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Extended Abstract

Chemical Analysis of Composition of Raw Soybean Oil Deodorized Distillates by GC-MS

Parisa Jafarian Asl[1], Razieh Niazmand[2*], Moslem Jahani[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- Assistant Professor, Department of Food Chemistry, Research Institute of Food Science and Technology, Mashhad, Iran

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Abstract

Deodorizer distillate is regarded as a waste material of the edible oil industries. It has intricate nature that consists of a valuable source of bioactive and nutritive compounds including phytosterols, tocopherols and squalene that are valuable economically. In this study instead of using sample pretreatments like saponification and derivatization, (using toxic solvents and tedious labor work) which are commonly utilized in previously published papers/manuscrpits, we used ultrasonic extraction of soybean deodorizer distillated with methanol, then centrifugation and the supernatant was directly injected into the GC-MS system. Optimization of the temperature profile of the oven was done to get a baseline separation of the different distillate components including, tocopherols, sterols, squalene. According to the results the most abundant compound in soybean distillate sample was phytosterols (22.5-24.3%). While concentration ranges for squalene and tocopherol were 5.9-6% and 10-11.24%, respectively. High amount of free fatty acids (62.46%) was detected and triacylglycerol was the main glycerides of distillate. The main glycerides of soybean distillate was triglycerides (1.4%). The present study exposed that the un-saponifiable fraction of deodorizer distillate could be employed after purification in drug, food and cosmetic industry because of its considerable amount of bioactive compounds.

Keywords: Gas chromatography, Soybean oil, Sterol, Tocopherol

Introduction

The principal by-product of edible oil refining is the deodorizer distillate (DOD) achieved in the deodorization stage (Naz, Sherazi, Talpur, Talpur, & Kara, 2012). Deodorizer distillates are complex mixtures of different compounds such as: free fatty acids; monoglycerides, diglycerides, triglycerides, squalene, phytosterols and tochopherols (Naz *et al*., 2014). Phytosterols 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 (Naz *et al*., 2012). The principal dietary phytosterols are stigmasterol, β-sitosterol, and campesterol (Yang *et al*., 2010). The value of deodorizer distillate is mainly specified by the content of tochopherols (vitamin E). Vitamin E is typically a mixture of four isomers alpha (α), beta (β),

gamma (γ) and delta (δ) tochopherols, that are usually consumed as additives in several foods, pharmaceutical and cosmetic products (Naz *et al*., 2012). Squalene is a hydrocarbon consumed as a predecessor for cholesterol biosynthesis, and as a natural moisturizer in cosmetics (Moreda, Pérez-Camino, & Cert, 2001). Durant, Dumont, & Narine (2006) decomposed canola oil DOD by GC-MS using simple methods "in situ" for measurement of compositions. Also they used derivatization by silylation for characterization of flax and soybean soap stocks and soybean deodorizer distillate (Dumont & Narine, 2007). These methods include toxic derivatizing reagents with pretreatment steps for drying the deodorizer distillate by using toxic and carcinogenic solvents and taking a lot of time and very effortful. In this study, we have developed a chromatography method for the immediate specification of bioactive ingredients without derivatization. A full scan analysis has been performed in order to identify soybean oil deodorizer distillate (SODD) composition by GC–MS within a reasonable time which has never been totally analyzed before.

Material and methods

Physicochemical Characteristics

The color of the samples was determined using a Lovibondn tintometer in a 1-in. cell on the Lovibond scale in transmittance mode and expressed as (5R + Y) units. Free fatty acids value, saponification value, unsaponifiable matter and total tocopherols and sterols content were determined by AOCS (1998) methods.

Preparation of Deodorizer Distillate

About 10 g of Deodorizer distillate samples was separately put into 100 mL Erlenmeyer with rubber seal and 10 mL methanol was added. Mixture was mixed for 1 min then put in ultrasonic bath for 30 min. After centrifugation upper layer was used to inject to GC-MS.

Determination of natural lipids

The natural lipids composition was determined according to Kartha (1953). The HPLC system (model X-Agilent technologies, USA) was fitted with a Diol 5 (lichrosphere) column 250×4×5 mm id and 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.

