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Gas chromatographic characterization of vegetable oil deodorization distillate

T. Verleyen[a,*], R. Verhe[a], L. Garcia[a], K. Dewettinck[b], A. Huyghebaert[b], W. De Greyt[c]

[a]*Department of Organic Chemistry, University of Ghent, Coupure Links 653, 9000 Ghent, Belgium*

[b]*Department of Food Technology and Nutrition, University of Ghent, Coupure Links 653, 9000 Ghent, Belgium*

[c]*De Smet Engineering, Prins Boudewijnlaan 265, Edegem, Belgium*

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Abstract

Because of its complex nature, the analysis of deodorizer distillate is a challenging problem. Deodorizer distillate obtained from the deodorization process of vegetable oils consists of many components including free fatty acids, tocopherols, sterols, squalene and neutral oil. A gas chromatographic method for the analysis of deodorizer distillate without saponification of the sample is described. After a concise sample preparation including derivatization and silylation, distillate samples were injected on column at 60°C followed by a gradual increase of the oven temperature towards 340°C. The temperature profile of the oven was optimized in order to obtain a baseline separation of the different distillate components including free fatty acids, tocopherols, sterols, squalene and neutral oil. Good recoveries for δ-tocopherol, α-tocopherol, stigmasterol and cholesteryl palmitate of 97, 94.4, 95.6 and 92%, respectively were obtained. Repeatability of the described gas chromatographic method was evaluated by analyzing five replicates of a soybean distillate. Tocopherols and sterols had low relative standard deviations ranging between 1.67 and 2.25%. Squalene, mono- and diacylglycerides had higher relative standard deviations ranging between 3.33 and 4.12%. Several industrial deodorizer distillates obtained from chemical and physical refining of corn, canola, sunflower and soybean have been analyzed for their composition. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Vegetable oils; Sterols; Tocopherols; Vitamins; Fatty acids

1. Introduction

Deodorizer distillate obtained from the deodorization process of vegetable oils is a complex product consisting of many components including free fatty acids, tocopherols, sterols, squalene and neutral oil [1–3]. The value of deodorizer distillate has varied over the years. Historically, deodorizer distillate used to be sold for its sterol content, as phytosterols have been used as starting materials or as intermediates for the manufacturing of pharmaceutical drugs and steroids for medicinal purpose [4]. Recently an increasing interest in phytosterols is observed as phytosterols are used in functional foods (e.g., margarines, etc.) for their potential to inhibit intestinal cholesterol adsorption and serum low-density lipoprotein (LDL) lowering capacities [5,6]. Today, the value of the distillate is primarily determined by

*Corresponding author. Tel.: +32-9-2645-950; fax: +32-9-2646-243.

E-mail address: tom.verleyen@rug.ac.be (T. Verleyen).

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the tocopherol content. Tocopherols are natural antioxidants, which are valued for their antioxidative and vitamin E activity [7]. As the economical value of the distillate depends on its composition and content of tocopherols and sterols, an accurate quantification of these and other minor components present in the distillate is primordial.

Because of its complex nature, the analysis of deodorizer distillate is a challenging problem. Few analytical techniques are available to provide a detailed analysis of deodorizer distillate [1,8,20,21]. The AOCS Ce3-74 Official Method [9] describes a method for the determination of tocopherols and sterols in soy sludges by gas–liquid chromatography using a packed glass column. After a long sample preparation, which involves saponification of the distillate and extraction of the unsaponifiables, tocopherols and sterols are transformed into their butyrate esters and are roughly separated by gas chromatography (GC). The more recently published AOCS Ce7-87 recommended practice [9] analyzes the total tocopherol content in the deodorizer distillate by capillary GC on an apolar column. After a concise sample preparation, which consists of a silylation, a short temperature profile is applied which separates δ-, β/γ- and α-tocopherols. In this method δ-, β/γ- and α-tocopherols are separated but due to the short temperature profile other components present in the deodorizer distillate are not well resolved.

