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Rapid Determination of Bioactive Lipid-type Materials of Rapeseed Oil Deodorizer Distillate by GC-MS

Parisa Jafarian Asl[1], Razieh Niazmand[2]*, Syed Tufail Hussain Sherazi[3]

1- PhD. Student, Department of Food Chemistry, Research Institute of Food Science and Technology, Mashhad, Iran
2- Associate Professor, Department of Food Chemistry, Research Institute of Food Science and Technology, Mashhad, Iran
* Corresponding author (r.niazmand@rifst.ac.ir)
3- Professor, National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan

Abstract

Rapeseed oil Deodorizer distillate (RODD) is regarded as a waste material of the rapeseed oil industries obtained during the deodorization process. It contains valuable bioactive nutritive compounds such as phytosterols, tocopherols, and squalene. In the present study, some physical and chemical properties such as color, free fatty acid value (as % oleic), saponification value (mg KOH/g), neutral oil (%), unsaponifiable matter (%), total tocopherols (mg%), total phytosterols (%) were determined ultrasonic assisted separation was applied to quick fractionation of rapeseed oil deodorizer distillate into the lower polar unsaponifiable methanol fraction and upper non-polar saponifiable hexane fraction mainly containing the lipids. The composition of natural lipids found in the methanolic extract of RODD was determined by HPLC while the fatty acid composition was evaluated by gas chromatography-mass spectrometry (GC-MS). Squalene had higher relative standard deviations 3%, while Tocopherols and sterols had low relative standard deviations ranging between 0.11-0.36 and 0.04-0.28% in distillate sample respectively. Based on the results, the most abundant compounds in rapeseed distillate sample were phytosterols (15.5-22.14%). However, the concentration of squalene and tocopherol was 1.9-2.05% respectively. Triglycerides were also the main glycerides of distillate. The results of the present study indicated that the polar lipid bioactive fraction was effectively extracted with methanol without saponification using a nondestructive approach.

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Canola oil
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Introduction

Generally, crude vegetable oils are processed in the industries via different stages before being used for edible purposes due to the presence of undesirable color, and odor (Jafarian Asl, Niazmand, & Jahani, 2020a). Degumming, refining is the deodorizer distillate (DOD), achieved during deodorization stage (Naz, neutralization, bleaching, and deodorization are common industrial treatments that generate by-products such as gums, soap stocks, spent bleaching clays and deodorizer distillates, respectively (Durant, Dumont, & Narine, 2006). The most valuable by-product of edible oil Sherazi, Talpur, Kara, Uddin, & Khaskheli, 2014).

Deodorization is the last step of the refining process that is responsible for the elimination of the volatile compounds that produce undesirable odor, color, and flavor in the oil. DOD is a complex mixture of different compounds such as free fatty acids (FFA); monoglyceride (MAG), diglyceride (DAG), and triglyceride (TAG) esters of the fatty acids that exist in the oil (predominantly the mono and diglyceride esters); terpenic and aliphatic alcohols; waxes; squalene; carotenoid pigments; free phytosterols and phytosterol esters of fatty acids; and tocopherols (Jafarian Asl, Niazmand, & Yahyavi, 2020b).

Saponifiable part of DOD including considerable amounts of FFAs, acylglycerols while the unsaponifiable section contains a high concentration of valuable components such as tocopherols, sterols and squalene that have high interest to use as nutraceuticals and functional food (Sherazi & Mahesar, 2016). The estimation of the amount of components in DOD is an effective factor for determining the exact extent of their ability to become valuable products (Durant *et al.*, 2006).

