

RECOVERY OF TOCOPHEROLS/TOCOTRIENOLS, CAROTENOIDS, GLYCEROLS, STEROLS AND FATTY ACID ESTERS FROM CRUDE VEGETABLE OIL AND THE PROCESS THEREOF

5 Field Of Invention

The present invention relates to recovery of valuable constituents from oils. More particularly, the invention pertains to a process involving a pre-treatment step, such as molecular distillation or neutralization, to reduce free fatty acid content in crude palm
10 oil or other vegetable oil, in which the pre-treated oils are further processed to recover high quality tocotrienols/tocopherols, carotenoids, sterols and optionally glycerols as well as free fatty acids in form of ester.

Background Of The Invention

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Over the years, crude vegetable oils such as palm, palm kernel, rice bran, soy bean, sunflower, canola, rapeseed, cottonseed, safflower and corn oil have been identified as containing high levels of phytonutrients or constituents beneficial to human health. These beneficial constituents may include but not limited to tocopherols, tocotrienols,
20 carotenoids and steroids.

Tocopherols and tocotrienols are valuable constituents of vegetable oils because of their abilities to act as antioxidants and to provide protection against cell damage in the brain, tumors and various types of cancers, as well as to assist in the rehabilitation
25 of damaged cells. Unique molecular structure of the tocotrienols further imparts hypocholesterolemic characteristics, thereby helping to maintain a healthy cardiovascular system. Specifically, tocotrienols can help lower blood cholesterol level through cleansing of the arteries of accumulated cholesterol.

Carotenoids are natural pigments synthesized by plants imparting yellow, orange or red colour. Of all the carotenoids, alpha-carotene, beta-carotene and beta-cryptoxanthin are precursors to vitamin A (or retinol), or interchangeably known as provitamin A, serving as a source of vitamin A. Other carotenoids such as lutein, 5 lycopene and zeaxanthin cannot be converted to vitamin A but they are still of special interest because they are good antioxidants.

Plant based sterols, on the other hand, are recognized for their abilities to block absorption of cholesterol and reduce blood cholesterol level. Because the plant 10 sterols are nearly identical to the cholesterol, they compete with each other for absorption in the small intestines. However, plant sterols are poorly absorbed by humans and they appear to block the absorption of the dietary cholesterol, thereby reducing the blood cholesterol level, as well as the risk of coronary heart disease. Some research studies have also demonstrated that the plant sterols possess anti- 15 cancer, anti-inflammatory, anti-atherogenic and antioxidant characteristics.

In view of the foregoing, it is desired to extract or recover these beneficial constituents from the crude vegetable oils. Numerous recovery techniques have been presented in the past, such as solvent extraction, solvent fractionation, ion exchange 20 resin treatment and chromatography method. However, these techniques have several drawbacks, including low yield, substantial degradation of constituents throughout the process, use of carcinogenic organic solvent, etc. Accordingly, there exists a need for an improved method for recovery of tocotrienols/tocopherols, carotenoids and sterols from oils.

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The present invention provides a process for isolating tocotrienols/tocopherols, carotenoids and sterols from crude oils, particularly palm oils and refined palm oil distillates, and simultaneously recovering glycerols and free fatty acids in form of esters. In order to further improve quality of the recovered/isolated components, either

molecular distillation or neutralization is utilized to the present invention as a pre-treatment step so as to reduce fatty acid content in the crude oils prior to recovery of components. Consequently, it facilitates to produce higher yield of phytonutrient (based on purity and mass yield) and a cleaner starting material for downstream processes. More importantly, various oil compositions with high free fatty acid could be used in the present invention for recovery of phytonutrients, which imparts versatility to it as well as the type of oil used.

Summary Of Invention

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One of the objects of the invention is to provide a process for isolating tocotrienols, tocopherols, carotenoids and sterols from crude oils, particularly palm oils and refined palm oil distillates, and simultaneously recovering glycerols and free fatty acids in form of esters.

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Another object of the invention is to provide a process utilizing molecular distillation or neutralization as a pre-treatment step for reducing free fatty acid content in the crude oils before being processed further, thereby improving quality of the components recovered or isolated from the oils.

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Still another object of the invention is to provide a process for isolation and recovery of tocotrienols, tocopherols, carotenoids and sterols without being degraded or modified throughout the process.

25 Yet another object of the invention is to utilize transesterification as a post-processing step so as to further increase the carotenoid content therein to more than 20 wt%.

At least one of the preceding objects is met, in whole or in part, by the invention, in which one of the embodiments of the invention describes a process for recovering

tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to less than 3.50% by weight.

- 5 Preferably, the amount of the free fatty acids in the oil is reduced by distillation or neutralization.

One of the preferred embodiments of the invention describes a process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil,
10 characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to less than 3.50% by weight by distillation. Preferably, the distillation is performed to produce a first fraction enriched with carotenoids and a second fraction enriched with tocotrienols/tocopherols. The recovery process comprises the steps of transesterifying the first fraction in the presence of an alcohol
15 and a basic catalyst to convert the glycerides therein to fatty acid esters and glycerine, forming a transesterified mixture comprising glycerine, fatty acid esters and carotenoids, followed by distilling the transesterified mixture to separate carotenoids; and esterifying the second fraction in the presence of an alcohol and an acid catalyst to convert fatty acids therein to fatty acid esters, forming an esterified mixture
20 comprising fatty acid esters and tocotrienols/tocopherols, followed by distilling the esterified mixture to separate tocotrienols/tocopherols.

A further embodiment of the invention describes a process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil,
25 characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to less than 3.50% by weight by neutralization. Preferably, the neutralization is performed such that a basic solution is added to the crude oil to convert the fatty acids to salts which are to be separated by using one or more filters, centrifuge or separators.

The recovery process according to the further embodiment of the invention comprises the steps of, after neutralizing the crude oil to reduce its free fatty acid content, transesterifying the neutralized oil in the presence of an alcohol and a basic catalyst to
5 convert the glycerides therein to fatty acid esters and glycerine, forming an ester-rich fraction and a glycerol-rich fraction; separating the ester-rich fraction from the glycerol-rich fraction; distilling the ester-rich fraction to produce a concentrated stream comprising tocotrienols/tocopherols, carotenoids and sterols; further transesterifying the concentrated stream in the presence of an alcohol and a basic
10 catalyst to form an ester-enriched fraction and a glycerol-rich fraction; and repeating the transesterification, separation and distillation sequentially and repeatedly to obtain a composition with pre-determined concentration of tocotrienols/tocopherols, carotenoids and sterols.

15 One skilled in the art will readily appreciate that the invention is well adapted to carry out the aspects and obtain the ends and advantages mentioned, as well as those inherent therein. The embodiments described herein are not intended as limitations on the scope of the invention.

20 **Detailed Description Of The Invention**

Hereinafter, the invention shall be described according to the preferred embodiments of the present invention and by referring to the accompanying description and drawings. However, it is to be understood that limiting the description to the preferred
25 embodiments of the invention and to the drawings is merely to facilitate discussion of the present invention and it is envisioned that those skilled in the art may devise various modifications without departing from the scope of the appended claim.

The present invention relates to a process involving a pre-treatment step, such as

molecular distillation and neutralization, to reduce free fatty acid content in crude vegetable oil, whereby the pre-treated oils with low fatty acid content are subjected to further processing for recovery of constituents including tocotrienols, tocopherols, carotenoids, sterols and optionally, glycerols as well as free fatty acids, in form of ester.

The crude vegetable oil referred herein preferably has a composition comprising tocotrienols/tocopherols, carotenoids, sterols, fatty acids and glycerides including monoglycerides, diglycerides and triglycerides, wherein the free fatty acids are present in an amount of more than 3.50% by weight of total composition. Other types of crude oil as crude palm oil, red palm oil, red palm olein, red palm fiber oil and palm oil distillate can also be processed by the process depicted herein throughout the description.

It should be appreciated that the term “tocotrienol/tocopherol” or “tocopherol/tocotrienol” used herein throughout the description shall refer to any one or a combination of tocotrienol and tocopherol.

The present invention provides a process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to less than 3.50% by weight. Preferably, the amount of the free fatty acids in the crude oil is reduced by subjecting the crude oil to distillation or neutralization.

One of the preferred embodiments of the invention discloses a process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to less than 3.50% by weight by distillation. The distillation depicted in this step is preferably vacuum distillation, and may involve one or more

distillation columns to produce a first fraction enriched with carotenoids and a second fraction enriched with tocotrienols/tocopherols.

In one preferable embodiment, the distillation is performed in two distillation columns arranged in series, the first column operating at a temperature of 90 to 200°C and a pressure ranging from 10 to 100 kPa and the second column at 120 to 250°C and 0.10 to 10 kPa. Substantial proportion of tocotrienols/tocopherols, sterols and fatty acids is separated from the crude oil as distillate from the first distillation column, thereby reducing the amount of the free fatty acids in the oil. The remaining oil components leave the first distillation column as a residue stream for feeding into the second column for further separation of tocotrienols/tocopherols, sterols and fatty acids. In the second distillation column, tocotrienols/tocopherols, sterols and fatty acids are substantially separated to produce a bottom stream rich of carotenoids, referred to as the first fraction mentioned in the preceding description. Correspondingly, the tocotrienols/tocopherols, sterols and fatty acids recovered from the second distillation column as a distillate stream, as well as the distillate stream from the first distillation column, makes up the second fraction which is enriched with tocotrienols/tocopherols.

In a more preferable embodiment, prior to the distillation, the crude oil is heated, or subjected to a nozzle spray vacuum dryer or a column dryer to reduce the moisture content to below 0.30% by weight. Preferably, the drying process is performed at a temperature of 40 to 80°C under vacuum or a pressure of 100 kPa (equivalent to 76 cm Hg) for 0.5 to 24 hours.

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It should be appreciated that the term “distillation” used herein throughout the description shall not be limited to only molecular distillation, but also refer to other types of distillation, depending on the properties of the component(s) to be recovered.

The process according to the preferred embodiment of the invention comprises the recovery steps of transesterifying the first fraction in the presence of an alcohol and a basic catalyst to convert the glycerides therein to fatty acid esters and glycerine, forming a transesterified mixture comprising glycerine, fatty acid esters and carotenoids, followed by distilling the transesterified mixture to separate carotenoids; and esterifying the second fraction in the presence of an alcohol and an acid catalyst to convert fatty acids therein to fatty acid esters, forming an esterified mixture comprising fatty acid esters and tocotrienols/tocopherols, followed by distilling the esterified mixture to separate tocotrienols/tocopherols.

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Prior to transesterifying the first fraction, it is desirable to remove moisture contained therein by heating such that the moisture is evaporated, thereby reducing the moisture content of the first fraction to a level of less than 0.30% by weight.

15 Subsequently, the first fraction with reduced moisture content is transesterified in the presence of an alcohol and a basic catalyst to convert the glycerides in the oil to fatty acid esters and glycerine, thus forming a transesterified mixture comprising glycerine, fatty acid esters, carotenoids and other impurities, such as sterols and glycerides. The alcohol used in transesterification of the first fraction is preferably a lower alkyl alcohol such as methanol, ethanol, iso-propanol and butanol, whilst the basic catalyst is sodium methoxide, sodium hydroxide in methanol, potassium methoxide or potassium hydroxide in methanol. Also referring to the preferred embodiment of the invention, the transesterification is conducted at a temperature of 35 to 80°C for 0.5 to 12 hours under atmospheric pressure, with or without agitation, but preferably with continuous agitation at 30 to 80 rpm. The ratio of the carotenoid-rich first fraction to the transesterification solution (i.e. the mixture of basic catalyst and alcohol) may range from 0.5-10:1.

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In accordance with the preferred embodiment of the invention, it is desirable to

separate the glycerine from the transesterified mixture. It can be achieved by subjecting to centrifugation or gravitational settling to produce an ester-rich portion and a glycerine-rich portion comprising 20 to 40% by weight of glycerine.

- 5 The glycerine-rich portion is neutralized using an acid such as hydrochloric acid, acetic acid or sulphuric acid, at a temperature of 35 to 90°C, to convert the excessive basic catalyst present therein to salt which can be easily separated from the glycerine, thereby producing 75 to 90% by weight of glycerine. Preferably, the neutralization is performed for a period of 0.5 to 24 hours, so as to ensure complete conversion.
- 10 Subsequently, the resultant mixture is subjected to a settling step for a period of 0.5 to 12 hours, for separating other possible residual oil matters from glycerine. Upon removal of these residual oil matters, the remaining product mixture is heated, while being agitated continuously, to a temperature of 70 to 100°C under vacuum (or a pressure equivalent to 100 kPa or 76 cm Hg), in order to remove the moisture or
- 15 solution present therein by evaporation.

The ester-rich portion obtained from centrifugation or gravitational settling of the first mixture is subjected to either direct-current or counter-current water washing, so as to substantially remove the excessive catalyst and possibly, unreacted alcohol and other

20 impurities such as soaps which may be formed during the process and aqueous soluble unwanted compounds. Hot or cold water can be used in this step but preferably, water heated to a temperature ranging from 40 to 80°C is used. As preferred by the present invention, the washing step is performed repeatedly until the pH of the ester-rich portion falls to a range of 6 to 8.

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After washing, the moisture content in the ester-rich portion may be increased to approximately 2% by weight or lower. Therefore, it is of particular interest to reduce the moisture content therein to less than 0.30% by weight prior to subjecting to further processing. It can be achieved by heating or vacuum drying the washed ester-rich

portion to a temperature of 35 to 80°C for 0.5 to 12 hours, under vacuum or a pressure of 100 kPa (equivalent to 76 cm Hg) substantially removing the moisture present therein.

