

Extraction Method and Solvent Effect on Safflower Seed Oil Production

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ABSTRACT
As a result of an increased world population, a new trend of producing vegetable oils from alternative vegetables/plants rather than sunflower and soy bean has arisen. Safflower, soybean, dotter (camelina), rapeseed (colza), canola are the examples of these alternative vegetables where specifically safflower attracted great interest owing to its linoleic acid rich content. In this study, we aimed to evaluate oil production from safflower seeds using different kinds of solvents (diethyl ether, petroleum ether, hexane, acetone and ethanol) and extraction methods (classical solvent extraction, Soxhlet extraction and ultrasonic extraction) and compare them in terms of the process viability and the composition of final product at various parameters. Our results showed that ultrasonic extraction method is more advantageous than traditional methods in terms of the time and solvent consumption in which the highest yield was obtained with the diethyl ether whereas ethanol showed the least.
Keywords: Safflower, ultrasonic, classicalsolvent, Soxhlet, extraction, linoleic acid

1. INTRODUCTION

Carthamus tinctorius L. or Safflower is a member of the Asteraceae family, which its seeds are noted for oil content. There are two types of safflower without thorns and barbed. Safflower is possibly originated in Southern Asia and is known to have been cultivated in China, India, Iran and Egypt. During the middle ages it was grown in Italy, France, and Spain, and afterward, it was introduced into North and South America from the Mediterranean region (Toma et al, 2014). In addition to this, especially in Turkey, due to their resistance to drought conditions, they can grow successfully in the drylands of Central Anatolia and surrounding regions with insufficient precipitation such as Ankara, Eskişehir, Konya and Çankırı (Ayas and Yılmaz, 2014). Safflower plant which is grown in many countries have many different uses; for example, it can be used as the colorant in the art, textile and lubricant industry (e.g. the petals of safflower are used for organic dye production), to flavor foods and also as therapeutic agent for many diseases Lijiano and Meili, 2013). Moreover, seed oil is used in food industry, for biodiesel production and as raw material for medicine and cosmetic industry.

Safflower seeds are rich in edible oil (40% dry matter weight) and contain several fatty acids including linoleic acid, oleic acid, margiric acid and palmitic acid, etc. The most important difference of safflower seeds from many other oilseeds is its high linoleic acid content (Sunflower oil contains 45% linoleic acid while safflower oil contains 80% linoleic acid) (Chorfa et al,2010). In the 40th Annual Meeting of the American College of Nutrition, researchers have united in five main benefits of linoleic acid which are inhibition of mammalian cancer cell growth, retardation and regression of atherosclerosis, modification of body fat metabolism and partitioning, anti-diabetic effect and stimulation of bone growth (Scimeca and Miller, 2000).

Methods used in vegetable oil production are as important as the content of the seed oil. There are many methods in the literature related to oil production which should be selected depending on the final product and process conditions. In general, these methods can be listed as Soxhlet extraction, water steam distillation, classical solvent extraction, supercritical fluid extraction, ultrasonic assisted extraction and microwave extraction (Sporring et al, 2005; Oniszczuk and Podorski, 2015).

Solvent extraction (SE) is a traditional method and classified as one of the most basic methods. SE method has the disadvantage of poor product quality, high investment, and energy requirement. It seeks the usage of organic solvents, in which the hexane is the most widely used one, that removal of these organic solvents through the process adds additional cost and labor (Gibbins et al, 2012).

Another conventional extraction method is the Soxhlet extraction (SXE). This method is widely used in laboratory scale. The disadvantages of Soxhlet extraction are high solvent requirements together with time and energy consumption. On the other hand, this method has the advantage of solvent recycling during the extraction over and over.

In order to eliminate afore mentioned disadvantages of the conventional methods, there has been an increasing tendency to alternative and greener extraction methods such as ultrasonic extraction, microwave extraction, supercritical fluid extraction, soxtec extraction etc. (Bampoili et al, 2014).

In microwave extraction, microwave energy is transmitted directly to the plant intra-structure through molecular interaction with the electromagnetic field. Once after the walls of the solid structure of the plant are destroyed, by thinning of the cell wall, higher yields could be reached. But this method is not always suitable for the plants since high microwave energy disrupts plant structure (Uqiche et al, 2008; Nde et al, 2015).