Therefore, the amount of phytosterols and tocopherols express commercial value (Estiasih, Ahmadi, Widyaningsih, Maligan, Mubarok, Zubaidah, Mukhlisiyyah, & Puspitasari, 2013). Phytosterols (also known as "plant sterols") are a group of steroid alcohol phytochemicals that are plentiful in nature, arising naturally in a large variety of fruits and vegetables and also are part of the human body. The main dietary phytosterols are Stigmasterol, β-sitosterol, and Campesterol (Naz *et al.*, 2014). These compounds play an important role in reducing cholesterol LDL and cardiovascular diseases in humans, which leads to the use of them in functional food (Yang, Yan, Wu, Huo, Li, Cao, & Jiang, 2010). The commercial value of DOD primarily determined by the amount of tocopherols (vitamin E). Tocopherols are a mixture of alpha (α), beta (β), gamma (γ) and delta (δ) isomers which are used as additives in some foods, healthy products and pharmaceuticals (Naz, Sherazi, Talpur, Talpur, & Kara, 2012). Squalene is a liner hydrocarbon that uses in cosmetic products and acts as a cholesterol biosynthesis precursor. It is found naturally in many tissues, especially shark liver and other fishes (Moreda, Pérez-Camino, & Cert, 2001). Acylglycerols are the other constituents of distillate. Triacylglycerols (TAGs), diacylglycerols (DAGs), and monoacylglycerols (MAGs) are known as acyl-glycerol and neutral fats (Shoaib, Mahesar, Jafarian, Niazmand, & Sherazi, 2019). Since DOD is a combination of different components with different polarities, gas chromatography coupled to mass spectrometry (GC-MS) for characterization and identification of the different by-products is an interesting task.

To the best of our knowledge, various analytical methods have been used to identify and recycle valuable chemical compounds such as tocopherols and sterols from DOD, but few studies have done a complete analysis of the distillation samples. Durant *et al.* (2006) decomposed canola oil DOD by GC-MS using simple method “in situ” for composition analysis.in another study, they also used the derivation method by silylation of the samples before direct injection into a non-polar column to examine the compounds in soybean soapstocks and soybean deodorizer distillate (Durant *et al.*, 2006). These methods include the use of toxic reagents such as N, O-bistrimethyl silyltrifluoro acetamide (BSTFA), hexamethyldisilazane and trifluoroacetic acid for derivatization process, among the other disadvantages of derivatization method used in previous studies, this method not only requires increasing of the volatility of tocopherols prior to injection to GC-MS and GC-FID But also needs a

lot of labor work and lead to undesirable side effects. American Oil Chemist's Society (AOCS, 1998) has provided standard methods for analyzing sterols and tocopherols. In these methods, DOD is saponified and the non-saponifiable section is separated and identified by GC that one of the disadvantages of this procedure being time-consuming.

Recently, Sherazi & Mahesar (2016) used a conventional saponification method for the determination of unsaponifiable constituents of canola and palm oil deodorizer distillate by GC-MS. Also, identification of compounds present in palm fatty acids distillates (PFADs) after saponification by GC-MS was done by Estiasih *et al.* (2013).

The main objective of this research is, introducing a quick, precise, and accurate method for determining sterols, hydrocarbons, and all the homologs of the tocopherol present in Rapeseed oil deodorizer distillate. In this study, not only the derivatization and conventional saponification were omitted, but also ultrasound was used as pre-treatment and bioactive compounds of Iranian Rapeseed oil distillate were isolated and detected only within a reasonable time which has never been thoroughly investigated by this method.

Materials and methods

Reagents

Rapeseed oil deodorizer distillate was provided by Shadgol Foods Ltd. (Neishaboor, Iran) and was stored at 4 °C until they were saponified. Octacosane, squalene, nonacosane, α-tocopherol, β-tocopherol, γ-tocopherol, brassicasterol, campesterol, stigmasterol, β-sitosterol, methanol, and cholesterol were obtained from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and solvents were of analytical grade.

Physicochemical characteristics

Color number

The color of the samples was determined using a Lovibond tintometer in a 1-in. the cell on the Lovibond scale in transmittance mode and expressed as (5R+Y) units (AOCS, 1998).

