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A Method of Chromatographic Isolation for Vitamin E Isomers**Field of the Invention**

The invention relates to a method of chromatographic isolation for Vitamin E isomers, particularly individual Vit E isomers such as α -tocopherol, β -tocopherol, γ -tocopherol, δ -tocopherol, α -tocotrienol, β -tocotrienol, γ -tocotrienol, δ -tocotrienol and /or the like from Vit E-comprising compounds such as crude palm oil, palm oil products and/or palm oil by-products, vegetable oils and/or the like.

Background of the Invention

This invention relates to a process of producing isomeric tocols or Vitamin E isomers (α -tocopherol, β -tocopherol, γ -tocopherol, δ -tocopherol; and α -tocotrienol, β -tocotrienol, γ -tocotrienol, δ -tocotrienol) from tocols or Vitamin E comprising compounds.

Tocols are Vitamin E and consist of tocopherols (T) and tocotrienols (T₃) which are also found in vegetable oils in varying quantity (see Table A). The tocols present in most vegetable oils are in the form of tocopherols (α -, β -, γ - and/or δ -tocopherols). On the other hand, palm oil is unique as the tocols present are mainly in the form of tocotrienols (α -T₃, γ T₃ and δ - T₃). Both of these tocols have shown interesting physiological properties.

Table A Tocopherols determined in vegetable oils (mg/kg)

Oil	α -T	β -T	γ -T	δ -T	α -T ₃	γ -T ₃	δ -T ₃
Castor	28	29	111	310	-	-	-
Cocoa butter	11	-	170	17	2	-	-
Coconut	-	-	-	2-4	20	-	-
Corn	134	18	412	39	-	-	-
Cottonseed	573	40	317	10	-	-	-
Groundnut	169	5	144	13	-	-	-
Linseed	-	-	-	-	-	-	-
Mustard	75	-	494	31	-	-	-
Olive	93	-	7	-	-	-	-
Palm from fibres	1662	-	-	-	456	485	142
Palm	150	-	-	-	1127	297	80
Palm	279	-	61	-	274	398	69
Rape	70	16	178	7	-	-	-
Rice bran	324	18	53	-	236	349	-
Safflower	477	-	44	10	-	-	-
Sesame	12	6	244	32	-	-	-
Soyabean	116	34	737	275	-	-	-
Sunflower	608	17	11	-	-	-	-
Wheat germ	1179	398	493	118	tr	-	-

Both the tocopherols and tocotrienols act as powerful nutritional antioxidants and help to reduce cellular damage due to free radicals arising from the body's normal oxidative energy metabolism or from the action of toxic chemicals and pollutants in our environment. Free radicals have been implicated in aging, chronic degenerative diseases and cancer.

Vitamin E is also a natural antioxidants, are present at approximately 600 - 1000 ppm in crude palm oil; the major components being the γ -tocotrienol which has recently been found to have anti-cancer properties besides its known antioxidant activity. Tocotrienols have been found to lower blood cholesterol.

A number of patents have been filed on the recovery of Vitamin E from palm oil and they are:

- i) Production of High Concentration of Tocopherols and Tocotrienols from Palm Oil By-Product. USP 5,190,618;
- ii) Integrated Process for Recovery of Carotenoids and Tocotrienols from oil. USP 5,157,132; and
- iii) Recovery of Carotenoids, Tocopherols, Tocotrienols, and Sterols from esterified Palm Oil GB 2,218,989.

The first patent discloses the extraction of Vitamin E from palm fatty acid distillates (PFAD) which involves conversion of a PFAD into methyl esters,

distillation of methyl esters followed by ion-exchange chromatography and molecular distillation to get Vitamin E concentrate of more than 90%.

The second patent discloses extraction of Vitamin E from crude palm oil (CPO) by converting CPO to methyl esters. Thereafter remove the esters by molecular distillation to yield carotene-rich and vitamin E-rich fractions.

The third patent discloses conversion of crude palm oil into methyl esters followed by subjecting the methyl esters in the presence of solvents into chromatographic separation with an adsorbent to yield carotene-rich, vitamin E-rich and sterol-rich fractions.

The above three inventions only describe the isolation of vitamin E from palm oil and do not include any work on the isolation of individual vitamin E isomers, which have exhibit important physiological properties.

To date, there has not been any method which discloses the use of **supercritical fluid** in the adsorption/desorption chromatography isolation/separation of the Vitamin E isomers from plant source such as CPO (crude palm oil), palm oil products and/or palm oil by-products as well as other vegetable oils and fats. All known methods previously disclosed in the adsorption/desorption chromatographic separation solely involve isolation of mixture of Vitamin E isomers and with the use of solvents (which are costly and hazardous).

