

FEATURE

PROOF

Natural vitamin E: activities and sources

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The superiority of natural vitamin E over synthetic α -tocopherol lies in two aspects. First, natural d - α -tocopherol has a higher bioavailability than synthetic *rac*- α -tocopherol. Second, natural vitamin E usually consists of different tocopherol homologues as well as tocotrienols, whereas synthetic sources contain mainly α -tocopherol. The non- α -tocopherol homologues, especially γ -tocopherol and tocotrienols play a different but important role in human health. Most of these effects are not related to vitamin E activity. Foods of plant origin such as vegetable oils, margarine, oilseeds, nuts, cereals and green vegetables are the major dietary sources of natural tocopherols and tocotrienols. At present, commercial natural vitamin E is produced almost exclusively from deodorizer distillates from the refining of vegetable oils. Oils from a series of edible berries are new potential sources of natural vitamin E. New developments in chemical synthesis and genetic engineering will probably provide new alternatives for an economical production of stereoisomers identical to natural tocopherols and tocotrienols in the future.

Introduction

The importance of vitamin E in animal and human nutrition is increasingly recognized. As the most important lipid-soluble antioxidants in living tissues, the biological activities of these compounds reach far beyond the vitamin E activity originally defined using rat foetus resorption studies. Vitamin E concentrates are widely used in feed, food, nutraceutical, pharmaceutical and cosmetic products. The global annual production of vitamin E exceeds 20 000 tonnes and is increasing at a rate of up to 7% a year. While synthetic vitamin E represents up to 90% of total vitamin E production, recent scientific investigations strongly suggest greater biological activities for the natural forms.

Vitamin E and its biological activity

Vitamin E refers to a group of compounds comprising four (α , β , γ and δ) tocopherols and four (α , β , γ and δ) tocotrienols (Figure 1). These compounds have a common structure of a chroman head attached to a phytyl tail. The four tocopherols have saturated phytyl tails and differ only in the number and pattern of methyl substitution on the chroman head. Due to the

presence of three chiral centres at positions 2, 4', 8', each tocopherol has eight stereoisomers (*RRR*, *RRS*, *RSR*, *RSS*, *SSS*, *SRR*, *SSR*, *SRS*).

Tocotrienols differ from the corresponding tocopherols by the presence of three isolated double bonds in the phytyl tail. Theoretically, each tocotrienol has eight isomers (*R*, *cis-cis*; *R*, *cis-trans*; *R*, *trans-cis*; *R*, *trans-trans*; *S*, *cis-cis*; *S*, *cis-trans*; *S*, *trans-cis*; *S*, *trans-trans*) because of the chiral centre at position 2 and the double bonds at positions 3' and 7'.

Naturally occurring tocopherols have a uniform *RRR* configuration (referred as *d*-tocopherols, e. g. *d*- α -tocopherol), whereas a synthetic tocopherol is a mixture of approximately equal amounts of the eight stereoisomers (referred to as *all-rac*-tocopherols or [*d*]-tocopherols, e. g. *all-rac*- α -tocopherol). Natural tocotrienols are exclusively *REE* isomer.

Vitamin E activity (expressed in International Units, IU) defines the biological efficiency of these

compounds in a standardized rat foetus resorption-gestation study; one IU equals the activity of 1 mg of (*all-rac*)- α -tocopheryl acetate. Vitamin E activities of natural tocopherols and tocotrienols relative to *d*- α -tocopherol fall into the following order, α -tocopherol > β -tocopherol > α -tocotrienol > γ -tocopherol > β -tocotrienol > δ -tocopherol. The relative activities of γ - and δ -tocotrienols are not known for certain. Among the stereoisomers of α -tocopherol, the natural (*RRR*) isomer is clearly