- 5 After reducing the moisture content of the ester-rich portion to less than 0.30%, the resulting ester-rich portion is distilled for recovery of carotenoids from the ester-rich fraction. It should be appreciably noted that the distillation in this step is similar to that for reducing the fatty acid content, as depicted in the foregoing. Specifically, the distillation is vacuum distillation conducted in two distillation columns arranged in
- 10 series, the first column operating at a temperature of 120 to 180°C and at a pressure of 10 to 100 kPa, and the other column (positioned after the first column) operating at 120 to 180°C and 0.1 to 10 kPa. Those of ordinary skill in the art should appreciate the fact that the first distillation column has an operating temperature lower than the subsequent column.

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- From the first column, fatty acid esters and tocotrienols/tocopherols are primarily recovered as distillate, whilst all other components remained unrecovered are discharged as a residue stream which is to be fed into the next column for separation of carotenoids. Substantial portion of fatty acid esters and tocotrienols/tocopherols is,
- 20 again, removed from the next column as distillate, with tocotrienols/tocopherols present in an amount of 3 to 8 wt%. At the same time, a residue stream concentrated in carotenoids is produced, in which the carotenoids are present in an amount of 8 to 20% by weight.

- 25 In a more preferable embodiment, prior to distillation, the ester-rich portion is heated, or subjected to a column dryer or by vacuum distillation, to reduce the moisture content to below 0.01% by weight.

In another preferable embodiment, the distillate streams obtained in this distillation

step, comprising fatty acid esters and tocotrienols/tocopherols, can be subjected to further processing to produce streams with higher concentration of tocotrienols/tocopherols or sold to the global market as that obtained.

- 5 The residue stream concentrated in carotenoids, or referred interchangeably to as “distilled transesterified mixture”, is subjected to further processing. Preferably, the distilled transesterified mixture is treated with an organic solvent such as hexane, heptane or pentane. The resulting mixture is continuously agitated and chilled, forming crystallised carotenoids. Subsequently, the mixture is centrifuged, producing
10 a liquid top layer and a semi-solid bottom layer, in which the bottom layer comprises crystallised carotenoids in an amount of 28 to 45% by weight.

Preferably, the bottom layer is subjected to evaporation using a conventional evaporator to remove the solvent content therein for recovery of carotenoids. Upon
15 heating to a temperature of 45 to 80°C under vacuum condition of 70 to 75 cm Hg, the solvent is substantially evaporated, thereby reducing the solvent content to a level of 1% or lower. In one preferred embodiment, the evaporation is conducted for duration of 0.5 to 96 hours, where upon removal of solvent, it produces a sludge-like compound, which is thick and viscous in terms of texture and physical appearance.

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The top layer may optionally be transesterified in the presence of an alcohol and a basic catalyst. It should be appreciated that this additional transesterification step aids to increase the carotenoid content in the top layer to a level of more than 20% by weight. The alcohol used in this step is preferably a lower alkyl alcohol, more
25 preferably methanol, whilst either sodium methoxide or potassium methoxide is used as basic catalyst. Still referring to the preferred embodiment, transesterification of the top layer is conducted at a temperature of 40 to 60°C at atmospheric pressure, with or without agitation, but preferably with agitation. It is also preferred that the ratio of the top layer to the transesterification solution (i.e. the mixture of methoxide salt and

methanol) is 4:5.

After transesterifying the top layer, the transesterified product mixture formed thereof is washed by contacting it with a methanolic acidic solution, preferably containing 1 to 2% by weight of hydrochloric acid, to remove undesirable impurities present therein. One should appreciate the fact that such washing step is performed at a slightly heated condition, particularly at a temperature of 28 to 50°C under atmospheric pressure, and that the ratio of methanolic acidic solution to the transesterified product mixture is in a range of 4-7:1. Subsequently, the washed product mixture is dried, forming a product comprising carotenoids in an amount of 20 to 30% by weight.

In accordance with the preceding description, the second fraction enriched with tocotrienols/tocopherols is esterified in the presence of an alcohol and an acid catalyst to convert the fatty acids present in the oil to fatty acid esters, thus forming an esterified mixture comprising essentially glycerides, fatty acid esters, sterols and tocopherols/tocotrienols. Preferably, the alcohol used in this esterification step is a lower alkyl alcohol such as methanol, ethanol, isopropanol and butanol, whilst hydrochloric acid, phosphoric acid, citric acid or other suitable acid can be used as the acid catalyst. As preferred by the present invention, the acidic esterification is conducted at a temperature of 5 to 90°C for 0.5 to 6 hours, with or without agitation, but preferably with agitation. The ratio of the acid catalyst to the lower alkyl alcohol in the esterification solution may range from 0.005-5:1.

Subsequently, the esterified mixture is subjected to direct-current or counter-current water washing so as to substantially remove the excessive catalyst, unreacted alcohol and other potential impurities. More preferably, the esterified mixture is repeatedly washed until the pH of the mixture achieves a range of 6 to 8. Hot or cold water can be used in this step but preferably, water heated to a temperature ranging from 35 to

90°C is used.

The washed esterified mixture is then heated so as to evaporate and substantially remove the moisture contained therein. The heating step is preferably performed at a
5 temperature of 35 to 80°C for 0.5 to 10 hours under atmospheric condition. Consequently, the moisture content is reduced to an amount of less than 0.30% by weight.

In accordance with the preferred embodiment, the esterified mixture is, upon drying,
10 distilled for recovery of tocopherols/tocotrienols. Like the distillation steps depicted in the foregoing, the distillation mentioned in this step is also vacuum distillation. Preferably, this distillation step is conducted in two distillation columns arranged sequentially, one column operating at 120 to 180°C and 0.01 to 10 kPa, and the next column operating at 120 to 180°C and 0.01 to 3 kPa. Those of ordinary skill in the art
15 should appreciate the fact that the first distillation column has an operating temperature lower than the subsequent column.

From the column operating at 0.01 to 10 kPa, fatty acid esters are primarily recovered as distillate. The rest of the components leaving the column as a residue stream are
20 fed into the other column, which operates at 0.01 to 3 kPa, for separation of tocopherols/tocotrienols. Similarly, fatty acid esters are substantially removed from the column as distillate, whereas a residue stream comprising tocotrienols/tocopherols, sterols and glycerides is recovered, whereby the tocotrienols/tocopherols are present in an amount ranging from 1 to 5 wt%. More
25 preferably, prior to distillation, the second fraction is further heated, or subjected to a column dryer or by vacuum distillation, to further reduce the moisture content to below 0.01% by weight.

The residue stream from the distillation, or referred interchangeably to as “distilled

esterified mixture”, is subjected to another transesterification process in the presence of an alcohol and a basic catalyst to convert the glycerides contained therein to fatty acid esters and glycerine, thus forming a product mixture comprising glycerine, fatty acid esters, sterols, tocopherols and tocotrienols. Preferably, the alcohol used in the transesterification step is a lower alkyl alcohol such as methanol, ethanol, isopropanol and butanol, whilst the basic catalyst can be either sodium methoxide or potassium methoxide. It is preferred that the transesterification is conducted at a temperature of 5 to 90°C for 0.5 to 6 hours, with or without agitation, but preferably with agitation. It is also preferable that the ratio of the product mixture to the esterification solution (i.e. the mixture of basic catalyst and alcohol) may range from 0.5-10:1.

Still referring to the preferred embodiment of the invention, the product mixture obtained from transesterification of the distilled esterified mixture is subjected to another multi-stage distillation for further recovery of tocopherols/tocotrienols.

More preferably, prior to distillation, the product mixture is subjected to direct-current or counter-current water washing so as to remove the excessive catalyst, unreacted alcohol and other potential impurities such as sterols. It is also desired to wash the product mixture repeatedly until the pH of the ester-rich portion achieves a range of 6 to 8. In this washing step, hot or cold water can be used but preferably, water heated to a temperature ranging from 40 to 80°C is used.

Subsequently, the washed product mixture is heated to reduce the moisture content to an amount of less than 0.30% by weight. It should be appreciated that the heating condition in this step is identical to that mentioned in the foregoing, in which the washed product mixture is heated to 35 to 80°C under atmospheric pressure for 0.5 to 10 hours.

Upon removal of moisture from the product mixture, the dried product mixture is distilled for further recovery of tocopherols/tocotrienols. The distillation is preferred to be conducted as that depicted for the distillation in the previous steps. The columns are arranged sequentially, one column operating at a temperature of 120 to 180°C and a pressure of 0.01 to 1 kPa and the subsequent column operating at 120 to 180°C and 0.01 to 3 kPa. Those of ordinary skill in the art should, again, appreciate the fact that the first distillation column has an operating temperature lower than the subsequent column. Like the distillation operations in the preceding description, fatty acid esters are primarily recovered as distillate from the column operating at the pressure of 0.01 to 1 kPa. The remaining components are removed as a residue stream which is to be fed into the subsequent column to recover tocopherols/tocotrienols. From the subsequent distillation column, fatty acid esters are substantially removed as distillate, whereas the residue stream is a composition having pre-determined concentration of tocotrienols/tocopherols, sterols and other potential impurities. In the present invention, the composition preferably comprises tocotrienols/tocopherols in an amount ranging from 5 to 35% by weight.

In a more preferable embodiment, prior to distillation, the product mixture is heated, or subjected to a column dryer, to reduce the moisture content to below 0.01% by weight.

Since there is presence of sterols in the desired composition, it is, therefore, preferred to subject the desired composition to crystallisation in the presence of an alcohol at low temperature to separate sterols and any glycerides present therein (such as monoglycerides, diglycerides or triglycerides) in the form of crystal. The alcohol used in this step is preferably a lower alkyl alcohol, such as methanol, ethanol, propanol, butanol or a combination thereof. The composition comprising tocotrienols/tocopherols in 5 to 35 wt% forms a mixture upon addition of an alcohol and the mixture formed thereof is chilled to a temperature of -30 to 0°C for a period

of 12 hours to 3 days. During the crystallisation step, the mixture is optionally, but preferably, subjected to continuous agitation.

Resulting from the crystallisation, the sterol crystals and the glyceride crystals are formed. By repeatedly performing the crystallisation step, it facilitates to ensure optimum recovery of sterols and glycerides. The crystals are subsequently filtered from the solution. It should be appreciated that any solid-liquid separation means can be utilised in this step. The crystals are then further processed to evaporate the solvent contained therein through heating or by other drying means.

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The remaining liquid solution is heated to remove the alcohol present therein, thereby a concentrated composition comprising tocotrienols/tocopherols in an amount of 30 to 90% by weight. The concentrated composition may also contain other compounds such as squalene, sterols, carotenoids and CoQ10.

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One can possibly appreciate that the steps depicted in the preceding description can be performed for more than one time, or repeatedly, under the same operating conditions specified herein, to produce an output with desirable composition or content. The steps referred herein may include transesterification, esterification, evaporation, washing, settling, separation, crystallization or a combination of two or more steps; however, it should not be limited thereto or thereby.

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A further embodiment of the present invention is a process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to 3.50% by weight, by neutralization. Preferably, the neutralization is performed such that a basic solution, such as caustic solution, is added to the crude oil to convert the fatty acids to salts which can be separated easily by filtration. It should be appreciated that preparation of the caustic solution is common in the art,

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whereby dissolution of caustic soda (sodium hydroxide flakes) in water forms the desired caustic solution.

In accordance with the further embodiment of the invention, the ratio of the crude oil
5 and the caustic solution added to the crude oil should be in a range of 1:0.030-0.035. Upon adding the caustic solution to the crude oil, neutralization takes place at a temperature of 40 to 80°C under atmospheric pressure, with or without agitation, but preferably under continuous agitation, for a duration of 20 to 90 min. The subsequent
10 resulting mixture is then subjected to separation by centrifugation or gravitational settling, followed by decanting, in order to recover the oil phase (also referred interchangeably to as “neutralized oil” hereinafter) from the aqueous phase which contains unreacted caustic solution, water formed during neutralization and the salts of fatty acids. It should be noted that after neutralization, the free fatty acid content in the oil phase has decreased to less than 3.50% by weight, as mentioned in the
15 preceding description.

The process according to the further embodiment of the invention comprises the steps of transesterifying the neutralized oil in the presence of an alcohol and a basic catalyst to convert the glycerides therein to fatty acid esters and glycerine, forming an ester-
20 rich fraction and a glycerol-rich fraction; separating the ester-rich fraction from the glycerol-rich fraction; distilling the ester-rich fraction to produce a concentrated stream comprising tocotrienols/tocopherols, carotenoids and sterols; further transesterifying the concentrated stream in the presence of an alcohol and a basic catalyst to form an ester-enriched fraction and a glycerol-rich fraction; and repeating
25 the transesterification, separation and distillation sequentially and repeatedly to obtain a composition with pre-determined concentration of tocotrienols/tocopherols, carotenoids and sterols.

Prior to transesterification, it is preferably to subject the neutralized oil to a heating

step, because the neutralized oil as obtained in the preceding step may still contain undesirable aqueous substances, such as moisture. In a preferred embodiment, the heating step is performed by subjecting the neutralized oil to a temperature of 40 to 80°C under vacuum condition or at a pressure equivalent to 100 kPa (or 76 cm Hg) under agitation, for duration of 0.5 to 12 hours, in order to separate the aqueous compounds from the neutralized oil, as well as to reduce the moisture content in the neutralized oil to a level of less than 0.20% by weight.