Hence, there is a need to provide a separation, particularly an isolation method for Vitamin E isomers which avoids or eliminates the sole use of solvents and consequently, rendering the process "non-hazardous" .

Summary of the Invention

In accordance with the present invention, there is provided a method of chromatographic isolation for Vitamin E isomers from Vitamin E-comprising compounds.

In particular, the present invention stipulates the use of supercritical fluid (such as SC-CO₂) in combination with adsorbents (such as silica gel or C18 reverse phase silica gel) to effect adsorption and/or desorption of Vit E isomers from Vit-E comprising compounds.

Most preferably, the present invention is subsequently applied for isolating Vit E isomers from;

a) crude vegetable oil esters (via catalytic alcoholic esterification/trans-esterification of vegetable oils) alone or by distilling the esters to yield vitamin E concentrate,

OR

b) unsaponifiable matters of vegetable oils and fats (through saponification process)

OR

- c) Vitamin E concentrate obtained through any/various processes including chromatography (solvent and supercritical) as well as supercritical fluid extraction.

A main advantage of the invention lies in the use of liquefied gas at supercritical conditions, hence avoiding or reducing the prior requirements for hazardous solvents.

Detailed Description of the Invention

According to the present invention there is provided a method of chromatographic isolation of Vitamin E isomers from crude palm oil (CPO), palm oil products and/or palm oil by-products, vegetable oils and/or Vitamin E- comprising compounds, said method comprising

- (I) Introducing the Vitamin E comprising-compounds onto selected adsorbents to effect adsorption of Vitamin E isomers; and
- (II) subsequently desorbing the adsorbed Vitamin E isomers

wherein,

the adsorption/desorption of the vitamin E isomers are carried out under a supercritical fluid environment.

The present invention may be applied subsequent to the following steps and thus allowing Vit E isomers to be isolated from Vit E concentrate as derived through,

EITHER

- (i) Esterifying the free fatty acid component of the Vit. E isomers-comprising oils with one or more monohydric alcohols into esters. The esterification may be carried out via employing (a) a solid alkali metal bisulfate or a sulfate acid strongly acidic-ion exchange resin or any effective acid as a catalyst;
- (ii) Trans-esterifying the glyceride components of Vit. E isomers-comprising oils with one or more monohydric alcohols (C₁ to C₈) into monoesters. The trans-esterification may be carried out via employing a basic catalyst. [Both steps i and ii may be carried out via employing an enzyme e.g. candida rugosa and/or the like effective catalyst];
- (iii) Removing the formed esters in step (i-ii) by vacuum distillation or molecular distillation thereby yielding vitamin E concentrate. The esters may be removed via vacuum distillation or molecular distillation.

OR

Supercritical and/or any chromatographic method which are effective in extracting Vitamin E concentrate from vegetable oils.

Alternatively, the present invention may be applied to unsaponifiable materials as obtained through saponification of vegetable oils.

Adsorbents: Selective adsorbent for the adsorption of the Vitamin E isomers comprise silica gel, C18 reverse phase silica gel and/or the like which are

equivalently effective in adsorbing Vit E isomers from any Vit E comprising compounds.

Solvents: The solvents employed for the present method comprises ;

- i) supercritical fluid, e.g. SC-CO₂ (100%). Other applicable solvents include propane, propene, ethylene and/or the like;

OR

- ii) supercritical fluid (e.g. SC-CO₂) in combination with an entrainer (such as alcohol and/or the like organic solvent)

Operating conditions Individual Vitamin E isomers may be isolated with substantially higher purity (e.g >50%) in a single run by varying pressures from 50 kg/cm² to 600 kg/cm² and temperatures 30°C -100°C.

It shall therefore be illustrated, by means of the following examples, that supercritical fluid applied to solid adsorbents (silica gel and/or C18 reverse phase silica gel), provides a very satisfactory means for isolating Vitamin E isomers (α -T, β -T, γ -T, δ -T and α -T₃, β -T₃, γ -T₃ and δ -T₃) from CPO (crude palm oil) , palm oil products, vegetable oil and/or the like Vitamin E comprising compounds.

The Vitamin E isomers recovered are useful in pharmaceutical compositions, food and health food formulations, dietary supplements and cosmetics formulations. They can also be used as fine chemicals and standard reference materials .

Example 1: Isolation of Palm Vitamin E isomers

Crude palm oil (containing 600- 1000 ppm of vitamin E) was transesterified into methyl esters . The methyl esters were removed through molecular distillation at a temperature of 90-180 degree C to yield a vitamin E concentrate (> 50%).

