *α*-glucosidase were determined using the method of Lam et al.¹⁸ The *α*-glucosidase (type IV) obtained from *Bacillus stearothermophilus* was prepared in 0.5 M phosphate buffer (pH 6.5).

Safflower oil samples were prepared by dissolving in 100% MeOH at concentrations of 1, 0.5, and 0.25 mg/mL. The solutions were incubated with *α*-glucosidase in a 96 well microplate reader at 37°C. Subsequently, substrate solution (10 µL, 20 mM *p*-nitrophenyl-*α*-D-glucopyranoside) was added and the reaction was allowed to occur at 37°C. At the end of the

period, color intensity was measured with an ELISA (Versamax Tunable Microplate Reader, USA) plate reader at a wavelength of 405 nm was used as the reference substance. Percentages of inhibition were calculated using equation 2.

$$\text{Inhibition (\%)} = [1 - (A \text{ (oil sample)} / A \text{ (control)})] \times 100 \quad (\text{equation 2})$$

A: Absorbance

Statistical analysis

While evaluating the test results, standard deviations (SD) were calculated in MS Excel program on the Windows XP operating system. All the results were given for at least as triplicate and values were expressed as mean \pm SD.

RESULTS

Acid and peroxide values of the oil samples

Acid and peroxide values of the safflower oil samples are given in Table 2. According to European Pharmacopoeia 7.0, acid value of the safflower oils should be at most 0.5 and the peroxide value should be 10. Except for n_1 and n_5 oils, acid value of 9 other oils is above the reference value of 0.5. On the other hand, only 2 (n_1 and n_4) out of 11 oil samples have the peroxide value below the reference value.

Table 2. Acid and peroxide values of the oil samples

Samples	n_1	n_2	n_3	n_4	n_5	n_6	n_7	n_8	n_9	n_{10}	n_{11}
$V_A^a \pm SD^c$	0.2 \pm 0.1	1.4 \pm 0.1	1.3 \pm 0.2	1.2 \pm 0.0	0.4 \pm 0.0	0.7 \pm 0.1	0.8 \pm 0.2	1.3 \pm 0.1	1.9 \pm 0.1	0.8 \pm 0.1	1.1 \pm 0.1
$V_P^b \pm SD^c$	7.5 \pm 1.6	28.6 \pm 0.2	14.6 \pm 0.3	8.2 \pm 0.4	12.8 \pm 2.1	14.8 \pm 1.5	157.7 \pm 6.1	25.4 \pm 3.4	18.4 \pm 1.9	87.8 \pm 0.0	30.4 \pm 1.5

^a: Acid value, ^b: Peroxide value, ^cSD: Standard deviation, n= 3

Table 3. Fatty acid composition of safflower oil (type I) according to European Pharmacopoeia monograph 7.0¹⁴

FAMES	% (w/w)
C16:0	4-10
C18:0	1-5
Σ saturated FAMES (C16:0 and C18:0)	5-15
C18:1	8-21
C18:2	68-83
Σ unsaturated FAMES (C18:1 and C18:2)	76-105

Table 4. Fatty acid compositions of the oil samples by using GC-MS

FAMES	Samples (% w/w)										
	n_1	n_2	n_3	n_4	n_5	n_6	n_7	n_8	n_9	n_{10}	n_{11}
C16:0	3.27	4.08	12.01	5.32	3.57	6.16	0.54	2.24	2.21	2.43	2.73
C18:0	0.05	0.43	0.17	1.35	1.28	0.52	0.04	0.23	0.09	0.46	0.58
Σ saturated FAMES (C16:0 and C18:0)	3.32	4.51	12.18	6.67	4.85	6.68	0.58	2.47	2.30	2.89	3.31
C18:1	4.09	6.32	32.06	40.34	24.51	23.17	9.71	9.61	6.25	12.29	9.75
C18:2	92.59	87.73	47.04	49.69	68.20	69.52	89.64	89.92	91.45	84.81	86.90
Σ unsaturated FAMES (C18:1 and C18:2)	96.68	94.05	79.10	90.03	92.71	92.69	99.35	99.53	97.70	67.10	96.65

Fatty acid composition of the oil samples

The pharmacopoeia mentions two types of safflower oil. The oil obtained from the seeds of *C. tinctorius* from expression and/or extraction is called type I and the oil obtained from the seeds of the hybrids of this plant is called type II fixed oil. Since it is not stated on the packages of the purchased oil samples whether the plant from which the oil is obtained is hybrid or not, the results of the fatty acid analysis were evaluated according to the results of type I oil (Table 3).

