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Supercritical Fluid Extraction of Tobacco Seed Oil and its Comparison with Solvent Extraction Methods

S. Majdi[1], M. Barzegar[2*], A. Jabbari[1], and M. AghaAlikhani[3]

ABSTRACT

Tobacco (*Nicotiana tabacum* L.) is an important industrial crop and its seeds contain significant amounts of oil. The extraction of oil components using solvent at high pressure, or supercritical fluid (SCF), has received much attention. In the present study, statistical analyses showed that the average extraction yield of seed oil of five tobacco varieties using SFE was 9.33%, which was higher than Sonication (7.75%) and DGF (Deutsche Gesellschaft f_r Fettwissenschaft) standard method B-I5(87) (8.48%), but lower than Soxhlet (13.72%). Also, fatty acids profile of each extracted oil was determined by gas chromatography. Various saturated and unsaturated fatty acids such as lauric (C12:0), myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and eicosanoic (C20:0) acids were observed in the extracted oils.

Keywords: Fatty acids profiles, Soxhlet, Sonication, Supercritical fluid extraction, Tobacco (*Nicotiana tabacum* L.).

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is an important industrial crop grown in 119 countries of the world. This crop is an unbranched annual plant growing to a height of 3 to 6 ft with long oval leaves. Although tobacco leaves are the main economic product of this crop, the crop also produces small seeds as an important byproduct. The seeds are so small that 1 g seed contains 10,000-18,000 grains. At the end of the growing season, tobacco plants produce a lot of small seeds (~ 750 kg ha^{-1}) that contain significant amounts of oil. Since tobacco seed oil is inedible, it is not used as a commercial product in food industry. It has been found that tobacco seed oil is fairly suitable for biodiesel production (Deng *et al*., 1998; Usta, 2005a; Usta, 2005b). Several studies have been devoted to determining tobacco seed oil composition. Fatty acids such as myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) have been found in tobacco seed oil obtained from different areas (Baydar and Turgut, 1999; Eshetu, 2005; Ogunniyi and Odetoye, 2008).

The traditional method of oil extraction for analytical purposes is expelling the oil by crushing the seed, followed by solvent extraction (Yermanos *et al*., 1972).

The extraction of the oil using solvent at high pressure, or supercritical fluid (SCF), has received much attention in the past several years, especially in food, pharmaceutical and cosmetic industries, because it presents an alternative method

[1]Department of Chemistry, K. N. Toosi University of Technology, Tehran, Islamic Republic of Iran.
[2] Department of Food Science and Technology, Tarbiat Modares University, P. O. Box: 14115-336, Tehran, Islamic Republic of Iran.
[*] Corresponding author; e-mail: mbb@modares.ac.ir
[3] Department of Agronomy, Tarbiat Modares University, Tehran, Islamic Republic of Iran.

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for conventional processes such as organic solvent extraction and steam distillation. In food section, the most frequently employed supercritical solvent is CO_2 due to its low toxicity, good safety, and low critical temperature (Pourmortazavi *et al*., 2005).

Supercritical fluid extraction (SFE) has several distinct advantages: (1) the fluid has relatively lower viscosity and higher diffusivity compared to the conventional solvent, (2) a fresh fluid is continuously forced to flow through the samples; resulting in quantitative or complete extraction, (3) the extraction power of the fluid can be manipulated by changing the pressure (P) and/or temperature (T), thereby achieving a remarkably high selectivity, (4) solutes dissolved in supercritical CO_2 can be easily separated by depressurization, (5) SFE uses no, or significantly less, environmental hostile organic solvents, (6) in large scale SFE processes, the fluid, usually CO_2, might be recycled or reused to minimizing waste generation, and (7) SFE is fast; for instance , the extraction time can be reduced from hours or days for a liquid-solid extraction (LSE) to a few tens of minutes for SFE (Lang and Wai, 2001; Khajeh *et al*., 2004; Bergeron *et al*., 2005; Yin *et al*., 2005; Rajaei *et al*., 2005).