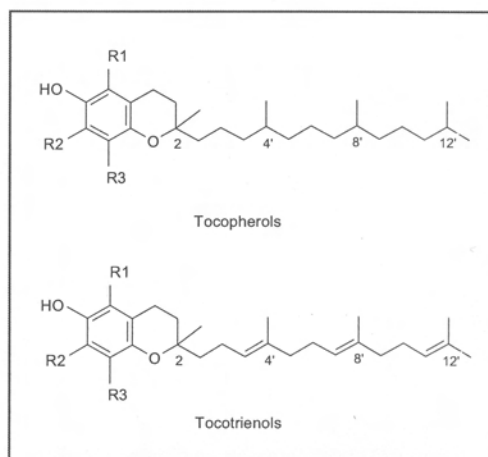


Figure 1. The chemical structures of tocopherols and tocotrienols. For α -tocopherol and α -tocotrienol, $R_1 = R_2 = R_3 = \text{CH}_3$; for β -tocopherol and β -tocotrienol, $R_1 = R_3 = \text{CH}_3$, $R_2 = \text{H}$; for γ -tocopherol and γ -tocotrienol, $R_1 = \text{H}$, $R_2 = R_3 = \text{CH}_3$; for δ -tocopherol and δ -tocotrienol, $R_1 = R_2 = \text{H}$, $R_3 = \text{CH}_3$.

Table 1. Vitamin E activity of tocopherols^a and tocotrienols^b and factors used for conversion of these compounds into international units (IU) as well as α -tocopherol equivalent in evaluation of the intake and requirement of vitamin E (1, 2). T, tocopherol; T₃, tocotrienol.

Activity relative to d - α -T (%) ^c		Conventional calculation per mg		New recommendation ^f			
		IU ^d	α -T ^e	IU/mg		mg α -T/mg	
<i>RRR</i> - α -T	100	1.49	1.0	<i>RRR</i> - α -T	1.49	<i>RRR</i> - α -T	1.0
<i>RRS</i> - α -T	90	1.34		<i>RRR</i> - α -T acetate	1.36	<i>RRS</i> - α -T	1.0
<i>RSS</i> - α -T	73	1.09		<i>RRR</i> - α -T succinate	1.21	<i>RSR</i> - α -T	1.0
<i>SSS</i> - α -T	60	1.10		<i>all-rac</i> - α -T	1.10	<i>RSS</i> - α -T	1.0
<i>RSR</i> - α -T	57	0.85		<i>all-rac</i> - α -T acetate	1.00	<i>all-rac</i> - α -T	0.5
<i>SRS</i> - α -T	37	0.55		<i>all-rac</i> - α -T succinate	0.89	<i>SSS</i> - α -T, <i>SSR</i> - α -T, <i>SRS</i> - α -T, <i>SRR</i> - α -T, β -T, γ -T, δ -T	0.0
<i>SRR</i> - α -T	31	0.46		^a Natural tocopherols are <i>RRR</i> -isomers, synthetic (<i>all-rac</i> -) tocopherols are mixtures of equal amounts of <i>RRR</i> -, <i>RRS</i> -, <i>RSR</i> -, <i>RSS</i> -, <i>SSS</i> -, <i>SRS</i> , <i>SSR</i> , and <i>SSR</i> -isomers. ^b Natural tocotrienols are <i>REE</i> -isomers. ^c Activity determined by the rat foetus resorption study. ^d 1 IU equals the vitamin E activity of 1 mg <i>all-rac</i> - α -tocopheryl acetate. ^e Factors for conversion to α -tocopherol equivalent which is used for evaluation of vitamin E intake and requirement. ^f Recommendation by the Food and Nutrition Board of the United States National Academy of Sciences in 2000.			
<i>SSR</i> - α -T	21	0.31					
<i>RRR</i> - β -T	57	0.75	0.5				
<i>RRR</i> - γ -T	31	0.15	0.1				
<i>RRR</i> - δ -T	1.4	0.04	0.03				
<i>REE</i> - α -T ₃		0.45	0.3				
<i>REE</i> - β -T ₃		0.08	0.05				

the most efficient as vitamin E (1).

The vitamin E activities of various tocopherol and tocotrienol isomers are summarized in Table 1, together with factors used for conversion of these compounds into international units and α -tocopherol equivalents in diet and food (1,2). The conventional way of conversion is challenged by the latest research evidence, which strongly suggests that d - α -tocopherol is at least twice as efficient as *all-rac*- α -tocopherol. In 2000 the Food and Nutrition Board of the US National Academy of Sciences recommended that only *RRR*-tocopherol (d - α -tocopherol) and three other synthetic 2*R*- (*RRS*-, *RSR*-, *RSS*-) stereoisomers of α -tocopherol should be included in the calculation of vitamin E daily requirement and intake. In this recommendation, the 2*S*- stereoisomers of α -tocopherol as well as the other (β , γ and δ) tocopherols and tocotrienols are not included in the estimation of the vitamin E intake.