Vit. E concentrate (0.01g; concentration: ~50%) was dissolved in organic solvent, such as dichloromethane or ethanol and loaded onto a column (silica gel, with internal diameter 20mm and length 250mm). The Vitamin E concentrate mixture was eluted using a mixture of supercritical carbon dioxide (5ml, 95.2%) and an entrainer, ethanol (0.25ml, 4.8%) with a flowrate of 5.25ml/min. The pressure of the supercritical fluid chromatography system was kept constant right through the run at $P=180\text{kg/cm}^2$, keeping temperature constant at 70°C. The four vitamin E isomers were collected at different time intervals and are shown in Table 1. The presence and concentration of Vitamin E isomer were confirmed by HPLC .

Example 2

The procedure of example 1 was repeated except vitamin E concentrate obtained from palm fatty acid distillate was used. The results are shown in Table 2.

Example 3

The procedure of example 1 was repeated except palm pressed fiber oil vitamin E concentrate was used. The results are shown in Table 3.

Example 4

The procedure of example 1 was repeated except soybean oil vitamin E concentrate was used. The results are shown in Table 4.

Example 5

The procedure of example 1 was repeated except corn oil vitamin E concentrate was used. The results are shown in Table 5.

Example 6

The procedure of example 1 was repeated except vitamin E from Canola (grapeseed oil) oil was used. The results are shown in Table 6.

Example 7

The procedure of example 1 was repeated except vitamin E from olive oil was used. The results are shown in Table 7.

Example 8

The procedure of example 1 was repeated except vitamin E from cottonseed oil was used. The results are shown in Table 8.

Example 9

The procedure of example 1 was repeated except vitamin E from groundnut oil was used. The results are shown in Table 9.

Example 10

The procedure of example 1 was repeated except vitamin E from ricebran oil was used. The results are shown in Table 10.

Example 11

The procedure of example 1 was repeated except vitamin E from sunflower seed oil was used. The results are shown in Table 11.

Example 12

The procedure of example 1 was repeated except C18 reverse phase silica gel was used as adsorbent instead of silica gel. Other conditions used are : Pressure : 180 kg/cm², CO₂ : ethanol ratio 93.75% : 6.25%. The four vitamin E isomers (alpha-tocopherol, alpha- tocotrienol, gamma- tocotrienol and delta- tocotrienol) were eluted in the same elution order as in example 1 and collected in high purity as in example 1. The same procedures using

C18 reverse phase silica gel as adsorbents were repeated for other palm oil products, palm oil by-products and other vegetable oils as mentioned in Examples 2-11. Similar elution order of vitamin E isomers was observed for both types of adsorbents used.

Table 1: Isolation of Palm Vit.E isomers (α -T, α -T₃, γ -T₃ and δ -T₃)

Fractions	Collection Time (min)	Vit.E			
		α -T	α -T ₃	γ -T ₃	δ -T ₃
S/M	-	12.7	11.4	19	5.8
1	-	-	-	-	-
2	41-47	100	-	-	-
3	47-54	-	100	-	-
4	54-61	-	-	100	-
5	61-77	-	-	99	1
6	77-145	-	-	3	97
7	145-	-	-	-	-

- a. S/M : Starting Material (vit. E concentrate ~50%)
- b. T: tocopherol
- c. T₃: tocotrienol
- d. Conditions: P=180kg/cm², T= 70°C
- e. SC-CO₂: ethanol = 5ml:0.25ml (95.2% : 4.8%)

Table 2: Isolation of Palm Vit.E isomers (α -T, α -T₃, γ -T₃ and δ -T₃) from Palm Fatty Acid Distillate

Fractions	Collection Time (min)	Vit.E			
		α -T	α -T ₃	γ -T ₃	δ -T ₃
S/M	-	22.7	21.4	39	9.8
1					
2	41-47	100			
3	47-54		100		
4	54-61			100	
5	61-77			98	2
6	77-145			2	98
7	145-				

- a. S/M : Starting Material (vit. E concentrate ~90%)

- b. T: tocopherol
- c. T₃: tocotrienol
- d. Conditions: P=180kg/cm², T= 70°C
- e. SC-CO₂: ethanol = 5ml:0.25ml (95.2% : 4.8%)

Table 3: Isolation of Palm Vit.E isomers (α -T, α -T₃, γ -T₃ and δ -T₃) from Palm Pressed Fibre Oil

Fractions	Collection Time (min)	Vit.E			
		α -T	α -T ₃	γ -T ₃	δ -T ₃
S/M	-	42.7	11.4	29	10.8
1					
2	41-47	100			
3	47-54		100		
4	54-61			100	
5	61-77			99	1
6	77-145			2.2	97.8
7	145-				

