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Vegetable Oil Deodorizer Distillate: Characterization, Utilization and Analysis

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Abstract: Depending on the sources, deodorizer distillates usually have significantly different characteristics, uses, and value. Soybean oil deodorizer distillate has been suggested as an alternative to marine animals as natural source of squalene and as a good raw material for the production of fatty acid steryl esters, tocopherols, free phytosterols and fatty acids. The aim of this review paper is to discuss the characteristics, uses, and value of vegetable oil deodorizer distillate (VODD), isolation and separation techniques of bioactive compounds from VODD, and analysis of VODD, as well as their future perspectives. The development of new separation techniques for isolating bioactive compounds and methods of analysis are highlighted and discussed.

Keywords: Analysis methods, characterization, deodorizer distillates, utilizations

INTRODUCTION

Practically all commercial vegetable oils produced worldwide sold are refined oils (pure triacylglycerols, TAGs). Without refining, most vegetable oils could not be used for nutritional purpose. This is because crude vegetable oils contain impurities or by-product such as gums (phospatides), waxes, metals, free fatty acids (FFAs), colored particles

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and substances, diacylglyecrols (DAGs), monoacylglycerols (MAGs), odoriferous components (aldehydes and ketones), pesticides, herbicides, and polycyclic aromatic hydrocarbons (PAHs) (1). The amount of these impurities depends on the kind of oil sources, the seed treatment, the extraction process, and the storage conditions. They can negatively influence the taste and smell of oils. Also, they can limit the use and complicate the processing of oils.

During the processing of vegetable oils, substances that usually give a bad taste and/or foul odor (aldehydes and ketones) are mostly removed *via* steam-stripping distillation. Also removed in this steam-stripping distillation are tocopherols, phytosterols, FFAs, TAGs, DAGs, MAGs, FASEs and hydrocarbons (2–6). The distillate obtained is termed as vegetable oil deodorizer distillates (VODD). Depending on the sources, the deodorizer distillates usually have significantly different characteristics, uses, and value. They can be a good raw material for the production of tocopherols, free phytosterols, FASEs, fatty acids and squalene (7–12).

The concerted effort to recover VODD began in earnest in the late 1960s for two reasons (13): (a) Increasing demand for phytosterols and tocopherols (natural vitamin E) by the pharmaceutical companies, and (b) The refiner's interest in cleaning up their messy soap stock skimming operations. Unfortunately, the value of VODD seems to follow the general economy closely and, as a result, has fluctuated quite violently. When the tocopherols and phytosterols market is strong, disposal of distillate is profitable. However, at other times it must be sold as a soap stock or can be a disposal problem. Some countries permit the use of distillates as an animal feed extender (14), but in others it is prohibited because of the distillation and concentration of pesticides in the deodorization process (13).

This paper discusses the characteristics, uses, and value of VODD, isolation and separation techniques of bioactive compounds from VODD, analysis of VODD, and their future perspectives. The development of new separation techniques for isolating bioactive compounds and methods of analysis are highlighted and discussed.

VEGETABLE OILS REFINING

Refining can be done by conventional processes, which include chemical refining or, following more recent techniques, by using physical refining (Figure 1). For chemical refining, the best known and the most widely used chemical refining process is the caustic soda process. Reaction of alkaline solution with fatty acids leads to the formation of soap. The soap

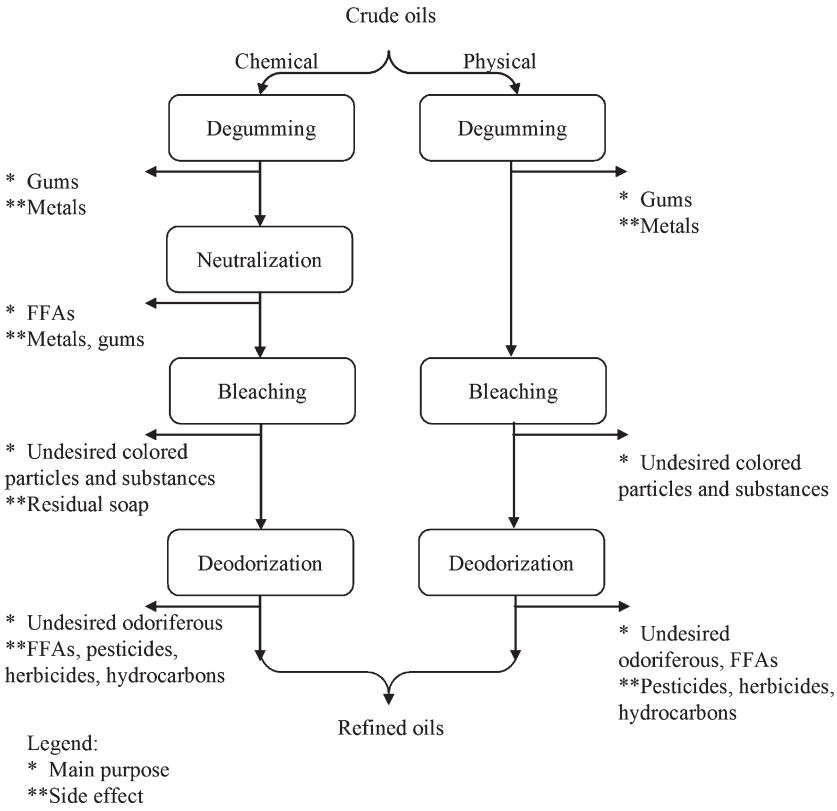


Figure 1. Full refining processing steps and their influence on the product.

stock created is then continuously separated from crude oil by centrifugation. The physical refining process is technically more costly. However, it requires only three processing steps, whereas the chemical refining may need up to four. The main advantage of physical refining is its environmental friendliness. The effluent is drastically reduced, FFAs are recovered as such without soap splitting and refining loss is lower. The disadvantage is that for some oils more careful degumming is required (1, 15, 16).

In physical refining, FFAs are vaporized during the deodorization process. Consequently the deodorizer distillates contain mainly FFAs (>70%) with only small amounts of unsaponifiables matters (5–10%) and is usually sold as a source of technical fatty acids (6). In chemical refining, FFAs are neutralized by caustic solution and washed out of the oil before deodorization. The deodorizer distillates from chemical refining thus have a lower FFA contents (30–50%) and a higher level of unsaponifiables ranging between 25 and 33% (17).

CHARACTERISTICS, USES, AND VALUE OF VODD

Typical compositions of VODD are shown in Table 1 and Table 2. SODD, CODD, SuODD, RODD, OODD and PFAD stand for soybean, corn, sunflower, rapeseed, olive oil deodorizer distillate and palm fatty acid distillate, respectively. It is shown that SODD is the most VODD studied in the literatures. This is because soybean oil is the most consumed vegetable oils in the world, representing 30% of the consumption in the worldwide market. Palm oil is expected to continue to lead soybean oil (18). SODD represents about 3% of refined soybean oil or 0.6% of soybean seed as feed in the refining process (19).

Free Fatty Acids (FFAs)

FFAs in VODD have low quality due to harsh conditions in the processing steps which resulted in reactions such as oxidation, *cis-trans* conversion etc. (28). These fatty acids contents of VODD have limited use such as for the production of biodiesel and as fluidizing agents for lecithin.

trans Fatty acids (TFAs) are mono- or polyunsaturated fatty acids with one or more double bonds in the *trans* configuration. In crude vegetable oils, double bonds are nearly always in the *cis* configuration with the hydrogen atoms sterically located on the same side of the double bond (Figure 2). Because of the low activation energy (125 kJ/mol), TFAs are relatively easily formed at elevated temperatures (e.g., during deodorization or hydrogenation). TFAs have structures comparable to saturated fatty acids. Consequently, they have a higher melting point than the corresponding *cis* isomer, as shown in Table 3 (30).

TFAs formation during deodorization was expressed on a relative basis by use of the term "Degree of Isomerization (DI)". The DI of a given fatty acids expresses the percentage present in *trans* configuration compared to the initial content (*cis* + *trans*) in crude oil. Depending on process conditions, 3 to 24% of α-linolenic acid will be converted into *trans* configuration. The DI of linoleic acid is clearly lower. Even under the most extreme process conditions, at most 2% of linoleic acid will be isomerized. In general, the rate of *cis* / *trans*-isomerization of α-linolenic acid (C18:3) is about 10 and 100 times higher than that of linoleic (C18:2) and oleic acid (C18:1), respectively. In practice, this means that TFAs formation during refining will be inevitably higher in oils rich in α-linolenic acid such as soybean and rapeseed oil (30).

In general, 3 different dietary sources of TFAs can be distinguished: ruminant fats, partially hydrogenated fats, and refined oils. Ruminant fats are the only natural source of TFAs with vaccenic acid (11-*trans*

Table 1. Typical compositions (wt %) of VODD obtained from chemical refining.