The objective of the research was to introduce a method combining a baseline separation of the different components present in deodorizer distillate (free fatty acids, squalene, tocopherols, free and esterified sterols and neutral oil) within a reasonable analysis time of maximally 1 h by optimization of the temperature profile.

2. Experimental

2.1. Materials

Deodorizer distillates originating from industrial- and laboratory-scale deodorization of several vegetable oils (soybean, canola, sunflower and corn oil) were obtained from Extraction De Smet (Edegem, Belgium). All samples were stored in the refrigerator at 4°C for a maximum of 4 months until analysis. The free fatty acid (FFA) content of the samples was determined by titration according to the AOCS Official Method Ca 5a-40 [9] and expressed as oleic acid ($C_{18:1}$).

Analytical-grade solvents were purchased from Merck (Darmstadt, Germany). All analytical-grade reference substances, squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene), stigmasterol (3β-hydroxy-24-ethyl-5,22-cholestadiene), cholesteryl stearate (5-cholesten-3β-yl-octadecanoate), monoacylglycerol olein, diacylglycerol olein and triacylglycerol olein, betulinol (lup-20[29]-ene-3β,28-diol) were purchased from Sigma (Bornem, Belgium) and were at least 96% pure. A tocopherol kit consisting of α-, β-, γ- and δ-tocopherols was obtained from Merck.

The internal standard heptadecanyl stearate (HDS) was prepared by condensation of heptadecanol and stearoyl chloride both purchased from Aldrich (Bornem, Belgium). Heptadecanol (50 g) was weighed into a 1-l flask and stearoyl chloride (30 g) was added under slow agitation. The mixture was dissolved in hot ethanol (500 ml), cooled to room temperature, crystallized during the night and filtrated. HDS crystals were crystallized twice from ethanol (500 ml).

2.2. Analysis of non-saponified deodorizer distillate

Deodorizer distillate samples (0.1–0.15 g of dry and homogeneous distillate) were directly weighed in a screw-capped test tube. Deodorizer distillate samples were derivatized in order to increase the volatility of the components. After dissolving the sample in 0.5 ml of pyridine and addition of 1 ml *N*,*O*-bis-(trimethylsilyl)trifluoracetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) solution as derivatizating and silylating agent, the test tube was placed in an oven at 70°C for 20 min for completion of the silylation. Afterwards 1 ml of HDS solution with known concentration (±400 mg HDS/100 ml chloroform) was added as internal standard and the sample was further diluted with 8 ml of chloroform. After transferring 15 μl of derivatized sample to a vial and dilution with 1.5 ml of chloroform the sample was ready for injection. After derivatization all samples were analyzed within 6 h of preparation.

2.3. Analysis of saponified deodorizer distillate samples

Deodorizer distillate samples (0.7 g) were saponified with 10 *M* KOH (5 ml) in 45 ml ethanol, together with 5 mg betulinol as internal standard to quantify losses during saponification and extraction. The solution was heated for 30 min at 70°C. After saponification, 100 ml water was added and the unsaponifiables were extracted twice with 100 ml diethyl ether. The combined diethyl ether fractions were washed twice with 20 ml of 0.5 *M* KOH, four times with 20 ml water and dried over anhydrous sodium sulfate. The residue obtained after evaporation was derivatized according to the procedure described in Section 2.2.

2.4. Capillary column gas–liquid chromatography

Separations were performed on a Hewlett-Packard 5890 series gas chromatograph with cold on-column injection on a capillary column CP-Sil 8 CB Low bleed/MS 15 m×0.25 mm, 0.1 μm (Chrompack, Middelburg, The Netherlands) with detection by flame ionization detection.

A temperature program with injection at 60°C, rising at 30°C/min to 140°C and continuing the oven heating at a speed of 5°C/min to 235°C with 7 min hold and further heating at 15°C/min to 340°C with 15 min hold, was found optimal for a good separation of the different components. The detector temperature was set at 360°C. Helium was used as a carrier gas at a pressure of 41.3 kPa. The peaks were computed using a HP 3396 series II integrator.