Ultrasonic extraction (UE) method is another method which reported as a suitable method for oil extraction. In UE, solvent contact with a solid structure (plant) is increased through vibration and heat and rigid cell walls are destroyed with high cavitations, which makes the method ideal for plant structure. The major advantages of this method are lower energy consumption and being eco-friendly (Hashemi et al, 2015; Tian et al, 2013). In the ultrasonic extraction method, the extraction efficiency is comparable with traditional methods, however, in this method, there is a significant reduction in the extraction time (Stainisavljevic et al, 2007). On the other hand, in comparison with the so called methods, ultrasonic extraction method reveals an increased extraction yield, increased rate of extraction by a reduction in extraction time and higher processing throughput. This method could be promising in order to alter the existing extraction processes and may allow new industrial extraction opportunities and processes (Vilkhu and Mawson, 2007). Li et al. (2004) have studied the oil production from soybean by ultrasonic extraction method where hexane was used as a solvent. Their results implied that ultrasound method has the potential to be used in oil extraction processes to improve efficiency and to reduce the process time which may have a significant impact on edible oil industry.

In this study, we aimed to evaluate oil production from safflower seeds using five different solvents with changing chemical composition and the performance of various extraction methods in terms of the process viability and the composition of the final product. Extracted final products were further characterized by gas chromatography and compared with content.

2. MATERIALS AND METHOD

2.1. Materials

The safflower seeds harvested from Kütahya territory (Turkey, TR) were obtained from a local company in Mersin, TR.

2.2. Method

2.2.1. Determination of Moisture Content

Moisture content of in plant material that was weighed approximately 5 g each was determined following the standard procedure provided at the section entitled as Method B of TS 1607 EN ISO 662. The calculations were performed as depicted in the method as well. Each experiment was repeated at least three times.

2.2.2. Grinding and Screen Analysis

Bosch MKM 6000 brand coffee grinder was used to decrease the particle size in the safflower seed samples and each particulate size was separated by a Fritsch brand vibratory sieving machine. Seed size for following experimental studies was chosen in between 500µm and 1mm.

2.2.3. Classic Solvent Extraction

2 g of safflower seed was grounded with classic solvent extraction method by using different solvents with a final volume of 20 mL. Ethanol, diethyl ether, petroleum ether, hexane and acetone were used as organic solvent. The extraction was performed with three interconnected operations at different times.

2.2.4. Soxhlet Extraction

Soxhlet extraction, which is traditionally considered as the extraction method resulting with the maximum yield, was carried out in a classic Soxhlet extractor (Figure 2.1) in the presence of ethanol, diethyl ether, petroleum ether, hexane or acetone as a solvent. 10 g of safflower seed mixed with 200 mL solvents was processed until the oil in ground seeds were all played out. After the operation completed, the solvent was removed until the oil came to the constant weighing. Oil yield was further calculated at the dry material base.

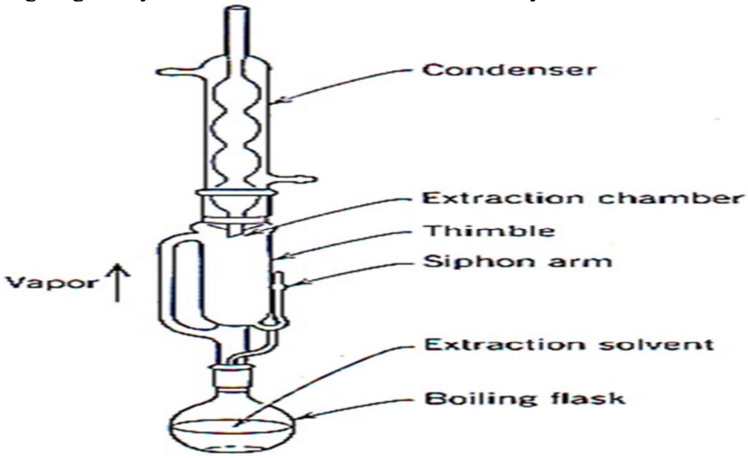

Figure 2.1 Soxhlet extraction system

2.2.1. Ultrasonic Extraction
Ultrasonic extraction was pursued with 2 g of ground safflower and 20 ml solvent mixture which exposed to ultrasonic vibration using Bandelin brand water-bath device. Experimental were conducted within different extraction times. Ethanol, diethyl ether, petroleum ether, hexane and acetone were also used as a solvent.