VODD types	FFAs	Acylglycerols	Tocopherols	Free phytosterols	FASEs	Squalene	Others [b]	Ref.
OODD	34.20	N.A [a]	N.A	4.60	N.A	28.00	N.A	(12)
SODD	23.62	N.A	12.74	11.39	N.A	2.62	N.A	(20)
SODD	N.A	N.A	10.20	11.20	N.A	1.90	N.A	(21)
SODD	33.00	9.07	16.48	17.06	2.59	1.28	20.52	(6)
SODD	32.00	11.63	18.01	18.81	2.33	2.09	15.13	(6)
SuODD	39.20	5.31	5.06	13.90	0.30	0.73	35.50	(6)
RODD	39.2	13.52	4.19	13.36	5.33	0.40	24.00	(6)
RODD	42.80	13.52	3.67	8.63	1.35	0.07	29.96	(6)
SODD	30.10	17.1	10.40	10.30	12.80	N.A	19.30	(22)
SODD	45.00	20.00	15.00	20.00	N.A	N.A	N.A	(23)
SuODD	45.00	16.30	6.00	5.10	N.A	N.A	N.A	(24)
SODD	2.70	N.A	49.70	18.80	N.A	3.10	N.A	(25)
PFAD	40.00	52.20	1.00	0.30	0.50	N.A	N.A	(26)
SODD	45.38	23.30	6.40	5.36	3.91	1.83	15.23	(10)
SODD	46.46	17.80	7.73	6.20	1.80	1.80	18.18	(11)
SODD	40.80	17.41	4.81	6.09	3.37	1.79	25.25	(11)
SODD	41.63	10.37	14.89	11.25	4.12	2.05	15.69	(11)

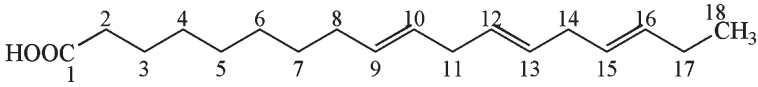
[a]Not available.
[b]Hydrocarbons, aldehydes, ketones, pesticides, herbicides, breakdown product of tocopherols and phytosterols.

Table 2. Typical compositions (wt %) of VODD obtained from physical refining.

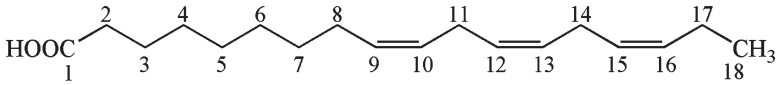
VODD types	FFAs	Acylglycerols	Tocopherols	Free phytosterols	FASEs	Squalene	Others [b]	Ref.
SODD	73.8	7.67	7.51	6.32	4.45	0.65	N.A [a]	(6)
CODD	81.2	0.72	1.42	2.71	0.62	0.21	13.12	(6)
CODD	77.1	2.20	3.31	5.42	N.A	0.99	10.98	(6)
SuODD	70.82	3.33	1.28	3.67	0.09	1.00	19.81	(6)
SODD	57.80	N.A	8.97	N.A	N.A	N.A	N.A	(27)
SuODD	82.00	N.A	10.00	2.00	2.00	4.00	0	(28, 29)

[a]Not available.

[b]Hydrocarbons, aldehydes, ketones, pesticides, herbicides, breakdown product of tocopherols and phytosterols.



α-Linolenic acid (9,12,15-*trans* 18:3)



α-Linolenic acid (9,12,15-*cis* 18:3)

Figure 2. Fatty acids with double bonds occurring in the *cis* or *trans* configuration.

18:1) as the predominant one. On the other hand, partially hydrogenated fats are known as major dietary source of TFAs. Besides saturation of double bonds, partial hydrogenation is also characterized by the positional and geometrical isomerization of double bonds, resulting in large amounts of *trans* 18:1 isomers. Refined oils contain only a limited amount of TFAs, mainly *trans* 18:2 and 18:3 isomers formed during deodorization. Interest in TFAs is due to the epidemiological evidence that linked TFAs intake with higher plasma total and low-density-lipoprotein-cholesterol concentrations and increased mortality from coronary heart disease (31–33). Although there is still a lot of controversy on the possible health effects of TFAs, most health organizations advice not to increase current average intakes.

Acylglycerols

TAGs, DAGs, and MAGs are known also as acylglycerols and most commonly as neutral fats. SODD obtained from chemical refining

Table 3. Overviews of melting points of some fatty acids.

Trivial name	Shorthand notation	Melting point (°C)
Stearic acid	18:0	70
Elaidic acid	9-*trans* 18:1	44
Oleic acid	9-*cis* 18:1	13.4
Linolelaidic acid	9,12-*trans* 18:2	56
	9-*trans*, 12-*cis* 18:2	11.9
	9-*cis*, 12-*trans* 18:2	−6.3
Linoleic acid	9,12-*cis* 18:2	−6.5
α-Linolenic acid	9,12,15-*cis* 18:3	−11
	9,12,15-*trans* 18:3	71

typically contains higher level of acylglycerols (10–20%) than that of physical refining (<8%). TAGs are the major components of acylglycerols in VODD and their types depend on the kind of oil sources. These compounds, if well separated, may have more commercial value than FFAs. The potential value of recovering TAGs from VODD will impact the planning of future modifications to vegetable oils manufacturing facilities. Although there is no information about the isomerization of FFAs in acylglycerols, most researchers convert acylglycerols to fatty acid methyl esters (FAME).

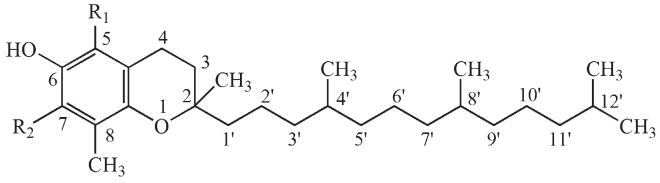
MAGs are the most polar components of simple lipids and, thus, need care to prevent their loss in hydrophilic solutions and in chromatographic columns. Furthermore, MAGs and DAGs have detergent property; hence they easily form micelles in water solutions.

Tocopherols / Tocotrienols

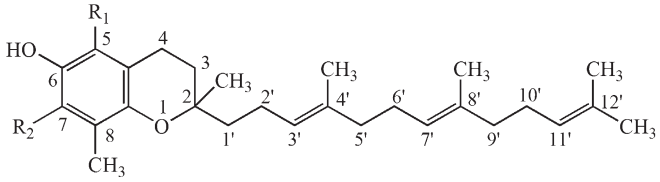
Tocopherols and tocotrienols (known as tocochromanols) are organic compounds that are found in plant material. These compounds are important because they retard the oxidation and spoilage of plant matter. Tocochromanols are also components of vitamin E and posses similar general structural features. Tocochromanols generally have an aromatic chromanol head and 16-carbon hydrocarbon tail. The number and position of methyl substituents in the chromanol nucleus give rise to the α−,β−,γ− and δ−homologues. Saturation of the hydrocarbon chain differentiates tocopherols from tocotrienols with an unsaturated chain as shown in Figure 3 (34). SODD, CODD, SuODD, RODD, and OODD are sources of tocopherols, while tocotrienols are found in PFAD.

Each form has its own biological activity, which is the measure of potency or functional use in the body (35). Such activities include platelet aggregation and antioxidant functions (36, 37). Alpha tocopherol is the most active form of vitamin E in humans (d-α-tocopherol, d-β-tocopherol, d-γ-tocopherol, d-δ-tocopherol, and d-α-tocotrienol showing, respectively, 1.49, 0.75, 0.30, 0.15 and 0.45 units of activity (38). It is also a powerful biological antioxidant (39, 40). Non-α-tocopherol can be converted into α-tocopherol by well-known techniques, such as methylation (25). Vitamin E in supplements is usually sold as α-tocopheryl acetate, a form that protects its ability to function as an antioxidant. The synthetic form is labeled "D,L" while the natural form is labeled "D". The synthetic form is only half as active as the natural form (41). A mixture of α-, γ- and δ- isomers containing 60 wt% tocopherols is widely utilized as an additive to various kinds of foods including fats and oils.

Antioxidants such as vitamin E act to protect cells against the effect of free radicals, which are potentially damaging by-products of energy



Tocopherols (T)



Tocopherols (T3)

Tocopherols	Tocotrienols	R_1	R_2
α-Tocopherol (α-T) (5,7,8-Trimethyltocol)	α-Tocotrienol (α-T3) (5,7,8-Trimethyltocotrienol)	CH_3	CH_3
β-Tocopherol (β-T) (5,8-Dimethyltocol)	β-Tocotrienol (β-T3) (5,8-Dimethyltocotrienol)	CH_3	H
γ-Tocopherol (γ-T) (7,8-Dimethyltocol)	γ-Tocotrienol (γ-T3) (7,8-Dimethyltocotrienol)	H	CH_3
δ-Tocopherol (δ-T) (8-Monomethyltocol)	δ-Tocotrienol (δ-T3) (8-Monomethyltocotrienol)	H	H

Figure 3. *S*tructures and methyl positions of the eight natural E vitamins. The chemical abstract name for tocopherol is 2-methyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol and for tocotrienol is 2-methyl-2-(4′,8′,12′-trimethyltrideca-3′,7′,11′-trienyl)-6-chromanol.

metabolism. Free radicals can damage cells and may contribute to the development of cancer and cardiovascular diseases. Vitamin E has also been shown to play a role in immune function, in DNA repair, and other metabolic processes (39, 40).