Subsequently, the neutralized oil with reduced moisture content is transesterified in the presence of an alcohol and a basic catalyst to convert the glycerides in the oil to fatty acid esters and glycerine, thus forming a transesterified mixture comprising glycerine, fatty acid esters, carotenoids and other impurities, such as sterols and glycerides. The alcohol used in transesterification of the neutralized oil is preferably a lower alkyl alcohol such as methanol, ethanol, iso-propanol and butanol, whilst the basic catalyst is sodium methoxide, sodium hydroxide with methanol, potassium methoxide or potassium hydroxide with methanol. Also referring to the preferred embodiment of the invention, the transesterification is conducted at a temperature of 40 to 80°C for 20 to 90 min under atmospheric pressure, with or without agitation, but preferably with continuous agitation at 30 to 80 rpm. The ratio of the neutralized oil to the transesterification solution (i.e. the mixture of basic catalyst and alcohol) may range from 0.5-10:1.

In accordance with the preferred embodiment of the invention, it is desirable to separate the glycerides from the transesterified mixture. It can be achieved by subjecting to centrifugation or gravitational settling to produce an ester-rich fraction and a glycerine-rich fraction.

The glycerine-rich fraction is neutralized using an acid such as hydrochloric acid, acetic acid or sulphuric acid, at a temperature of 35 to 90°C, to convert the excessive

basic catalyst present therein to salt which can be easily separated from the glycerine, thereby producing 75 to 90% by weight of glycerine. Preferably, the neutralization is performed for a period of 0.5 to 24 hours, so as to ensure complete conversion. Subsequently, the resultant mixture is subjected to a settling step for a period of 0.5 to 12 hours, for separating other possible residual oil matters from glycerine. Upon removal of these residual oil matters, the remaining product mixture is heated, while being agitated continuously, to a temperature of 70 to 100°C under vacuum (or at a pressure equivalent to 100 kPa or 76 cm Hg), in order to remove the moisture or solution present therein by evaporation.

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At the same time, the ester-rich portion as obtained is subjected to either direct-current or counter-current washing with a washing agent such as water, so as to substantially remove the excessive catalyst and possibly, unreacted alcohol and other impurities such as soaps which may be formed during the process and aqueous soluble unwanted compounds. Hot or cold water can be used in this step but preferably, water heated to a temperature ranging from 40 to 80°C is used. As preferred by the present invention, the washing step is performed repeatedly until the pH of the ester-rich portion falls to a range of 6 to 8.

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After washing, the moisture content in the ester-rich portion may be increased to approximately 2% by weight. Therefore, it is preferred to reduce the moisture content therein to less than 0.30% by weight prior to being subjected to the next processing step. It can be achieved by heating or vacuum drying the washed ester-rich portion to a temperature of 40 to 70°C for 0.5 to 6 hours, under vacuum or a pressure of 100 kPa (equivalent to 76 cm Hg), preferably with recirculation, for substantially removing the moisture present therein.

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After reducing the moisture content of the ester-rich fraction to less than 0.30 wt%, the resulting ester-rich fraction is distilled for producing a concentrated stream

- comprising tocotrienols/tocopherols, carotenoids and sterols. It should be appreciably noted that the distillation in this step is similar to that in the preceding description. Specifically, the distillation is vacuum distillation which can be performed in a single stage or multiple stages, depending on the user's preference or the desired composition of the tocotrienols/tocopherols, carotenoids and/or sterols in the concentrated stream. Preferably, the vacuum distillation is performed in a distillation column operating at a temperature of 120 to 180°C and at a pressure of 0.001 to 0.01 kPa (0.001 to 0.1 mbar).
- 10 In a more preferable embodiment, two-step distillation is performed, where from the first distillation column operating at a temperature of 120 to 180°C and at a pressure of 0.001 to 0.01 kPa, fatty acid esters are primarily recovered as distillate, whilst all other components remained unrecovered are discharged as residue which is to be fed into the next column which operates at a temperature of 120 to 180°C and at a pressure of 0.001 to 0.01 kPa. Substantial portion of fatty acid esters is again removed as distillate, thereby producing a residue stream concentrated in tocotrienols/tocopherols, carotenoids and sterols.

In a more preferable embodiment, prior to distillation, the ester-rich fraction is heated, or subjected to a column dryer or by vacuum distillation, to reduce the moisture content to below 0.01% by weight.

In another further embodiment, the distillate streams obtained from the distillation step, comprising predominantly fatty acid esters, can be subjected to further processing to produce streams with higher purity or sold to the global market as that obtained.

Upon obtaining the concentrated stream comprising tocotrienols/tocopherols, carotenoids and sterols, it is further transesterified in the presence of an alcohol and a

basic catalyst to form an ester-enriched fraction and a glycerol-rich fraction. Preferably, the alcohol used in this step is a lower alkyl alcohol such as methanol, ethanol, iso-propanol and butanol, whilst the basic catalyst is sodium methoxide, sodium hydroxide with methanol, potassium methoxide or potassium hydroxide with
5 methanol. Also, it is preferred to perform the transesterification at a temperature of 40 to 80°C for 20 to 90 min under atmospheric pressure, with or without agitation, but preferably with continuous agitation at 30 to 80 rpm. The ratio of the neutralized oil to the transesterification solution (i.e. the mixture of basic catalyst and alcohol) may range from 0.5-10:1. It should be appreciated that the further transesterification
10 facilitates to increase the carotenoid content in the concentrated stream to a level of more than 20% by weight.

Alternatively, the ester-enriched fraction may be subjected to transesterification, separation and distillation steps repeatedly and sequentially until a composition with
15 pre-determined concentration of tocotrienols/tocopherols, carotenoids and sterols is obtained.

If the ester-enriched fraction obtained from the further transesterification step (and upon separation from the glycerol-rich fraction) comprises the predetermined
20 concentration of tocotrienols/tocopherols, carotenoids and sterols, it is preferred to subject the ester-enriched fraction to a washing step, where it is contacted with a methanolic acidic solution, preferably containing 1 to 2% by weight of hydrochloric acid, to remove undesirable impurities present therein. One should appreciate the fact that such washing step is performed at a slightly heated condition, particularly at a
25 temperature of 28 to 50°C under atmospheric pressure, and that the ratio of methanolic acidic solution to the ester-enriched fraction is in a range of 4-7:1. Subsequently, the washed ester-enriched fraction is dried, thereby forming a composition comprising an increased concentration of carotenoids, particularly an amount of 20 to 50% by weight.

Upon drying, it is preferred to subject the desired composition to crystallisation in the presence of an alcohol at low temperature to separate sterols and glycerides, if present therein, (such as monoglycerides, diglycerides or triglycerides) in the form of crystal.

5 The alcohol used in this step is preferably a lower alkyl alcohol, such as methanol, ethanol, propanol, butanol or a combination thereof. The mixture formed thereof is then chilled to a temperature of -30 to 0°C for a period of 12 hours to 3 days. During the crystallisation step, the mixture is optionally, but preferably, subjected to continuous agitation.

10

Resulting from the crystallisation, the sterol crystals and glyceride crystals, if present, are formed. By repeatedly performing the crystallisation step, it facilitates to ensure optimum recovery of sterols and glycerides. The crystals are subsequently filtered from the solution. It should be appreciated that any solid-liquid separation means can be utilised in this step. The crystals are then further processed to evaporate the alcohol contained therein through heating or by other drying means.

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The remaining liquid solution is heated to remove the alcohol present therein, thereby a concentrated composition comprising predominantly tocotrienols/tocopherols and carotenoids. The concentrated composition may also contain trace amounts of other compounds such as squalene, sterols, carotenoids and CoQ10. The concentrated composition may optionally be subjected to a further processing step in order to recover carotenoids using hexane, thus producing a composition comprising primarily tocotrienols/tocopherols.

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One can possibly appreciate that the steps depicted in the preceding description can be performed for more than one time, or repeatedly, under the same operating conditions specified herein, to produce an output with desirable composition or content. The steps referred herein may include transesterification, esterification, evaporation,

washing, settling, separation, crystallization or a combination of two or more steps; however, it should not be limited thereto or thereby.

In another further embodiment of the invention, the distillate streams obtained from the preceding distillation steps can be subjected to further process comprising the steps of esterifying the distillate streams with an alcohol in the presence of an acid catalyst to convert the fatty acids contained therein to produce an esterified mixture comprising fatty acid esters, thereby reducing fatty acid content in the oil to less than 3.50% by weight; distilling the esterified mixture for separating and removing the fatty acid esters therefrom; transesterifying the distilled mixture with an alcohol in the presence of a basic catalyst for converting glycerides present therein to fatty acid esters and glycerine, forming a transesterified mixture comprising glycerine and fatty acid esters; purifying the transesterified mixture to substantially remove glycerine therefrom; and distilling the purified mixture to substantially remove fatty acid esters, producing a composition comprising a predetermined concentration of tocotrienols/tocopherols.

The disclosure includes as contained in the appended claims, as well as that of the foregoing description. Although this invention has been described in its preferred form with a degree of particularity, it is understood that the disclosure of the preferred form has been made only by way of example and that numerous changes in the details of construction and the combination and arrangements of parts may be resorted to without departing from the scope of the invention.

Claims

1. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the
5 free fatty acids in the oil is reduced to 3.50% by weight.
2. A process according to claim 1, wherein the amount of the free fatty acids in the crude oil is reduced by distillation or neutralization.
- 10 3. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to 3.50% by weight by distillation.
4. A process according to claim 3, wherein the distillation is performed to produce a
15 first fraction enriched with carotenoids and a second fraction enriched with tocotrienols/tocopherols.
5. A process according to claim 3 or 4, wherein the distillation is performed using two distillation columns, one column operating at a temperature of 90 to 200°C and a
20 pressure ranging from 10 to 100 kPa and the subsequent column at 120 to 250°C and 0.10 to 10 kPa.
6. A process according to claim 3 or 4, wherein the process comprises the steps of
transesterifying the first fraction in the presence of an alcohol and a basic catalyst to
25 convert the glycerides therein to fatty acid esters and glycerine, forming a transesterified mixture comprising glycerine, fatty acid esters and carotenoids, followed by distilling the transesterified mixture to separate carotenoids; and esterifying the second fraction in the presence of an alcohol and an acid catalyst to convert fatty acids therein to fatty acid esters, forming an esterified mixture

comprising fatty acid esters and tocotrienols/tocopherols, followed by distilling the esterified mixture to separate tocotrienols/tocopherols.

7. A process according to claim 6, wherein the transesterifying step is conducted at a temperature of 35 to 80°C under atmospheric pressure, with or without agitation.

8. A process according to claim 7, wherein the transesterifying step is conducted for 0.5 to 12 hours.

9. A process according to claim 6, wherein the distilling step, after the transesterifying step, is performed using two distillation columns, in a sequential arrangement, each column operating under vacuum condition and at a temperature of 120 to 180°C, with the first column having an operating temperature lower than the subsequent column.

10. A process according to claim 6, wherein the esterifying step is conducted at a temperature of 5 to 90°C for 0.5 to 6 hours, with or without agitation.

11. A process according to claim 10, wherein the esterifying step is conducted for 0.5 to 6 hours.

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12. A process according to claim 6, wherein the distilling step, after the esterifying step, is performed using two distillation columns, in a sequential arrangement, each column operating under vacuum condition and at a temperature of 120 to 180°C, with the first column having an operating temperature higher than the subsequent column.

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13. A process according to claim 3 or 4, wherein the process further comprises a step of heating the crude oil to remove moisture contained therein, prior to reduction of fatty acids in the crude oil by distillation.

14. A process according to claim 13, wherein the heating step is performed at a temperature of 40 to 80°C under vacuum condition or a pressure of 100 kPa for 0.5 to 24 hours.
- 5 15. A process according to claim 6, wherein the process further comprises a step of reducing moisture content in the second fraction to less than 0.30 wt% prior to transesterification.
- 10 16. A process according to claim 6, wherein the process further comprises a step of separating the glycerine from the transesterified mixture to produce an ester-rich portion, prior to distilling the transesterified mixture.
- 15 17. A process according to claim 16, wherein the glycerine is separated from the transesterified mixture by centrifugation or gravitational settling, producing a glycerine-rich portion and the ester-rich portion, wherein the glycerine-rich portion is extracted and neutralized using an acid to recover glycerine therefrom.
- 20 18. A process according to claim 17, wherein the process further comprises a step of washing the transesterified mixture with a washing agent to remove catalyst, unreacted alcohols and other impurities.
- 25 19. A process according to claim 18, wherein the process further comprises a step of heating the washed transesterified portion to reduce moisture content therein to less than 0.30 wt%.
20. A process according to claim 19, wherein the heating step is conducted at a temperature of 35 to 80°C under vacuum or a pressure of 100 kPa.
21. A process according to claim 20, wherein the heating step is conducted for 0.5 to

12 hours.

22. A process according to claim 6, wherein the process further comprises a step of adding an organic solvent into the distilled transesterified mixture and chilling the mixture to produce crystallised carotenoids, followed by subjecting the resulting mixture to centrifugation thereby producing a top layer and a bottom layer; whereby the bottom layer comprises crystallised carotenoids in an amount of 28 to 45% by weight.
23. A process according to claim 22, wherein the bottom layer is heated to evaporate the solvent contained therein for recovery of carotenoids.
24. A process according to claim 23, wherein the heating step is performed at a temperature of 45 to 80°C to reduce the solvent content to a level of less than 1% or lower.
25. A process according to claim 24, wherein the heating step is performed under vacuum condition of 70 to 75 cm Hg.
26. A process according to claim 22, wherein the top layer is subjected to transesterification in the presence of an alcohol and a basic catalyst to increase the carotenoid content therein; and washing the mixture obtained thereof with a methanolic acid solution to remove the impurities present therein.
27. A process according to claim 6, wherein the process further comprises a step of washing the esterified mixture using a washing agent to remove catalyst, unreacted alcohols and other impurities therefrom, prior to distilling the esterified mixture.
28. A process according to claim 27, wherein the process further comprises a step of

heating the washed esterified mixture to reduce moisture content therein to less than 0.30% by weight.