3. Results and discussion

3.1. Analysis of non-saponified deodorization distillates: optimization of separation

Literature references analyze the tocopherol and sterol content of deodorizer distillate after a laborious sample preparation procedure including saponification and derivatization. In this research, a baseline separation of the different components present in vegetable oil deodorizer distillate was achieved by a direct derivatization of non-saponified deodorizer distillate followed by an optimized GC temperature profile. Comparing their GC retention times with standards was performed for the identification of the different peaks. Retention times and relative retention times using HDS as internal standard for the identified components are presented in Table 1. Chromatograms of non-saponified soybean and rapeseed distillate are shown in Figs. 1 and 2.

Upon optimization of the procedure the separation of two critical pairs was observed. Within the tested temperature profiles, co-elution of the β- and γ-tocopherols was always found (Fig. 2). However, this was expected as due to their similar chemical structures, a separation between the β- and γ-tocopherols homologues is hardly achieved by GC [1,10,11]. Baseline separation of the β- and γ-tocopherols can only be obtained by normal-phase high-performance liquid chromatography [11–14]. Actually this incomplete separation was not regarded as a problem as β-tocopherol is only present in most natural vegetable oils, and consequently in deodorizer distillate, in very low concentrations [15–17].

Another pair difficult to separate was α-tocopherol and brassicasterol (Fig. 1). By applying the proposed temperature profile a peak resolution of 0.75 was obtained for the separation between α-tocopherol and brassicasterol. This resolution factor is not completely perfect, as resolutions above 0.9 are necessary for making a good quantification possible. No further

Table 1
Retention times (t_R) and relative retention times (RRTs) of identified components present in deodorizer distillate

	t_R (min)	RRT[a]
Monoacylglycerides	19.3	0.58
Squalene	20.1	0.61
δ-Tocopherol	21.9	0.66
β-Tocopherol	22.8	0.69
γ-Tocopherol	22.9	0.69
α-Tocopherol	25.2	0.76
Brassicasterol	25.5	0.77
Campesterol	27.1	0.82
Stigmasterol	27.8	0.84
Sitosterol	29.3	0.89
Heptadecanyl stearate (HDS)	33.1	1
Diacylglycerides	35.4	1.07
Steryl esters	38.7	1.17
Triacylglycerides	47.3	1.43

[a] Retention time relative to HDS.