- a. S/M : Starting Material (vit. E concentrate ~90%)
- b. T: tocopherol
- c. T₃: tocotrienol
- d. Conditions: P=180kg/cm², T= 70°C
- e. SC-CO₂: ethanol = 5ml:0.25ml (95.2% : 4.8%)

Table 4: Isolation of Palm Vit.E isomers (α -T, β -T, γ -T and δ -T) from Soyabean Oil

Fractions	Collection Time (min)	Vit.E			
		α -T	β -T	γ -T	δ -T
S/M	-	11.6	3.4	53.7	17.5
1					
2	35-41	100			
3	42-47		99		
4	48-55			100	
5	58-65				100

- a. S/M : Starting Material (vit. E concentrate ~80%)
- b. T: tocopherol
- c. Conditions: P=180kg/cm², T= 70°C
- d. SC-CO₂: ethanol = 5ml:0.25ml (95.2% : 4.8%)

Table 5: Isolation of Palm Vit.E isomers (α -T, β -T, γ -T and δ -T) from Corn Oil

Fractions	Collection Time (min)	Vit.E α -T	Vit.E β -T	Vit.E γ -T	Vit.E δ -T
S/M	-	23.4	1.8	51.2	3.9
1					
2	35-41	100			
3	42-47		92		
4	48-55			100	
5	58-65				92

- a. S/M : Starting Material (vit. E concentrate ~80%)
- b. T: tocopherol
- c. Conditions: P=180kg/cm², T= 70°C
- d. SC-CO₂: ethanol = 5ml:0.25ml (95.2% : 4.8%)

Table 6: Isolation of Palm Vit.E isomers (α -T, β -T, γ -T and δ -T) from Canola Oil

Fractions	Collection Time (min)	Vit.E α -T	Vit.E β -T	Vit.E γ -T	Vit.E δ -T
S/M	-	20	8	58	2
1					
2	35-41	100			
3	42-47		85		
4	48-55			100	
5	58-65				80

- a. S/M : Starting Material (vit. E concentrate ~90%)
- b. T: tocopherol
- c. Conditions: P=180kg/cm², T= 70°C
- d. SC-CO₂: ethanol = 5ml:0.25ml (95.2% : 4.8%)

Table 7: Isolation of Palm Vit.E isomers (α -T, β -T, γ -T and δ -T) from Olive Oil

Fractions	Collection Time (min)	Vit.E	Vit.E	Vit.E	Vit.E
		α -T	B-T	γ -T	δ -T
S/M	-	83	7		
1					
2	35-41	100			
3	42-47		80		
4	48-55				
5	58-65				

- S/M : Starting Material (vit. E concentrate ~90%)
- T: tocopherol
- Conditions: P=180kg/cm², T= 70°C
- SC-CO₂: ethanol = 5ml:0.25ml (95.2% : 4.8%)

Table 8: Isolation of Palm Vit.E isomers (α -T, β -T, γ -T and δ -T) from Cottonseed Oil

Fractions	Collection Time (min)	Vit.E	Vit.E	Vit.E	Vit.E
		α -T	B-T	γ -T	δ -T
S/M	-	57.3	4	31.7	1
1					
2	35-41	100			
3	42-47		92		
4	48-55			100	
5	58-65				85

- S/M : Starting Material (vit. E concentrate ~90%)
- T: tocopherol
- Conditions: P=180kg/cm², T= 70°C
- SC-CO₂: ethanol = 5ml:0.25ml (95.2% : 4.8%)

Table 9: Isolation of Palm Vit.E isomers (α -T, β -T, γ -T and δ -T) from Groundnut oil

Fractions	Collection Time (min)	Vit.E	Vit.E	Vit.E	Vit.E
		α -T	B-T	γ -T	δ -T
S/M	-	42.9	1.5	34.4	3
1					
2	35-41	100			
3	42-47		80		

4	48-55			100	
5	58-65				85

- S/M : Starting Material (vit. E concentrate ~80%)
- T: tocopherol
- Conditions: P=180kg/cm², T= 70°C
- SC-CO₂: ethanol = 5ml:0.25ml (95.2% : 4.8%)

Table 10: Isolation of Palm Vit.E isomers (α -T, γ -T, α -T3 and γ -T3) from Ricebran Oil

Fractions	Collection Time (min)	Vit.E	Vit.E	Vit.E	Vit.E
		α -T	γ -T	α -T3	γ -T3
S/M	-	28.2	5	20.6	38.5
1					
2	35-41	100			
3	42-47		85		
4	48-55			100	
5	58-65				100