29. A process according to claim 6, wherein the process further comprises a step of
5 transesterifying the distilled esterified mixture in the presence of an alcohol and a basic catalyst to convert the fatty acids therein to fatty acid esters, forming a product mixture comprising glycerine and fatty acid esters.

30. A process according to claim 29, wherein the transesterifying step is conducted at
10 a temperature of 5 to 90°C with or without agitation.

31. A process according to claim 30, wherein the transesterifying step is conducted for 0.5 to 6 hours.

15 32. A process according to claim 29, wherein the process further comprises a step of washing the product mixture with a washing agent to remove catalyst, unreacted alcohols and other impurities.

33. A process according to claim 32, wherein the process further comprises a step of
20 heating the washed product mixture to reduce moisture content therein to less than 0.30% by weight.

34. A process according to claim 33, wherein the heating step is performed at a temperature of 35 to 80°C under atmospheric pressure.

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35. A process according to claim 34, wherein the heating step is performed for 0.5 to 10 hours.

36. A process according to claim 33, wherein the process further comprises a step of

distilling the product mixture with reduced moisture content to obtain a composition with pre-determined concentration of tocotrienols/tocopherols and sterols.

37. A process according to claim 36, wherein the distilling step is performed using
5 two distillation columns, in a sequential arrangement, one column operating at a temperature of 120 to 180°C and a pressure of 0.01 to 10 kPa and the subsequent column operating at 120 to 180°C and 0.01 to 3 kPa.

38. A process according to claim 36, wherein the process further comprises a step of
10 separating sterols from the composition.

39. A process according to claim 38, wherein the sterols are separated by treating the composition with an alcohol and chilling the resulting mixture to produce crystallised sterols which are to be filtered.
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40. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to 3.50% by weight by neutralization.

20 41. A process according to claim 40, wherein the neutralization is performed such that a basic solution is added to the crude oil to convert the fatty acids to salts which are to be separated by filtration.

42. A process according to claim 40 or 41, wherein the process comprises the steps of
25 transesterifying the neutralized oil in the presence of an alcohol and a basic catalyst to convert the glycerides therein to fatty acid esters and glycerine, forming an ester-rich fraction and a glycerol-rich fraction;
separating the ester-rich fraction from the glycerol-rich fraction;
distilling the ester-rich fraction to produce a concentrated stream comprising

tocotrienols/tocopherols, carotenoids and sterols;

further transesterifying the concentrated stream in the presence of an alcohol and a basic catalyst to form an ester-enriched fraction and a glycerol-rich fraction; and

repeating the transesterification, separation and distillation sequentially and

5 repeatedly to obtain a composition with pre-determined concentration of tocotrienols/tocopherols, carotenoids and sterols.

43. A process according to claim 42, wherein the process further comprises a step of heating the neutralized oil, prior to transesterification, to reduce the moisture content
10 in the neutralized oil to a level of less than 0.20% by weight.

44. A process according to claim 43, wherein the heating step is performed at a temperature of 40 to 80°C under vacuum condition or at a pressure equivalent to 100 kPa under agitation.
15

45. A process according to claim 44, wherein the heating step is performed for a duration of 0.5 to 12 hours.

46. A process according to claim 42, wherein the transesterification is conducted at a
20 temperature of 40 to 80°C under atmospheric pressure, with or without agitation.

47. A process according to claim 46, wherein the transesterification is conducted for 20 to 90 min.

25 48. A process according to claim 42, wherein the separating step is performed by centrifugation or gravitational settling.

49. A process according to claim 42, wherein the process further comprises a step of washing the ester-rich portion with a washing agent to remove catalyst, unreacted

alcohols and other impurities, prior to the distilling step.

50. A process according to claim 49, wherein the process further comprises a step of heating the washed ester-rich portion to reduce moisture content therein to less than
5 0.30 wt%.

51. A process according to claim 50, wherein the heating step is conducted at a temperature of 40 to 70°C under vacuum or a pressure of 100 kPa.

10 52. A process according to claim 42, wherein the distilling step is vacuum distillation performed in a single stage or multiple stages.

53. A process according to claim 53, wherein the vacuum distillation is performed in a distillation column operating at a temperature of 120 to 180°C and at a pressure of
15 0.001 to 0.01 kPa.

54. A process according to claim 42, wherein the process further comprises a step of washing the ester-rich portion with a washing agent to remove catalyst, unreacted alcohols and other impurities.
20

55. A process according to claim 42, wherein the process further comprises a step of washing the composition with predetermined concentration of tocotrienols/tocopherols, carotenoids and sterols with a methanolic acid solution to remove the impurities present therein.
25

56. A process according to claim 55, wherein the washing step is performed at a temperature of 28 to 50°C under atmospheric pressure,

57. A process according to claim 55, wherein the process further comprises a step of

drying the washed composition.

58. A process according to claim 57, wherein the process further comprises a step of crystallizing the dried composition in the presence of an alcohol to separate sterols
5 and glycerides, if present therein, in form of crystals.

59. A process according to claim 58, wherein the crystallization is performed at a temperature of -30 to 0°C for a period of 12 hours to 3 days.

10 60. A process according to claim 59, wherein the crystallization is performed for a period of 12 hours to 3 days.

61. A process according to claim 58, wherein the process further comprises a step of separating the sterol crystals and glyceride crystals, if present.
15

62. A process according to claim 61, wherein the process further comprises a step of evaporating the alcohol contained therein by heating.

63. A process according to claim 41, wherein the basic solution is caustic solution or
20 sodium hydroxide solution.

64. A process according to any one of the preceding claims, wherein the washing agent is fed in a fashion of a direct or a counter current.

25 65. A process according to any one of the preceding claims, wherein the washing agent is water heated to a temperature of 40 to 80°C.

66. A process according to any one of the preceding claims, wherein the basic catalyst is sodium methoxide, sodium hydroxide in methanol, potassium methoxide or

potassium hydroxide in methanol.

67. A process according to any one of the preceding claims, wherein the alcohol is any one or a combination of methanol, ethanol, propanol and butanol.

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68. A process according to claim 22 or 23, wherein the organic solvent is any one or a combination of hexane, heptane, iso-octane, acetone and ethyl acetate.

69. A process according to claim 6, wherein the acid is hydrochloric acid, acetic acid, sulphuric acid or citric acid.

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70. A process according to any one of the preceding claims, wherein the crude oil is crude palm oil, red palm oil, red palm olein, red palm fiber oil or any crude oil having free fatty acid of more than 3.50% by weight of total composition.

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Abstract**Recovery Of Tocopherols/Tocotrienols, Carotenoids, Glycerols, Sterols And Fatty Acid Esters From Crude Vegetable Oil And The Process Thereof**

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A process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to 3.50% by weight by distillation or neutralization.

10 *No illustrations for abstract.*

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PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

CORRECTED VERSION

PCT

NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Rule 71.1)

To:

CHOON HONG, Lok
PINTAS CONSULTING GROUP SDN BHD
NO. 19, JALAN SS 1/36, PETALING JAYA
47300 SELANGOR
Malaysia

Date of mailing
(day/month/year) 19 April 2017

Applicant's or agent's file reference
PT/5083/DAVIDHO/14/PCT

IMPORTANT NOTIFICATION

International application No.
PCT/MY2015/050030

International filing date (day/month/year)
07 May 2015

Priority date (day/month/year)
12 January 2015

Applicant
HO, David Sue San

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translations to those Offices.
4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the *PCT Applicant's Guide*.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed invention is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the IPEA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
Email address: pct@ipaaustralia.gov.au

Authorised officer

Karl Heys
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No. 0262832725

PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PT/5083/DAVIDHO/14/PCT	FOR FURTHER ACTION See Form PCT/IPEA/416																									
International application No. PCT/MY2015/050030	International filing date (<i>day/month/year</i>) 07 May 2015	Priority date (<i>day/month/year</i>) 12 January 2015																								
International Patent Classification (IPC) or national classification and IPC C11B 7/00 (2006.01) C11B 3/12 (2006.01) C11B 3/02 (2006.01) C11C 1/10 (2006.01)																										
Applicant HO, David Sue San																										
1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36. 2. This REPORT consists of a total of 5 sheets, including this cover sheet. 3. This report is also accompanied by ANNEXES, comprising: a. ☒ (<i>sent to the applicant and to the International Bureau</i>) a total of 33 sheets, as follows: ☒ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions). ☐ sheets containing rectifications not taken into account by this Authority because they were not available at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2). ☐ Superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)). b. ☐ (<i>sent to the International Bureau only</i>) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3bis of Annex C of the Administrative Instructions). 4. This report contains indications relating to the following items: <table style="width: 100%; border: none;"> <tr> <td style="width: 5%;">☒</td> <td style="width: 20%;">Box No. I</td> <td>Basis of the report</td> </tr> <tr> <td>☐</td> <td>Box No. II</td> <td>Priority</td> </tr> <tr> <td>☐</td> <td>Box No. III</td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td>☐</td> <td>Box No. IV</td> <td>Lack of unity of invention</td> </tr> <tr> <td>☒</td> <td>Box No. V</td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td>☐</td> <td>Box No. VI</td> <td>Certain documents cited</td> </tr> <tr> <td>☐</td> <td>Box No. VII</td> <td>Certain defects in the international application</td> </tr> <tr> <td>☐</td> <td>Box No. VIII</td> <td>Certain observations on the international application</td> </tr> </table>			☒	Box No. I	Basis of the report	☐	Box No. II	Priority	☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	☐	Box No. IV	Lack of unity of invention	☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement	☐	Box No. VI	Certain documents cited	☐	Box No. VII	Certain defects in the international application	☐	Box No. VIII	Certain observations on the international application
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☐	Box No. VIII	Certain observations on the international application																								
Date of submission of the demand 07 October 2016	Date of completion of this report 3 May 2017																									
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaaustralia.gov.au	Authorised officer Karl Heys AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262832725																									

Box No. I

Basis of the report

1. With regard to the **language**, this report has been established on the basis of:
- ☒ The international application in the language in which it was filed:
- ☐ A translation of the international application into , which is the language of a translation furnished for the purposes of :
- ☐ international search (under Rules 12.3(a) and 23.1 (b)).
- ☐ publication of the international application (under Rule 12.4(a)).
- ☐ international preliminary examination (Rules 55.2(a) and/or 55.3(a) and (b)).
2. With regard to the **elements** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):
- ☐ the international application as originally filed/furnished, or
- ☒ the description: pages **1-23** , as originally filed/furnished
pages , received by this Authority on with the letter of
- ☒ the claims: pages , as originally filed/furnished
pages , as amended (together with any statement) under Article 19,
pages **24-33** , received by this Authority on **07 October 2016** with the letter of **29 September 2016**
- ☐ the drawings: pages , as originally filed/furnished
pages , received by this Authority on with the letter of
- ☐ a sequence listing - see Supplemental Box Relating to Sequence Listing.
3. ☒ The amendments have resulted in the cancellation of:
- ☐ the description, pages
- ☒ the claims, Nos. **67-70**
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (specify):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (*c-bis*)):
- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (specify):
5. ☐ This report has been established:
- ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-*bis*) and 70.2(e)).
- ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.4*bis* and 70.2(e)).
6. With regard to top-up searches (Rules 66.1*ter* and 70.2(f)):
- ☒ A top-up search was carried out by this Authority on 3 January 2017.
- ☐ Additional relevant documents have been discovered during the top-up search.
- ☐ No top-up search was carried out by this Authority because it would serve no useful purpose.
7. ☐ Supplementary international search report(s) from Authority(ies)
has/have been received and taken into account in establishing this report (Rule 45*bis*.8(b) and (c)).

* If item 4 applies, some or all of those sheets may be marked "superseded."

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-66	YES
	Claims NONE	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-66	NO
Industrial applicability (IA)	Claims 1-66	YES
	Claims NONE	NO

Note: Details of earlier searches conducted following the filing of a demand can be found on Patentscope.

2. CITATIONS AND EXPLANATIONS:**CITATIONS**

D1: US 2011/0220483 A1 (MARGNAT et al.) 15 September 2011
D3: EP 1894913 A1 (DESMET BALLESTRA OLEO S.P.A.) 05 March 2008
D6: WO 2000/049116 A1 (CALGENE LLC) 24 August 2000
D7: US 2007/0238886 A1 (HO) 11 October 2007

NOVELTY (N)

This report has been written to address the letter from the applicant filed on the 03 March 2017, this letter was not accompanied by any amendments.

The applicant has argued that the present application is distinguished from the prior art primarily through a step of reducing free fatty acid (FFA) content below 3.5 wt % prior to recovery steps of phytonutrients and additionally in using two distillation columns in the process in sequential (series). However distillation columns are well known to be applied to separate hydrocarbon materials based on their boiling points and additional distillation columns would be only a further optimisation of this separation process.

Furthermore, the step of reducing free fatty acids to below 3.5 wt % appears to be a well-known goal in these methods to aid processes such as transesterification from being slowed/fouled up. The use of sequential distillation in the present application seems to be defined as involving two columns where the residue of a first step of a distillation is then moved onto a second column for a second step of treatment by either differing or the same temperatures. Although it is argued that the sequential nature of this use of two columns is advantageous over only using one column with two runs it is still going to achieve the same technical effect. Distillations at different stages of the purification process would be routinely carried out already, sequential distillations to enhance this separation would be an obvious enhancement of the separation process.