Free fatty acid value (acidity evaluation)

The amount of free fatty acids (FFAs) in the DOD was evaluated by standard titration method Ca 5a-40 (AOCS, 1998). To titrate the DOD sample, a standardized aqueous solution of sodium hydroxide (KOH), was applied. About 2.35±0.001 g of DOD with 10 mL of neutral ethanol was heated on a heating plate, shaken truly and titration was done against 1 N NaOH after the addition of a phenolphthalein indicator.

Saponification Value (SV)

SV is determined as the amount of potassium hydroxide in mg that is necessary to saponify one gram of fat and oil. The amount of SV is affected by the type of free fatty acids present in the oil. Refluxing about 2 g of the DOD samples with 25 mL of 95% ethanolic potassium hydroxide was performed at 80 °C for at least 60 min. After refluxing, titration of the sample after adding phenolphthalein as the indicator was done by the standardized solution of 0.5 N HCl (AOCS, 2010).

Unsaponifiable matters

About 0.5 g of oil was poured into a 15 mL test tube with 15 mL of ethanolic potassium (1 N) and then warmed at 95 °C for 1 h. After cooling, 10 mL of distilled water was added and mixed well. The extraction process was performed by adding 10 mL of ether to the separating funnel. The organic fraction of ether extraction was combined and washed twice with 10 mL of distilled water. 10 mL of ethanolic potassium was added to the organic phase and then saponified. After

that 10 mL of distilled water was added to remove the remaining soap substances. Sodium sulfate was then used to dry the organic phase. Filtration was done with the Whatman filter paper, and the solution was dried under vacuum at 45 °C (Jafarian Asl *et al.*, 2019).

Total sterol

A certain volume of chloroform was used to increase the volume of about 1 g of DOD to 10 mL. Then, 1 mL of that diluted with 10 mL of chloroform. 3 mL of the diluted sample solution was transferred to a 15 mL tube and 2 mL of Lieberman-Borchard reagent was added to each sample, and the volume of the solution was diluted to 7 with chloroform. The samples were kept in a dark place for 15 min. The absorption of the sample was then determined at 640 nm (Sabir, Hayat, & Gardezi, 2003) .

Total tocopherol

For Determination of total tocopherol, 210 mg of DOD was with 5 mL of toluene were mixed well. Then 3.5 mL of 2.2-bipyridine solution with 0.5 mL of $FeCl_3$ were added. The solution was kept for 1 min without shaking. The absorption of the sample was measured at 520 nm.

Separation of RODD by solvent-solvent (metahnol:hexane) extraction

About 10 g of DOD sample was weighed in 100 mL Erlenmeyer with rubber seal and 10 mL of solvents (methanol to hexane ratio 40% v/v) were added. The mixture was stirred for 1 min at room temperature and placed in the ultrasound bath for 30 min. then the mixture was separated using a separating funnel. The Hexane section was full of natural lipids and polar compounds. Unsaponifiable compounds were separated by methanol.

HPLC analysis of hexane fraction of RODD

The natural lipids composition was determined according to Jafarian Asl *et al.* (2019) using an HPLC system (model X-Agilent technologies, USA) that was fitted with a Diol 5 (lichrosphere) column 250×4×5 mm id and Evaporative Light Scattering (ELS) detector and the mobile phase consisted of methyl tertiobuthyll/acetic acid (0.1% by vol) at a flow rate of 1.0 mL/min was applied. The natural lipids were identified and quantitated using standards of MAG, TAG, DAGs.

Preparation of standard solutions

Standard solutions of octacosane, squalene, nonacosane, α-tocopherol, β-tocopherol, γ-tocopherol, brassicasterol, campesterol, stigmasterol, and β-sitosterol were prepared at a concentration of 10 mg/mL in chloroform and were kept at -20 °C. Cholesterol was chosen as the internal standard (IS) as its deficiency in the rapeseed oil deodorizer distillates. A combination of these standards solutions was injected into the GC-MS after saponification under the same chromatographic conditions as tracked by the deodorized samples. The separated components were applied for specifying the elution order of the compounds, and for MS cognition.