High serum cholesterol levels are implicated in numerous diseases and disorders including arteriosclerosis, atherosclerosis, cardiovascular diseases, diabetes mellitus, familial hypercholesterolemia, anorexia nervosa, cirrhosis of liver, hepatitis and obstructive jaundice. A decrease in low density lipoproteins (LDLs) and/or an increase in the ratio of high density lipoproteins (HDLs) to LDLs will lower the risk of heart disease and retard the progression of the abovementioned diseases and disorders. Tocopherols and tocotrienols are two classes of compounds that are known to have a beneficial effect on the level of cholesterol in the bloodstream (26).

In many foods, α- and γ- tocopherols account for most of the vitamin E activity. The primary form of vitamin E in dietary and animal feed supplements is α-tocopherol. However, it is widely known that γ-tocopherol is the major form present in SODD, CODD and RODD. It has been discovered that the individual members in tocopherols exhibit different biological properties from one another and play different roles in cells. Numerous studies recently have promoted the benefits of γ-tocopherols for human diet. Gamma tocopherol may be involved in the pathogenesis of prostate cancer (42), and is a superior trapping agent for electrophilic species, such as nitrogen oxides or NO (43) or peroxynitrite (44).

Phytosterols (Free and Conjugated Phytosterols)

Phytosterols (plant sterols) are members of the "triterpene" family of natural products. Phytostanols are a fully-saturated subgroup of phytosterols. Phytostanols occur in trace levels in many plant species and they occur in high levels in tissues of only a few cereal species. Common dietary sources of phytostanols are corn, wheat, rye, and rice. Phytosterols can be converted to phytostanols by chemical hydrogenation.

All triterpenes are synthesized via a pathway that starts with the reduction of HMG-CoA (6 carbons) to mevalonate (5 carbons). Six mevalonate units are then assembled into 2 farnesyl diphosphate molecules, which are combined to make squalene (30 carbons or "three terpenes"). Enzymatic ring closure steps then form cycloartenol (also 30 carbons), and additional enzymatic reactions form common plant triterpenes such as phytosterols, triterpene alcohols, and brassinosteroids (Figure 4) (45, 46).

Phytosterols nomenclature is confusing because international attempts at standardization have been only partially adopted. The two main nomenclature (Figure 5) currently utilized follow the IUPAC-IUB recommendations of 1976 and 1989 (47, 48). A convenient way to describe and catalog phytosterols is to divide them based on common forms (free and conjugated phytosterols). Moreover, free phytosterols can be classified based on the number of methyl groups on carbon-4 as shown in Figure 6.

4-Dimethyl phytosterols and 4-monomethyl phytosterols are metabolic intermediates in the biosynthetic pathway leading to end-product (4-desmethyl phytosterols). They are usually present at low levels in most plant tissues. Cycloartenol and cycloartanol are examples of 4-dimethylsterols, and gramisterol is an example of a 4-monomethylsterol.

4-Desmethyl phytosterols include the 27-carbon (such as cholesterols, ubiquitous and predominant in animals, but also generally present in

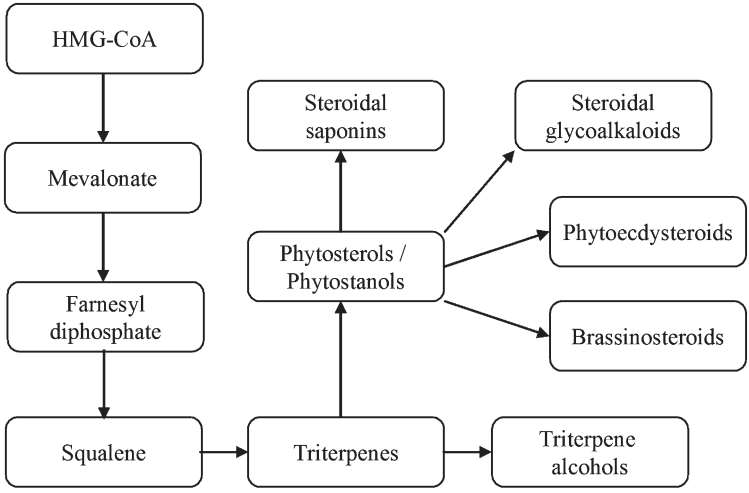


Figure 4. Biosynthesis of phytosterols / phytostanols and other triterpenes.

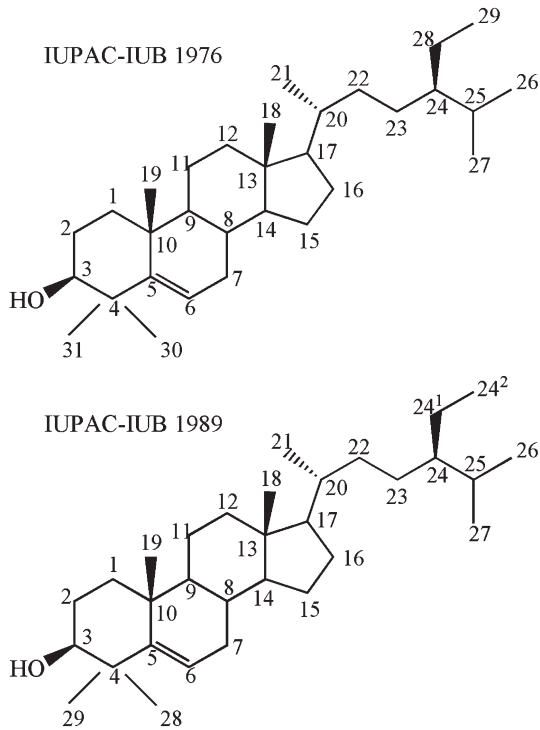


Figure 5. Nomenclature of phytosterols. (Example: β-sitosterols = stigmast-5-en-3b-ol = 24R- ethylcholest-5-en-3β-ol).

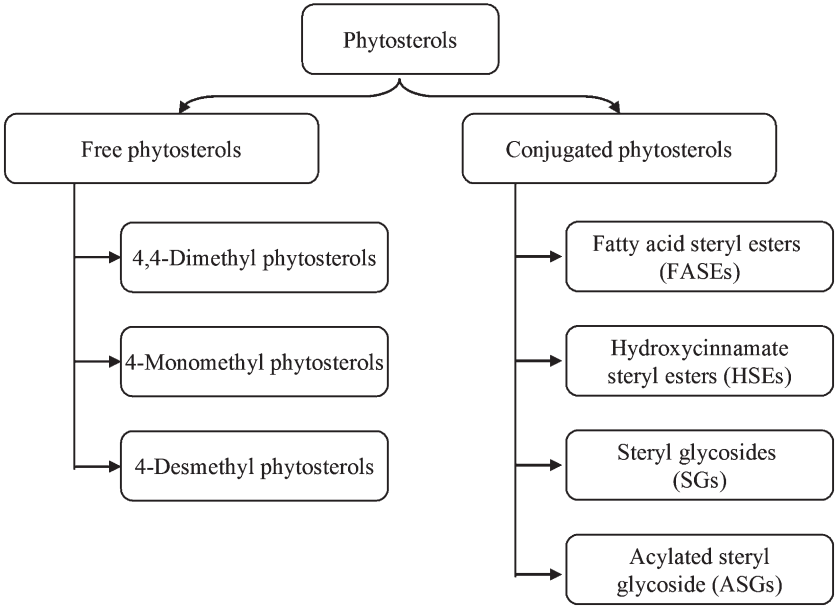


Figure 6. Classification of phytosterols based on the number of methyl groups on 4-carbon and based on common forms.

plants at low levels) and all of the common 28-carbon (Figure 7) and 29-carbon (Figure 8), which are typically major membrane structural components in plant cells. Most 4-desmethyl phytosterols have a double bond in 5-6 of the ring system and are thus called Δ^5 desmethyl phytosterols. However, another group of common 4-desmethyl phytosterols that are abundant in plants of certain families have a double bond in 7–8 instead of 5–6, and are hence referred to as Δ^7 desmethyl phytosterols. Both Δ^5 and Δ^7 desmethyl phytosterols can include a second double bond in the alkyl side chain, most frequently between carbons 22 and 23 or carbon 24 and 28.