Why natural vitamin E?

The superiority of natural vitamin E compared with synthetic sources lies in the following aspects.

- Natural α -tocopherol has higher biological activity than synthetic α -tocopherol.
- Synthetic vitamin E contains mainly α -tocopherol, whereas natural vitamin E usually consists of a mixture of different tocopherols and/or tocotrienols.
- Non- α -tocopherol isomers, especially (β -tocopherol and tocotrienols, also play an important role in human health.
- A balanced intake of different isomers is important: large doses of α -tocopherol may displace other tocopherols and tocotrienols in plasma and tissues.
- Under specific conditions, α -tocopherol may act as a prooxidant. Similar activity has not yet been found in other (β , γ and δ) tocopherols.

The superior activity of natural vitamin E results from its higher bioavailability, which is twice that

of synthetic d - α -tocopherol. This is mostly due to the preferential delivery to very-low-density lipoprotein by tocopherol transfer protein in the liver. In contrast, the synthetic isomers are mostly metabolized and excreted. The preferential transport probably also involves other three 2*R*-isomers.

γ -Tocopherol is another major form of natural vitamin E. Although the vitamin E activity of γ -tocopherol is much lower than that of α -tocopherol, it probably possesses multiple biological activities that cannot be replaced by α -tocopherol in the body.

Harmful reactive nitrogen species are trapped and detoxified by γ -tocopherol much more efficiently than by α -tocopherol. During chronic inflammation, peroxynitrite (ONOO⁻) is produced by macrophages. Nitric oxide (NO), nitrogen dioxide (NO₂) and nitrous acid (HNO₂) are present in the air due to pollution. These reactive nitrogen species are not only powerful lipid oxidants but also act as mutagenic agents by damaging DNA. The greater

efficiency of γ -tocopherol in quenching these reactive nitrogen molecules, with formation of stable adducts such as 5-NO₂- γ -tocopherol, results from the lack of substitution at the 5-position in the chromanol ring. This activity of γ -tocopherol is not influenced by the presence of α -tocopherol. Thus the two tocopherols probably play complementary roles in protection of cellular components from oxidative damage (3).

Recent research indicates that γ -tocopherol reduces the risk of cancer and cardiovascular disease. This may also be related to the anti-inflammatory property of this compound. Cyclooxygenase-catalysed synthesis of prostaglandin E₂ (PGE₂) plays an important role in inflammation and in diseases associated with inflammation. Studies *in vitro* showed that γ -tocopherol suppressed PGE₂ synthesis in lipopolysaccharide-stimulated macrophages and IL-1 β -treated human epithelial cells with an IC₅₀ of 7.5 and 4 mM, respectively. γ -CEHC [2,7,8-trimethyl-2-(β -carboxyethyl)-6-hydroxychroman], a major metabolite of dietary γ -tocopherol, exhibited similar effects with IC₅₀ being 30 mM. In contrast, α -tocopherol showed only slight inhibition at high concentration in macrophages, and no effect in epithelial cells (4).

Skin has an extraordinary need for antioxidants because of the constant exposure to environmental stress such as ultraviolet irradiation, air pollution, and harsh temperatures. Although γ -tocopherol is present at much lower levels than α -tocopherol in plasma, it accounts for over 50% of total vitamin E in skin. This is probably a result of a preferential transport and/or retention of this isomer into skin. Taken together with the high reactivity towards reactive nitrogen species and the anti-inflammatory properties,

this suggests an irreplaceable role for γ -tocopherol in skin protection.

A wide range of health effects beyond vitamin E activity has been demonstrated for tocotrienols in recent investigations. The beneficial effects include lowering plasma cholesterol level, reducing risk of cardiovascular disease, and protecting the skin, as well as anti-tumour and anti-cancer functions. Most of the health effects of tocotrienols are related to the high antioxidant activity of these compounds.

The radical-scavenging potency of vitamin E isomers is strongly influenced by their ability to reach free radicals and their efficiency in regenerating other antioxidants such as vitamin C and flavonoids in the body. In this respect, tocotrienols are much more efficient than tocopherols because they have three isolated double bonds in the phytol chain. This ensures higher antioxidative activity of tocotrienols compared with tocopherols (5).