Gas chromatography-mass spectrometry (GC-MS) analysis of methanol fraction

The bioactive compounds were separated and detected by a gas chromatograph (X-Agilent technologies, USA) with a quadrupole mass spectrometer detector (5977A-Agilent Technologies, USA). Data of the chromatography separations were acquired using an HP-5MS (5% phenyl methyl siloxane) 30 m×0.25 mm×0.25 μm capillary column (Agilent Technologies, Palo Alto, CA, USA). Helium was used as a carrier gas at a flow rate of 0.5 mL/min. The injection temperature was 310 °C. A sample injection of 2 μL was performed in

a split mode of 1:10. The oven temperature was programmed as follows: the initial temperature was set at 190 °C, The ionization energy was 70 eV, detection and data acquisition were performed in full scan mode from 50 to 500 Da. Bioactive compounds were identified by comparison of the data obtained in the GC-MS analysis with m/z values compiled in the spectrum library Wiley and corroborate with the NIST spectrum database. Data analysis was performed using the Agilent MassHunter software (Agilent Technologies, USA), quantification of the different compounds was carried out through the internal standard (cholesterol) (Jafarian Asl *et al.*, 2020a).

Statistical Analysis

All experiments and analysis were performed in triplicate and results were analyzed for standard deviations by INSTAT statistical program.

Results and discussion

Physicochemical characteristics of DOD

The DOD of rapeseed oil which was obtained from the Shadgol company was dark brown (75.4 Lovibond color units) and semisolid at room temperature. It was converted to a liquid by increasing the ambient temperature to 30 °C. Other Physicochemical characteristics are presented in Table (1). The high amount of saponification value in DOD (160.3) indicates a lower amount of glycerides (expressed as neutral oil, 6.7%). The FFA and saponification values were in good agreement with the stated research (Naz *et al.*, 2012). Sherazi & Mahesar (2016) reported that the amount of non-saponifiable matter in RODD was 33%. Also, their results showed that the content of tocopherols and phytosterols was 18 and 14.60% respectively. They also stated that difference in the range of values to the previous literature was due to variation in processing conditions and raw material variability.

Table 1. Composition and properties of rapeseed oil deodorizer distillate (mean±std, n=3)

Parameters	DOD
Physical appearance (visual)	Dark semi-solid
Color (1 in. cell, 5R+Y unit)	75.40±1.90
Free fatty acid value (as % oleic)	53.30±1.90
Saponification value (mg KOH/g)	160.30±1.40
Neutral oil* (wt%)	6.70±1.00
Unsaponifiable matter (wt%)	40.10±1.70
Total tocopherols (wt%)	14.10±2.20
Total phytosterols (wt%)	26.00±1.50

*Neutral oil % = [100-(%unsaponifiable matter+ %FFA)]

Natural lipids in RODD

Triacylglycerol is the main acylglycerol in vegetable oil that their types depend on the kind of oil source. If these compounds are extracted well, they will have a higher commercial value than FFA. The different families of neutral lipids are identified by comparing the retention times. The order of elution being: Esters>Waxes> TAG> FFA>DAG>MAG. Fig. (1) and Table (2) show the HPLC chromatogram and composition of neutral oil in Rapeseed oil DOD. RDOD included 3.2 wt% diglycerides, and the contents of mono- and triglycerides were 1.6 and 1.9% respectively. RODD from the chemical refining process contains a large amount of acyl glycerol which is reported by Verleyen, Verhé, Garcia, Dewettinck, Huyghebaert, & De Greyt (2001) to be 7% while the amount obtained in this study was 6.7%. They also reported that the content of TAG, DAG and MAG in RODD was 3, 3.85 and 1.42% respectively. Expressed that MAG and DAG were the main glycerids of RODD that were obtained from hydrolysis of TAG during chemical refining with an amount of 0.46 and 0.41% respectively.