In conjugated phytosterols, the 3β-OH is covalently bound with another constituent (Figure 9). The OH group is ester-linked with a fatty acid in fatty acid steryl esters (FASEs) and linked by a 1-*O*-β-glycosidic bond with a hexose (most commonly glucose) in steryl glycosides (SGs). The third group of phytosterol conjugates, acylated steryl glycosides (ASGs), differ from SGs by the addition of a fatty acid esterified to the 6-OH of the hexose moiety. Seed of corn and rice and other grains contains a fourth type of phytosterol conjugates, phytosteryl hydroxycinnamic-acid esters (HSEs), in which the sterol 3β-OH group is esterified to ferulic or p-coumaric acid. FASEs are storage products in cell and can be found in the cytosol and in droplets or vesicles (49).

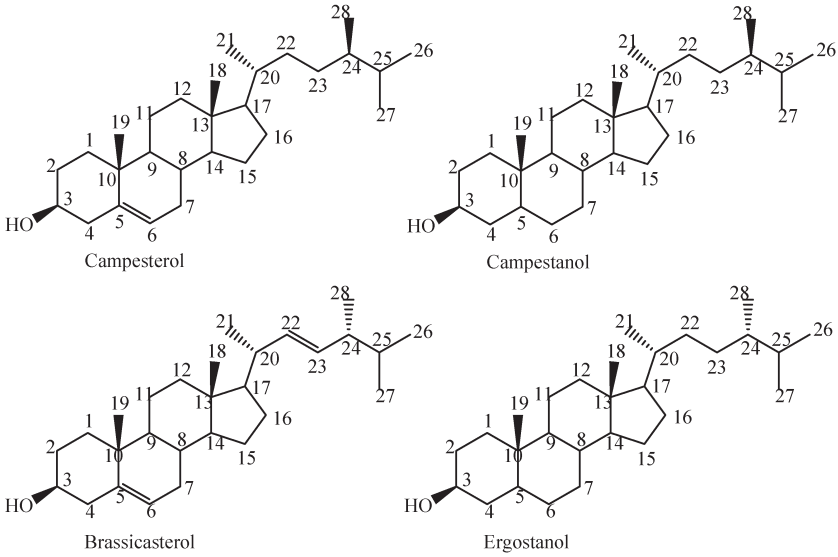


Figure 7. 28-Carbon 4-desmethyl phytosterols and phytostanols.

The composition of free phytosterols in VODD is frequently a mixture of sitosterol, campesterol, stigmasterol, and brassicasterol. In addition, FASEs are the conjugated phytosterols that present in the

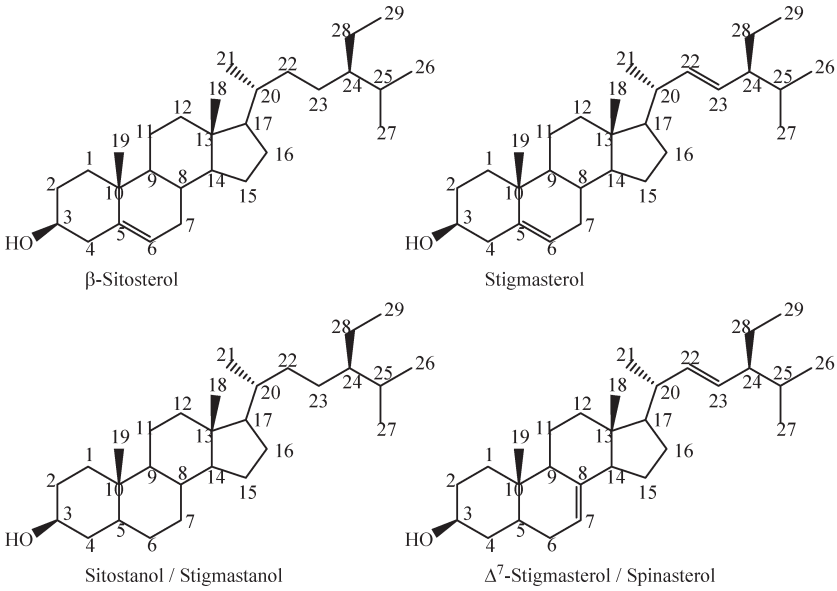


Figure 8. 29-Carbon 4-desmethyl phytosterols.

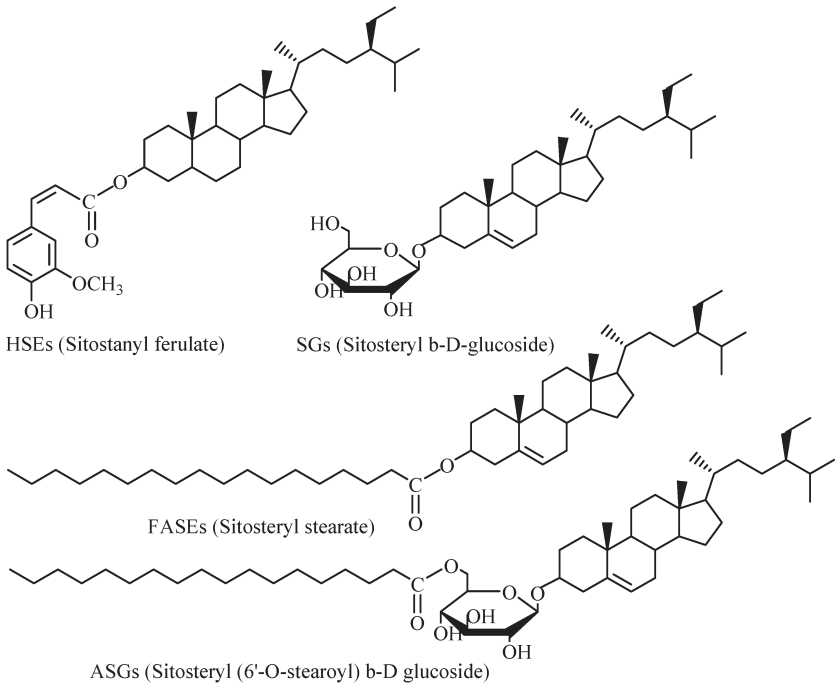


Figure 9. Structures of phytosterol conjugates.

VODD. Their composition is not available in the literatures. This is because FASEs have been usually characterized following the ester bond cleavage of the aliphatic and steryl ester by saponification. This approach gives useful information about their characteristics but prevents the recognition of the individual steryl ester and thus valuable information is lost.

Phytosterols and phytostanols are effective in lowering low density lipoprotein serum cholesterol (LDL-C), as long as they are formulated and delivered in a "bioavailable" physical state. Stigmasterol is also used in manufacturing progesterone and corticoids. Sitosterol is used to produce estrogens, contraceptives, diuretics, and male hormones (46). Moreover, FASEs are more effective at lowering serum cholesterol levels than free phytosterols. This effective physiological activity has led to the development of several functional foods, such as salad oils and dressings with added sterols and margarine blended with steryl esters. In particular, because FASEs and oils (TAGs) completely dissolve each other, a lot of attention is being focused on the addition of FASEs in oil-related foods (22). In addition, steryl esters are simpler and cheaper to synthesize than stanyl esters because no hydrogenation is required.

Nowadays, there are many products enriched with phytosterols or phytostanols on the commercial market and the enriched margarine have

been marketed worldwide (Table 4). Enriched products coming in the future are different cooking oils, new spreads, chocolates and chocolate beverages (50).

Hydrocarbons

Hydrocarbons are the least polar compounds of the unsaponifiable matter of vegetable oils. Crude vegetable oils contain elements of the

Table 4. Countries where phytosterols enriched products have been launched ((50) Moreau, 2004).

Country	Year introduced	Benecol[a] products	Becel[b] products	Other phytosterols enriched products other than margarine spreads and dietary supplements
Australia	2000		Y[c]	
Austria	2000		Y	
Belgium	2000	Y	Y	
Brazil	2000		Y	
Czech Republic	2000		Y	
Denmark	2000		Y	
Finland	1995	Y	Y	Cream cheese, spreads, milk, mayonnaise, meat products, cheese, pasta, yogurt, snack bars
France	2000		Y	
Germany	2000		Y	
Greece	2000		Y	
Ireland	2000		Y	
Japan	1999	Y	Y	Cooking oil, beverages
Korea	?		Y	Beverages
Netherlands	2000		Y	
New Zealand	2000		Y	
Poland	2000	Y		
Portugal	2000		Y	
South Africa	2000		Y	
Spain	2000		Y	Yogurt
Sweden	2000	Y	Y	Milk
UK	1999	Y	Y	Snack bars, mayonnaise, milk
US	1999	Y	Y	

[a]Benecol contains stanyl esters.
[b]Becel contains FASEs.
[c]Y means the product is commercially available in that country.

n-alkane series from C_{10} to C_{35}, the odd numbered elements being the most abundant. Other hydrocarbons were also detected in low concentration. Among them are n-alkenes, sesquiterpenenic (α-farnesene), terpenic (kaurene), carotenes, low-molecular-mass aromatics (from benzene to tetramethylbenzene, including styrene), and polycyclic aromatic hydrocarbon (mainly those of low molecular mass).