2.3. Determination of Extraction Yield
In all experiments, solvents were removed once after the extraction process was terminated and obtained extract was weighed. The extraction yield of each extraction procedure was calculated as described by Equation as the percentage of obtained extract mass to the initial sample based on dried weight.

$$\text{Extraction Yield (\%)} = \frac{\text{Mass of extract (g)}}{\text{Mass of dry matter (g)}} \text{x}100 \qquad (1)$$

2.4. Determination of Fatty Acid Composition
2.4.1. Preparation of Fatty Acid Methyl Esters (FAME)
10 mL hexane, 1.5 g of sodium sulphate and 0.75 mL potassium hydroxide with 2 N methanol were added to oil sample which is approximately 6 – 7 g and after that, it was shaken by hand. Obtained mixture was then centrifuged at 4000 rpm for ten minutes. 1 µL of this solution was taken from supernatant and analyzed by gas chromatography.
2.4.2. Analysis of Gas Chromatography
After fatty acid was turned into methyl esters, gas chromatography (Agilent 6890N) and fatty acid composition was determined. The condition of the gas chromatography was given in Table 2.1.

Table 2.1. Condition of the gas chromatography to determine FAME composition

System	Agilent 6890N
Column	HP 88 capillary column (100m x 250m x 0.20m)
Detector	FID
Carrier gas	Helium
Velocity of carrier gas flow	1.0ml/min
Injection heat	250°C
Detector heat	260°C
Split rate	40:1
Injection amount	1µl

3. RESULTS AND DISCUSSION
In this study, safflower seed was processed in order to obtained oil. The impact of solvent and the extraction methods on extraction yield was investigated by use of various extraction methods and solvents. Extraction times gathered from experimental studies for each parameter and yield (%) based on the dry matter were graphed. In the light of these results, extraction method used was found to be effective in the quality of the final oil and extraction yield. We further compared each method by means of their advantages and disadvantages.

3.1. Moisture content in safflower seed
As a result of the calculations, moisture content was designated as 5.28%. According to the procedure defined in TS 1607 EN ISO 662, drying process was not required since moisture content of the sample was under 10%.

3.2. Effect of particle size on extraction yield
Dry ground safflower seeds were sieved in the vibratory sifter. Particle size's impact on extraction yield was examined by using the seeds with particle size changing between 500 µm – 1 mm and 1 mm – 2 mm (Figure 3.1).