Like γ -tocopherol, tocotrienols are probably a major line of defence in the skin. Recent studies suggest that dietary tocotrienols are preferentially directed to skin, while topically-applied tocotrienols are readily absorbed by skin. Topical application of tocotrienols prior to UV-irradiation preserves the skin antioxidant pool and protects skin from oxidative stress from UV-light and ozone.

Some metal ions induce lipid oxidation by stimulating the decomposition of hydrogen peroxide and hydroperoxide. These metal ions at low valency state (Cu⁺ and Fe²⁺) decompose hydroperoxides much faster than the corresponding higher valency forms. Under certain conditions, α -tocopherol may reduce metal ions and act as a prooxidant. For example, in the copper-induced oxidation of methyl linoleate

micelles in aqueous dispersions, α -tocopherol and α -tocotrienol reduced Cu²⁺ to Cu⁺ and speeded up the oxidation of methyl linoleate. Under the same conditions, β -, γ - and δ -tocopherols did not show any prooxidant effect (5).

Vitamin E in food

Plants are the primary source of natural vitamin E. Tocopherols are present in oil seeds, pollens, leaves, and other green parts and fruits of higher plants. α -Tocopherol is present mainly in the chloroplast; β , γ and δ -homologues are usually found outside this organelle. Tocotrienols exist mainly in cereal grains and certain seeds. While all tocopherols and tocotrienols are present in plant materials, α -tocopherol is the primary vitamin E in materials of animal origin because of the preferential retention of the isomer in animal tissues.

Vegetable oils are the richest food source of natural vitamin E (Table 2). Most nuts are rich in tocopherols: almonds (27 mg/100 g) and peanuts (11 mg/100 g) are good sources of α -tocopherol; walnuts, pistachios and pecans of γ -tocopherol (20–30 mg/100 g). Many peas and beans also are rich in γ -tocopherol (4–13 mg/100 g). In fruits and green leafy vegetables α -tocopherol is the major vitamin E isomer present at levels varying from 0.1 to 3 mg/100 g fresh weight. Red (16 mg/100 g fresh weight) and green chili peppers (9 mg), celery (14 mg), and kale (7 mg) are outstanding sources of α -tocopherol among vegetables. Certain tropical vegetables contain exceptionally high levels of α -tocopherols.

Cereal grains normally contain α -tocopherol and α -tocotrienol, each at levels around 1 mg/100 g. In addition, wheat grain contains also β -tocopherol (1 mg/100 g) and

β -tocotrienol (3 mg), corn grain γ -tocopherol (4 mg).

Margarine is rich in γ -tocopherol (51 mg/100g) and α -tocopherol (7 mg), whereas butter (α -tocopherol 2 mg) and lard (α -tocopherol 1.2 mg, α -tocotrienol 0.7 mg) are much poorer in vitamin E. Meat, fish and milk contains mainly α -tocopherol at levels of 0.1–1 mg/100 g.

Vegetable oils and oil distillates as sources

Vegetable oils are the primary source of natural vitamin E, with α - and γ -tocopherols being the most abundant isomers (Table 2). Among the common vegetable oils, wheat germ oil has the highest content of α -tocopherol (120 mg/100 g) and total vitamin E (about 250 mg/100 g). Sunflower seed oil is also rich in α -tocopherol (60 mg/100 g). Soybean oil is the most abundant source of γ -tocopherol. Corn oil, canola oil, sesame oil, and cocoa butter are also good sources of γ -tocopherol. Cottonseed oil, palm oil, safflower oil, and peanut oil contain α - and γ -tocopherols at roughly equal levels of 20–40 mg/100 g.

Palm oil is the most abundant commercial source containing a full range of tocotrienol isomers. Commercial products containing palm tocotrienol fractions are available. Oils from rice bran, barley, oat, and wheat are also good sources of tocotrienols (Table 2).

The global production of natural vitamin E is estimated to be well over 2000 tonnes a year from vegetable oil distillates, mainly soy deodorizer distillate, which is a by-product of the oil refining process. The distillate accounts for 0.5–1% of the oil and normally contains 1–20% natural vitamin E, depending on oil sources and distillation conditions. Other major components of the distillates are

free fatty acids (30–60%), sterols and sterol esters (10–35%), hydrocarbons (10–30%), and triacylglycerols (10–20%).