Table 2. Composition of natural lipids of rapeseed deodorizer distillate

Parameters	Rt* (min)	Concentration** (mg/g)
Short chain esters	2.50-2.70	170±9
Long chair esters	3.40	230±10
Triglycerids	8.90	19±1
Free fatty acids	12.90-13.70	530±30
Diglycerids	15.30-15.80	32±2
Monoglycerids	27.90-28.30	16±2

*Retention time
**Means±standard deviation of triplicates

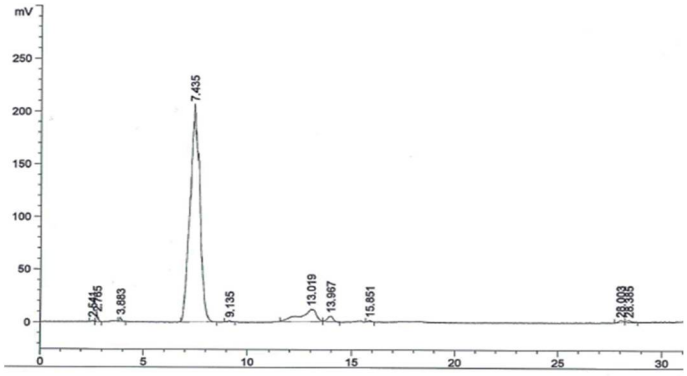

Fig. 1. HPLC chromatogram of monoacylglycerol (MAG), diacylglycerol (DAG), and triacylglycerol (TAG) in rapeseed deodorizer distillate

Validation of method and quantification

The method precision was assessed by a recovery study, which was demarcated as the percentage of the spiked amount regained. Preparation of samples was done in triplicate and the range of mean recoveries for rapeseed were 90-96%. The repeatability was examined within 1 day and reproducibility (between-run precision) was measured by analyzing five replicates of distillate samples including saponification and extraction. Quantification of the limit criteria was obtained from both, the precision study and linear least square regression. In the case of precision, which was supposed unacceptable when an R.S.D. of equal or more than 20% is obtained (this is true for very low concentration levels) it was not higher than 2.12% for the identified components existing in the rapeseed and soybean distillates. For linear least square regression the analysis is used to demonstrate the linearity of peak area responses against concentrations. These validation studies revealed good linearity, accuracy and precision. Therefore, the proposed method could be used effectively for quantitative analysis of the unsaponifiable part of rapeseed oil deodorizer distillates.

The calibration curves of the 5 standard solutions were used for quantification. For each standard, the linear regression by the least squares was used to determine the regression line. These regression line equations were applied to determine the concentration of each compound. The correlation coefficients (r) for all the standard curves were 0.996. The relative response factor method was also applied to validate the quantification

Qualitative analysis and concentration of bioactive compounds

The components of rapeseed oil are presented in Fig. (2). After optimization of GC-MS temperature programming, separation and identification of various components were performed as it can be seen, the analytes according to their carbon numbers are separated in compound classes. The first eluted one is squalene. The order of removal of the tocopherol

isomers is based on increasing numbers of methyl groups attached to the chromanol ring: first Б-tocopherol followed by the next isomer of three dimethyltocols, β-, γ- and finally α-tocopherol. Afterward brassicasterol, campesterol, stigmasterol, and β-sitosterol followed by the signals of cholesterol. Verleyen *et al.* (2001) referred that separation between β and γ- is hard to obtain in rapeseed oil deodorizer distillate and normal phase high-performance liquid chromatography was needed to achieve good separation, in our case, a proper separation of the compounds have been achieved with the proposed CG-MS method. Final signals are related to steryl esters. The characteristic composition and relative retention time (RRT) of the deodorized distillates of rapeseed oil are presented in Tables (3). The standard deviation (SD) was within an acceptable range of 0.09-0.41%.