In refined vegetable oils, new hydrocarbons are formed as consequences of reactions occurring during the refining process such as bleaching with acidic earth and deodorization at high temperature. These hydrocarbons include steroidal hydrocarbons from the dehydration of phytosterols, terpenic hydrocarbons from terpenic alcohols, and other compounds deriving from squalene isomerization (51). Each Δ^5-phytosterols gives rise to three sterene isomers (steroidal skeleton) with the two double bonds at 3,5-, 2,4- and 2,5- positions, the first one being the most abundant. Among these steroidal hydrocarbons, the stigmadienes are the most abundant in all refined vegetables oil since they derive from β-sitosterol by dehydration as shown in Figure 10.

The presence of hdrocarbons was detected in the 1940s, when Jasperson and Jones (52) encountered in the deodorization distillates

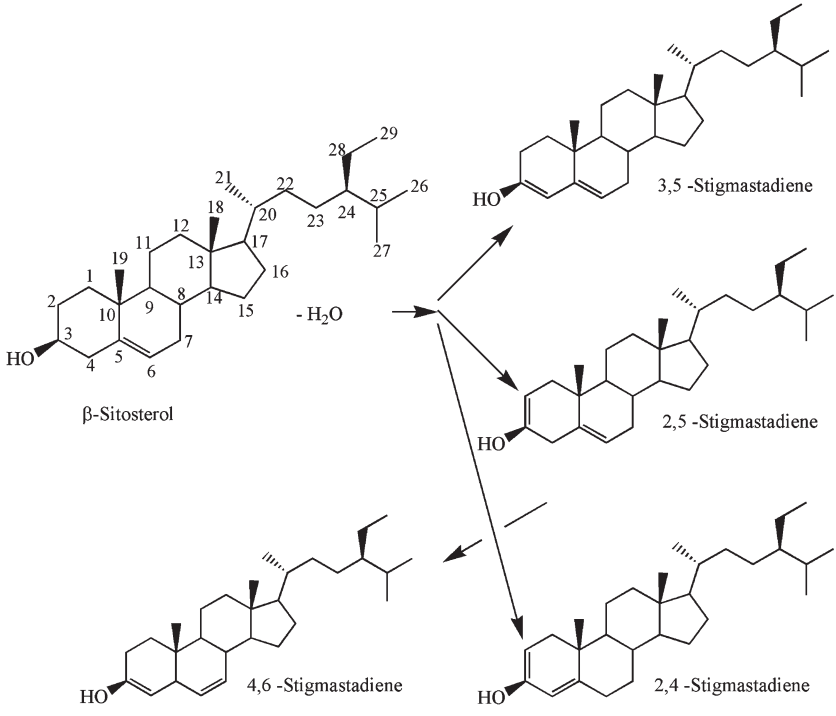


Figure 10. Steroidal hydrocarbons from the dehydration of β-sitosterols.

from several vegetable oils, large amounts of terpenic hydrocarbons accompanied by smaller amounts of *n*-alkanes. Goh et al. (53) reported that squalene, sesquiterpene, diterpene, paraffinic hydrocarbon and other volatile degradation or oxidized products are removed and concentrated in palm fatty acid distillate (PFAD) during the refining of palm oil. In another study, it was mentioned that aliphatic, steroidal, sesquiterpene and triterpene (squalene) hydrocarbons exist in SODD (9). Natural squalene can be found in many tissues, notably the livers of sharks (*Squalus*) and other fishes. Due to environmental concerns including the protection of marine life, the extraction of squalene from VODD is of great interest.

Squalene, with structure shown in Figure 11, is a linear hydrocarbon with applications in cosmetic preparation and in the biosynthesis of cholesterol (51). Squalane is prepared by the hydrogenation of squalene and is fully saturated, which means that it is not subject to autoxidation. Both squalene and squalane can be metabolized and have a good record in toxicology studies (54). Yarkoni and Rapp (55) used squalene as well as hexadecane and other lipids, with mycobacterium cell wall, to augment non-specific immunity against tumors.

Squalene and squalane were found to be equally effective and squalene was preferred because of its greater stability. Squalane is a free-flowing oil and has been used in pharmaceuticals and as skin lubricant, as ingredient in suppositories, and as a vehicle for lipophilic drugs. Squalene in Syntex Adjuvant Formulation (SAF) conforms to National Formulary guidelines and is used at a final concentration of 5% (w/v). Microfluidized squalene or squalene emulsions are efficient adjuvant, eliciting both humoral and cellular immune responses. Squalane or squalene emulsions have been administered in human cancer vaccines, with mild side effects and evidence of efficacy, in terms of both immune response and antitumor activity (56).

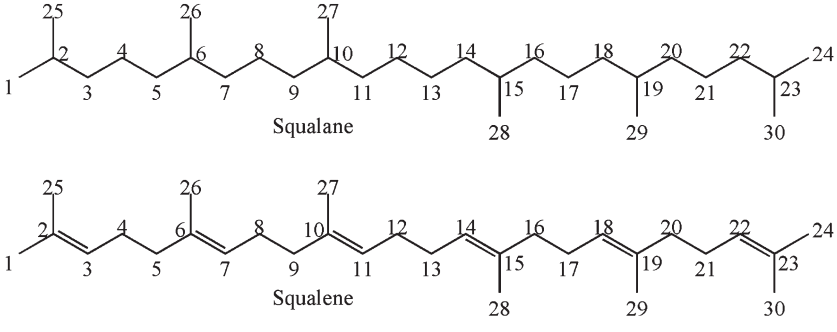


Figure 11. Structures of squalane and squalene.

ISOLATION OF BIOACTIVE COMPOUNDS FROM VODD

Deodorizer distillate typically contains high level of FFAs and acylglycerols depending on the type of raw materials and the conditions of the oil refining process, and the efficient removal of them is crucial for the enrichment and separation of bioactive compounds. Therefore, a convenient way to describe and catalog methods that have been proposed for treating deodorizer distillate is to divide the process based on FFAs and acylglycerols removal. There are several different methodologies for the removal of FFAs and acylglycerols: hydrolysis, esterification, transesterification, distillation, crystallization, adsorption, and liquid-liquid extraction. The first three methods are either chemical or enzymatic process, and the next three are physical process.

Chemical Process

In many of these methods, a first essential step involves subjecting deodorizer distillate to an esterification or saponification reaction. The reaction products were separated by either distillation or solvent extraction. For example, in an early work (57); deodorizer distillate was subjected to high vacuum, unobstructed path distillation. A distillate fraction containing tocopherols was treated with lime soda to saponify FFAs, followed by the extraction of unsaponifiable fraction (tocopherols and free phytosterols) with ethyl ether, in which the saponification products are insoluble. The extract is then washed and concentrated by removing solvent in a rotary evaporator, and then the residual oil dissolved in five times methyl alcohol and cooled to crystallize free phytosterols. Free phytosterols were removed by filtration, leaving a tocopherols fraction. The fatty acid soaps formed can be acidulated and converted into FFAs. The total recovery of tocopherols exceeded 50% with 25% purity.

Brown and Smith (58) isolated free phytosterols and tocopherols from deodorizer distillate by esterifying FFAs with a monohydric alcohol under strongly acidic condition for about 1–2 h. Free phytosterols and tocopherols were then fractionally extracted from the esterification product mixture with a combination of polar and nonpolar solvents.

Fizet (21) esterified free phytosterols in deodorizer distillate with FFAs at 180°C for 2.5 h or at 250°C for 1.5 h and then the resulting mixture was distilled at 120–150°C and 0.1 mbar to obtain a residue containing tocopherols and FASEs and a distillate containing fatty acids. This residue was then distillated at 200–220°C and 0.1 mbar to obtain a residue containing FASEs and a distillate containing tocopherols. Free phytosterols were obtained by an acid-catalyzed trans-esterification of

FASEs with methanol. Some tocopherols in the distillate were transformed into fatty acid tocopherol esters. Tocopherols were obtained by subjecting distillate from the 2nd distillation to an ion exchange chromatography, especially over a strongly basic resin.

Jacobs (26) proposed a method for recovering tocotrienols from PFAD (contained 1% tocopherols and 0.3% free phytosterols) by stripping FFAs (0.5–1.5 Torr, 180–240°C, and 0.5–1.5 min) to form a first stripped product. Short path distillation of the first stripped product gave a first distillate. Short path stripping of fatty acids from the first distillate yielded a second stripped product. Saponification of the second stripped product resulted in a saponified product. A second short path distillation of the saponified product yielded a second distillate. Solvent wintering (via filtration) of the second distillate gave a stripped filtrate. A third short path distillation of the stripped filtrate recovered the desired tocotrienols with 50% purity.

In another work (27), acylglycerols in SODD were converted into FFAs and glycerol through saponification at 65°C followed by acidulation and then the treated deodorized distillate was subjected to five stages of molecular distillation. Tocopherols (34.14 % purity) were enriched 5.8 times and MAGs (32.61 %) were the main impurity in the final product.