Processing of the distillates normally involves transesterification, distillation, crystallization, ion-exchange chromatography and extraction, yielding products with vitamin E content from 20–80%. The quality of the final product is strongly influenced by conditions of processing and storage such as exposure to heat, light, oxygen, metal ions and moisture. Lately, supercritical CO₂ fluid extraction (SFE) has also been applied in industrial fractionation of soy distillates. The process involves esterification, freezing and repeated SFE separation, yielding three separate products: natural vitamin E, fatty acid methyl esters and phytosterols. A Chinese company using this process produces a total amount of 700 tonnes a year of natural vitamin E and plant sterols (data from the company).

Concentrates containing mixtures tocopherol homologues are used as antioxidants. Mixed tocopherols from soy deodorizer distillates consist of about 60% γ -tocopherol and 10% α -tocopherol. Commercial products with varying compositions are available. A large proportion of vitamin E concentrate obtained from soy distillates is modified by permethylation reaction, transforming β , γ and δ -tocopherols to α -tocopherol. α -Tocopherol is often further converted to the more oxidation-stable α -tocopheryl acetate (sometimes succinate). Mixed isomers and α -tocopherol-rich forms of natural vitamin E are both available in the market as oil and powder forms for either direct encapsulation or incorporation into different products.

At present, the largest proportion (around 80%) of natural vitamin E concentrate is

used in pharmaceutical products and food supplements, and only about 20% goes to cosmetics and food. The demand for natural vitamin E is estimated to be increasing at a rate of over 10–20% a year, clearly exceeding the supply. Global production of soybean oil is around 30 million tonnes per year. The total annual production of vegetable oils is 100 million tonnes. This provides considerable growth potential for production of natural vitamin E. On the other hand, deodorization removes 20–50% of tocopherols from the oils, as well as some other valuable components such as plant sterols. Considering the importance of vegetable oils as a primary dietary source of natural vitamin E, and the health effects of plant sterols, this refining process should perhaps be regulated by the relevant authorities. It is also important to look for potential alternative sources of tocopherols and tocotrienols.

Oils from edible berries are excellent sources

Wild and cultivated berries of various types of plant are an important dietary source of vitamins and polyphenols in many countries. The total harvest of such berries is estimated to be on the scale of several million tonnes per year. These berries can be processed into juices, jams, jellies and wines by the food industry.

Seed oils from some of these berries contain attractive levels of tocopherols and tocotrienols (Table 2). The oils are also rich in beneficial fatty acids as well as other natural vitamins, antioxidants and plant sterols. The crude oils are rarely used for isolation of vitamin E, but rather as sources of mixed, natural, bioactive lipids for health-care products and cosmetics. In production, replacing conventional oil-isolation methods with supercritical CO₂

Table 2. Content of tocopherols and tocotrienols in vegetable oils (mg/100 g) (1–3, 6). T, tocopherol; T₃, tocotrienol. Blank entries indicate that the compounds are not detectable or the content is less than 0.5 mg/100 g.

Oils	α -T	β -T	γ -T	δ -T	α -T ₃	β -T ₃	γ -T ₃	δ -T ₃	Total
Wheat germ	121	65	24	25	2	17			254
Sunflower	60	2	1	1					64
Safflower	39	1	17	24					81
Cottonseed	36		36	4					76
Peanut	13		22	2					37
Palm	28		32	7	14	3	29	7	120
Olive	20	1	1						22
Soybean	10	1	80	30					121
Maize (corn)	11	5	60	2					78
Rapeseed (canola)	17		35	1					53
Sesame	1	1	24	3					29
Hemp			150						150
Rice bran	12	4	5		18	2	57		98
Barley	35	5	5		67	12	12		136
Oat	18	2	5	5	18		3		51
Rye	71	17		104					192
Sea buckthorn pulp	200	10	15	10	3	5	3	1	247
Sea buckthorn seed	100	20	100	10	5	5	5	2	247
Gooseberry	19		55	5					79
Jostaberry	59		73	4					136
Blackcurrant	60		103	7					170
Red-, whitecurrant	26	3	87	29					145
Cloudberry	60		140						200
Raspberry	50		240	40					330
Cranberry	20		10				150		180
Lingonberry	20		10				120		150
Bilberry	20		10				70		100
Evening primrose	16		42	7					65

extraction guarantees both high yield and superior quality of the oils. (Preparative and plant-scale supercritical CO₂ extraction of substances such as tocopherols is reviewed by Jerry W. King in the new *Advances in Lipid Methodology—Five* published by The Oily Press in November 2003.)