Table 3. GC-MS identification of the different components of rapeseed distillate with cholesterol as internal standard

Matters	Components	Rt (min)	RRT*	SD** (min)
Saponificable	Phytol	10.20	0.66	0.30
	Methyl oleate	10.85	0.70	0.50
	Linoleic acid	11.57	0.75	0.14
	Squalene	12.36	0.80	0.25
Unsaponificable	Б-tochopherol	13.80	0.89	0.09
	β-tochophero	13.88	0.90	0.17
	γ-Tocopherol	14.18	0.95	0.19
	A-Tocopherol	14.71	1.04	0.19
	Cholesterol (I.S)	15.41	1.00	0.13
	Brassicasterol	15.52	0.99	0.41
	Campesterol	15.96	0.96	0.28
	β-Sitosterol	27.44	0.56	0.31

* Relative Retention Time
** Standard Deviation

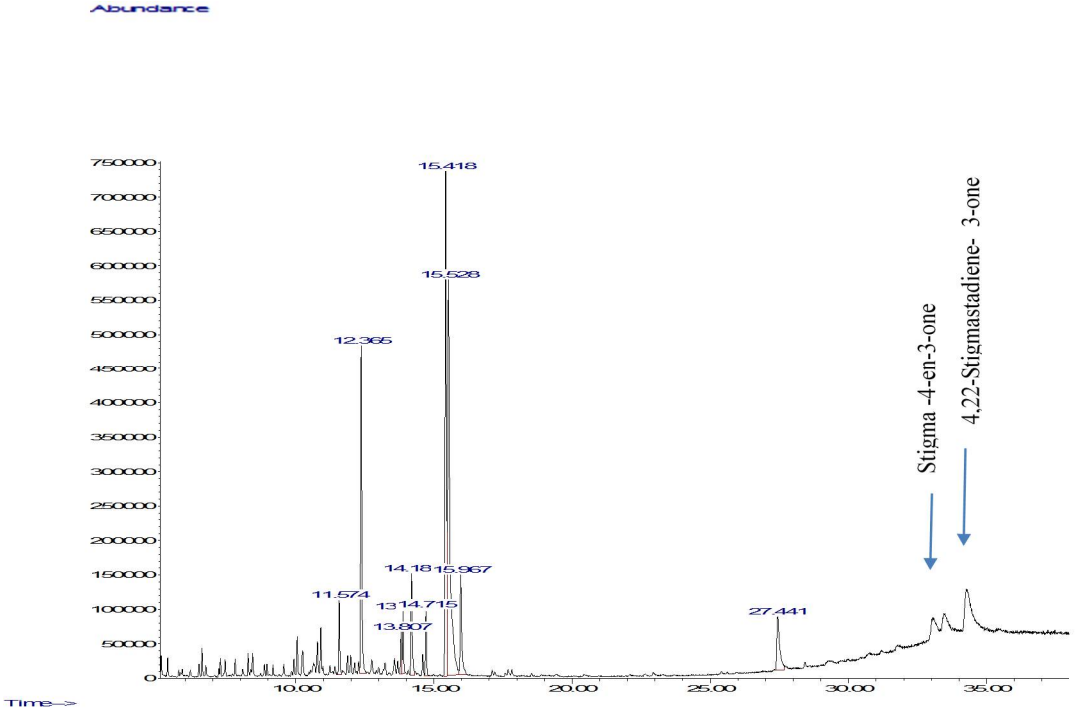

Fig. 2. Chromatogram of rapeseed oil deodorizer distillate with cholesterol as internal standard by GC-MS. Time scale in min

Rapeseed oil is the most used vegetable oil in the world, especially in the Middle East. Depending on the sources, RODD usually has different specifications, usages and costs. It is suitable for the production of tocopherols, and phytosterols (Jafarian-asl, Niazmand, & Jahani, 2020).

The composition of soybean and rapeseed oil distillates in terms of tocopherols and the free sterols is shown in Table (3). The concentration of free sterols in both samples was high meanwhile non-saponifiable matter was found to be the main part of the total composition (24.0-26.14%).

In Western countries, the average daily consumption of phytosterols is almost 250 mg/day, which is principally derived from vegetable oils, cereals, and nuts, etc. (Naz *et al.*, 2014). In human usage, phytosterols act as mediators for the synthesis of steroidal hormones and also reduce blood cholesterol levels (Khatoon, Rajan, & Krishna, 2010).