It is further argued by the applicant that the independent claims involve crude vegetable oils which differs from the treated oils of the prior art. However as the processes of claims 1 and 3 comprise the steps anywhere before the final separation of phytonutrients, it does not limit the starting materials. Although the prior art documents might contain additional steps prior to achieving a treated oil, they will have started with crude vegetable oils.

D1 discloses a process for separation of sterols, hydrocarbons (including carotenes), tocotrienols/tocopherols from crude vegetable oil. Free fatty acids in the oil material are esterified to reduce the amount of free fatty acids, before transesterification of the glycerides. Example 7 shows a vegetable oil which has been reduced to 0.8 wt % of free fatty acids as percentage of the oil. The esterified free fatty acid components are separated via distillation with numerous distillation steps leading to removal of the esterified fatty acids, and subsequent separation of sterols and tocopherol/tocotrienols from a fraction containing squalene and other hydrocarbons. The process of D1 uses three distillation processes with increasing temperature and reducing pressure to allow the separation of the oil components (see. page 6 col 1 & 2, examples). D1 also includes steps of separating the carotene fraction and the tocopherol/tocotrienol and sterol components [0079]. However, D1 fails to teach directly sequential distillation would be used in the process, as a result it is not considered relevant for novelty.

D3 discloses a process for preparing biofuel materials from vegetable oil with additional disclosures to remove vitamin components, sterols and carotenoids during the process to sell separately. This discloses that for efficient transesterification of the glycerides present in the oil, it is best to remove the water and free fatty acids present to reduce the amount of catalyst, and soap formation

during these processes (see [0003]-[0009]). Examples teach oils with greater than 4% free fatty acid content which are reduced to below 1% in pre-treatment steps (see examples 1) with other examples showing FFA acid content being reduced. Also the free fatty acids are removed through distilling the crude oil material ([0023], examples). D3 teaches the importance of FFA removal in processes where free fatty acids or nutrients are to be extracted, but fails to teach directly sequential steps of distillation.

D7 discloses a process of separating tocotrienols or tocopherols, carotenoids, sterols from vegetable oils leading to efficient separation of these components without degradation of the phytonutrients. D7 fails to teach reduction of FFA below 3.5 wt % so is not relevant to consideration of novelty of the present invention.

D6 is considered to be the closest prior art as it teaches the use of sequential distillation steps to either purify the samples arriving at the final product. D6 discloses a process of refining crude vegetable oil to reduce FFA content and separate tocotrienols, sterols and carotenes. The crude oils are treated leading to final oil extracts with very low FFA concentrations (examples). The extraction of free fatty acids is either done via neutralisation with a base prior to separation, or distillation at a temperature below 200°C and reduced pressure (page 13 lines 3-line 25, claims). Furthermore the isolated deacidified oil is concentrated through transesterification with an acid catalyst and short chain alcohol, before the glycerine component is extracted and the resulting fatty esters distilled from the phytonutrient components (page 17 lines 9-14, example 6) . However D6 differs as it fails to teach two different columns are used in this distillation which is a feature of independent claims 1, 3 and 39.

Claims 1-66 are therefore novel and comply with PCT Article 33(2).

INVENTIVE STEP (IS)

Claims 1-66 do not involve an inventive step and therefore do not comply with PCT Article 33(3).

The applicant has argued that the present application is distinguished from the prior art primarily through a step of reducing free fatty acid (FFA) content below 3.5 wt % prior to recovery steps of phytonutrients and additionally in using two distillation columns in the process in sequential (series). However, distillation columns are well known to be applied to separate hydrocarbon materials based on their boiling points and additional distillation columns would be only a further optimisation of this separation process. Furthermore, the step of reducing free fatty acids to below 3.5 wt % appears to be a well-known goal in these methods to aid processes such as transesterification from being slowed/fouled up. It is further argued that the applicant has worked to optimise the temperatures and pressures of the invention, however the independent claims 1, 3 and 39 either fail to define any temperature/pressure values (as in claim 1) that are covered by the broad ranges of temperatures and pressures overlapping those used in the prior art documents for the same types of separations.

The use of sequential distillation in the present application seems to be defined as involving two columns where the residue of a first step of a distillation is then moved onto a second column for a second step of treatment by either differing or the same temperatures, which at least seem to overlap. Although it is argued that the sequential nature of this use of two columns is advantageous over only using one column with two runs it is still going to achieve the same technical effect. Distillations at different stages of the purification process would be routinely carried out already, sequential distillations to enhance this separation would be an obvious optimisation of the separation process. The skilled person would routinely alter temperature and/or pressure between subsequent runs to further purify the materials in these steps, this is the general use of distillation as a separation/purification process for hydrocarbon-based materials with differing boiling points. As a result it is not considered the invention as currently claimed provides a technological advancement over the prior art.

Additionally the applicant argues that the present process leads to a system where no contamination of the materials occurs through this process due to the sequential distillation. As distillation is commonly used as a purification technique, it would be one of the desired outcomes of the process well known in the art that contaminants are routinely avoided (for example ensuring methanol impurity is not present in distilled alcohol).

It is further argued by the applicant that the independent claims involve crude vegetable oils which differs from the treated oils of the prior art. However as the processes of claims 1 and 3 comprise the steps anywhere before the final separation of phytonutrients, it does not limit the starting materials. Although the prior art documents might contain additional steps prior to achieving a treated oil, they will have started with crude vegetable oils.

The closest prior art is considered to be D6 which teaches the process of extracting a nutrient rich oil component from a crude oil material. The processes taught in D6 involve a degumming step, followed by a reduction in free fatty acid (FFA) content via either distillation or neutralisation followed by filtering of the oil component leaving a reduced FFA oil for subsequent purification. The purification of the carotenoid containing oil is carried out using 2 passes of distillation using with modifications to the temperatures and pressures of the distillation apparatus to control the separation of the components (examples). This document includes examples where transesterification of fatty acids to fatty acid methyl esters (example 6 & 7) is done prior to distillation procedures (two steps at the same conditions as in the present claim 39) leaving an enriched residue component.

The processes taught in D6 contain the use of the reduction in free fatty acids and sequential distillation process to purify the nutrient rich oil from the free fatty acid fractions. It differs from the present invention by failing to explicitly teach that these

sequential distillation steps must be carried out on two different columns. However D6 teaches two steps of distillation with the subsequent distillation being carried on the residue of the first, and carried out at either the same conditions or varied temperature/pressure conditions compared with the first step of distillation. The use of 2 different columns is seen as a mere optimisation of the process carried out with the skilled person routinely and does not involve an inventive step. Furthermore as in the present claim 1 it is expected that the person skilled in the art would be able to determine the temperatures and pressures most suited to these separations as a result none of the independent claims 1, 3 or 39 contains an inventive step.

The features of the remaining claims 2, 4-38 & 40-66 are either taught in D6 alone or would be common general knowledge in the art of purifying phytonutrient such as carotene, tocotrienol/tocopherols and sterols as evidenced by D1, D3 and D7 that were raised previously, and as a result are not considered to contain non-obvious features.

INDUSTRIAL APPLICABILITY (IA)

The invention defined in the claims is considered to meet the requirements of Industrial Applicability under Article 33(4) of the PCT because it can be made by, or used in, industry.

Your Ref: PCT/MY2015/050030
Our Ref: PT/5083/DAVIDHO/14/PCT
29 September 2016

Australian Patent Office
PO Box 200, Woden Act 2606,
Australia.
Attn: Karl Heys

Dear Sir/Madam,

**AMENDMENT AND RESPONSE TO WRITTEN OPINION OF THE INTERNATIONAL SEARCH REPORT UNDER
ARTICLE 34**

APPLICANTS : DAVID HO SUE SAN
TITLE : RECOVERY OF TOCOPHEROLS/TOCOTRIENOLS, CAROTENOIDS, GLYCEROLS, STEROLS
AND FATTY ACID ESTERS FROM CRUDE VEGETABLE OIL AND THE PROCESS THEREOF
APPLICATION NO. : PCT/MY2015/050030

In the matter of the above-identified application for the Written Opinion of International Searching Authority dated 10 August 2015, attached are:

1. Amendment to the claims; and
2. Remarks.

A) AMENDMENT(S)

- (I) Original claim 1 is amended to include novel and inventive feature of the application.
- (II) Original claim 3 is amended to incorporate feature as claimed in originally filed claim 5.
- (III) Original claim 40 is amended to incorporate features as claimed in originally filed claims 42, 52 and 53.
- (IV) Original set of claims are renumbered due to amendment as mentioned in (I) to (III).

CLAIMS

1. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to less than 3.50 % by weight, the process comprising the step of:
distilling the crude oil or fractions thereof using two distillation columns, in a sequential arrangement, each column operating under a pressure and at a temperature suitable for separating tocotrienols/tocopherols, carotenoids or sterols from the fractions.
2. A process according to claim 1, wherein the amount of the free fatty acids in the crude oil is reduced by distillation or neutralization.

3. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to 3.50% by weight by distillation, wherein the distillation is performed using two distillation columns, one column operating at a temperature of 90 to 200°C and a pressure ranging from 10 to 100 kPa and the subsequent column at 120 to 250°C and 0.10 to 10 kPa.

4. A process according to claim 3, wherein the distillation is performed to produce a first fraction enriched with carotenoids and a second fraction enriched with tocotrienols/tocopherols.

~~5. A process according to claim 3 or 4, wherein the distillation is performed using two distillation columns, one column operating at a temperature of 90 to 200°C and a pressure ranging from 10 to 100 kPa and the subsequent column at 120 to 250°C and 0.10 to 10 kPa.~~

~~5~~ 6. A process according to claim 3 or 4, wherein the process comprises the steps of:

transesterifying the first fraction in the presence of an alcohol and a basic catalyst to convert the glycerides therein to fatty acid esters and glycerine, forming a transesterified mixture comprising glycerine, fatty acid esters and carotenoids, followed by distilling the transesterified mixture to separate carotenoids; and

esterifying the second fraction in the presence of an alcohol and an acid catalyst to convert fatty acids therein to fatty acid esters, forming an esterified mixture comprising fatty acid esters and tocotrienols/tocopherols, followed by distilling the esterified mixture to separate tocotrienols/tocopherols.

~~6~~ 7. A process according to claim ~~5~~ 6, wherein the transesterifying step is conducted at a temperature of 35 to 80°C under atmospheric pressure, with or without agitation.

~~7~~ 8. A process according to claim ~~6~~ 7, wherein the transesterifying step is conducted for 0.5 to 12 hours.

~~8~~ 9. A process according to claim ~~6~~ 7, wherein the distilling step, after the transesterifying step, is performed using two distillation columns, in a sequential arrangement, each column operating under vacuum condition and at a temperature of 120 to 180°C, with the first column having an operating temperature lower than the subsequent column.

~~9~~ 10. A process according to claim ~~5~~ 6, wherein the esterifying step is conducted at a temperature of 5 to 90°C for 0.5 to 6 hours, with or without agitation.

10-11. A process according to claim 9-10, wherein the esterifying step is conducted for 0.5 to 6 hours.

11-12. A process according to claim 5-6, wherein the distilling step, after the esterifying step, is performed using two distillation columns, in a sequential arrangement, each column operating under vacuum condition and at a temperature of 120 to 180°C, with the first column having an operating temperature higher than the subsequent column.

12-13. A process according to claim 3 or 4, wherein the process further comprises a step of heating the crude oil to remove moisture contained therein, prior to reduction of fatty acids in the crude oil by distillation.

13-14. A process according to claim 12-13, wherein the heating step is performed at a temperature of 40 to 80°C under vacuum condition or a pressure of 100 kPa for 0.5 to 24 hours.

14-15. A process according to claim 5-6, wherein the process further comprises a step of reducing moisture content in the second fraction to less than 0.30 wt% prior to transesterification.

15-16. A process according to claim 5-6, wherein the process further comprises a step of separating the glycerine from the transesterified mixture to produce an ester-rich portion, prior to distilling the transesterified mixture.

16-17. A process according to claim 15-16, wherein the glycerine is separated from the transesterified mixture by centrifugation or gravitational settling, producing a glycerine-rich portion and the ester-rich portion, wherein the glycerine-rich portion is extracted and neutralized using an acid to recover glycerine therefrom.

17-18. A process according to claim 16-17, wherein the process further comprises a step of washing the transesterified mixture with a washing agent to remove catalyst, unreacted alcohols and other impurities.

18-19. A process according to claim 17-18, wherein the process further comprises a step of heating the washed transesterified portion to reduce moisture content therein to less than 0.30 wt%.

19-20. A process according to claim 18-19, wherein the heating step is conducted at a temperature of 35 to 80°C under vacuum or a pressure of 100 kPa.

20-21. A process according to claim 19-20, wherein the heating step is conducted for 0.5 to 12 hours.

21-22. A process according to claim 5-6, wherein the process further comprises a step of adding an organic solvent into the distilled transesterified mixture and chilling the mixture to produce crystallised carotenoids, followed by subjecting the resulting mixture to centrifugation thereby producing a top layer and a bottom layer; whereby the bottom layer comprises crystallised carotenoids in an amount of 28 to 45% by weight.

22-23. A process according to claim 21-22, wherein the bottom layer is heated to evaporate the solvent contained therein for recovery of carotenoids.

23-24. A process according to claim 22-23, wherein the heating step is performed at a temperature of 45 to 80°C to reduce the solvent content to a level of less than 1% or lower.

