The Effect of Different Solvent Types and Extraction Methods on Oil Yields and Fatty Acid Composition of Safflower Seed

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Abstract: The aim of this study was to determine the effect of different extraction solvents (petroleum benzene, hexane, diethyl ether and acetone) and extraction methods (hot and cold) on oil yield of safflower seeds and its fatty acid compositions. Oil contents of safflower seeds extracted by hot extraction system were changed between 37.40% (acetone) and 39.53% (petroleum benzene), while that of cold extraction was varied between 39.06% (petroleum benzene) and 39.40% (diethyl ether). Regarding the extraction solvents, the highest oil yield (39.53%) was obtained with petroleum benzene, while the minimum value (37.40%) was found with acetone under hot extraction condition. The main fatty acids observed in all extracted oil samples were linoleic, oleic and palmitic acids. Oleic acid contents of safflower oils extracted by hot extraction system was ranged between 41.20% (acetone) and 42.54% (hexane), its content in oils obtained by cold extraction method was varied between 40.5% (acetone) and 41.20% (hexane and diethyl ether). Linoleic content of safflower oil extracted by hot extraction system was found between 48.23% (acetone) and 49.62% (hexane), while that oil extracted by cold method range from 48.07% (hexane) to 49.09% (acetone). The fatty acid composition of safflower seeds oil showed significant (p < 0.05) differences depending on solvent type and extraction method. The results of this study provide relevant information that can be used to improve organic solvent extraction processes of vegetable oil.

Key words: safflower, oil content, extraction method, solvent type, fatty acid composition, GC

1 Introduction

Safflower (Carthamus tinctorius L.) is a member of the Asteraceae family. Its seeds are used for production of cooking oil and also used as salad dressing and colorant¹. Safflower seeds contain 38–48% oil, 15–22% protein and 11–22% fiber. Moreover, the fatty acid composition of safflower seed oil is 6–8% palmitic, 2–3% stearic, 16–20% oleic and 71–75% linoleic acids². It is well known that consumption of oily foods having high content of polyunsaturated fatty acids could help in prevention atherosclerosis and cardiovascular diseases³.

Commercial production of vegetable oils is based mainly on mechanical pressing and solvent extraction. Mechanical pressure method is the most common technique for oil extraction form plant seeds as it could eases the release of oil droplets from oil-bearing matrix in seeds⁴. However, the presently available mechanical oil-extraction equipment and processes has low extraction efficiency (<70% oil extraction) and are thus considered inadequate to extract all oil from the seeds⁵. Conventional oil extraction methods have certain disadvantages which has encouraged the development of alternate modern and green techniques⁶. Numerous extraction methods such as cold press, Soxhlet extraction, water steam distillation, classical solvent extraction, supercritical fluid extraction, ultrasonic assisted extraction, and microwave extraction has been used for vegetable oil extraction⁷, ⁸. Supercritical fluid extraction technique has been extensively studied as an alternative to conventional oil extraction methods⁹. The most frequently used supercritical oil extraction method is supercritical carbon dioxide (SC-CO₂) because it is nontoxic, nonflammable, inexpensive, and easily separated from the extract¹⁰, ¹¹.

Solvent extraction has great advantage as high yield (>
99 wt.%) can be obtained at economically reasonable costs. In this method, extraction of oil is performed using organic solvents such as acetone, chloroform, petroleum benzene, diethyl ether and hexane. The amount of polar and non-polar lipids in the sample together with the type of lipids could significantly affect solubility and extractability of lipids in the organic solvent. Soxhlet extraction is a standard method, which provides higher oil yield in comparison to several techniques such as cold press. In addition, this extraction method has serious disadvantages as necessity high process time and temperature, which has adverse effects on oil quality. Besides, conventional soxhlet apparatus cannot agitate and addition evaporation step must apply following extraction process. However, cold extraction method could give high oil yield without major effect on the quality of the extracted oil. In addition, reports on the comparison of cold and hot extraction methods using various solvents to extract oil from safflower seeds are scarce. The purpose of this study was to determine the effect of both cold and hot extraction (Soxhlet) methods on oil yield and fatty acid composition of safflower seeds by using different solvents.