The composition of rapeseed oil distillates in terms of tochopherols and the free sterols is shown in Table (4). The main compound that has been detected in the rapeseed distillate was β-sitosterol (11.3 g/100 g), which was fairly higher than other sterols. β-Sitosterol is used for heart disease to decreasing cholesterol levels and also for improving the immune system and for inhibiting colon cancer (Jafarian Asl *et al.*, 2019). Besides, stigmasterol was not quantified in rapeseed distillate whereas in the case of soybean distillate it was 5.8 g/100 g. In this sense, results obtained for free sterols (Gunawan & Ju, 2009) are not comparable with previously reported studies because of the minor differences in the concentration among the deodorized distillates composition that relies on the deodorization settings, the variety of seeds and agricultural conditions.

In the case of tocopherol, all their isomers were detected in rapeseed distillate being γ-tocopherol in higher concentration. Also, concentrations of α-tocopherol (5.5 g/100 g) were found in rapeseed distillate. γ-Tocopherol is one of the chemical compounds that is considered vitamin E. α-Tocopherol inhibits the production of new radicals, while γ-tocopherol neutralizes existing free radicals. Only two isomers of tocopherol were found in the case of soybean distillate, α-tocopherol and Б-tocopherol, being Б-tocopherol found in a higher amount (7.6 g/100 g).

Dumont & Narine (2007), have reported two types of tocopherols (б and γ) with an amount of nearly 19.1% on a mass basis in SODD. Also, Verleyen *et al.* (2001) have already found the high mass percentage of tocopherols in soybean deodorizer distillates. Finally, squalene as the hydrocarbon fraction of deodorize distillate samples was in very high concentrations followed by nonacosane (6.5 g/100 g). Squalene was identified approximately high in the case of rapeseed distillate. Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) has antioxidant activity so potentially delaying the oxidation reaction of different vegetable oils. Moreover, it can protect the skin from harmful environmental effects (Nandi, Gangopadhyay, & Ghosh, 2008). Since squalene has an anticancer and antitumor activity (Loganathan, Selvaduray, Nesaretnam, & Radhakrishnan, 2010), rapeseed distillate could be used as a valuable and cost-effective source of squalene.

In the present study oxidized isomers and dehydrated products, i.e. sterenes and steradienes were not found. In both distillate samples two constituents i.e. 4, 22-stigmastadiene- 3-one and stigmast-4-en-3-one were detected in rapeseed distillate. These components may be the consequence of stigmasterol, and β-sitosterol dehydrogenation during deodorization. No literature references

could be found to show the presence of these two components in rapeseed and soybean deodorizer distillate.

Table 4. GC-MS quantification of different components of rapeseed oil deodorizer distillate with their relative standard deviation (RSD)

Components	Concentration	
	g/100 g	RSD (%)
Phytol	0.01	0.38
Methyl oleate	0.14	0.32
Linoleic acid	0.94	1.20
Hexacosane	1.20	1.71
Heptacosane	0.13	0.55
Octacosane	0.01	0.76
Squalene	6.05	1.60
Nonacosane	0.50	0.91
Б-tochopherol	1.70	0.36
β-tochopherol	1.00	1.24
γ–Tocopherol	6.40	0.12
α-Tocopherol	5.50	0.24
Brassicasterol	6.80	0.28
Stigmasterol	ND	ND
Campesterol	7.90	0.10
β-Sitosterol	11.30	0.07
Stigma -4-en-3-one	1.33	0.48
4,22-Stigmastadiene- 3-one	3.01	1.12

ND: not detected

Conclusions

Nowadays deodorizer distillates are a challenge due to their different characteristics, consumption and commercial values. One of the main advantages of analyzing of deodorizer distillate is a good separation of minor components present in it and obtaining acceptable results within the minimum requirement of preparation of sample without using toxic and carcinogenic solvents within a reasonable time. The results of this study showed that, due to the presence of natural valuable compounds such as squalene, tocopherol, phytosterol, acylglycerols, and free fatty acids in considerable quantity, DOD could be used in various applications. The unsaponifiable portion of this DOD is recommended for use in the medicine and cosmetic industry because of the high content of bioactive compounds and the saponifiable portion is recommended for the production of biodiesel due to the high amount of FFA.