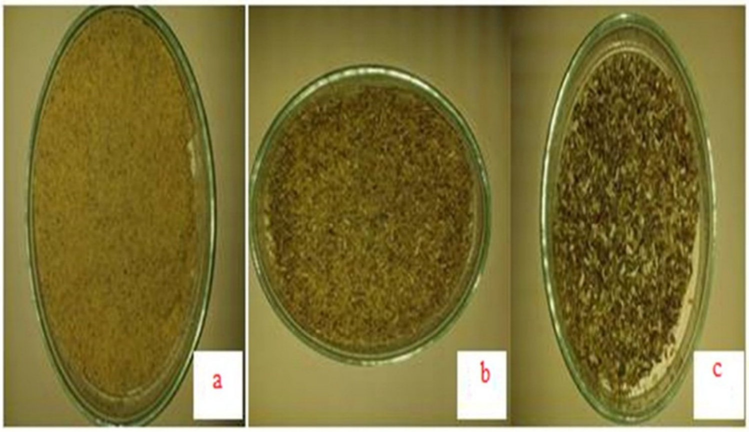

Figure 3.1. Particle size a) Undersize b) 500µm- 1mm c) 1mm – 2mm

Soxhlet extraction believed to be a traditional method was chosen as an extraction method. On the other hand, hexane which is the most frequently used was preferred as a solvent. In consequence of the experiments, as the particle size was getting smaller, firstly yield increased but then it decreased (Figure 3.2.). In the applied experiment by using 1 mm – 2 mm particle size seeds, the extraction yield was 27.13%; with 500 µm – 1 mm particle size seeds, the extraction yield was 34.08% and finally the experiment by using undersize, the extraction yield was 31.22%. As is seen from the Figure 3.2, shell rate of the ground safflower seed whose particle size was between 1 mm – 2 mm was higher than the others and it caused to increase dead zone and decrease the extraction yield. As 500 µm – 1 mm particle size and particle size in the undersize were getting smaller, there was a small reduce in the extraction yield. The reason for this decrease was thought the oil loss in the experiment during the milling process. In the subsequent studies, the samples were between 500µm – 1mm particle size. In the Soxhlet extraction method of Sluaiman and his colleagues in 2013, they studied to obtain hexane and coconut oil. Looking at the results, in a similar vein, when particle size was getting smaller, there is firstly a rise and then there is a decrease in oil yield% (Sulaiman et al, 2013).

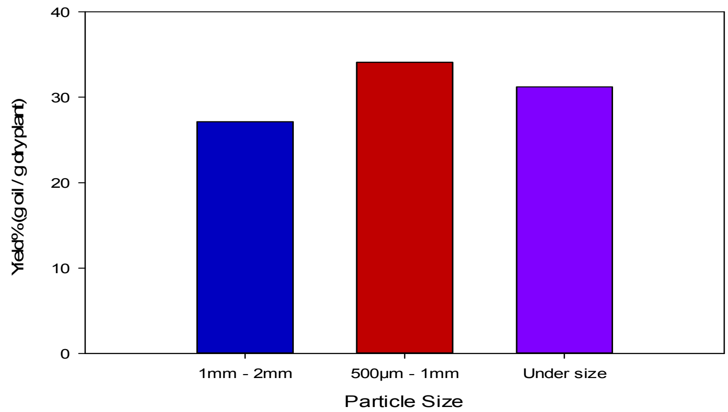

Figure 3.2. The effect of particle size on the extraction yield

3.3 *Classic Solvent Extraction*

As a traditional method, the classic solvent method was made of 5 different solvents (diethyl ether, petroleum ether, hexane, acetone, and ethanol) on the basis of solid - liquid extraction. The experiments were carried out at different extraction times (Figure 3.3). Advantages and disadvantages of the classic solvent extraction method were investigated. In consequence, of the experiments, the highest yield (20.72%) was reached in the experiment conducted with diethyl ether. On the other hand, as a result of the experiment conducted with ethanol, the least yield was reached (Figure 3.3).

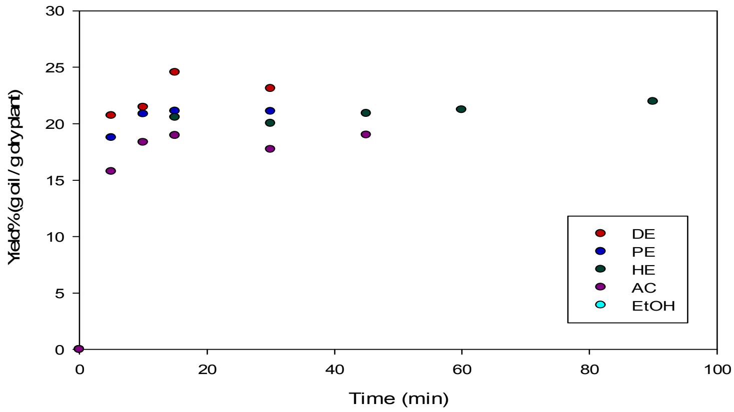

Figure 3.3. The effect of solvent type on conventional solvent extraction

3.4 Soxhlet Extraction

By the Soxhlet extraction method, oil was obtained from safflower seeds by using five different solvents which have different chemical structures (diethyl ether, petroleum ether, hexane, acetone, and ethanol). In all experiments a ratio of 1:20 solid solvent is used. The experiments conducted with 10 g safflower seed were performed by using 200 ml solvent. The Soxhlet extraction carried out with diethyl ether which has a low boiling point was completed with 8 syphons in 4 hours. Extraction yield was calculated as 37.36% on the basis of dry material. In the experiments with petroleum ether, the extraction lasted 4 hours was terminated by the end of 8 syphons and it was calculated as 35.76%.