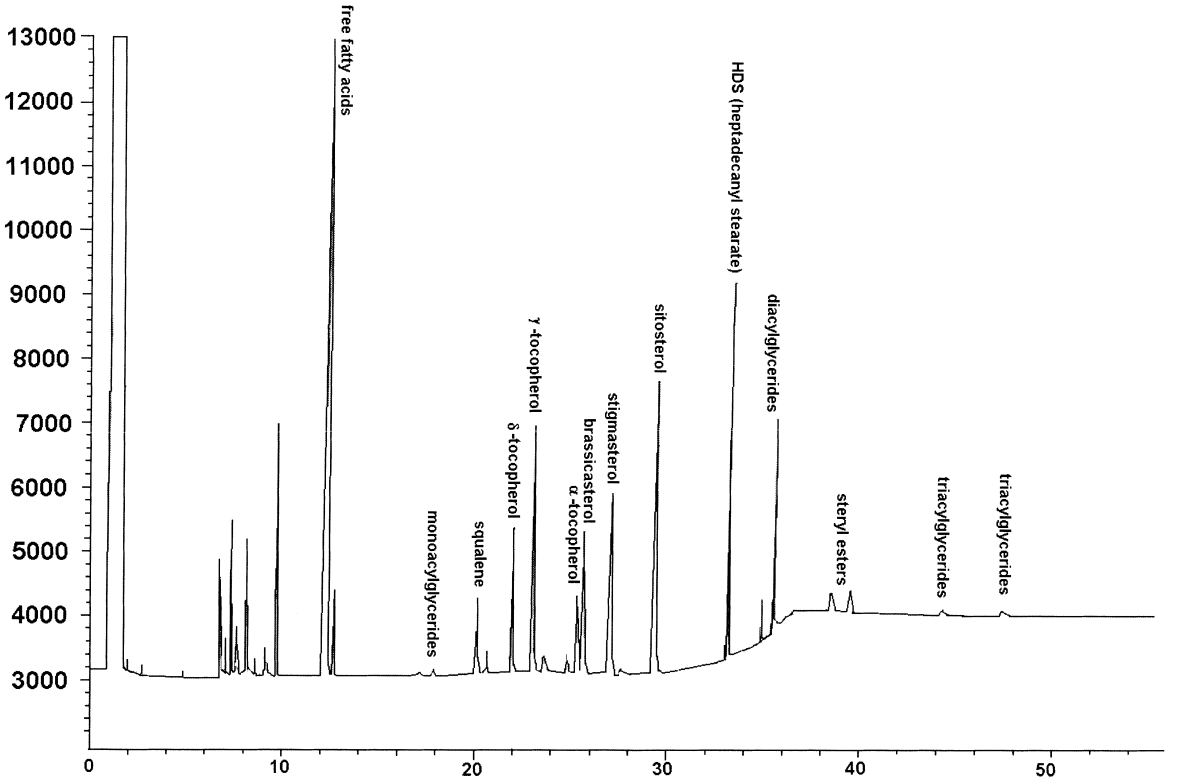

Fig. 1. Chromatogram of non-saponified rapeseed deodorizer distillate analyzed by gas chromatography. Time scale in min.

research was undertaken to improve this separation, as brassicasterol is only present in rapeseed or canola cultivars.

3.2. Determination of response factors

Quantification of the different components present in deodorizer distillate was done by use of an internal standard. HDS was selected as internal standard as no components eluted in this region. Response factors (RFs) were determined by accurately weighing 10–15 mg of pure standard component into a test tube, followed by the derivatization procedure described.

Response factors were determined for α- and δ-tocopherols. The mean of these response factors was used as the estimated response factor for β- and γ-tocopherols. Stigmasterol was selected for the determination of the sterol response factor and was used for all free sterols. The response factor of cholesteryl stearate was used to quantify the whole steryl ester group. Monooleylglycerol, diaoleylglycerol and trioleylglycerol are the standards used for the response factors of mono-, di-, triacylglycerides. The response factors determined for the different standards are listed in Table 2. All response factors were determined fivefold and showed a good repeatability as indicated by the low standard deviation (SD) and relative standard deviation (RSD) ranging between 3.5 and 4.6%. A good stability of the response factors over a period of several months was observed. An accurate determination of the response factors is necessary for obtaining a good quantification of the different distillate components.

3.3. Recovery

In order to exclude an incorrect quantification of deodorizer distillate samples the recovery of the analysis method was evaluated. A ‘‘synthetic’’ dis-