2 Material and Methods

2.1 Materials

Safflower seeds were obtained from Selcuk University, Faculty of Agriculture, Department of Field Crops, Turkey. The seed and kernels were transferred to the laboratory in polypropylene bags under cold conditions. The seeds were cleaned of any adhering residue and dried at 70°C for 24 h. Dried seeds were ground in a mill and screened through a mesh of 0.5 mm diameter. The powdered samples were put into colored bottle and kept in a refrigerator before use. All solvent and other chemicals were purchased from Sigma-Aldrich.

2.2 Methods

2.2.1 Oil extraction

Oil content of sample was determined according to AOAC method. Petroleum benzene, hexane, diethyl ether and acetone were used for extraction the total oil from safflower seed under hot (Soxhlet extraction for 5 h) and cold (rinsing bath at room temperature for 12 h) conditions. The solvents were removed with a rotary vacuum evaporator at 50°C.

2.2.2 Fatty acid composition

Fatty acid methyl esters of safflower seed oils were esterified according to ISO-5509 (ISO-International Organization for Standardization) method. The esterified samples were analyzed using gas chromatography (Shimadzu GC-2010) equipped with flame-ionization detector (FID) and capillary column (Tecnocroma TR-CN100, 60 m × 0.25 mm, film thickness: 0.20 μm). The injection block and detector temperatures were set as 260°C. Nitrogen gas was used as a mobile phase and run at a flow rate of 1.51 mL/min. Total flow and split rates were 80 mL/min and 1/40, respectively. Column temperature was programmed as 120°C for 5 min, and then increased 240°C at 4°C/min and held for 25 min at 240°C.

2.3 Statistical Analysis

Analysis of variance (ANOVA) was carried out using JMP software, version 9.0 (SAS Inst. Inc., Cary, N.C.U.S.A). All analyses were carried out in triplicates and the results expressed as means ± standard deviation (MSTAT C).

3 Results and Discussion

Oil contents of safflower seeds extracted with different solvents are presented in Fig. 1. The oil contents of safflower seeds extracted by hot extraction system were changed between 37.40% (acetone) and 39.53% (petroleum benzene), oil contents of seed obtained by cold extraction system were varied between 36.96% (petroleum benzene) and 39.40% (diethyl ether). Regarding the influence of the extraction solvents, the highest oil yield (39.53%) was achieved with petroleum benzene, while the minimum value was obtained with acetone (37.40%) under hot extraction conditions using Soxhlet apparatus. These findings suggested that the polarity of the solvents affect the extraction of oils from safflower seeds under hot extraction conditions, in which, marginally non-polar solvents (petroleum benzene and diethyl ether) were more efficient than polar solvent (acetone). However, under cold extraction conditions, diethyl ether was the most convenient solvent to extract oil as it yields about 39.40% oil. Petroleum benzene and hexane were improper for cold extraction because the oil yield of these two solvents was low at cold extraction system. Concerning Soxhlet extraction, petroleum benzene and diethyl ether provided higher oil yield in comparison to other solvents (hexane and acetone). It is clear that solvent type and extraction conditions significantly (p < 0.05) affected the oil yield from safflower seeds. Similarly, previous reports indicated that slightly non-polar solvents were more effective for extraction of oils from sesame seeds and Niger seeds. It is generally accepted that both the nature of the oil and solvent polarity affect the oil yield and composition.

Fatty acid compositions of safflower seed oils are presented in Table 1. Palmitic acid contents of safflower oils extracted by hot extraction method varied between 2.14% (diethyl ether) and 2.36% (petroleum benzene) while palmitic acid contents of safflower oils obtained by cold extraction system were determined between 2.17% (petroleum benzene) and 2.30% (acetone). While oleic acid
contents of safflower oils extracted by hot extraction system change between 41.20% (acetone) and 42.54% (hexane), oleic acid contents of seed oils obtained by cold extraction method varied between 40.58% (acetone) and 42.10% (hexane and diethyl ether). In addition, linoleic contents of safflower seed oils extracted by hot extraction system were determined between 48.23% (acetone) and 49.62% (hexane) while linoleic acid contents of safflower oil by cold extraction method between 48.07 (hexane) and 49.09% (acetone). As seen in Table 1, depending on extraction types, fatty acid compositions of safflower seed oils showed partial differences. Statistically differences were observed among fatty acid compositions depending on extraction method and solvent types ($p < 0.05$). 