GC–MS analysis of bioactive compounds

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 a HP-5MS (5% phenyl methylsiloxane) 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. Injection temperature was 310 °C. A sample injection of 2 μL was performed in a split mode of 1:10. The oven temperature was program as follows: 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. Standard solutions were prepared at a concentration of 10 mg ml^{-1} in chloroform and were kept at -20 °C. Cholesterol was chosen as the internal standard (IS).

Results and discussion

The DOD of soybean oil which was obtained from the company was dark semisolid at room temperature and converted to liquid when heated to 30 °C. It had a dark brown color (137.4 Lovibond color units), and other physicochemical characteristics are presented in Table (1). Difference in the range of values in literatures was due to variation in processing conditions

and raw material variability. Triacylglycerol is the main acylglycerol in vegetable oil whose types depend on the kind of oil source. The different families of neutral lipids are identified by comparing the retention times (Fig. 1). The order of elution being: Esters>Waxes>TAG> FFA>DAG>MAG. High amount of free fatty acids in SODD (62.46±9) indicates a lower amount of glycerides (Table 2).

Table 1. Composition and properties of Soybean oil deodorizer distillate*

Parameters	DOD
Color (1 in. cell, 5R+Y unit)	137.40±1.00
Free fatty acid value (as % oleic)	62.46±2.10
Neutral oil** (%)	2.00±0.80
Unsaponifiable matter (%)	35.54±2.70
Total tocopherols (%)	11.24±1.20
Total phytosterols (%)	24.30±1.50
Esterified sterol (%)	35.54±2.70

* Means and standard deviation of triplicates.
** Neutral oil % = [100 - (%unsaponifiable matter + %FFA)]

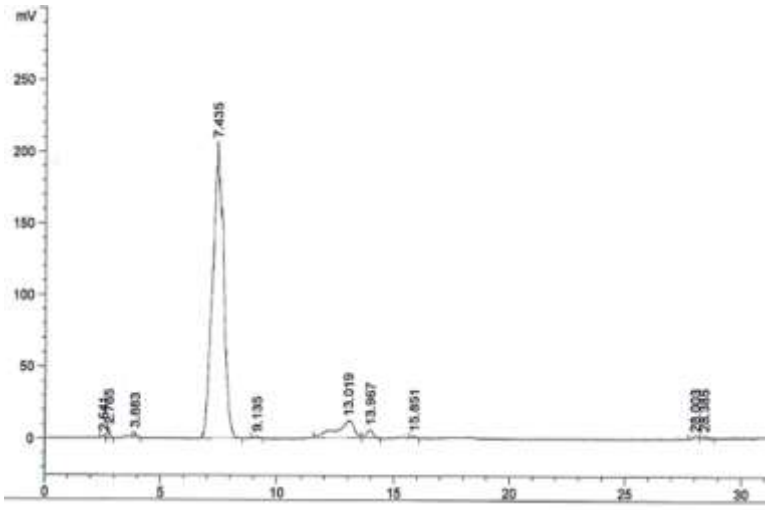

Fig. 1. HPLC chromatogram of monoacylglycerol (MAG), diacylglycerol (DAG), and triacylglycerol (TAG) in rapeseed deodorizer distillate (relative area %)

Table 2. Composition of natural lipids of SODD (relative area %)

Parameters	Rt (min)	Concentrations[1] %
Short chain esters	2.5-2.8	19.00±0.90
Long chair esters	3.4-3.8	16.14±0.30
Triglycerides	6.8-9.2	1.40±0.09
Diglycerides	15.2-18.2	0.70±0.01
Monoglycerides	27.9-28.3	0.30±0.06
Free fatty acids	12-13.9	62.46±9.00

Means and standard deviation of triplicates

Qualitative analysis of unsaponifiable section of SODD

Separation and identification of various components were performed as it can be seen, the analytes according to their carbon numbers are parted 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 δ - tocopherol followed by the next isomer of three dimethyltocols, β-, γ- and finally α-tocopherol. Afterward campesterol stigmasterol and β sitosterol followed by the signals of cholesterol. Final signals are related to steryl esters. However, there is no brassicasterol peaks in soybean deodorized distillate and

also just two types of tocopherols (α, γ) have been found. The standard deviation (SD) was within the acceptable range of 0.05–0.25% (Table 3).