Over the last few years, numerous application of SFE have been reported in the literature for oily substances extraction from different raw materials such as celery seed (Papamichail *et al*., 2000), coriander seed (Illes *et al*., 2000), cherry seed (Bernardo *et al*., 2001), grape seed (Cao and Ito, 2003), sunflower seed (Antolin *et al*., 2002; Bravi *et al*., 2002), hazelnut, sesame seed, rapeseed (Bernardo *et al*., 2002) ,cotton, olive, and soybean seeds (Hajimahmoodi *et al*., 2005), and tea seed (Rajaei *et al*., 2005).

The main aim of the present work is to evaluate the use of SFE in oil extraction from tobacco seeds, optimize extraction conditions, and compare the extraction yield and fatty acids profile of the oil with solvent extraction.

MATERIALS AND METHOS

Plant Material

Five cultivars of tobacco seeds [Virginia (coker 347), Basma (187-2), Barley (21), Tiklak (TK.F.K 40-1) and Trabuzan (Ch.T 269-12)] were obtained from Tobacco Research Center of Iran (Tirtash), which is located in Mazandaran Province. Seed samples were cleaned and air dried to reduce their moisture to 14%. Then, the seeds were milled with a blender, since seed coat is almost impermeable and does not allow the oil to be extracted. The seeds were ground to pass through 40 mesh screen.

Chemicals

Standards of lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and eicosanoic acids were obtained from Sigma-Aldrich (USA). Hexane, petroleum benzene (bp 40-60^{o}C), methanol, sodium hydroxide and methanolic boron trifluoride were of analytical grade and were purchased from Merck Chemical Co. (Germany).

Soxhlet Extraction

Three grams of the milled and dried tobacco seeds were placed in an extraction thimble and extraction was carried out for 8 hours using 100 ml petroleum benzene (bp 40-60 ^{0}C). Then, the solvent was evaporated by a rotary evaporator at 40^{0}C and, after that, cooled for 30 minutes in desiccators and weighed.

Sonication Extraction

An Elma Trassonic model 690/H ultrasonic bath (Germany) was used to extract the tobacco seed (mesh 40) oil by Sonication. Three grams of the milled seeds were mixed with 70 ml petroleum benzene (bp 40-60 ^{0}C). The mixture was then

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sonicated in an ultrasonic bath for 30 minutes. After that, oil was recovered by evaporating the solvent in the same way as mentioned previously.

DGF Standard Method B-I (87)

Three grams of the milled seeds were mixed with 70 ml of petroleum benzene (bp 40-60°C). The extraction was then carried out for 4 hours in Twisselmann extraction apparatus. After extraction, the solvent was evaporated by a rotary evaporator and the extracted oil was dried at 103°C to remove residual solvent, cooled for 30 minutes in a desiccator, and weighed. This procedure was repeated until a constant weight was obtained (Matthaus and Bruhl, 1999).

Supercritical Fluid Extraction

SFE was carried out in Suprex MPS/225 system (Pittsburg, USA). The supercritical CO_2 flow rate through the Duraflow restrictor was approximately 0.3–0.4 ml min^{-1} (compressed). Exactly 2.0 g (±0.1 mg) of the milled seeds were mixed with glass beads which were added in order to reduce the dead space in the extractor vessel (10 ml) and allow the uniform distribution of the solvent flow. Actually, CO_2 with purity of 99.9% was used as a solvent. The operating conditions of each experiment are reported in Table 1. The extracted oils were collected in 3 ml hexane in 5 ml volumetric flask. The final volumes of the extracted oils were adjusted to 5 ml with hexane, at the end of the extraction. In order to increase collection efficiency, during the dynamic time, the volumetric flask was placed in an ice bath. For all the modifier studies, ethanol was added directly into the extraction vessel with charged samples prior to extraction.