The experiments with hexane lasted 6 hours with 10 syphons. After the solvent was removed from setting, the yield was calculated as 34.08% according to the amount of residual oil. In the experiments that were used acetone and lasted 8 hours with 10 syphons, the yield was 33.65% and the experiments with ethanol were completed with 9 syphons in 10 hours. At the end of the study, attained yield was 24.21%. As is seen in Figure 3.4, in the experiments conducted by using the solvents which have different chemical structures while the highest yield was obtained from the experiments with diethyl ether, the least yield was obtained from the experiments with ethanol.

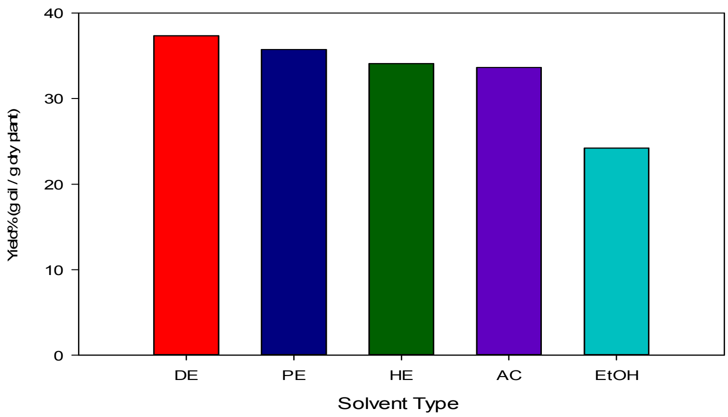

Figure 3.4. The effect of solvent type on conventional Soxhlet extraction

3.5 Ultrasonic Extraction

Ultrasonic extraction is an alternative way that aims to increase the yield by making cell wall thinner and enhancing the interaction of the solvent through ultrasonic sound waves. 5 different solvents were used in the experiments conducted with this method. The yields, which were obtained from the experiments conducted at different batch times, were shown in Figure 3.5.

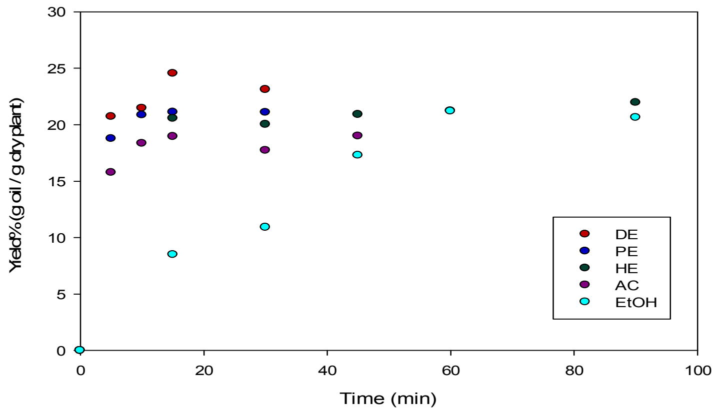

Figure 3.5. Effect of the solvent and extraction time on the ultrasonic extraction

According to the results, at dry matter base, the highest yield obtained from the experiment with diethyl ether was 24.51% and the least yield obtained from the experiment with acetone was 18.99% (Figure 3.6).