Bondioli et al. (12) described a supercritical carbon dioxide process for recovering squalene from OODD. FFAs and fatty acid methyl and ethyl esters in OODD were converted into their corresponding triacylglycerides. The pre-treated OODD was then extracted with supercritical carbon dioxide to yield a highly enriched squalene fraction in high purity (~90%) and high yield (~90%). A summary of chemical processes used in the isolation and purification of bioactive compounds from deodorizer distillate is shown in Table 5.

Enzymatic Process

Enzymatic methods were developed in the last decade to purify and/or pre-concentrate deodorizer distillate to recover tocopherols and free phytosterols. Ramamurthi and McCurdy (20) concentrated free phytosterols and tocopherols from canola deodorizer distillate (contained 1% tocopherols and 1.58% free phytosterols) and SODD (contained 12.74% tocopherols and 11.39% free phytosterols). FFAs were converted into FAMEs by employing non-specific SP 382 lipase. Free phytosterols and tocopherols were then concentrated in the residue fraction by distillation. The conversion of FAMEs was 96%, while a small amount of FFAs was left in the residue. Free phytosterols and tocopherols were enriched 1.5 times and 1.7 times, respectively. The total recoveries of free physterols and tocopherols were 17.56% and 21.48%, respectively.

Table 5. Summary of chemical processes used in the purification of bioactive compounds.

Samples	Procedures	Ref.
Scum, sludge, and the like	Path distillation, saponification of fatty acids, and solvent extraction (tocopherols)	(57)
Deodorizer sludge and the like	Esterification of fatty acids, solvent extraction (tocopherols)	(58)
Deodorizer sludge	Esterification of free phytosterols, two-stages short path distillation, transesterification of FASEs in residue fraction (free phytosterols), esterification of trace fatty acids in distillate fraction, ion exchange (tocopherols)	(20)
Deodorizer distillate	FFAs Stripping, first short path distillation, short path FFAs stripping, saponification, second short path distillation, solvent wintering and third short path distillation (tocotrienols)	(25)
SODD	Saponification of acylglycerols, acidulation, five stages of molecular distillation (tocopherols)	(27)
OODD	Transesterification of FFAs, supercritical carbon dioxide extraction (squalene)	(11)

Desired compounds reported in parentheses.

Ghosh and Bhattacharyya (59) proposed a method for the purification of tocopherols and free phytosterols from SuODD. Hydrolysis by *Candida cylindracea* lipase was applied to convert acylglycerols into fatty acids and glycerol. *Mucor miehei* lipase was then applied to the hydrolyzed deodorizer distillate to esterify fatty acids into their corresponding butyl ester. Butyl esters were then separated from the esterified deodorizer distillate *via* fractional distillation at 180–230°C for 45 min. A second fraction containing tocopherols and free phytosterols was obtained at 230–260°C for 15 min. The total recovery of tocopherols is about 70.2% with 30.1 wt% purity. The total recovery of free phytosterols is about 41.9% with 36.4 wt % purity.

Purification of tocopherols from SODD was carried out by Shimada et al. (60). SODD was distilled using molecular distillation at 250°C and 0.02 mmHg and the resulting distillate was used as a starting material. *Candida rugosa* or *Pseudomonas* sp lipase was then applied to the mixture to efficiently esterify free phytosterols into their corresponding esters and concurrently MAGs were hydrolyzed at 35°C for 24 h. FASEs were recovered as residue from the reaction mixture *via* molecular distillation at 250°C and 0.2 mmHg. However, the last molecular distillation failed to separate FFAs and tocopherols. A second esterification of free phytosterols was applied at 35°C for 24 h, followed by another four-stage molecular distillation (160°C and 0.2 mmHg, 200°C and 0.2 mmHg,

230°C and 0.04 mmHg, and 255°C and 0.03 mmHg), tocopherols with purity and recovery of about 65.3 wt% and 54.6%, respectively were obtained.

Hirota et al. (22) isolated naturally occurring FASEs from SODD. SODD was distilled using molecular distillation at 250°C and 0.02 mmHg and the resulting residue was rich in DAGs and TAGs. Lipolysis was then conducted to selectively hydrolyze DAGs and TAGs at 35°C for 24 h, resulting in a mixture from which fatty acid steryl esters were readily purified using two-stage molecular distillation (180°C and 0.2 mmHg, and 250°C and 0.02 mmHg). The recovery and purity of FASEs were about 87.7% and 97.3 wt%, respectively.

Watanabe et al. (8) purified tocopherols and free phytosterols as their esters from SODD. SODD was distilled using molecular distillation at 240°C and 0.02 mmHg and the resulting distillate was rich in FFAs, free physterols and MAGs. A two-step in situ reaction catalyzed by *Candida rugosa* lipase was then applied to the distillate. In the first step, the esterification of free phytosterols with FFAs and the hydrolysis of acylglycerols were carried out at 30°C for 16 h. In the second step, the esterification of FFAs with methanol was conducted 30°C for 24 h. FASEs were not affected by the reaction. FAMEs were separated from tocopherols (and fatty acid steryl asters) *via* molecular distillation at 160°C and 0.2 mmHg.

Tocopherols were separated from fatty acid steryl asters *via* molecular distillation at 240°C and 0.02 mmHg. The total recovery of free phytosterols as their corresponding esters is about 86.3% with 97.2 wt % purity. The resulting tocopherol-rich distillate was subjected to a two-step *in situ* reaction. In the first step, esterification of free phytosterols with FFAs and hydrolysis of acylglycerols were carried out at 30°C for 16 h. In the second step, esterification of FFAs with methanol was conducted 30°C for 6 h. Tocopherols were readily purified from the product of the last reaction by using a three-stage molecular distillation (160°C and 0.2 mmHg, 175°C and 0.2 mmHg, and 240°C and 0.02 mmHg). The total recovery of tocopherols is about 84.26% with 76.4 wt% purity.

Nagao et al. (9) obtained purified tocopherols and free phytosterols as their esters from SODD. SODD was distilled using molecular distillation at 240°C and 0.02 mmHg and the resulting tocopherols, free physterol, FFAs and MAGs–rich distillate was used as a starting material in a two-step *in situ* reaction catalyzed by *Candida rugosa* lipase. In the first step, esterification of free phytosterols with FFAs and hydrolysis of acylglycerols were carried out at 40°C at 20 mmHg for 24 h with dehydration. In the second step, esterification of FFAs with methanol was conducted 30°C for 8 h without dehydration. The oil layer was recovered by standing the reaction mixture and the oil layer separated

was dehydrated at 80°C/5 mmHg for 30 min. FAMEs were separated from tocopherols and FASEs *via* a two-stage molecular distillation (160°C and 0.2 mmHg, and 175°C and 0.2 mmHg).

Tocopherols were then separated from FASEs *via* molecular distillation at 230°C and 0.02 mmHg. Tocopherols with a purity of 72.3 wt% were recovered in the distillate. The total recovery of the tocopherols is 87.6%. The FASE-rich residue was subjected to another molecular distillation at 240°C and 0.02 mmHg to recover FASEs with a purity of 97%. The total recovery of free phytosterols as their corresponding esters is about 97%. A summary of enzymatic processes used in the isolation and purification of bioactive compounds from deodorizer distillate is shown in Table 6.

Physical Process

Extractive separation methods have been employed in treating deodorizer distillate to isolate one or more bioactive components. For example, Copeland and Belcher (23) recovered fatty acids, tocopherols and free

Table 6. Summary of enzymatic processes used in the purification of bioactive compounds.

Samples	Procedures	Ref.
Deodorizer distillate	Fatty acid esterification catalyzed by lipase, distillation (tocopherols and free phytosterols)	(19)
SuDD	Neutral glycerides hydrolysis by lipase, fatty acid esterification by lipase, fractional distillation (tocopherols and free phytosterols)	(59)
SODD	Molecular distillation, free phytosterols esterification by lipase, molecular distillation, free phytosterols esterification by lipase, four-stages molecular distillation (tocopherols)	(60)
SODD	Molecular distillation, glycerides hydrolysis by lipase, 2-stages molecular distillation (FASEs)	(21)
SODD	Molecular distillation, a two-step *in situ* enzymatic reaction, two-stages molecular distillation (free phytosterols as their esters), a two-step *in situ* enzymatic reaction of resulting distillate, three-stage molecular distillation (tocopherols)	(7)
SODD	Molecular distillation, a two-step *in situ* enzymatic reaction with dehydration, three-stages molecular distillation (tocopherols), molecular distillation of the resulting residue (free phytosterols as their esters)	(8)

Desired compounds reported in parentheses.

phytosterols from a deodorizer distillate by contacting deodorizer distillate into a scrubber. The scrubber had at least two condensing zones and operated at a pressure of less than about 10 mmHg.

Deodorizer distillate (contained 12% tocopherols and 14% free phytosterols) was contacted into first condensing zone (166–232°C, 2.5–4 mmHg), thereby producing a first condensate stream with relatively high concentration of tocopherols (25%) and free phytosterols (30%). The remaining uncondensed vaporized distillate flowed to a second condensing zone, producing a second condensate stream with high concentration of FFAs (90%).