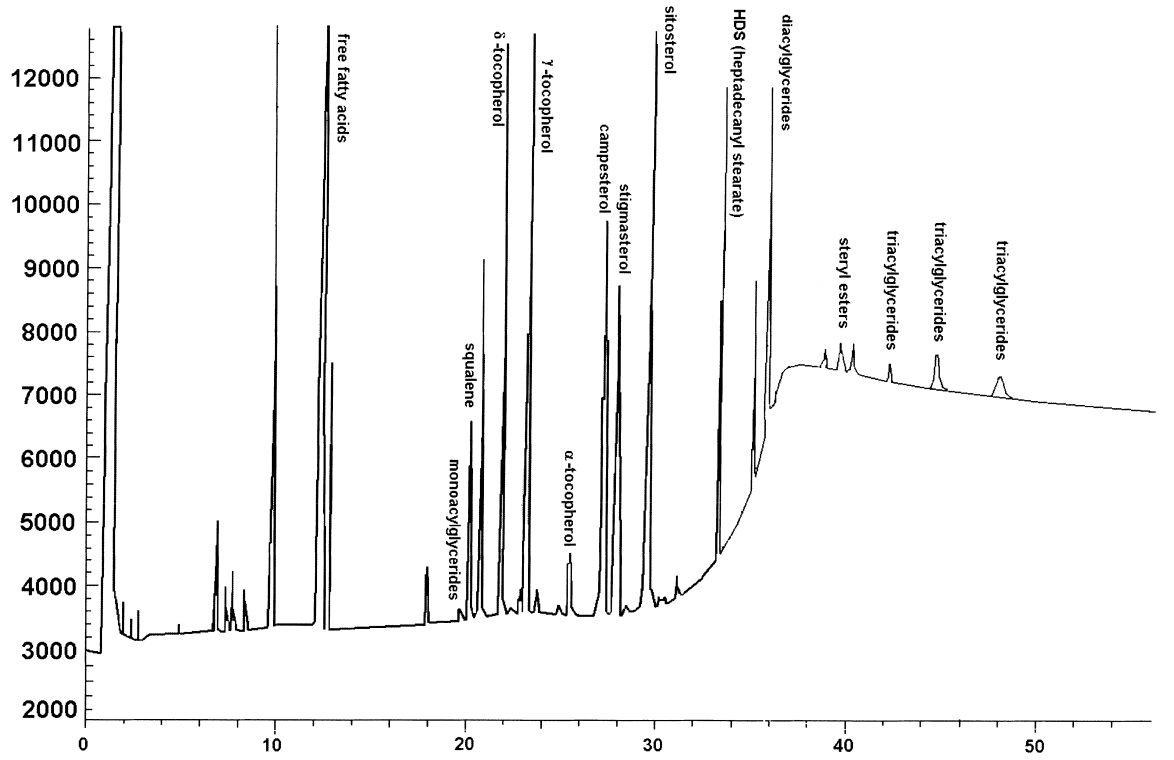

Fig. 2. Chromatogram of non-saponified soybean deodorizer distillate analyzed by gas chromatography. Time scale in min.

tillate was prepared by dissolving δ- and α-tocopherols, stigmasterol and cholesteryl stearate in a free fatty acid matrix, which consisted of oleic acid ($C_{18:1}$). Three distillates with different tocopherol and sterol content were prepared. Tocopherol and sterol concentrations are expressed as g/100 g of distillate for both gravimetric and gas chromatographic data. Upon analyzing the distillates good recoveries of 94–97% for the tocopherol and sterol contents were found (Table 3). A perfect recovery was expected due to the minimal sample preparation. The current recovery levels for tocopherol and sterol concentration are in accordance with literature reports [10,11,18]. A lower but still acceptable recovery of 92% was observed for cholesteryl stearate. Lechner et al. [10] also reported a lower analyte recovery factor for steryl esters. This lower analyte recovery for steryl esters was explained by degradation on the GC column due to the high temperature applied in GC.

Table 2
Response factors (RFs) obtained of standards relative to heptadecanyl stearate (HDS) as internal standard

	RF mean[a]	SD	RSD (%)
Monoacylglycerides	0.76	0.03	3.56
Squalene	0.76	0.03	3.56
δ-Tocopherol	0.87	0.03	3.58
α-Tocopherol	0.78	0.03	4
Stigmasterol	0.74	0.02	2.88
Diacylglyceride	0.94	0.04	4.26
Cholesteryl palmitate	1.16	0.05	4.62
Triacylglycerides	2.5	0.1	4

[a] Response factor calculated as mean of five replicates.