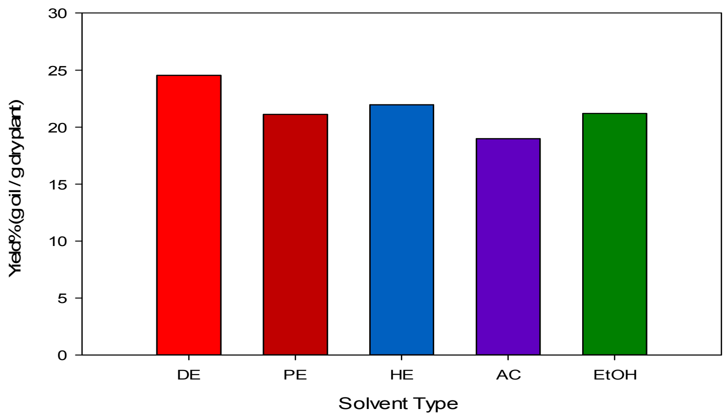

Figure 3.6. The effect of solvent type on extraction yield at ultrasonic extraction method

3.6 Effect of Extraction Method

Classical solvent extraction is the oldest known method. In this extraction method, the average yield was reached at the end of a long period such as 15 hours. Diffusion was quite slow and the contact between solid and solvent is minimal. In order to overcome these disadvantages Soxhlet extraction was developed. The main feature of Soxhlet extraction is a syphon. In this way, the unsaturated solvent is sent to the system regularly and by this way provides removal of the remaining oil from the solid structure in each siphon. However, in this method, experiments have been realized at long extraction times (4 – 10 hours). Solvent consumption and, consequently, the elapsed time for solvent removal was found to be too long that causes longer retardance of the organic solvent in solid structures (Wang and Weller, 2006).

The ultrasonic extraction method is one of the new methods in which less solvent was used compared to Soxhlet extraction method. Despite the Soxhlet extraction with lower extraction yield, in ultrasonic extraction one can reach an average yield in a shorter time. This can be due to sound waves and vibrations which increased the contact between solid matter and the solvent which as a result provides the same yield achieved at 20% shorter process time. Samaram and his friends analyzed the oil production from papaya seeds by both ultrasonic extraction and solvent extraction in 2014 (Samaram et al, 2014). They reported that conventional solvent extraction lasted 12 hours while ultrasonic extraction lasted 30 minutes which are close to the findings of this report as depicted in Table 3.1.

Table 3.1 The effect of extraction method and solvent type on extraction yield

EXTRACTION METHOD	SOLVENT TYPE				
	DE	PE	HE	AC	EtOH
SE	21.73%	20.21%	19.55%	16.86%	8.15%
SXE	37.36%	35.76%	34.08%	33.65%	24.21%
UE	20.72%	20.10%	21.95%	19.00%	20.65%

3.7 Effect of Solvent Type

In this study, five different type of solvent was chosen due to their physical properties and polarization index (PI) which are different from each other. Overall yield obtained for each solvent can be ranged as diethyl ether (PI: 2.8)> petroleum ether (PI: 0.1)> hexane (PI: 0.1)> acetone (P: 5.1)> ethanol (PI: 5.2). According to the principle of solubility, nonpolar compounds are soluble in nonpolar solvents and polar compounds are soluble in polar solvents. Oil is apolar while hexane and petroleum ether are more polar than diethyl ether. However, diethyl ether was shown to give higher yield which might be due to its viscosity where diffusion takes place easier in this solvent and thus a faster extraction process occurs. Acetone and ethanol have also similar polarities, lowest yield has been achieved studies with ethanol, though. The Higher viscosity of ethanol compared to acetone might also be the reason. Considering these results, we can conclude that viscosity is as effective as the solvent polarity and overall, the physical properties of the solvent (density, polarity, viscosity, etc.) has a distinguishable effect on extraction yield.

Rezaie and friends have studied the antioxidative compounds obtained from the mastic tree by ultrasonic extraction in which they have studied the solvent with different physical properties and the yield variation in each solvent. They reported that the extraction yield depends not only on polarity but also dielectric constant and viscosity of the solvent. Similarly, the principle of non-polar compounds are soluble in non-polar solvents and polar compounds are soluble in polar solvents is emphasized (Rezaie et al, 2015).

3.8 Determination of Fatty Acid Composition

Firstly, obtained safflower seed oil was turned into fatty acid methyl esters and then the oil composition was determined by gas chromatography (Figure 3.7).