- S/M : Starting Material (vit. E concentrate ~90%)
- T: tocopherol
- T3: tocotrienol
- Conditions: P=180kg/cm², T= 70°C
- SC-CO₂: ethanol = 5ml:0.25ml (95.2% : 4.8%)

Table 11: Isolation of Palm Vit.E isomers (α -T, β -T, γ -T and δ -T) from Sunflowerseed Oil

Fractions	Collection Time (min)	Vit.E	Vit.E	Vit.E	Vit.E
		α -T	β -T	γ -T	δ -T
S/M	-	80.8	3.3	1.1	
1					
2	35-41	100			
3	42-47		85		
4	48-55			85	
5	58-65				

- S/M : Starting Material (vit. E concentrate ~80%)
- T: tocopherol
- Conditions: P=180kg/cm², T= 70°C
- SC-CO₂: ethanol = 5ml:0.25ml (95.2% : 4.8%)

Claims

1. A method of chromatographic isolation of vitamin E isomers (α -tocopherol, β -tocopherol, γ -tocopherol, δ -tocopherol, α -tocotrienol, β -tocotrienol, γ -tocotrienol and δ -tocotrienol) from Vitamin E-comprising compounds, said method including the steps of
 - (a) introducing the Vitamin E concentrate onto selective adsorbents to effect adsorption Vitamin E isomers and subsequently,
 - (b) effecting an individual desorption of the adsorbed Vitamin E isomers,wherein the adsorption and/or desorption of the Vit E isomers are carried out under a supercritical fluid environment.
2. A method of chromatographic isolation of claim 1, wherein the selective adsorbents include silica gel, reverse phase C18 silica gel and/or the like.
3. A method of chromatographic isolation of claim 1, wherein the adsorption/desorption of the Vit E isomers are carried out under a supercritical fluid environment comprising an effective amount of CO₂ as a solvent.
4. A method of chromatographic isolation of claim 1, wherein the adsorption/desorption of the Vit E isomers are carried out under a



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supercritical fluid environment comprising an effective amount of a combination of CO₂ and an entrainer.

5. A method of chromatographic isolation of claim 4, wherein the entrainer comprises an alcohol.
6. A method of chromatographic isolation of anyone of claims 3-5 wherein the supercritical fluid conditions comprise operating temperatures of T=30-100°C and pressures of P=50-600kg/cm².
7. A method of chromatographic isolation of Vit E isomers from (α -tocopherol, β -tocopherol, γ -tocopherol, δ -tocopherol, α -tocotrienol, β -tocotrienol, γ -tocotrienol and δ -tocotrienol) from Vitamin E-comprising compounds, said method including the steps of
 - i. esterifying free fatty acid components of the Vit E-comprising compounds into esters with one or more monohydric alcohols;
 - ii. trans-esterifying glyceride components of the Vit E-comprising compounds into esters with one or more monohydric alcohols;
 - iii. removing the formed esters and thereby yielding a Vit E concentrate;
 - iv. introducing the Vit E concentrate onto selective adsorbents to effect adsorption of Vit E isomers; and
 - v. effecting an individual desorption of the adsorbed Vit E isomers,wherein,
the adsorption and/or desorption of the Vit E isomers are carried out under a supercritical fluid environment.



8. A method of chromatographic isolation of claim 7, wherein the selective adsorbents include silica gel, reverse phase C18 silica gel and/or the like.
9. A method of chromatographic isolation of claim 7, wherein step (i) is carried out via employing a solid alkali metal bisulfate and/or a sulfate acid strongly acidic-ion exchange resin as a catalyst; step (ii) is carried out via employing a basic catalyst; or wherein anyone or both the esterification and/or trans-esterification are carried out via employing an enzyme.
10. A method of chromatographic isolation of claim 7, wherein the esterification and/or trans-esterification comprises the use of one or more C₁-C₈ alcohols.
11. A method of chromatographic isolation of claim 10 wherein the esterification and/or trans-esterification comprises the use of methanol.
12. A method of chromatographic isolation of claim 7, wherein the adsorption/desorption of the Vit E isomers are carried out under a supercritical fluid environment comprising an effective amount of, a combination of CO₂ and an entrainer.



13. A method of chromatographic isolation of claim 12 wherein the entrainer comprises an alcohol.
14. A method of chromatographic isolation of anyone of claims 12-13 comprises an alkyl alcohol.
15. A method of chromatographic isolation of anyone of claims 12-13 wherein the supercritical fluid conditions comprise operating temperatures of $T=30-100^{\circ}\text{C}$ and pressure $P=50-600\text{kg/cm}^2$.