3.4. Repeatability

Repeatability of the described gas chromatographic method was evaluated by analysing five replicates of a soybean distillate including derivatization procedure (Table 4). Only identifiable peaks were taken into consideration and concentration was expressed

Table 3
Recovery of δ- and α-tocopherols, stigmasterol and cholesteryl palmitate spiked at three concentration levels in oleic acid ($C_{18:1}$)

	Concentration (g/100 g distillate)						Recovery (%)		
	Distillate A		Distillate B		Distillate C		Mean	SD	RSD
	Grav[a]	GC[b]	Grav	GC	Grav	GC			(%)
δ-Tocopherol	1.52	1.48	3	2.86	5.31	5.19	96.97	0.5	0.6
α-Tocopherol	2.04	1.93	4.08	3.77	6.97	6.7	94.37	0.71	0.76
Stigmasterol	0.47	0.45	2.88	2.71	2.71	2.61	95.61	1.57	1.7
Cholesteryl palmitate	0.44	0.38	1.21	1.1	1.01	1	91.98	1.31	1.42

[a] Grav, concentration calculated on gravimetric basis.
[b] GC, concentration determined by GC analysis.

as g/100 g of distillate. Taking into account the short sample preparation a good repeatability was observed with similar RSDs to another analytical report on tocopherol and sterol quantification [11]. The four tocopherols and different sterols had similar RSDs ranging between 1.67 and 2.25%. Only for β-tocopherol a higher RSD was obtained, probably due to the incomplete separation between β- and γ-tocopherols. Squalene, mono- and diacylglycerides had higher (3.33–4.12%) but still acceptable RSD values.

3.5. Overview of different deodorization distillate samples

Tocopherols and phytosterols were identified as the most important minor components and were present in varying concentrations in the distillate.

Table 4
Repeatability of the quantitative gas chromatographic analysis of a soybean deodorizer distillate obtained by repeated analysis ($n=5$)

	Mean[a]±SD (g/100 g distillate)	RSD (%)
Monoacylglycerides	1.59±0.03	3.33
Squalene	0.9±0.06	3.77
δ-Tocopherol	4.28±0.07	1.67
β-Tocopherol	0.31±0.02	7.06
γ-Tocopherol	10.31±0.25	2.46
α-Tocopherol	1.26±0.03	2.61
Campesterol	4.08±0.08	2.02
Stigmasterol	3.83±0.09	2.25
Sitosterol	7.35±0.16	2.12
Diacylglycerides	2.78±0.11	4.12
Steryl esters	3.94±0.07	1.68
Triacyglycerides	5.37±0.1	1.9

[a] Mean of five replicates.

The tocopherol and sterol content of deodorizer distillate strongly depends on the initial free fatty acid content and the applied deodorization process conditions including temperature, steam and pressure [4]. The quantitative composition of the different distillates obtained from the deodorization of soybean, corn, sunflower and rapeseed oil is listed in Table 5 and is largely affected by the refining technique (chemical or physical). Results are expressed as g/100 g of oil. In the physical refining (numbers 3, 4, 5, 6) free fatty acids are vaporized during the deodorization process. Consequently the distillate contains mainly free fatty acids (>80%) with only small amounts of unsaponifiables (5–10%) and is usually sold as a source of technical fatty acids. In the chemical refining (numbers 1, 2, 7, 8) free fatty acids are neutralized by a caustic solution and washed out of the oil before deodorization. Deodorizer distillates obtained from a chemical refining obviously have lower free fatty acid content (30–50%) and a higher level of unsaponifiables ranging between 25 and 33% [19]. Due to this higher tocopherol and sterol content deodorizer distillates obtained from a chemical refining are a good source of vitamin E and phytosterols. Especially the distillate of chemically refined soybean oil is an attractive source of tocopherols and sterols [4]. For every type of oil, a sterol-to-tocopherol ratio exists which is characteristic. Monitoring the sterol over tocopherol ratio is a way to determine if degradation is occurring during processing or storage of the distillate [4].