Fatty Acid Analysis

The fatty acid methyl esters (FAMEs) were prepared by the procedure described by Metcalf *et al*. (1966). Fifty milligram of extracted oil was saponified with 5 ml methanolic NaOH (2%) solution by refluxing for 10 minutes at 90°C. After addition of 2.2 ml BF_3-methanolic, the sample was boiled for 5 minutes. The FAMEs were extracted from a salt-saturated mixture with hexane. The FAMEs were then analyzed using a gas chromatography (UNICAM model 4600, England) coupled with a FID detector. The column used for fatty acid separation was a fused silica BPX70 column, 30 m×0.22 mm id×0.25 μm film thickness (from SGE, UK). The oven temperature was held at 180°C during separation; the injector and detector temperatures were 240 and 280°C, respectively. The carrier gas (helium) flow rate was 1 ml min^{-1}. One microliters of methyl esters of free fatty acids were injected into the split injector. The split ratio

Table 1. The four factors, three level (L9) orthogonal array design for SCF extraction of Virginia tobacco seed oil.

Run No.	Pressure (atm)	Temperature (°C)	Dynamic time (min)	Modifier (%)	Yield (%)[a]
1	300	55	30	0	4.5
2	300	65	40	5	4.8
3	300	75	50	10	5.0
4	350	55	40	10	6.2
5	350	65	50	0	8.0
6	350	75	30	5	7.0
7	400	55	50	5	9.5
8	400	65	30	10	10.0
9	400	75	40	0	11.5

[a] Relative standard deviation (RSD %) for three replicate extractions were in the range 2-4.

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was adjusted to 1:20. The fatty acids in the tobacco oil were identified by comparing their retention times with the standard fatty acids. For quantitative analysis external standard method was applied.

Statistical Analysis

Experimental data was analyzed using analysis of variance (ANOVA) and significant differences among the means (from triplicate analyses) were determined by LSD (least significant difference) at ($P< 0.01$) using the Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

Supercritical fluid extraction (SFE) has several distinct advantage compared to conventional solvent extraction methods (as mentioned in Introduction). Over the last few years, numerous application of SFE for oily substances extraction from different raw materials such as grape seed (Cao and Ito, 2003), sunflower seed (Antolin *et al*., 2002; Bravi *et al*., 2002), and rapeseed (Bernardo *et al*., 2002) have been reported in the literature. In this study, we intended to compare the efficiency of the SFE with the other extraction methods. Since various parameters potentially affect the extraction process efficiency, optimization of the experimental conditions was a critical step in the development of a SFE method. In fact, the fluid pressure and temperature, types and percentage of modifier and the extraction time are generally considered as the most important factors.

Optimization of the Experimental Conditions of SFE

Optimization of the method can be carried out step-by-step or by using an experimental design. Table 1 shows different conditions of the experiments carried out with SFE for extraction of tobacco seed oil (Virginia cultivar, extraction yield of other cultivars not shown here because, their results were similar) according to the Taguchi experimental design (Roy, 1990). In this study, interactions among variables were not incorporated in the matrix, and emphasis was placed on the main effects of the four most important factors. The results of the SFE experiments, based on the extraction yields, are given in Table 1. Also, the extraction yields of different methods (SFE, Soxhlet, Sonication and DGF method B-I 5(87)) are shown in Table 2.

Analysis of variance (ANOVA) was carried out on the results in Table 1 (data not shown). The ANOVA results of this experiment indicate that the pressure plays an important role in SFE. Pressure was found to be the most important factor, where higher pressure increased significantly the yield of oil (from 4.8 to 10.3%). At lower pressure, the solubility of oil was affected by vapor pressure of the oil; apparently, at this stage CO_2 relatively acted as an ideal gas that does not have any special characteristic of a solvent. However, at higher pressures, the solubility of the oil increased due to the increase in density of CO_2. As the density

Table 2 Comparison of extraction yield of oils obtained by SFE and other methods [a].

	Variety				
Method	Virginia	Tiklak	Basma	Barley	Trabuzan
Soxhlet	17.9 a	15.4 b	14.3 c	10.6 e	10.4 e
Sonication	8.2 ijk	7.4 m	8.2 ijkl	7.5 klm	7.5 klm
DGF	8.6 ij	7.0 m	9.4 fgh	7.9 jkl	9.5 fg
SFE	11.5 d	8.7 ghi	9.5 f	8.7 hij	8.2 ijk

[a] Values expressed are mean of triplicate measurements; Values with different letters in the columns and rows were significantly different ($P< 0.01$).