24-25. A process according to claim 23-24, wherein the heating step is performed under vacuum condition of 70 to 75 cm Hg.

25-26. A process according to claim 21-22, wherein the top layer is subjected to transesterification in the presence of an alcohol and a basic catalyst to increase the carotenoid content therein; and washing the mixture obtained thereof with a methanolic acid solution to remove the impurities present therein.

26-27. A process according to claim 5-6, wherein the process further comprises a step of washing the esterified mixture using a washing agent to remove catalyst, unreacted alcohols and other impurities therefrom, prior to distilling the esterified mixture.

27-28. A process according to claim 26-27, wherein the process further comprises a step of heating the washed esterified mixture to reduce moisture content therein to less than 0.30% by weight.

28-29. A process according to claim 5-6, wherein the process further comprises a step of transesterifying the distilled esterified mixture in the presence of an alcohol and a basic catalyst to convert the fatty acids therein to fatty acid esters, forming a product mixture comprising glycerine and fatty acid esters.

29-30. A process according to claim 28-29, wherein the transesterifying step is conducted at a temperature of 5 to 90°C with or without agitation.

30-31. A process according to claim 29-30, wherein the transesterifying step is conducted for 0.5 to 6 hours.

31 32. A process according to claim 30 31, wherein the process further comprises a step of washing the product mixture with a washing agent to remove catalyst, unreacted alcohols and other impurities.

32 33. A process according to claim 31 32, wherein the process further comprises a step of heating the washed product mixture to reduce moisture content therein to less than 0.30% by weight.

33 34. A process according to claim 32 33, wherein the heating step is performed at a temperature of 35 to 80°C under atmospheric pressure.

34 35. A process according to claim 33 34, wherein the heating step is performed for 0.5 to 10 hours.

35 36. A process according to claim 32 33, wherein the process further comprises a step of distilling the product mixture with reduced moisture content to obtain a composition with pre-determined concentration of tocotrienols/tocopherols and sterols.

36 37. A process according to claim 35 36, wherein the distilling step is performed using two distillation columns, in a sequential arrangement, one column operating at a temperature of 120 to 180°C and a pressure of 0.01 to 10 kPa and the subsequent column operating at 120 to 180°C and 0.01 to 3 kPa.

37 38. A process according to claim 35 36, wherein the process further comprises a step of separating sterols from the composition.

38 39. A process according to claim 38, wherein the sterols are separated by treating the composition with an alcohol and chilling the resulting mixture to produce crystallised sterols which are to be filtered.

39 40. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to less than 3.50% by weight by neutralization, the process comprising the steps of:

neutralizing crude oil by adding basic solution thereinto so as to convert fatty acids to salts which are to be separated by filtration;

transesterifying the neutralized oil in the presence of an alcohol and a basic catalyst to convert the glycerides therein to fatty acid esters and glycerine, forming an ester-rich fraction and a glycerol-rich fraction;

separating the ester-rich fraction from the glycerol-rich fraction;

distilling the ester-rich fraction to produce a concentrated stream comprising tocotrienols/tocopherols, carotenoids and sterols, in which the distilling step is performed using distillation columns arranged sequentially, each column operating at a pressure of 0.001 kPa to 0.01 kPa and at a temperature of 120 to 180°C;

further transesterifying the concentrated stream in the presence of an alcohol and a basic catalyst to form an ester-enriched fraction and a glycerol-rich fraction; and

repeating the transesterification, separation and distillation sequentially and repeatedly to obtain a composition with pre-determined concentration of tocotrienols/tocopherols, carotenoids and sterols.

40 44. A process according to claim 39 40, wherein the neutralization is performed such that a basic solution is added to the crude oil to convert the fatty acids to salts which are to be separated by filtration.

~~42. A process according to claim 40 or 41, wherein the process comprises the steps of
transesterifying the neutralized oil in the presence of an alcohol and a basic catalyst to convert the glycerides therein to fatty acid esters and glycerine, forming an ester rich fraction and a glycerol-rich fraction;
separating the ester rich fraction from the glycerol-rich fraction;
distilling the ester rich fraction to produce a concentrated stream comprising tocotrienols/tocopherols, carotenoids and sterols;
further transesterifying the concentrated stream in the presence of an alcohol and a basic catalyst to form an ester-enriched fraction and a glycerol-rich fraction; and
repeating the transesterification, separation and distillation sequentially and repeatedly to obtain a composition with pre-determined concentration of tocotrienols/tocopherols, carotenoids and sterols.~~

41 43. A process according to claim 39 42, wherein the process further comprises a step of heating the neutralized oil, prior to transesterification, to reduce the moisture content in the neutralized oil to a level of less than 0.20% by weight.

42 44. A process according to claim 41 43, wherein the heating step is performed at a temperature of 40 to 80°C under vacuum condition or at a pressure equivalent to 100 kPa under agitation.

43 45. A process according to claim 42 44, wherein the heating step is performed for a duration of 0.5 to 12 hours.

44 46. A process according to claim 39 42, wherein the transesterification is conducted at a temperature of 40 to 80°C under atmospheric pressure, with or without agitation.

45 47. A process according to claim 44 46, wherein the transesterification is conducted for 20 to 90 min.

46 48. A process according to claim 39 42, wherein the separating step is performed by centrifugation or gravitational settling.

47 49. A process according to claim 39 42, wherein the process further comprises a step of washing the ester-rich portion with a washing agent to remove catalyst, unreacted alcohols and other impurities, prior to the distilling step.

48 50. A process according to claim 47 49, wherein the process further comprises a step of heating the washed ester-rich portion to reduce moisture content therein to less than 0.30 wt%.

49 54. A process according to claim 48 50, wherein the heating step is conducted at a temperature of 40 to 70°C under vacuum or a pressure of 100 kPa.

~~52. A process according to claim 42, wherein the distilling step is vacuum distillation performed in a single stage or multiple stages.~~

~~53. A process according to claim 53, wherein the vacuum distillation is performed in a distillation column operating at a temperature of 120 to 180°C and at a pressure of 0.001 to 0.01 kPa.~~

50 54. A process according to claim 39 42, wherein the process further comprises a step of washing the ester-rich portion with a washing agent to remove catalyst, unreacted alcohols and other impurities.

51 55. A process according to claim 39 42, wherein the process further comprises a step of washing the composition with predetermined concentration of tocotrienols/tocopherols, carotenoids and sterols with a methanolic acid solution to remove the impurities present therein.

52 56. A process according to claim 51 55, wherein the washing step is performed at a temperature of 28 to 50°C under atmospheric pressure,

53 57. A process according to claim 51 55, wherein the process further comprises a step of drying the washed composition.

54 ~~58~~. A process according to claim 53 ~~57~~, wherein the process further comprises a step of crystallizing the dried composition in the presence of an alcohol to separate sterols and glycerides, if present therein, in form of crystals.

55 ~~59~~. A process according to claim 54 ~~58~~, wherein the crystallization is performed at a temperature of -30 to 0°C for a period of 12 hours to 3 days.

56 ~~60~~. A process according to claim 55 ~~59~~, wherein the crystallization is performed for a period of 12 hours to 3 days.

57 ~~64~~. A process according to claim 54 ~~58~~, wherein the process further comprises a step of separating the sterol crystals and glyceride crystals, if present.

58 ~~62~~. A process according to claim 57 ~~64~~, wherein the process further comprises a step of evaporating the alcohol contained therein by heating.

59 ~~63~~. A process according to claim 40 ~~44~~, wherein the basic solution is caustic solution or sodium hydroxide solution.

60 ~~64~~. A process according to any one of the preceding claims, wherein the washing agent is fed in a fashion of a direct or a counter current.

61 ~~65~~. A process according to any one of the preceding claims, wherein the washing agent is water heated to a temperature of 40 to 80°C.

62 ~~66~~. A process according to any one of the preceding claims, wherein the basic catalyst is sodium methoxide, sodium hydroxide in methanol, potassium methoxide or potassium hydroxide in methanol.

63 ~~67~~. A process according to any one of the preceding claims, wherein the alcohol is any one or a combination of methanol, ethanol, propanol and butanol.

64 ~~68~~. A process according to claim 21 ~~22~~ or 22 ~~23~~, wherein the organic solvent is any one or a combination of hexane, heptane, iso-octane, acetone and ethyl acetate.

65 ~~69~~. A process according to claim 5 ~~6~~, wherein the acid is hydrochloric acid, acetic acid, sulphuric acid or citric acid.

66 70. A process according to any one of the preceding claims, wherein the crude oil is crude palm oil, red palm oil, red palm olein, red palm fiber oil or any crude oil having free fatty acid of more than 3.50% by weight of total composition.

(B) REMARKS

In the written opinion of international search report issued, subject matter of the originally filed application is deemed lack of novelty and inventiveness in view of prior art namely: D1: US 2011/0220483 A1; D2: US 6407306 B1; D3: EP 1894913 A1; D4: US 6585201 B1; D5: EP 1426368 A1; D6: WO 2000/049116 A1; and D7: US 2007/0238886 A1. The Examiner's comment was respectfully traversed and remarks below shall distinguish the current application from the prior art.

NOVELTY

According to the written opinion, the examiner finds that claims 5-12, 14-39, 42-63 & 68-69 are novel. Amendments are made to originally filed independent claims 1, 3 and 40. As such, dependent claims appended to the amended independent claims shall deemed novel.

INVENTIVENESS

The Examiner commented that D1 is considered the closest prior art to claims 5-12, 14-39 & 68-69 in the present application because:

(a) the originally filed claim 6 disclosed:

- ❖ D1 involves the distillation of crude vegetable oil material to both reduce fatty acid content and separate carotene and tocotrienols/tocopherols and sterols.
- ❖ D1 disclosed the process of transesterifying glycerides in the presence of a base catalyst to gain glycerine and alkyl esters of the fatty acids side chains.
- ❖ D1 also disclosed the esterification of free fatty acids in the presence of a base catalyst to achieve a solution with reduced free fatty acid content which can be subsequently be separated into its components.

(b) Claims 5, 9, 12 and 37 in the present application are considered design choices;

(c) Claims 14, 15, 19-21, 34-35 in the present application are considered design choices; and

(d) Claims 7, 8, 10 & 11, 24-26, 28, 30 & 31 in the present application are considered optimizations of the process.

The applicant begs to differ, especially on the part where the Examiner commented that original claims 5, 9, 12 and 37 of the present application are considered design choices. The applicant wishes to highlight that the current application claimed two embodiments of a single novel and inventive concept, in which both embodiments comprises a process whereby distillation of different mixture can be performed in **distillation columns arranged in a sequential manner**.

Particularly, the present claimed subject matter uses two distillation columns at different stages, including during:

- (a) reducing free fatty content in crude oil;
- (b) separating crude oil into two fractions : a first fraction enriched with carotenoids and a second fraction enriched with tocotrienols/tocopherols;
- (c) separating carotenoids from transesterified first fraction comprising glycerine, fatty acid esters and carotenoids;
- (d) separating tocotrienols/tocopherols from esterified second fraction comprising fatty acid esters and tocotrienols/tocopherols;
- (e) concentrating a mixture (known as 'the product mixture') comprising tocotrienols/tocopherols and sterols; and
- (f) concentrating an ester-rich fraction to produce a concentrated stream comprising carotenoids, tocotrienols/tocopherols and sterols.

D1 cannot be considered relevant because staged distillation (see [0041] to [0045] and Fig. 1, b.1 to b.3) is not a similar process to the sequential distillation as claimed in the present application. Main differences are laid down below:

- ❖ Starting material used in D1 are **condensates from physical refining (OPRCs) and/or deodorization distillates (DD)** of vegetable oils. However, due to the use of OPRCs and DDs, there is a need to remove odorous, light hydrocarbons (C_8 to C_{15}) from esterified OPRCS and DDs. This being carried out under a distillation condition described in [0074] of D1. This step is not a concern of the present application because **crude vegetable oil** is used as starting material. This is a huge step in the field because extraction of tocopherols/tocotrienols and sterols from crude vegetable oil has been said not practical due to its small percentage. This point was also supported by [0007] of D1. Having said that, the present claimed subject matter provides technological know-how in extracting tocopherols/tocotrienols and sterols from **crude vegetable oil**.
- ❖ Staged distillation of D1 is a continuous process flowing from first distillation, second distillation to third distillation. [0041] of D1 showed that the staged distillation is conducted for:
 - (a) extracting vegetable hydrocarbons and alkyl esters;
 - (b) further extracting alkyl esters; and
 - (c) separating residual alkyl esters, squalene and residual vegetable hydrocarbons from sterols and Vitamin E.

However, there is no indication that each distillation above is conducted by means of two distillation columns. Further, the starting material, residue and distillate for each distillation in D1 are not the same as the present application. Particularly, D1 did not explicitly includes extraction of **carotenoids**. Further perusal of D1 and its Fig. 1 also showed that the staged distillation does not start with separation of crude oil into a first fraction enriched with **carotenoids** and a second fraction enriched with tocopherols/tocotrienols.

The Examiner further commented that it is obvious to reduce free fatty acid (FFA) content by distillation prior to subsequent recovery steps to improve yield of phytonutrients, as evidenced by D1-D6. However, there is no found evidence showing that a distillation to reduce FFA content can be conducted by means of two sequential distillation columns. Hence, D1-D6 cannot be seen as introducing the idea of distillation by means of two sequential distillation columns.

D7 disclosed multiple steps of distillation with increasing temperature and vacuum pressure to refine oil components present, coupled with crystallization steps to extract carotenoids and sterols from the distillate components. It shall be noted that the 'step-wise/multi-stage molecular distillation' disclosed by D7 is not the same as the 'distillation conducted by means of two sequential distillation columns' as claimed in the present application.