3.6. Analysis of saponified deodorization distillates

Distillate samples can also be analyzed by a

Table 5
Composition of non-saponified deodorizer distillates analyzed by quantitative gas chromatography

	Soybean			Corn		Sunflower		Rapeseed	
	1	2	3	4	5	6	7	8	9
Squalene	1.28	2.09	0.65	0.21	0.99	1	0.73	0.4	0.07
δ-Tocopherol	4.41	5.59	2.01	0.12	0.12	nd	nd	0.18	0.31
β-Tocopherol	0.52	0.36	0	0.06	0.08	nd	nd	0.18	0.14
γ-Tocopherol	10.73	11.26	4.96	1.09	2.75	0.07	0.3	2.48	2.33
α-Tocopherol	0.82	0.81	0.54	0.15	0.36	1.21	4.76	1.35	0.89
Brassicasterol	nd[b]	nd	nd	nd	nd	nd	nd	2.75	1.64
Campesterol	5.06	5.66	1.91	0.84	1.67	0.45	1.58	4.37	2.93
Stigmasterol	4.1	4.81	1.38	0.19	0.37	0.62	2.04	nd	0.01
Sitosterol	7.9	8.34	3.03	1.68	3.38	2.6	8.60	6.24	4.05
Other sterols[a]	nd	nd	nd	nd	nd	0.62	1.68	nd	nd
Steryl esters	2.59	2.33	4.45	0.62	nd	0.09	0.3	5.33	1.35
Monoacylglycerides	1.24	1.93	1.85	0.04	0.13	0	0.86	1.42	2.11
Diacylglycerides	2.7	3.79	8.06	0.54	1.26	0.66	1.89	3.85	3.87
Triacylglycerides	5.13	5.91	3.76	0.14	0.81	2.67	2.56	3.01	7.54
FFA ($C_{18:1}$)	33	32	73.8	81.2	77.1	70.82	39.2	39.2	42.8

Concentrations expressed as g/100 g of distillate.
[a] Sum of Δ5 avenasterol, Δ7 avenasterol and Δ5 stigmasterol.
[b] nd, Not detected.