Quantitative analysis of fatty acids contained in the samples was carried out over the peak areas by comparing the retention times of standard fatty acids and the results are given in Table 4. According to this, safflower oil samples are rich in monounsaturated (oleic acid) and polyunsaturated (linoleic acid) fatty acids (67.10-99.53%) of total fatty acids in its content are oleic, linoleic, palmitic, and stearic acids. Saturated fatty acids are 0.58 to 12.18 % of the total FAMES in oils. The fatty acid composition in safflower oils consisted of 47.04 to 92.59% linoleic acid (C18:2), 4.09 to 40.34% oleic acid (C18:1), 0.54 to 12.01% palmitic acid (C16:0), and 0.05 to 1.35% stearic acid (C18:0). Considering all these results and the values in the monograph, it was seen that none of the oil samples were suitable for the pharmacopoeia.

α -Amylase and α -glucosidase enzyme inhibitory activities of the oil samples

All oil samples were tested at concentrations of 0.25, 0.5, and 1.0 mg/mL to evaluate the inhibitory effects on both enzymes (Table 5). The results showed that safflower oil samples had no activity on α -glucosidase, which is an enzyme that digest carbohydrates. A feeble α -glucosidase inhibitory effect was detected only in sample number 11. The inhibition values of this sample were calculated as $6.70 \pm 2.55\%$ at a concentration of 0.25 mg/mL and $4.58 \pm 1.03\%$ at a concentration of 1 mg/mL. Inhibition values of acarbose used as the reference was determined as $98.19 \pm 0.53\%$ (0.25 mg/mL), $99.53 \pm 0.04\%$ (0.5 mg/mL), and $99.57 \pm 0.04\%$ (1 mg/mL).

Generally, it was found that the oil samples did not show a significant inhibitory effect on α -amylase. Only sample number 1 showed a moderate inhibitory activity ($52.13 \pm 2.87\%$) at a concentration of 1 mg/ml. At the same concentration, acarbose displayed 100% inhibitory activity.

DISCUSSION

Acid value is used as a shelf-life monitoring parameter in the quality control of oils. The high free fatty acidity is one of the rancidity indicators of any oil and an increase in the oxidation potential of the oil and decreases stability. Except for oil samples 1 and 5, all samples were found to be rancid and oxidized. Peroxide value is a measure of the amount of active oxygen in oils, and the amount of peroxide in 1 kg oil in milliequivalent grams of oxygen. Oxygen causes smaller molecule fatty acids. Oils can deteriorate due to various factors (storage conditions, metal ions, temperature, light, etc.). The peroxide value shows oxidation state of the oil. It also indicates whether the deodorization process has been done effectively.^{17,18} Again, it was determined that all samples were highly oxidized except for oil samples 1 and 4.

Palmitic, stearic, oleic, and linoleic acids were detected in all oil samples. It was determined that oleic acid (8.0-21.0%) and linoleic acid (68.0-83.0%), which were reported as the main fatty acids in the European Pharmacopoeia 7.0, were not among the desired amounts in the samples. The contradictory situation we detected in the samples for these two fatty acids led us to predict that oleic acid is oxidized into linoleic acid.

There are many *in vitro* and *in vivo* methods to evaluate the effects of pure compounds or plant extracts on weight control. In this study, the effects of safflower oils, which have been in high demand from pharmacies for weight control recently, on α -amylase and α -glucosidase that help carbohydrate digestion were evaluated and the oil samples did not show any inhibitory effect. However, the fact that none of the oil samples met the quality standards may have caused unpredictable interactions in activities of the enzymes.