By employing molecular distillation at 160°C, 7.5×10^{-4} mmHg and with 10.4 g/min of feed flow rate, it was possible to obtain a product that contains 6.4% FFAs and 18.3% tocopherols from a feed that contains 57.8% of FFA and 8.97% tocopherols. A FFAs elimination of 96.16% and tocopherols recoveries of 81.23% were achieved (61). Lee et al. (62), Mendes et al. (28, 29), Nagesha et al. (63), Chang et al. (64) and Gast et al. (25) recovered tocopherols from SODD using supercritical CO_2 extraction. Supercritical fluid extraction is an alternative technique to conventional processes (such as molecular distillation), especially for treating and processing temperature sensitive components.

Starting with a feed that contains 48.3% tocopherols, Gast et al. (25) were able to obtain tocopherols with a purity of 94.4 % in the bottom by supercritical CO_2 extraction at 23 MPa and 353 K, with a solvent-to-feed ration of 110 and a reflux ratio of 4.6. Squalene was completely recovered in the top phase.

The simulation of the vitamin E enrichment from deodorizer distillate using supercritical carbon dioxide was carried out in a semi batch mode at 40°C to separate linoleic acid (90 bar), stigmasterol (250 bar) and squalene (350 bar) from tocopherols. The separation between stigmasterol and squalene was the most difficult among the others (29).

Adsorption is an energy efficient process, suitable for isolating components with similar boiling point. It is competitive with distillation for bulk separation when relative volatility is less than about 1.25 (65). General features which distinguish physisorption from chemisorption are shown in Table 7. Chu et al. (66) separated vitamin E from palm fatty acid distillate using silica in batch adsorption. It was found that Redlich-Peterson and Langmuir isotherm models described the equilibrium data reasonably well and thermodynamic parameters of the adsorption further confirmed the exothermic nature of the adsorption process. The overall Gibbs free energy change during the adsorption process was negative for the experimental range of temperatures (35–50°C), corresponding to spontaneous adsorption. Two main adsorption mechanisms were involved during vitamin E adsorption, namely external mass transfer and intraparticle diffusion. It was noted that increasing the initial vitamin

Table 7. General features that distinguish physisorption from chemisorption.

Features	Physisorption	Chemisorption
Activation energy	Low	High
Enthalpy	Low ($\Delta H < 20$ KJ/mol)	High ($\Delta H \approx 400$ KJ/mol)
Process	Rapid and reversible	Slow and irreversible
Temperature	Only significant at relatively low temperature	Possible over a wide range of temperature
Type of interaction	Relatively weak intermolecular forces (van der Waals forces)	Strong (covalent bond between adsorbate and surface)
Type of layer	Monolayer or multilayer	Monolayer only
Type of phenomenon	Non specific	Highly specific
Electron transfer	No electron transfer although polarization of sorbate may occur	Electron transfer leading to bond formation between sorbate and surface
Dissociation	No dissociation of adsorbed species	May involve dissociation of adsorbed species

E concentration resulted in a decrease in k_f (external mass transfer coefficient) values, but an increase in k_{id} (rate constant of intraparticle diffusion).

Gunawan et al. (10) applied a modified soxhlet extraction on SODD to obtain one fraction enriched with FASEs (12.19%, recovery 94.32%) and squalene (6.29%, recovery 100%), while tocopherols, free phytosterols, TAGs, and FFAs were enriched in another fraction. By combining a modified soxhlet extraction with a silica gel column chromatography, squalene with a high purity (95.90%) and a high recovery (93.09%) was obtained from SODD with an initial squalene content of 1.83%. Although modified soxhlet extraction and silica gel column chromatography require large amounts of organic solvents, the solvents can be recovered for reuse easily.

By combining a modified soxhlet extraction, a modified silica gel column chromatography, and water-acetone extractions, FASEs fraction could be obtained with a high purity (86.74%) and a high total recovery (85.32%) from SODD with initial FASEs contents of 4.12%. This separation process can yield FASEs fraction from SODD without degradation of FASEs (11, 67). A summary of physical processes used in the isolation and purification of bioactive compounds from deodorizer distillate is shown in Table 8.

Numerous methods have been proposed for treating deodorizer distillate to isolate one or more components. None of the above methods for isolating one or more components from a deodorizer distillate has proved satisfactory. Tocopherols and free phytosterols are among the

Table 8. Summary of physical processes used in the purification of bioactive compounds.

Samples	Procedures	Ref.
Deodorizer distillate	Scrubber with two condensing zone (fatty acids, tocopherols, free phytosterols)	(22)
SODD	Molecular distillation (tocopherols)	(61)
SODD	Supercritical carbon dioxide extraction (tocopherols)	(24, 28, 29, 62)
PFAD	Batch adsorption on silica (tocotrienols)	(66)
SODD	Modified soxhlet extraction and modified silica gel column chromatography (squalene)	(9)
SODD	Modified soxhlet extraction, modified silica gel column chromatography, and mixture of acetone and water extraction (FASEs)	(10, 67)

Desired compounds reported in parentheses.

compounds of nutritional interest that are frequently purified from deodorizer distillate.

Methods employing chemical reactions, such as esterification or saponification of FFAs or acylglycerols, introduce processing complexity and require later processing steps that often involve the use of strong mineral acids in order to recover fatty acids. Mineral acids can be dangerous in handling and can induce discoloration or other degradation of distillate components (23). During esterification of FFAs in deodorizer distillate, the addition of strong acid as catalyst greatly accelerates the esterification, which however also causes the undesired esterification of tocopherols and degradation of FASEs (21). In addition, tocopherols (68) and FASEs (46) are prone to degradation if alkaline condition is involved. Furthermore, it has been reported that prolonged exposure to alkaline conditions resulted in significant losses of squalene (51).

The main disadvantage of using enzyme is the cost due to its stability and reusability. However, denaturization of bioactive compounds can be suppressed (24). Unfortunately, converting FFAs and free phtosterols by enzymatic reaction in VODD to FASEs are not preferable because FFAs in SODD have low quality due to harsh conditions during soybean oil refining, which resulted in reactions such as in oxidation and *cis-trans* conversion.

A simple distillation is not preferred because it introduces degradation of bioactive compounds. Tocopherols (3, 21, 23) and FASEs (11) are prone to degradation if high-temperature is involved. Tocopherols levels decreased about 11%, 25%, 38% and 61% for ½ hour processing during deodorization step at 240, 260, 280 and 300°C, respectively (Figure 12) (3). Methods employing extractive steps are expensive and risk contamination by residual solvent. Molecular distillation is by far the

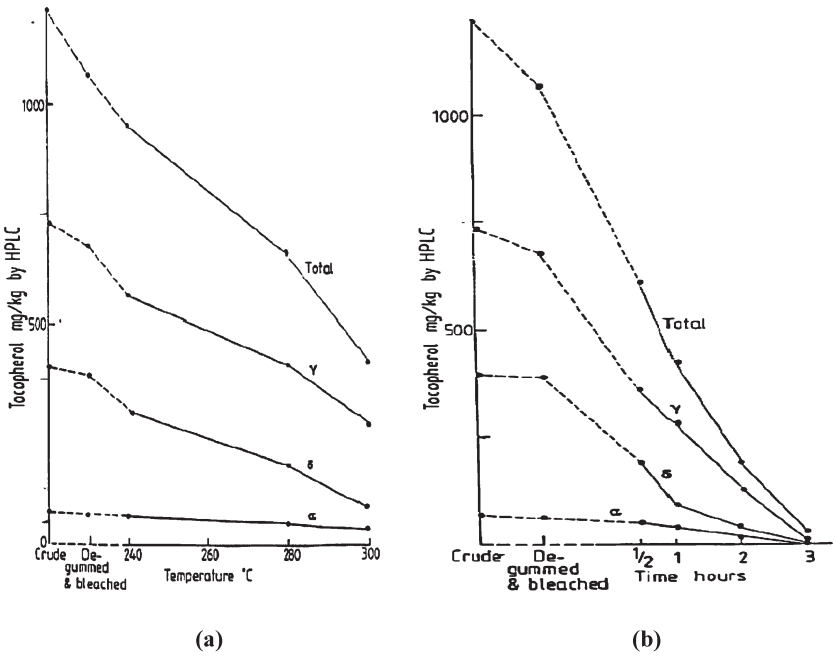


Figure 12. Tocopherol levels in physically refined soybean oil, (a) for ½ hour processing, (b) processed at 280°C.

preferred method to isolate both thermo sensitive and high-molecular-weight compounds from deodorizer distillate. Molecular distillation requires operation under high temperature and high vacuum with expensive equipment.