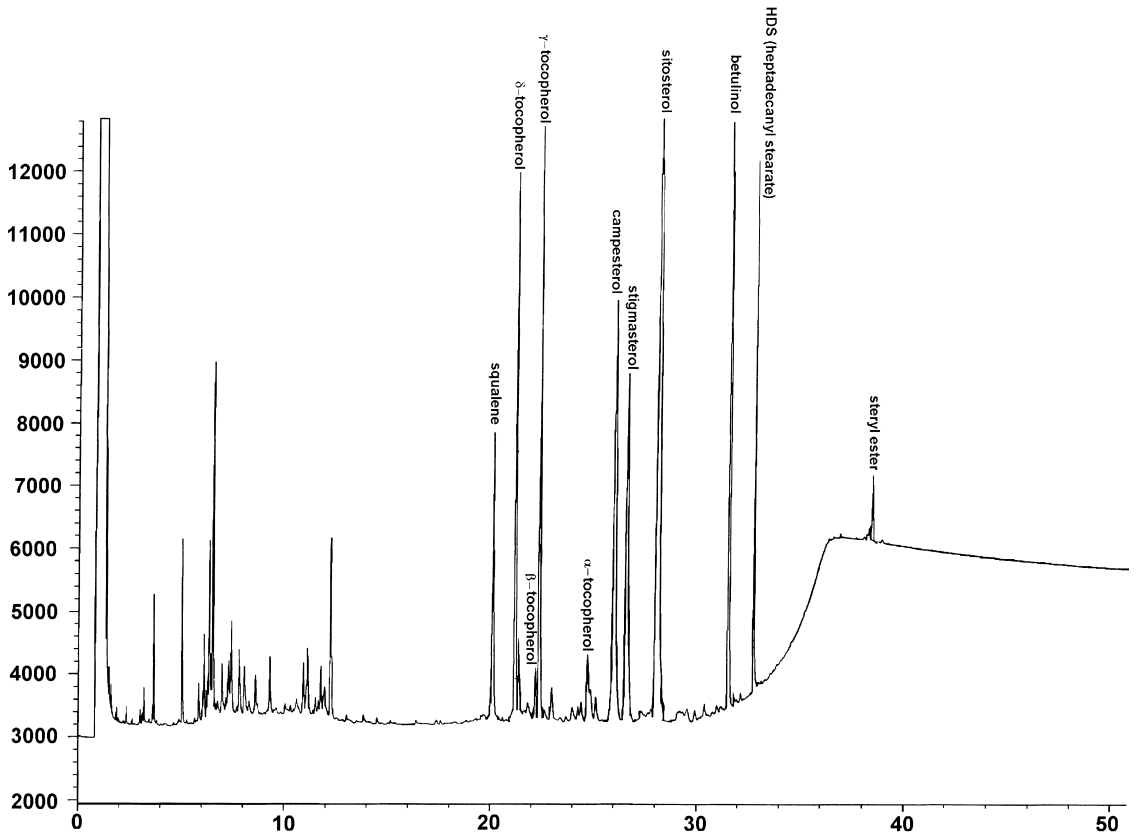

Fig. 3. Chromatogram of saponified soybean deodorizer distillate analyzed by gas chromatography. Time scale in min.

Table 6
Comparison between saponified and non-saponified deodorizer distillate composition analyzed by quantitative gas chromatography and expressed as g/100 g distillate[a]

	Rapeseed		Rapeseed		Soybean	
	Non-saponified	Saponified	Non-saponified	Saponified	Non-saponified	Saponified
Squalene	0.4	0.38	0.38	0.39	0.26	0.29
δ-Tocopherol	0.18	0.32	1.94	2.04	3.66	3.27
β-Tocopherol	0.18	nd	nd	nd	0.27	nd
γ-Tocopherol	2.48	2.99	3.91	3.97	8.7	8.93
α-Tocopherol	1.35	1.56	1.56	1.68	0.9	1.03
Brassicasterol	2.75	3.61	2.82	2.56	nd	nd
Campesterol	4.37	5.82	4.24	5.27	3.36	3.78
Stigmasterol	0	0.2	0	0.1	3.16	3.69
Sitosterol	6.24	8.43	6.27	7.63	5.78	6.73
Steryl esters	5.33	0.25	1.67	nd	2.47	nd

[a] Monoacylglycerides, diacylglycerides and triacylglycerides could not be detected anymore after saponification.

preceding saponification, as explained in Section 2.3, followed by the same derivatization procedure and gas chromatographic analysis procedure. Saponification of distillate samples neutralizes free fatty acids and hydrolyses neutral oil and esterified minor components. As expected, free fatty acids, mono-, di-, triacylglycerides could not be detected anymore in the gas chromatogram after saponification as shown in Fig. 3. Sometimes part of the components eluting in the steryl esters region remained present after saponification. Chromatograms obtained after saponification showed much more interferences of small components appearing in the tocopherol and sterol region. Probably this can be explained by an enrichment of some tocopherol and sterol oxidation products during saponification.

A comparison between the analysis method without preceding saponification and the method including preceding saponification was made for some deodorizer distillate samples (Table 6). The saponified distillates have a tocopherol content which is rather similar to the straight analysis indicating tocopheryl esters are hardly present in the distillate. The sterol content of saponified deodorizer distillate samples is significantly higher due to a hydrolysis of steryl esters during saponification.

4. Conclusion

In conclusion, advantage is given to an analysis non-saponified deodorizer distillate by capillary column GC as it gives a good separation of the different distillate components and a perfect result is obtained with minimal sample preparation. In further research the relationship between the deodorization process condition and the composition of the deodorizer distillate will be studied.

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