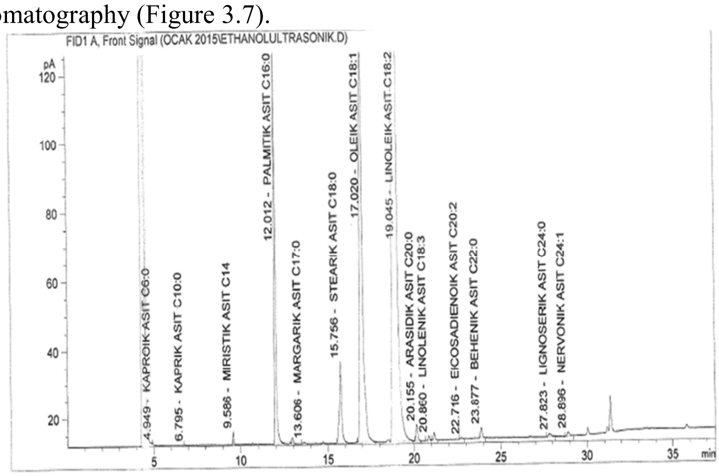

Figure 3.7. Example of gas chromatography result obtained from ultrasonic extraction method (EtOH)

Safflower seed oil has a wide range of fatty acid and we determined 37 different fatty acids from the oil samples. Out of 37 molecules, palmitic acid, stearic acid, oleic acid, linoleic acid and arachidonic acid were give in Table 3.2 which were present in high amounts. According to the type of solvent, the composition of essential oils from safflower seeds did not show any significant difference.

Table 3.2 Result of gas chromatography obtained from ultrasonic extraction

FATTY ACID METHYL ESTER	SOLVEN TYPE				
	ETHANOL	ACETONE	HEXANE	PETROLEUM ETHER	DIETHYL ETHER
PALMITIC ACID (%)	6.673	6.422	6.997	7.000	6.577
STEARIC ACID (%)	2.254	2.253	2.440	2.468	2.268
OLEIK ACID (%)	15.520	15.682	16.526	16.602	15.644
LIMOLEIK ACID (%)	**74.298**	**74.248**	**72.579**	**72.563**	**74.166**
ARACHIDONIC ACID (%)	0.314	0.331	0.363	0.363	0.338
OTHERS (%)	0.941	1.064	1.095	1.004	1.007

4. CONCLUSION

In this study, we aimed to evaluate oil production from safflower seeds using different kinds of solvents (diethyl ether, petroleum ether, hexane, acetone and ethanol) and extraction methods (classical solvent extraction, Soxhlet extraction and ultrasonic extraction), compare them in terms of the process viability and the composition of final product at various parameters and we showed that the ultrasonic extraction method in oil extraction from safflower seed is more advantageous compared to the traditional methods. Compared to other methods studied, ultrasonic extraction resulted in shorter extraction time with less solvent use. Although the highest yield was reached with Soxhlet extraction, the solvent amount used was higher and process time was 10 times longer than ultrasonic method while the yield of ultrasonic extraction was closed to that of Soxhlet extraction. In the industry, time and yield are very important and we have showed that the conventional extraction methods result in lower yield at longer extraction times. Thus, ultrasonic extraction is a more suitable in terms of the time consumption and the yield which also provides ease of operation at milder operation temperatures. Thanks to the developing technology, new methods took place of traditional methods.

Another parameter, effect of solvent type was also examined and as a result, solvent type, viscosity and polarity were found to have a strong impact on the extraction yield. The highest yield was obtained from the diethyl ether and the least yield was obtained in ethanol. Experiments conducted with diethyl ether resulted with the highest yield in a shorter time due to the low boiling point of the solvent. Although the low boiling point is advantageous for time, in terms of the disadvantage of diethyl ether is that it has a low boiling and flash point for extraction methods. However, it is not suitable for field applications due to its toxicity and flammability. On the other hand, ethanol is more environment friendly but has higher viscosity and polarity which turns out with lower extraction yields lower than other solvents. Instead of these two solvents, petroleum ether and hexane were considered to be more suitable solvents in terms of extraction yield and availability.

In this report, alternative methods were optimized and process conditions were compared and provided for safflower oil extraction which might open new insight for the development of new technologies in oil industry.

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