### Table 1  
Fatty acid compositions of safflower oils extracted with different solvent and extraction methods.

| Fatty acids | Petroleum benzine | Hexane | Diethyl ether | Acetone       |
|------------|-------------------|--------|---------------|---------------|
| Myristic   | 0.08 ± 0.00a**    | 0.07 ± 0.01b | 0.08 ± 0.00b | 0.07 ± 0.01a  |
| Palmitic   | 5.88 ± 0.04a***   | 5.60 ± 0.29ab | 5.50 ± 0.02bc | 5.36 ± 0.11c  |
| Stearic    | 2.36 ± 0.08a      | 2.20 ± 0.01b | 2.14 ± 0.04bc | 2.19 ± 0.02b  |
| Oleic      | 41.98 ± 0.42a     | 41.20 ± 0.10b | 42.50 ± 0.24c | 42.54 ± 0.10b |
| Linoleic   | 48.31 ± 0.42a     | 49.62 ± 0.24c | 48.46 ± 0.17b | 48.23 ± 0.29a |
| Arachidic  | 0.37 ± 0.00c      | 0.35 ± 0.01c | 0.35 ± 0.01c  | 0.36 ± 0.00b  |
| Linolenic  | 0.13 ± 0.09c      | 0.11 ± 0.05c  | 0.10 ± 0.07bc | 0.13 ± 0.10a  |
| Arachidonic| 0.12 ± 0.00c      | 0.12 ± 0.00a  | 0.12 ± 0.00a  | 0.13 ± 0.00b  |

| Fatty acids | Petroleum benzine | Hexane | Diethyl ether | Acetone       |
|------------|-------------------|--------|---------------|---------------|
| Myristic   | 0.08 ± 0.00       | 0.08 ± 0.00 | – ****        | –             |
| Palmitic   | 5.62 ± 0.02b      | 5.72 ± 0.09b | 5.73 ± 0.13c  | 6.55 ± 0.01e  |
| Stearic    | 2.17 ± 0.03a      | 2.20 ± 0.03b | 2.18 ± 0.02c  | 2.30 ± 0.06e  |
| Oleic      | 42.02 ± 0.21a     | 42.10 ± 0.31a | 42.10 ± 0.01a | 40.58 ± 0.25b |
| Linoleic   | 48.25 ± 0.34a     | 48.07 ± 0.04b | 48.75 ± 0.08b | 49.09 ± 0.00e |
| Arachidic  | 0.36 ± 0.00a      | 0.26 ± 0.13d  | 0.35 ± 0.00b  | 0.27 ± 0.07e  |
| Linolenic  | 0.15 ± 0.12d      | 0.21 ± 0.05a  | 0.16 ± 0.01f  | 0.18 ± 0.01b  |
| Behenic    | 0.23 ± 0.00c      | 0.21 ± 0.02b  | 0.18 ± 0.02d  | 0.20 ± 0.01f  |
| Arachidonic| 0.14 ± 0.02a      | 0.12 ± 0.00b  | –             | –             |