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اندازه‌گیری سریع مواد فعال زیستی لیپیدی موجود در تقطیرات بوگیری روغن کُلزا با استفاده از دستگاه کروماتوگراف گازی-طیف‌سنج جرمی (GC-MS)

پریسا جعفریان اصل[1]، راضیه نیازمند[2*]، سیدحسین طفیل شیرازی[3]

1- دانشجوی دکتری، گروه شیمی مواد غذایی، مؤسسه پژوهشی علوم و صنایع غذایی، مشهد، ایران
2- دانشیار ،گروه شیمی مواد غذایی، مؤسسه پژوهشی علوم و صنایع غذایی، مشهد، ایران
* نویسندهٔ مسئول (r.niazmand@rifst.ac.ir)
3- استاد، مرکز ملی تجزیه و آنالیز شیمی، دانشگاه سند، جامشور، پاکستان

چکیده

تقطیرات بوگیری روغن کلزا یکی از محصولات فرعی و ضایعات کارخانجات فراوری روغن‌های گیاهی می‌باشد که حاوی ترکیبات مغذی باارزش و زیست‌فعال مانند فیتواسترول‌ها، توکوفرول‌ها و اسکالن می‌باشد. در مطالعهٔ حاضر برخی از خصوصیات فیزیکی و شیمیایی مانند رنگ (1 اینچ سلول، واحد R + Y5)، مقدار اسید چرب آزاد (به عنوان درصد اولئیک)، عدد صابونی (میلی‌گرم پتاسیم هیدروکسید/گرم)، روغن خنثی (درصد)، مواد صابونی ناشونده (درصد)، توکوفرول‌های کل (میلی‌گرم درصد)، فیتواسترول‌های کل (درصد) تعیین گردید. از تکنیک فراصوت جهت جزءبه‌جزء کردن و جداسازی تقطیرات بوگیری کُلزا به بخش متانولی صابونی ناشوندهٔ کمتر قطبی و بخش هگزانی که به‌طور عمده حاوی لیپیدها بود، استفاده شد. ترکیب چربی‌های طبیعی موجود در عصارهٔ متانولیک تقطیر بی‌بوکنندهٔ کُلزا توسط کروماتوگرافی مایع با کارایی بالا (HPLC) و ترکیب اسیدهای چرب توسط طیف‌سنجی جرمی کروماتوگرافی گازی (GC-MS) موردبررسی قرار گرفت. اسکوآلن انحراف استاندارد نسبی بالاتری داشت (3 درصد). درحالی‌که توکوفرول‌ها و استرول‌ها انحراف استاندارد نسبی پایین‌تری به‌ترتیب بین 0/36-0/11 و 0/28-0/04 درصد نشان دادند. بر اساس نتایج به‌دست‌آمده فراوان‌ترین ترکیبات در تقطیرات روغن سویا فیتواسترول‌ها بودند (22/14-15/5 درصد). این در حالی است که غلظت اسکوآلن و توکوفرول به‌ترتیب 2/05-1/9 درصد بود. همچنین تری‌گلیسیریدها اصلی‌ترین گلیسریدهای تقطیرات بودند (1/4 درصد). نتایج این پژوهش نشان داد که بخش حاوی لیپیدهای قطبی با فعالیت زیستی، با استفاده از حلال متانول تحت یک روش غیرمخرب (بدون استفاده از فرایند صابونی‌شدن) به‌طور مؤثری قابلیت جداسازی و استخراج دارد.

واژه‌های کلیدی: استرول، تقطیرات بوگیری روغن کانولا، توکوفرول، کروماتوگرافی گازی