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increases, the distance between molecules decreases and the interaction between oil and CO_2 increases, leading to greater oil solubility in CO_2 (De Castro *et al*., 1994).

Depending on the properties of the samples and the desired compounds, the best modifier usually can be determined based on preliminary experiments. As our preliminary experiments showed, methanol was not a good modifier and was not used in other experiments. Therefore, ethanol, which is GRAS, was used because of its availability and comparatively low cost (Cao and Ito, 2003). When the modifier was used, the extraction yield was lower (decreasing from 8.0 to 7.1%). Temperature and dynamic extraction time did not influence significantly the yields of extracted seed oil. The optimum extraction conditions by SF CO2 were as follows: P= 400 atm; T= 75^{o}C; Dynamic time= 40 minutes, Percent of modifier= 0%.

Soxhlet extraction has usually been used for quantitative extraction of the oil. The yield of oil by Soxhlet extraction (13.70%) was found to be higher than the other methods. The SFE efficiency was higher than Sonication and DGF Standard Method B-I (87) methods (as seen in Table 2), but lower than extraction yield of Soxhlet. In spite of this lower recovery, SFE is more acceptable, not only for environmental considerations but also for health and safety reasons. It could be stated that SFE is a useful method to extract valuable tobacco seed oil without any residues of organic solvents. The yield of extracted oil by SFE depended on the main parameter, i.e. pressure, which was applied during extraction. Based on our experience, SFE`s extraction efficiency might be improved to the same level by increasing fluid pressure up to 600 atm.

In order to compare the seed oil quality in different extraction methods, the oils were analyzed by GC. The composition of the oils (for 5 tobacco cultivars) from the SFE and the other methods are listed in Table 3, which shows, different FAs were identified in different cultivars of tobacco seed oils by the gas chromatography, as previously reported (Baydar and Turgut, 1999; Giannelos *et al*., 2002). The fatty acids composition of tobacco seed oils was dominated by linoleic acid (59.8 – 66.82%), although the other fatty acids such as palmitic, stearic, oleic and linolenic, were also present. As seen in Table 3, the content of saturated (SFA) and unsaturated fatty acids (UFA) were about 24.66 and 75.30%, 20.63 and 79.04%, 20.02 and 79.90%, and 27.19 and 72.24% for SFE, Soxhlet, Sonication and DGF Standard method, respectively. By comparing the fatty acid profiles of the oils obtained by various extraction methods with the previously published works (Table 4), one could conclude that the fatty acid profiles of the oils obtained by the studied methods were not different from those of the oils recovered elsewhere. The total saturated fatty acid compositions of tobacco seed oils extracted by SFE (24.66%) and DGF (27.19%) were higher than that of Soxhlet (20.63%) and Sonication (20.02%). A small decrease in unsaturated fatty acid and an increase in the content of saturated fatty acid were observed in the case of soybean oil, which was explained by the oxidation of unsaturated fatty acid (Li *et al*., 2004). It was also observed that ultrasound (0.65 W g^{-1}) affected the fatty acid composition of sunflower oil by decreasing the content of linoleic acid, due to oxidation of the oil during Sonication (Chemat *et al*., 2004).

It is obvious that PUFAs are very important for human nutrition. The fatty acids profile of the oils showed that PUFAs are predominant in tobacco seed oil ranging from 72.79-79.94%. Linoleic acid is an essential fatty acid for humans. The saturated to unsaturated fatty acids ratios of the studied cultivars ranged from 0.250 to 0.374 in all samples. These ratios show that these oils are ready for autoxidation and polymerization. Tobacco seed oil is classified as linoleic oil and, therefore, it is comparable to other oils and can be used in pain industries. Also, based on our experiences, it can be fractionated and used in food industries.

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Table 3. Comparison of fatty acids profile of tobacco seed oils extracted by different extraction methods [a].