The basic principle of separation in modified Soxhlet extraction can be understood in terms of the coating and selective desorption processes. Coating is a process that occurs when VODD accumulates on the surface of a silica gel, forming a thin film. Selective desorption occurs when some compounds in VODD that is coated on the surface of silica gel dissolve in hexane. That is because nonpolar compounds (such as hydrocarbons and FASEs) are weakly attached on the silica gel surface whereas polar compounds are strongly attached on the silica gel surface, so that compounds with different polarity can be separated. Due to disposal as well as economic considerations, regeneration of silica gel is favorable. Finding suitable method for the regeneration and reactivation of used silica gel remains a challenge.

Tocopherols have been purified from deodorizer distillate in industrial scale by a combination of molecular distillation, ethanol fractionation, chemical alcoholysis, and ion-exchange chromatography. Extractive isolation of tocopherols by cold ethanol did not produce high

yield and purity. The recoveries of tocopherols are less than 75%, because a part of tocopherols coprecipitates with free phytosterols (60).

The development of new separation techniques has gained increasing importance in chemical, food, and pharmaceutical industries, due to the imposed environmental regulations and the necessity of minimizing energy requirement. Difficulties associated with separating tocopherols and / or free phytosterols from deodorizer distillate are that their boiling points are roughly in the same range as shown in Table 9 (23), and they have similar molecular weight (Table 10) (7). One difficulty associated with separating squalene and / or FASEs from deodorizer distillate are that their polarities are roughly in the same range. The isolation of squalene, FASEs, tocopherols and free phytosterols from deodorizer distillates in high yield and purity remains a challenge.

ANALYSIS OF VODD

The analysis of the deodorizer distillates is a challenging problem. Several analytical techniques are available to provide a detailed analysis of one or more compounds in the deodorizer distillates. Tocopherols and free phytosterols are among the lipids of nutritional interest that are frequently determined in the deodorizer distillates. The two species are commonly analyzed by GC as well as by HPLC method. A general advantage of HPLC compared to GC is that derivatization and silylation

Table 9. Boiling points of tocopherols and free phytosterols as function of pressure.

Pressure (Pa)	Tocopherols b.p. (°C)	Free Phytosterols b.p. (°C)
133	~ 232	~ 243
267	~ 243	~ 246
400	~ 254	~ 263
533	~ 277	~ 271

Table 10. Molecular weight and relative volatility of some key components of deodorizer distillate.

Component	Molecular weight (g/mol)	Relative volatility
Linoleic acid	280	2.5
Squalene	411	5
Tocopherols (γ-tocopherol)	417	1
Free phytosterols (β-sitosterol)	415	0.6
Fatty acid steryl esters	675	0.038
Oil (triolein)	885	Small (< 0.038)

(in order to increase the volatility of the compounds) is not necessary. However, the application of HPLC is limited by the lack of separation of tocopherols and free phytosterols under the same conditions.

If data on both tocopherols and free phytosterols are required it is possible to determine them in the same analysis by GC under similar conditions (69). Therefore, GC is the most applied technique in the analysis of the deodorizer distillates. A convenient way to describe and catalog analytical procedures that have been proposed for analyzing the deodorizer distillates is to divide the procedure to two groups: indirect and direct analysis.

Indirect Analysis

The quantification of compounds presents in the deodorizer distillates by direct analysis is difficult when the constituents overlap or present at low levels. A pretreatment for the elimination of interfering substances is necessary. The AOCS Ce3-74 (70) proposes a method to determine tocopherols and free phytosterols contents in soy sludge by GC using a packed glass column. The preparation involves saponification of the distillate and extraction of the unsaponifiable matters. Tocopherols and free phytosterols are converted into their butyrate esters and separated by GC. In this procedure, saponification may cause degradation of tocopherols that have to be taken into account during quantification.

Another analytical procedure (71), combining silica gel column chromatography, high-performance size-exclusion chromatography (HPSEC) and GC was developed. Silica gel column chromatography was used to separate the distillate into two fractions. Further separation of both fractions by HPSEC, allowed separation and quantification of the main groups of compounds (TAGs, DAGs, MAGs, FASEs, free phytosterols, FFAs, hydrocarbons, and alkyl esters). A detail quantification of the specific compounds of interest present in the fractions could be further achieved by GC after silylation using a mixture of pyridine, hexamethyldisilazane and TMCS (9:3:1, v/v/v) to increase the volatility of the components. Within the analysis of GC, TAGs peak was not detected. Moreover, the separation of individual FASEs were not obtained.

Direct Analysis

A GC method for the analysis of deodorizer distillate without pretreatment to reduce analysis time was proposed (6, 10, 11, 67, 72, 73). Total tocopherols contents in deodorizer distillate are analyzed by

using capillary GC on a polar column (72). After sample preparation, which consists of a silylation, a short temperature profile is applied. This method separates δ-, β/γ-, and α-tocopherol but other compounds present in deodorizer distillate are not well resolved.

Verleyen et al. (6) proposed a GC method for the analysis of TAGs, DAGs, MAGs, squalene, tocopherols, free phytosterols and FASEs in deodorizer distillate without saponification. After a concise sample preparation including derivatization and silylation (using a mixture of pyridine and *N,O*-bis-(trimethyl) trifluoracetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS)), distillate samples were injected into GC. Within the tested temperature profile, co-elution of β- and γ- tocopherol was found. The resolution factor of α-tocopherol and brassicasterol was not perfect, and the separation of individual FASEs was not achieved.

GC with a flame ionization detector has been used for the characterization of SODD (73). Derivatization by silylation of the samples prior to their injection into a medium polar column enabled a peak resolution of the different components, which led to unambiguous identification of most of the major components (FFAs, free phytosterols and tocopherols) of SODD. The identification and quantification of other components, such as FASEs and acylglycerols, were not reported.

Recently, a direct analysis method without sample derivatization and silylation, for the identification and quantification of different compounds present in soybean oil deodorizer distillate (squalene, tocopherols, free phytosterols, FASEs and acylglycerols) by optimization of temperature and linear velocity profile within reasonable analysis time was proposed (10, 11, 67).

None of the above analysis techniques for identifying and quantifying components in a deodorizer distillate has proved satisfactory. The overlapping of different groups of compounds (such as α-tocopherols and brassicasterol) and different individual compounds (such as individual FFAs and FASEs) in the chromatogram were found. Saponification prior to GC analysis caused degradation of bioactive compounds (such as squalene, tocopherols and FASEs) in deodorizer distillate, which have to be taken into account during quantification. Detail analysis of the original individual FASEs of SODD has not been reported yet.

CONCLUSIONS AND FUTURE PERSPECTIVES

Depending on the sources, the deodorizer distillates usually have significantly different characteristics, uses, and value. The utilization of deodorizer distillates remains a challenge. Due to their high content, the efficient removal of FFAs and acylglycerols is crucial for the concentration of bioactive compounds in SODD. Most of the methods that have

been developed for converting fatty acids into biodiesel (FAMEs) and isolating tocopherols and free phytosterols show promising results. However, no economical study has been assessed and no comparison between the methods has been made.

The development of new separation techniques has gained increasing importance in chemical, food, and pharmaceutical industries, due to the imposed environmental regulations and the necessity of minimizing energy requirement. Adsorption is an alternative method of separation and regeneration of the used adsorbent is favorable due to disposal as well as economic considerations.

The analysis of deodorizer distillate is a challenging problem. Few analytical techniques are available to provide a detailed analysis of compounds in deodorizer distillates. It will be of great importance if a method to identify individual FFAs, tocopherols, free phytosterols, and FASEs and to evaluate the suitability of GC analysis by optimizing temperature profile in reasonable analysis time can be established.

LIST OF ABBREVIATIONS

AOCS: American Oil Chemists' Society
ASGs: Acylated steryl glycosides
BSTFA: *N,O*-bis-(trimethyl)trifluoracetamide
VODD: Vegetable oil deodorizer distillates
CODD: Corn oil deodorizer distillate
DAGs: Diacylglycerols
DDT: Dichloro-diphenyl-trichloroethane
DI: Degree of isomerization
DNA: Deoxyribonucleic acid
FAMEs: Fatty acid methyl esters
FASEs: Fatty acid steryl esters
FFAs: Free fatty acids
FID: Flame ionization detector
GC: Gas chromatography
HDLs: High-density lipoproteins
HPLC: High-performance liquid chromatography
HPSEC: High-performance size-exclusion chromatography
IUB: International union of biochemistry and molecular biology
IUPAC: International union of pure and applied chemistry
LDLs: Low density lipoproteins
MAGs: Monoacylglycerols
OODD: Olive oil deodorizer distillate
PAHs: Polycyclic aromatic hydrocarbons
PFAD: Palm fatty acid distillate

RODD: Rapeseed oil deodorizer distillate
SAF: Syntex adjuvant formulation
SGs: Steryl glycosides
SODD: Soybean oil deodorizer distillate
SuODD: Sunflower oil deodorizer distillate
TAGs: Triacylglycerols
TFAs: Trans fatty acids
TMCS: Trimethylchlorosilane

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