Examples of the seeds and berries include:

sea buckthorn seeds and berries which are highly enriched with α - and γ -tocopherols;

seed oils of some species of *Ribes* (e.g. redcurrant) and *Rubus* (e.g. raspberry) containing high levels of γ -tocopherol; and

seeds of some *Vaccinium* species (e.g. cranberry) which are the richest natural source of γ -tocotrienol.

Sea buckthorn (*Hippophaë rhamnoides*) is native to Eurasia. Oil content in the seed is about 10%, and the fresh fruit flesh and peel contains 2–5% pulp oil. Both seed and pulp oils are rich in natural vitamin E, commonly within the range of 100–300 mg/100 g (Table 2); in extreme sources the level is as high as 1% (1000 mg/100 g). α -Tocopherol represents 80% of the total vitamin E in pulp oil, whereas α - and γ -tocopherols have equal predominance in seed oil.

Production is limited at the moment, and the oils are therefore primarily used in food supplements for strengthening skin and mucous membrane and for improving cardiovascular health. A small proportion is used in personal care products. The

global resource of sea buckthorn — including both wild and cultivated plants — is estimated to represent a theoretical yield of about 300 000 tonnes of fresh berries per year. Planting is increasing at a rate of 100 000 hectares per year and will lead to a significant increase in overall yield of berries (6).

The seeds from currants of *Ribes* species contain 20–30% oil. These oils are a known source of γ -linolenic acid and stearidonic acid. They are excellent sources of both α - and γ -tocopherols. Seed oils of blackcurrant (*R. nigrum*), redcurrant and whitecurrant (*R. rubrum*), and gooseberry (*R. grossularia*) contain vitamin E at levels of 140–170 mg/100 g (Table 2).

In the genus *Rubus*, raspberry

(*R. idaeus*) and cloudberry (*R. chamaemorus*) have seed yielding an oil rich in γ -tocopherol (Table 2), linoleic and α -linolenic acids. SFE oils are available and are mainly used in personal-care products.

Vaccinium includes more than 200 species, of which some bear edible fruit. The seeds of these berries contain 20–30% oil. In seed oil of cranberry (*V. oxycoccos* and *V. macrocarpon*), lingonberry (*V. vitis-idaea*) and bilberry (*V. myrtillus*), the content of γ -tocotrienol is as high as 150 mg/100 g (Table 2). In addition, the oils are excellent sources of linoleic (30–40%) and α -linolenic (up to 50%) acids.

Selective synthesis of stereoisomers

Effort has been put into the stereoselective synthesis of the *RRR*-isomer. By using optical resolution, biocatalysis, and chiral auxiliaries, *RRR*- α -tocopherol and *REE*- α -tocotrienol have been successfully synthesized. However, an economical synthesis on the industrial scale has yet to be reached.

Gene manipulation provides new prospects

Genes of several enzymes in the biosynthetic pathway of tocopherols and tocotrienols in plants have been identified. Over-expression of γ -tocopherol methyltransferase in *Arabidopsis thaliana* led to 80-fold increase in α -tocopherol content in the seed. A cyanobacterial (*Synechocystis*) knock-out mutant for the corresponding methyltransferase gene eliminated the biosynthesis of α -tocopherol and resulted in accumulation of the biosynthetic precursor γ -tocopherol.

In the future, regulation of the expression of these genes will make it possible to increase both total content and proportion of

specific isomers of tocopherols and tocotrienols in plants. Alternatively, cloning these genes into microbes could allow production of vitamin E isomers using fermentation technology. However, consumer attitudes toward genetically-modified products are as challenging as the technical questions facing the scientists working in this area.

Conclusions

Natural vitamin E has superior biological activities compared to the synthetic isomers. The demand for natural vitamin E is increasing rapidly, exceeding the supply of this material. Vegetable oils are the major source for both dietary intake and commercial production of natural vitamin E. While the global production of vegetable oils provides potential for growth in production of natural vitamin E, removal of these compounds from vegetable oils should be regulated. It is important to explore new potential natural sources as well as new technologies for economical production of stereoisomers identical to natural tocopherols and tocotrienols.

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