D7 showed that 'step wise/multi-stage distillation/' is conducted at two stages: stage one performed after the first transesterification (see [0019] & [0034]); and stage two performed after the second transesterification (see [0023] & [0042]). According to [0034] and [0042] of D7, there is no found indication on the number of distillation column applied. Also, there is no indication that **two sets of distillation conditions** may be applied. Therefore, D7 did not suggest the idea where two distillation columns can be applied at different stages during recovery of phytonutrients (especially tocopherol/tocotrienols, sterols and carotenoids) from crude oil. Hence, it cannot be merely a design choice.

In view of the above, the present claimed subject matter shall deemed inventive in view of D1 to D7, alone or in combination. Advantages of the present claimed method are listed below:

- ❖ Two different sets of distillation conditions can be applied. There is no need to recalibrate the operating distillation column (operating under a first set of distillation condition) in order to carry out a subsequent distillation (which requires a second set of distillation condition).
- ❖ Purified or concentrated distillates can be obtained via one time calibration of distillation conditions in each stage.
- ❖ Versatility in adapting such arrangement in different stages of recovery.

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2. Purely as a matter of precaution, however we request further corresponding in the event the Examiner forms the intention to object the application. Accordingly, the applicant respectfully requests reconsideration and further examination of the application.

3. Any amendments made during the objection of this application are intended solely to expedite prosecution of the application. Unless expressly stated otherwise, the cancellation or amendment of any claim does not amount to abandonment of any subject matter in the application.

Yours faithfully,
PINTAS CONSULTING GROUP



LOK CHOON HONG

Claims

1. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount
5 of the free fatty acids in the oil is reduced to less than 3.50 % by weight, the process comprising the step of: distilling the crude oil or fractions thereof using two distillation columns, in a sequential arrangement, each column operating under a pressure and at a temperature suitable for separating tocotrienols/tocopherols, carotenoids or sterols from the fractions.
10
2. A process according to claim 1, wherein the amount of the free fatty acids in the crude oil is reduced by distillation or neutralization.
3. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from
15 crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to 3.50% by weight by distillation, wherein the distillation is performed using two distillation columns, one column operating at a temperature of 90 to 200°C and a pressure ranging from 10 to 100 kPa and the subsequent column at 120 to 250°C and 0.10 to 10 kPa.
20
4. A process according to claim 3, wherein the distillation is performed to produce a first fraction enriched with carotenoids and a second fraction enriched with tocotrienols/tocopherols.
- 25 5. A process according to claim 3 or 4, wherein the process comprises the steps of: transesterifying the first fraction in the presence of an alcohol and a basic catalyst to convert the glycerides therein to fatty acid esters and glycerine, forming a transesterified mixture comprising glycerine, fatty acid esters and carotenoids, followed by distilling the transesterified mixture to separate carotenoids; and

esterifying the second fraction in the presence of an alcohol and an acid catalyst to convert fatty acids therein to fatty acid esters, forming an esterified mixture comprising fatty acid esters and tocotrienols/tocopherols, followed by distilling the esterified mixture to separate tocotrienols/tocopherols.

5

6. A process according to claim 5, wherein the transesterifying step is conducted at a temperature of 35 to 80°C under atmospheric pressure, with or without agitation.

7. A process according to claim 6, wherein the transesterifying step is conducted for
10 0.5 to 12 hours.

8. A process according to claim 6, wherein the distilling step, after the transesterifying step, is performed using two distillation columns, in a sequential arrangement, each column operating under vacuum condition and at a temperature of 120 to 180°C, with
15 the first column having an operating temperature lower than the subsequent column.

9. A process according to claim 5, wherein the esterifying step is conducted at a temperature of 5 to 90°C for 0.5 to 6 hours, with or without agitation.

20 10. A process according to claim 9, wherein the esterifying step is conducted for 0.5 to 6 hours.

11. A process according to claim 5, wherein the distilling step, after the esterifying step, is performed using two distillation columns, in a sequential arrangement, each
25 column operating under vacuum condition and at a temperature of 120 to 180°C, with the first column having an operating temperature higher than the subsequent column.

12. A process according to claim 3 or 4, wherein the process further comprises a step of heating the crude oil to remove moisture contained therein, prior to reduction of

fatty acids in the crude oil by distillation.

13. A process according to claim 12, wherein the heating step is performed at a temperature of 40 to 80°C under vacuum condition or a pressure of 100 kPa for 0.5 to
5 24 hours.

14. A process according to claim 5, wherein the process further comprises a step of reducing moisture content in the second fraction to less than 0.30 wt% prior to transesterification.
10

15. A process according to claim 5, wherein the process further comprises a step of separating the glycerine from the transesterified mixture to produce an ester-rich portion, prior to distilling the transesterified mixture.

15 16. A process according to claim 15, wherein the glycerine is separated from the transesterified mixture by centrifugation or gravitational settling, producing a glycerine-rich portion and the ester-rich portion, wherein the glycerine-rich portion is extracted and neutralized using an acid to recover glycerine therefrom.

20 17. A process according to claim 16, wherein the process further comprises a step of washing the transesterified mixture with a washing agent to remove catalyst, unreacted alcohols and other impurities.

18. A process according to claim 17, wherein the process further comprises a step of
25 heating the washed transesterified portion to reduce moisture content therein to less than 0.30 wt%.

19. A process according to claim 18, wherein the heating step is conducted at a temperature of 35 to 80°C under vacuum or a pressure of 100 kPa.

20. A process according to claim 19, wherein the heating step is conducted for 0.5 to 12 hours.
- 5 21. A process according to claim 5, wherein the process further comprises a step of adding an organic solvent into the distilled transesterified mixture and chilling the mixture to produce crystallised carotenoids, followed by subjecting the resulting mixture to centrifugation thereby producing a top layer and a bottom layer; whereby the bottom layer comprises crystallised carotenoids in an amount of 28 to 45% by weight.
- 10 22. A process according to claim 21, wherein the bottom layer is heated to evaporate the solvent contained therein for recovery of carotenoids.
- 15 23. A process according to claim 22, wherein the heating step is performed at a temperature of 45 to 80°C to reduce the solvent content to a level of less than 1% or lower.
- 20 24. A process according to claim 23, wherein the heating step is performed under vacuum condition of 70 to 75 cm Hg.
- 25 25. A process according to claim 21, wherein the top layer is subjected to transesterification in the presence of an alcohol and a basic catalyst to increase the carotenoid content therein; and washing the mixture obtained thereof with a methanolic acid solution to remove the impurities present therein.
26. A process according to claim 5, wherein the process further comprises a step of washing the esterified mixture using a washing agent to remove catalyst, unreacted alcohols and other impurities therefrom, prior to distilling the esterified mixture.

27. A process according to claim 26, wherein the process further comprises a step of heating the washed esterified mixture to reduce moisture content therein to less than 0.30% by weight.

5

28. A process according to claim 5, wherein the process further comprises a step of transesterifying the distilled esterified mixture in the presence of an alcohol and a basic catalyst to convert the fatty acids therein to fatty acid esters, forming a product mixture comprising glycerine and fatty acid esters.

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29. A process according to claim 28, wherein the transesterifying step is conducted at a temperature of 5 to 90°C with or without agitation.

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30. A process according to claim 29, wherein the transesterifying step is conducted for 0.5 to 6 hours.

31. A process according to claim 30, wherein the process further comprises a step of washing the product mixture with a washing agent to remove catalyst, unreacted alcohols and other impurities.

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32. A process according to claim 31, wherein the process further comprises a step of heating the washed product mixture to reduce moisture content therein to less than 0.30% by weight.

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33. A process according to claim 32, wherein the heating step is performed at a temperature of 35 to 80°C under atmospheric pressure.

34. A process according to claim 33, wherein the heating step is performed for 0.5 to 10 hours.

35. A process according to claim 32, wherein the process further comprises a step of distilling the product mixture with reduced moisture content to obtain a composition with pre-determined concentration of tocotrienols/tocopherols and sterols.

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36. A process according to claim 35, wherein the distilling step is performed using two distillation columns, in a sequential arrangement, one column operating at a temperature of 120 to 180°C and a pressure of 0.01 to 10 kPa and the subsequent column operating at 120 to 180°C and 0.01 to 3 kPa.

10

37. A process according to claim 35, wherein the process further comprises a step of separating sterols from the composition.

15

38. A process according to claim 38, wherein the sterols are separated by treating the composition with an alcohol and chilling the resulting mixture to produce crystallised sterols which are to be filtered.

20

39. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to less than 3.50% by weight by neutralization, the process comprising the steps of:

neutralizing crude oil by adding basic solution therein so as to convert fatty acids to salts which are to be separated by filtration;

25

transesterifying the neutralized oil in the presence of an alcohol and a basic catalyst to convert the glycerides therein to fatty acid esters and glycerine, forming an ester-rich fraction and a glycerol-rich fraction;

separating the ester-rich fraction from the glycerol-rich fraction;

distilling the ester-rich fraction to produce a concentrated stream comprising tocotrienols/tocopherols, carotenoids and sterols, in which the distilling step is

performed using distillation columns arranged sequentially, each column operating at a pressure of 0.001 kPa to 0.01 kPa and at a temperature of 120 to 180°C;

further transesterifying the concentrated stream in the presence of an alcohol and a basic catalyst to form an ester-enriched fraction and a glycerol-rich fraction; and

- 5 repeating the transesterification, separation and distillation sequentially and repeatedly to obtain a composition with pre-determined concentration of tocotrienols/tocopherols, carotenoids and sterols.

40. A process according to claim 39, wherein the neutralization is performed such that
10 a basic solution is added to the crude oil to convert the fatty acids to salts which are to be separated by filtration.

41. A process according to claim 39, wherein the process further comprises a step of heating the neutralized oil, prior to transesterification, to reduce the moisture content
15 in the neutralized oil to a level of less than 0.20% by weight.

42. A process according to claim 41, wherein the heating step is performed at a temperature of 40 to 80°C under vacuum condition or at a pressure equivalent to 100 kPa under agitation.
20

43. A process according to claim 42, wherein the heating step is performed for a duration of 0.5 to 12 hours.

44. A process according to claim 39, wherein the transesterification is conducted at a
25 temperature of 40 to 80°C under atmospheric pressure, with or without agitation.

45. A process according to claim 44, wherein the transesterification is conducted for 20 to 90 min.

46. A process according to claim 39, wherein the separating step is performed by centrifugation or gravitational settling.
47. A process according to claim 39, wherein the process further comprises a step of
5 washing the ester-rich portion with a washing agent to remove catalyst, unreacted alcohols and other impurities, prior to the distilling step.
48. A process according to claim 47, wherein the process further comprises a step of heating the washed ester-rich portion to reduce moisture content therein to less than
10 0.30 wt%.
49. A process according to claim 48, wherein the heating step is conducted at a temperature of 40 to 70°C under vacuum or a pressure of 100 kPa.
- 15 50. A process according to claim 39, wherein the process further comprises a step of washing the ester-rich portion with a washing agent to remove catalyst, unreacted alcohols and other impurities.
51. A process according to claim 39, wherein the process further comprises a step of
20 washing the composition with predetermined concentration of tocotrienols/tocopherols, carotenoids and sterols with a methanolic acid solution to remove the impurities present therein.
52. A process according to claim 51, wherein the washing step is performed at a
25 temperature of 28 to 50°C under atmospheric pressure,
53. A process according to claim 51, wherein the process further comprises a step of drying the washed composition.

54. A process according to claim 53, wherein the process further comprises a step of crystallizing the dried composition in the presence of an alcohol to separate sterols and glycerides, if present therein, in form of crystals.
- 5
55. A process according to claim 54, wherein the crystallization is performed at a temperature of -30 to 0°C for a period of 12 hours to 3 days.
56. A process according to claim 55, wherein the crystallization is performed for a
- 10 period of 12 hours to 3 days.
57. A process according to claim 54, wherein the process further comprises a step of separating the sterol crystals and glyceride crystals, if present.
- 15 58. A process according to claim 57, wherein the process further comprises a step of evaporating the alcohol contained therein by heating.
59. A process according to claim 40, wherein the basic solution is caustic solution or sodium hydroxide solution.
- 20 60. A process according to any one of the preceding claims, wherein the washing agent is fed in a fashion of a direct or a counter current.
61. A process according to any one of the preceding claims, wherein the washing
- 25 agent is water heated to a temperature of 40 to 80°C.
62. A process according to any one of the preceding claims, wherein the basic catalyst is sodium methoxide, sodium hydroxide in methanol, potassium methoxide or potassium hydroxide in methanol.

63. A process according to any one of the preceding claims, wherein the alcohol is any one or a combination of methanol, ethanol, propanol and butanol.

5 64. A process according to claim 21 or 22, wherein the organic solvent is any one or a combination of hexane, heptane, iso-octane, acetone and ethyl acetate.

65. A process according to claim 5, wherein the acid is hydrochloric acid, acetic acid, sulphuric acid or citric acid.

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66. A process according to any one of the preceding claims, wherein the crude oil is crude palm oil, red palm oil, red palm olein, red palm fiber oil or any crude oil having free fatty acid of more than 3.50% by weight of total composition.