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

Components	Rt (min)	RRT	SD
Phytol	10.9	0.70	0.05
Linoleic acid	11.57	0.75	0.04
Squalene	12.36	0.80	0.25
δ-Tochopherol	13.81	0.89	0.12
α-Tochopherol	14.71	0.95	0.10
Cholesterol (I.S)	15.42	1	0.13
Campsterol	15.52	1.01	0.04
Stigmasterol	15.97	1.03	0.15
β-Sitosterol	27.44	1.77	0.20

Validation of Method and Quantification

The method precision was assessed by 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 and soybean distillates 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. The correlation coefficients (r) for all the standard curves were 0.996. The relative response factor method was also applied to validate the quantification (Table 4).

Table 4. The least squares regression line parameters and correlation coefficients* (R^2) of calibration curves

Components	m	b	R^2
Octacosan	1E-05	0.0228	0.9965
Nonacosan	7E-06	0.1212	0.9977
α-Tochopherol	1E-05	0.0471	0.9981
β-Sitosterol	9E-07	0.129	0.9964

* y=mx+b (where y: is the concentration in mg mL^{-1} and x: is the area of the chromatogram peak)

Unsaponifiable compounds of SODD

The composition of soybean oil distillates in terms of tocopherols and the free sterols is shown in Table (5). The concentration of free sterols in the sample was high meanwhile non-saponifiable matter was found to be the main part of the total composition (24.3%). The main compound that has been detected was β-sitosterol, which was fairly higher than other sterols. β-sitosterol is used for heart diseases to decrease cholesterol levels and also for improving the immune system and for inhibiting colon cancer. Only two isomers of tocopherol were found in the soybean distillate, α-tocopherol and δ-tocopherol, being δ-tocopherol found in higher amount (7.6 g/100 g). Also stigmasterol was quantified that was 5.8 g/100 g. In this sense, results obtained for free sterols are not comparable with previously reported studies because of the minor differences in the concentration among the deodorized distillates composition that rely on the deodorization settings, the variety of seeds and agricultural conditions. Finally, squalene as the hydrocarbon fraction of deodorize distillate samples was in very high concentrations (5.9 g/100 g) followed by nonacosane (0.01 g/100 g). Oxidized isomers and dehydrated products, i.e. sterenes and steradienes were not found. In the distillated sample one constituents i.e. 4,22-stigmastadiene-3-one was detected. This component 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 5. GC-MS quantification of different components of Soybean oil deodorizer distillate with their relative standard deviation (RSD)

Components	SODD	
	g/100 g	RSD (%)
Phytol	0.02	0.38
Ethyl oleate	0.10	0.20
Linoleic acid	0.63	0.71
Hexacosane	0.12	0.66
Heptacosane	0.12	0.57
Octacosane	0.08	0.13
Squalene	5.90	3.00
Nonacosane	0.01	1.20
δ-tochopherol	7.60	0.11
β-tochopherol	---	---
γ -Tocopherol	---	---
α-Tocopherol	3.64	0.14
Brassicasterol	---	---
Stigmaesterol	5.8	0.04
Campesterol	6.8	0.19
β-Sitosterol	10.7	0.04
4,22-Stigmastadiene-3-one	2.4	1.12

ND not detected; RSD relative standard deviation

Conclusions

The deodorizer distillates usually have significantly different characteristics, usages, and cost. The main advantage of analysis of unsaponified deodorizer distillate by gas chromatography mass spectroscopy is a good separation of the different distillate components and achieving a perfect result with minimal sample preparation and without using toxic and carcinogenic solvents. Results of the present study showed that due to the existence of natural valuable components such as squalene, sterols, tocopherols and fatty acid in considerable quantity, the unsaponifiable section of DOD could be applied in medicine and cosmetic industry because it is a rich source of natural bioactive compounds while the saponifiable section could be used for biodiesel production due to high FFA contents.

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