Previously, Takahashi and Miyazawa¹⁹ studied the effects of MeOH extracts of safflower seeds on α -glucosidase enzyme. As a result, compounds with stronger inhibitory effects than acarbose (*N-p*-coumaroyl serotonin and *N-feruloyl* serotonin) were isolated from the extract.¹⁹ However, it is clear that the secondary metabolite contents of seed oils will be different from seed MeOH extracts. In a study examining the activities of 10 fatty acids on enzymes known to be associated with diabetes, Su et al.²⁰ showed that oleic and linoleic acids have potent glucosidase enzyme inhibitory activity. In short, the fact that the oils analyzed in this study were highly oxidized and the fatty acid content was not between the desired values should be a factor to be considered in determining the effectiveness.²⁰

Safflower oils are among the commonly used oils because they have many activities (antioxidant, antiulcer, cardioprotective, antinociceptive, anti-inflammatory, hepatoprotective, anti-

Table 5. α -Amylase inhibition results of the oil samples

Samples	Inhibition % \pm SD		
	0.25 mg/mL	0.5 mg/mL	1 mg/mL
n ₁	-	5.37 \pm 2.87	52.13 \pm 2.87
n ₂	13.81 \pm 3.57	-	7.73 \pm 0.86
n ₃	-	15.53 \pm 3.26	6.32 \pm 1.89
n ₄	13.96 \pm 3.50	3.42 \pm 1.05	13.81 \pm 1.06
n ₅	8.59 \pm 3.50	4.91 \pm 1.77	-
n ₆	-	11.52 \pm 1.07	-
n ₇	8.11 \pm 2.86	5.38 \pm 1.18	21.08 \pm 7.68
n ₈	16.09 \pm 3.73	17.23 \pm 1.05	24.45 \pm 5.06
n ₉	28.37 \pm 4.90	20.93 \pm 2.40	24.66 \pm 2.81
n ₁₀	40.38 \pm 3.25	18.15 \pm 4.14	16.98 \pm 2.98
n ₁₁	-	7.54 \pm 1.83	4.01 \pm 5.50
Acarbose	92.70 \pm 5.80	98.45 \pm 6.66	100.00 \pm 0.04

-: No inhibition, SD: Standard deviation, n= 3

cancer, antidiabetic, and weight control activities²¹⁻²⁶ as well as their use in food and cosmetics, while most of these health benefits are associated with the oil content and the fatty acid composition of the respected oils. These oils, which contain significant number of oxygen radicals, will cause serious health problems after chronic exposure, especially considering that they are used daily by people who buy them for weight control. Meanwhile, evaluating the effectiveness of safflower oils on weight control with other methods can be considered another research topic. In conclusion, the high oxidation rates and significant variability in fatty acid content (especially for unsaturated fatty acids) detected in 11 purchased samples indicated that more attention should be paid to the quality control of these oils, which points out to fact that they would be beneficial in terms of the health benefits expected from safflower oils.

CONCLUSION

Our literature survey has shown that quality control analysis of the safflower oils in Turkish market and their effectiveness on two enzymes (α -amylase and α -glucosidase) was performed in this study for the first time. Within this study, we conducted in 11 safflower oil samples we bought from the market; it was concluded that these oil samples did not meet the quality criteria required by European Pharmacopoeia for safflower oil. Interestingly, it was observed that the amount of oleic acid was very low, while the amount of linoleic acid was high. This result suggested that oleic acid in oils was oxidized to linoleic acid due to the production methods and storage conditions. The results of enzyme inhibitory activities of the safflower oil samples showed that these oils could not have any effect on weight control by inhibiting the enzymes that provide carbohydrate digestion.

Safflower, whose market cap and production are low, may be the hope of closing the vegetable oil gap for the present/future of the growing world's population demand. In addition to increasing oil production, the end product also meets the expectations in terms of quality. Likewise, if the oil is used or at least marketed with health claims (with or without pharmaceutical usage), to establish the necessary quality criteria and show no harm to the user, the safflower oil (if for human use) needs to be encouraged to be European Pharmacopoeia compliance.

Ethics

Ethics Committee Approval: There is no requirement for ethical approval.

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: D.D.O., Design: D.D.O., Data Collection or Processing: D.D.O., D.S., S.P., B.Ö., A.B., O.K.U., Analysis or Interpretation: D.D.O., D.S., S.P., B.Ö., A.B., O.K.U., Literature Search: D.D.O., O.K.U., Writing: D.D.O., O.K.U.

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