Method	Cultivar	Fatty acids (w%)										
		C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	∑SFA	∑USFA
Soxhlet	Virginia	0.69k	0.13fgh	18.18d	0.22f	3.97de	13.20ef	61.76ij	1.03bc	0.24g	23.21fg	76.20h
	Tiklak	ND	ND	17.44f	0.10h	4.02de	15.00b	61.90i	1.00c	0.19g	21.64i	78.00d
	Basma	1.83g	1.04c	15.277i	0.16g	3.69fg	14.30c	61.95i	1.16a	0.15g	21.98hi	77.50de
	Barely	0.15m	0.19fg	13.25l	0.29e	4.60b	12.60gh	68.22d	0.48d	0.14g	18.33k	81.60b
	Trabuzan	0.64k	0.03gh	13.58k	0.09hi	3.52g	8.25l	72.52a	1.12ab	0.18g	17.95k	81.90b
	Mean	0.66d	0.28c	15.55c	0.17d	3.96c	12.71ab	65.27b	0.96a	0.18c	20.63c	79.12b
Sonication												
	Virginia	0.64k	0.04gh	12.51m	0.11h	4.35c	12.40h	68.85c	0.19fg	0.68e	18.22k	81.60b
	Tiklak	ND	5.16a	14.12j	0.37d	3.50g	12.80g	62.90h	0.13g	0.95d	23.74e	76.20h
	Basma	1.10h	0.06gh	13.20l	0.44c	3.59g	13.40de	66.60e	0.18fg	1.36a	19.30j	80.60c
	Barely	3.52d	0.75d	13.29l	0.29e	3.28h	12.60gh	64.62f	0.14g	1.39a	22.24h	77.70d
	Trabuzan	0.36l	0.19fg	11.76n	0.42c	3.26h	11.70j	71.12b	0.15g	1.00d	16.58l	83.40a
	Mean	1.13c	1.24a	12.97d	0.33b	3.59d	12.63b	66.82a	0.16c	1.07a	20.02d	79.94a
DGF												
	Virginia	3.85b	0.53e	14.11j	0.64b	4.35c	13.60d	62.12i	0.20fg	0.48f	23.32f	76.60gh
	Tiklak	ND	3.16b	17.76e	0.04j	4.17cd	13.40de	59.93l	0.19fg	1.17b	26.26c	73.60j
	Basma	3.75c	0.63de	16.97g	0.06ij	4.14d	14.20c	59.26l	0.19fg	0.93d	26.42c	73.70j
	Barely	0.83j	0.25f	22.26a	0.92a	5.18a	12.10i	56.70l	0.34e	1.28ab	29.81a	70.10l
	Trabuzan	4.97a	0.29f	18.89c	0.37d	4.99a	8.16l	60.98k	0.25ef	0.98d	30.13a	69.7l
	Mean	2.68a	0.97b	18.00a	0.41a	4.56a	12.34c	59.80d	0.24b	0.97b	27.19a	72.79d
SFE												
	Virginia	2.53e	0.23f	15.07i	0.25f	4.60b	11.70j	64.83f	0.20fg	0.53f	22.96fg	77.00fg
	Tiklak	ND	3.07b	16.94g	0.09hi	4.04de	13.10f	61.33jk	0.18fg	1.00d	25.06d	74.70i
	Basma	2.13f	0.57e	15.07i	0.11h	3.85ef	16.60a	60.20l	0.19fg	1.20b	22.83g	77.10ef
	Barely	0.93i	0.48e	20.41b	0.68b	4.00de	12.10i	59.88l	0.22fg	1.15bc	26.99b	72.90k
	Trabuzan	3.82bc	0.18fg	16.40h	0.37d	4.03de	10.10k	63.78g	0.21fg	1.01cd	25.44d	74.50i
	Mean	1.88b	0.91b	16.78b	0.30c	4.10b	12.77a	62.00c	0.20bc	0.98b	24.66b	75.28c

[a] Values expressed are mean of triplicate measurements, Values with different letters in the same column were significantly different (P< 0.01).

Table 4. Comparison of fatty acids profile of tobacco seed oil with other studied on tobacco seed oil (wt %).