*mean ± standard deviation; **Values within each column followed by different letters are significantly different ($p < 0.05$); ***Not detected.
Concerning the unsaturated fatty acids, linoleic acid was the dominant acid in all oils, which varied from 48.07 to 49.62\% from 40.58 to 42.54\% in safflower seed oils. Among the saturated fatty acids, palmitic acid was determined as the major acid, with a content varying from 5.36 to 6.55\%. Results of fatty acid analysis showed that fatty acid compositions of seed oils were significantly affected by using different extraction solvents and methods. In hot extraction, the highest oleic acid (42.54\%) and the lowest linoleic acid (48.23\%) contents were observed in sample extracted with acetone (p<0.05). Contrary to this, the minor oleic acid (40.58\%) and the major linoleic acid (49.09\%) amounts were determined using acetone with cold extraction. In addition, oleic and linoleic acid contents of oil samples extracted by petroleum benzene and hexane solvents in cold extraction method did not show significant differences. Apparently, the polarity of the solvent and nature of oil affected the quality of extracted oils as the quantity of fatty acids in safflower oils are differed significantly between different extracting solvents and methods. Several previous reports have studied the impacts of solvents types and extraction methods on the quantity and quality of vegetable oils\textsuperscript{21-24}. According to Matthaus \textit{et al.}\textsuperscript{25}, oil contents of safflower seeds ranged from 23.10 to 36.50\% and the dominant fatty acids were linoleic (54.30-77.00\%), oleic (12.50-35.20\%) and palmitic (5.74-6.81\%) acids. Abdolshahi \textit{et al.}\textsuperscript{26} determined the fatty acid profile of pistachio oil extracted with different solvents such as hexane, dichloromethane, ethyl acetate and ethanol. The major unsaturated fatty acid amount was found using Soxhlet apparatus with ethyl acetate, with the range of 88.49\%. Aquino \textit{et al.}\textsuperscript{13} extracted the oil from pequi pulp with several solvents (hexane, acetone and ethyl alcohol) and their mixtures, and extraction using acetone and hexane had higher yield. Silva \textit{et al.}\textsuperscript{14} reported that the highest oil content of chia (\textit{Salvia hispanica} L.) was observed using hexane and ethyl acetate and fatty acid profile did not effect change of solvent. Results showed some minor differences compared to literature values. These differences can be probably due to extraction method, sample composition (hull and/or non-hull), nature of the oil in the seeds, and solvent types.

Multivariate analysis using HJ-Biplot method\textsuperscript{27} clearly indicated the combined effects of solvent type (acetone, petroleum benzene, diethyl ether, and hexane) and extraction method (hot and cold) on oil yield and fatty acid composition of safflower seed oil (Fig. 2). The results indicated a great contribution of the principle components axes (PC1 and PC2) to the total variability (70.29\%) of the blotted data. Clustering analysis clearly showed three distinct clusters with hot extraction method of all solvent types formed single cluster, while cold extraction formed two clusters. With different dependencies of the solvent type used, hot extraction resulted in higher oil contents and values of arachidic, arachidonic, myristic, and oleic compared to cold extraction. Whereas, cold extraction with hexane and to lesser extend petroleum benzene resulted in oil with higher values of linolenic and behenic acid than other solvents and extraction methods. However, cold extraction with diethyl ether and acetone extract oil with higher palmitic, stearic and linoleic acids. These findings clearly demonstrated that both solvent types and extraction method have

![HJ-biplot based on principle component analysis (PCA) showing the interactive effects of solvent types and extraction method on the oil yield and fatty acid profile of safflower seeds oil.](image-url)
significant impacts on the oil yield and fatty acid composition of safflower seeds. Similarly, numerous studies indicated that both solvent type and extraction methods influenced the oil yield and fatty acid profile of various seed oils.  

Conclusion

Diethyl ether was a convenient solvent to obtain high oil yield (39.40%) from safflower seeds in cold extraction. The fatty acid composition of seed oils in hot extraction showed significant differences depending on solvent types. However, the fatty acid composition partly changed according to solvent types in cold extraction. Oleic and linoleic acid contents of oil samples extracted by Petroleum benzene and hexane solvents did not show significant differences. The authors would like to extend their sincere appreciation to the "Researchers Supporting Project" number (RSP-2019/83), King Saud University, Riyadh, Saudi Arabia.

Acknowledgements

The authors would like to extend their sincere appreciation to the "Researchers Supporting Project" number (RSP-2019/83), King Saud University, Riyadh, Saudi Arabia.

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