Fatty acid	Tobacco				
	This work (Soxhlet)	This work (SFE)	Baydar and Turgut (1999)	Giannelos *et al.* (2002)	Stanisavljevic, *et al.* (2009)
Myristic (C14:0)	0.00-1.04	0.18-3.07	0.09	0.17	-
Palmitic (C16:0)	13.25-18.18	15.07-20.41	10.96	8.87	8.42
Palmitoleic (C16:1)	0.09-0.29	0.09-0.68	0.2	0.0	-
Stearic (C18:0)	3.52-4.60	3.85-4.60	3.34	3.49	3.78
Oleic (C18:1)	8.25-15.00	10.10-16.60	14.25	12.4	11.78
Linoleic (C18:2)	61.76-72.52	59.88-64.83	69.49	67.75	72.22
Linolenic (C18:3)	0.48-1.16	0.18-0.22	0.69	4.20	0.84
Others	0.19-2.07	0.53-5.02	0.69	3.12	2.96

CONCLUSIONS

For obtaining oil from tobacco seed, several extraction techniques such as Soxhlet, Sonication, DGF and SFe were compared with respect to both the extraction efficiency, measured by the oil yield, and the fatty acids profile. The maximum oil yield of 13.70 % was obtained by Soxhlet, followed by SFE that had higher yield than the other methods. In spite of lower recovery, SFE is more acceptable than the Soxhlet method, not only for environmental considerations but also for health and safety reasons. It could be stated that SFE is a useful method to extract valuable tobacco seed oil without any residues of organic solvents. The yield of extracted oil by SFE depended on the main parameter, i.e. pressure, which was applied during extraction. The extraction efficiency by SF CO2 under optimum conditions (P= 400 atm; T= 75^{o}C; Dynamic time= 40 minutes, Percent of modifier=0%) was lower than Soxhlet (for all cultivars), which, based on our experience, might be improved to the same level by increasing fluid pressure up to 600 atm. Results showed that profile of fatty acids were the same as other published works, with UFAs higher than percentage of SFAs, which is characteristics of vegetable oil. The results also imply that tobacco seed oil may be used as a potential alternative source of lipid. This study has provided a basis for future studies in this area. It is recommended that other properties of the extracted oils be determined and effect of stage of maturity, growing region, and harvesting and storage conditions be examined on the characteristics of the oil and the nutritive values of the plant seeds.

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استخراج روغن بذر تنباکو با سیال فوق بحرانی و مقایسه آن با روش های استخراج با حلال

س. مجدی، م. برزگر، ع. جباری و م. آقا علیخانی

چکیده

تنباکو (*Nicotiana tabacum* L.) یکی از محصولات صنعتی مهم است که در بیش از ۱۱۹ کشور کشت می شود. بذرهای تنباکو حاوی مقدار زیادی روغن است. در چند سال گذشته استخراج روغن با روش حلال با فشار بالا و سیال فوق بحرانی مورد توجه قرار گرفته است. روغن پنج رقم بذر تنباکو (Virginia, Tiklak, Basma, Barely and Trabuzan)با سیال فوق بحرانی استخراج شد و با نتایج روش های استخراج با حلال (Soxhlet, Ultrasonic and DGF standard method B-I5 (87)) در شرایط آزمایشگاهی مقایسه شد. تجزیه ی آماری نشان داد که راندمان استخراج روغن با سیال فوق بحرانی (میانگین راندمان استخراج برای پنج رقم ۹.۳۳ درصد بود) بیشتر از سونیکیشن (۷.۷۵ درصد) و روش استاندارد B-I5 87 (۸/۴۸ درصد) بود ولی از روش سوکسله (۱۳/۷۲ درصد) کمتر بود. همچنین, پروفایل اسیدهای چرب روغن های استخراج شده با کروماتوگرافی گازی تعیین شدند. اسیدهای چرب اشباع و غیر اشباع مختلفی همچون لوریک (C12:0), میرستیک (C14:0), پالمیتیک (C16:0), پالمیتولئیک (C16:1), استئاریک (C18:0), اولئیک (C18:1), لینولئیک (C18:2), لینولنیک (C18:3)و ایکوزانوئیک اسید (C20:0) در روغن های استخراج شده مشاهده شدند.

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