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Claims

1. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount
5 of the free fatty acids in the oil is reduced to less than 3.50 % by weight, the process comprising the step of: distilling the crude oil or fractions thereof using two distillation columns, in a sequential arrangement, each column operating under a pressure and at a temperature suitable for separating tocotrienols/tocopherols, carotenoids or sterols from the fractions.
10
2. A process according to claim 1, wherein the amount of the free fatty acids in the crude oil is reduced by distillation or neutralization.
3. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from
15 crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to 3.50% by weight by distillation, wherein the distillation is performed using two distillation columns, one column operating at a temperature of 90 to 200°C and a pressure ranging from 10 to 100 kPa and the subsequent column at 120 to 250°C and 0.10 to 10 kPa.
20
4. A process according to claim 3, wherein the distillation is performed to produce a first fraction enriched with carotenoids and a second fraction enriched with tocotrienols/tocopherols.
- 25 ~~5. A process according to claim 3 or 4, wherein the distillation is performed using two distillation columns, one column operating at a temperature of 90 to 200°C and a pressure ranging from 10 to 100 kPa and the subsequent column at 120 to 250°C and 0.10 to 10 kPa.~~

- 5 6. A process according to claim 3 or 4, wherein the process comprises the steps of:
transesterifying the first fraction in the presence of an alcohol and a basic catalyst to
convert the glycerides therein to fatty acid esters and glycerine, forming a
transesterified mixture comprising glycerine, fatty acid esters and carotenoids,
5 followed by distilling the transesterified mixture to separate carotenoids; and
esterifying the second fraction in the presence of an alcohol and an acid catalyst to
convert fatty acids therein to fatty acid esters, forming an esterified mixture
comprising fatty acid esters and tocotrienols/tocopherols, followed by distilling the
esterified mixture to separate tocotrienols/tocopherols.
- 10
- 6 7. A process according to claim 5 6, wherein the transesterifying step is conducted
at a temperature of 35 to 80°C under atmospheric pressure, with or without agitation.
- 7 8. A process according to claim 6 7, wherein the transesterifying step is conducted
15 for 0.5 to 12 hours.
- 8 9. A process according to claim 6 7, wherein the distilling step, after the
transesterifying step, is performed using two distillation columns, in a sequential
arrangement, each column operating under vacuum condition and at a temperature of
20 120 to 180°C, with the first column having an operating temperature lower than the
subsequent column.
- 9 10. A process according to claim 5 6, wherein the esterifying step is conducted at a
temperature of 5 to 90°C for 0.5 to 6 hours, with or without agitation.
- 25
- 10 11. A process according to claim 9 10, wherein the esterifying step is conducted
for 0.5 to 6 hours.
- 11 12. A process according to claim 5 6, wherein the distilling step, after the

esterifying step, is performed using two distillation columns, in a sequential arrangement, each column operating under vacuum condition and at a temperature of 120 to 180°C, with the first column having an operating temperature higher than the subsequent column.

5

12 13. A process according to claim 3 or 4, wherein the process further comprises a step of heating the crude oil to remove moisture contained therein, prior to reduction of fatty acids in the crude oil by distillation.

10 13 14. A process according to claim 12 13, wherein the heating step is performed at a temperature of 40 to 80°C under vacuum condition or a pressure of 100 kPa for 0.5 to 24 hours.

14 15. A process according to claim 5 6, wherein the process further comprises a step
15 of reducing moisture content in the second fraction to less than 0.30 wt% prior to transesterification.

15 16. A process according to claim 5 6, wherein the process further comprises a step
20 of separating the glycerine from the transesterified mixture to produce an ester-rich portion, prior to distilling the transesterified mixture.

16 17. A process according to claim 15 16, wherein the glycerine is separated from
the transesterified mixture by centrifugation or gravitational settling, producing a
glycerine-rich portion and the ester-rich portion, wherein the glycerine-rich portion is
25 extracted and neutralized using an acid to recover glycerine therefrom.

17 18. A process according to claim 16 17, wherein the process further comprises a
step of washing the transesterified mixture with a washing agent to remove catalyst,
unreacted alcohols and other impurities.

18 19. A process according to claim 17 18, wherein the process further comprises a step of heating the washed transesterified portion to reduce moisture content therein to less than 0.30 wt%.

5

19 20. A process according to claim 18 19, wherein the heating step is conducted at a temperature of 35 to 80°C under vacuum or a pressure of 100 kPa.

20 21. A process according to claim 19 20, wherein the heating step is conducted for
10 0.5 to 12 hours.

21 22. A process according to claim 5 6, wherein the process further comprises a step of adding an organic solvent into the distilled transesterified mixture and chilling the mixture to produce crystallised carotenoids, followed by subjecting the resulting
15 mixture to centrifugation thereby producing a top layer and a bottom layer; whereby the bottom layer comprises crystallised carotenoids in an amount of 28 to 45% by weight.

22 23. A process according to claim 21 22, wherein the bottom layer is heated to
20 evaporate the solvent contained therein for recovery of carotenoids.

23 24. A process according to claim 22 23, wherein the heating step is performed at a temperature of 45 to 80°C to reduce the solvent content to a level of less than 1% or lower.

25

24 25. A process according to claim 23 24, wherein the heating step is performed under vacuum condition of 70 to 75 cm Hg.

25 26. A process according to claim 21–22, wherein the top layer is subjected to

transesterification in the presence of an alcohol and a basic catalyst to increase the carotenoid content therein; and washing the mixture obtained thereof with a methanolic acid solution to remove the impurities present therein.

5 26 27. A process according to claim 5 6, wherein the process further comprises a step of washing the esterified mixture using a washing agent to remove catalyst, unreacted alcohols and other impurities therefrom, prior to distilling the esterified mixture.

10 27 28. A process according to claim 26 27, wherein the process further comprises a step of heating the washed esterified mixture to reduce moisture content therein to less than 0.30% by weight.

15 28 29. A process according to claim 5 6, wherein the process further comprises a step of transesterifying the distilled esterified mixture in the presence of an alcohol and a basic catalyst to convert the fatty acids therein to fatty acid esters, forming a product mixture comprising glycerine and fatty acid esters.

20 29 30. A process according to claim 28 29, wherein the transesterifying step is conducted at a temperature of 5 to 90°C with or without agitation.

30 30 31. A process according to claim 29 30, wherein the transesterifying step is conducted for 0.5 to 6 hours.

25 31 32. A process according to claim 30 31, wherein the process further comprises a step of washing the product mixture with a washing agent to remove catalyst, unreacted alcohols and other impurities.

32 32 33. A process according to claim 31 32, wherein the process further comprises a step of heating the washed product mixture to reduce moisture content therein to less

than 0.30% by weight.

33 34. A process according to claim 32 ~~33~~, wherein the heating step is performed at a temperature of 35 to 80°C under atmospheric pressure.

5

34 35. A process according to claim 33 34, wherein the heating step is performed for 0.5 to 10 hours.

35 36. A process according to claim 32 ~~33~~, wherein the process further comprises a
10 step of distilling the product mixture with reduced moisture content to obtain a composition with pre-determined concentration of tocotrienols/tocopherols and sterols.

36 37. A process according to claim 35 ~~36~~, wherein the distilling step is performed
15 using two distillation columns, in a sequential arrangement, one column operating at a temperature of 120 to 180°C and a pressure of 0.01 to 10 kPa and the subsequent column operating at 120 to 180°C and 0.01 to 3 kPa.

37 38. A process according to claim 35 ~~36~~, wherein the process further comprises a
20 step of separating sterols from the composition.

38 39. A process according to claim 38, wherein the sterols are separated by treating the composition with an alcohol and chilling the resulting mixture to produce crystallised sterols which are to be filtered.

25

39 40. A process for recovering tocotrienols/tocopherols, carotenoids and sterols from crude vegetable oil, characterised in that prior to the recovery steps, the amount of the free fatty acids in the oil is reduced to less than 3.50% by weight by neutralization, the process comprising the steps of:

neutralizing crude oil by adding basic solution thereinto so as to convert fatty acids to salts which are to be separated by filtration;

transesterifying the neutralized oil in the presence of an alcohol and a basic catalyst to convert the glycerides therein to fatty acid esters and glycerine, forming an ester-rich fraction and a glycerol-rich fraction;

separating the ester-rich fraction from the glycerol-rich fraction;

distilling the ester-rich fraction to produce a concentrated stream comprising tocotrienols/tocopherols, carotenoids and sterols, in which the distilling step is performed using distillation columns arranged sequentially, each column operating at a pressure of 0.001 kPa to 0.01 kPa and at a temperature of 120 to 180°C;

further transesterifying the concentrated stream in the presence of an alcohol and a basic catalyst to form an ester-enriched fraction and a glycerol-rich fraction; and repeating the transesterification, separation and distillation sequentially and repeatedly to obtain a composition with pre-determined concentration of tocotrienols/tocopherols, carotenoids and sterols.

41. A process according to claim 39 40, wherein the neutralization is performed such that a basic solution is added to the crude oil to convert the fatty acids to salts which are to be separated by filtration.

~~42. A process according to claim 40 or 41, wherein the process comprises the steps of transesterifying the neutralized oil in the presence of an alcohol and a basic catalyst to convert the glycerides therein to fatty acid esters and glycerine, forming an ester-rich fraction and a glycerol-rich fraction;~~
~~separating the ester-rich fraction from the glycerol-rich fraction;~~
~~distilling the ester-rich fraction to produce a concentrated stream comprising tocotrienols/tocopherols, carotenoids and sterols;~~
~~further transesterifying the concentrated stream in the presence of an alcohol and a~~

~~basic catalyst to form an ester enriched fraction and a glycerol rich fraction; and repeating the transesterification, separation and distillation sequentially and repeatedly to obtain a composition with pre-determined concentration of tocotrienols/tocopherols, carotenoids and sterols.~~

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41 43. A process according to claim 39 42, wherein the process further comprises a step of heating the neutralized oil, prior to transesterification, to reduce the moisture content in the neutralized oil to a level of less than 0.20% by weight.

10 42 44. A process according to claim 41 43, wherein the heating step is performed at a temperature of 40 to 80°C under vacuum condition or at a pressure equivalent to 100 kPa under agitation.

15 43 45. A process according to claim 42 44, wherein the heating step is performed for a duration of 0.5 to 12 hours.

44 46. A process according to claim 39 42, wherein the transesterification is conducted at a temperature of 40 to 80°C under atmospheric pressure, with or without agitation.

20

45 47. A process according to claim 44 46, wherein the transesterification is conducted for 20 to 90 min.

25 46 48. A process according to claim 39 42, wherein the separating step is performed by centrifugation or gravitational settling.

47 49. A process according to claim 39 42, wherein the process further comprises a step of washing the ester-rich portion with a washing agent to remove catalyst, unreacted alcohols and other impurities, prior to the distilling step.

48 ~~50~~. A process according to claim 47 ~~49~~, wherein the process further comprises a step of heating the washed ester-rich portion to reduce moisture content therein to less than 0.30 wt%.

5

49 ~~51~~. A process according to claim 48 ~~50~~, wherein the heating step is conducted at a temperature of 40 to 70°C under vacuum or a pressure of 100 kPa.

~~52. A process according to claim 42, wherein the distilling step is vacuum distillation performed in a single stage or multiple stages.~~

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~~53. A process according to claim 53, wherein the vacuum distillation is performed in a distillation column operating at a temperature of 120 to 180°C and at a pressure of 0.001 to 0.01 kPa.~~

15

50 ~~54~~. A process according to claim 39 ~~42~~, wherein the process further comprises a step of washing the ester-rich portion with a washing agent to remove catalyst, unreacted alcohols and other impurities.

20 51 ~~55~~. A process according to claim 39 ~~42~~, wherein the process further comprises a step of washing the composition with predetermined concentration of tocotrienols/tocopherols, carotenoids and sterols with a methanolic acid solution to remove the impurities present therein.

25 52 ~~56~~. A process according to claim 51 ~~55~~, wherein the washing step is performed at a temperature of 28 to 50°C under atmospheric pressure,

53 ~~57~~. A process according to claim 51 ~~55~~, wherein the process further comprises a step of drying the washed composition.

54 58. A process according to claim 53 57, wherein the process further comprises a step of crystallizing the dried composition in the presence of an alcohol to separate
5 sterols and glycerides, if present therein, in form of crystals.

55 59. A process according to claim 54 58, wherein the crystallization is performed at a temperature of -30 to 0°C for a period of 12 hours to 3 days.

10 56 60. A process according to claim 55 59, wherein the crystallization is performed for a period of 12 hours to 3 days.

57 61. A process according to claim 54 58, wherein the process further comprises a step of separating the sterol crystals and glyceride crystals, if present.
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58 62. A process according to claim 57 61, wherein the process further comprises a step of evaporating the alcohol contained therein by heating.

59 63. A process according to claim 40 41, wherein the basic solution is caustic
20 solution or sodium hydroxide solution.

60 64. A process according to any one of the preceding claims, wherein the washing agent is fed in a fashion of a direct or a counter current.

25 61 65. A process according to any one of the preceding claims, wherein the washing agent is water heated to a temperature of 40 to 80°C.

62 66. A process according to any one of the preceding claims, wherein the basic catalyst is sodium methoxide, sodium hydroxide in methanol, potassium methoxide or

potassium hydroxide in methanol.

63 67. A process according to any one of the preceding claims, wherein the alcohol is any one or a combination of methanol, ethanol, propanol and butanol.

5

64 68. A process according to claim 21 ~~22~~ or 22 ~~23~~, wherein the organic solvent is any one or a combination of hexane, heptane, iso-octane, acetone and ethyl acetate.

65 69. A process according to claim 5 ~~6~~, wherein the acid is hydrochloric acid, acetic acid, sulphuric acid or citric acid.

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66 70. A process according to any one of the preceding claims, wherein the crude oil is crude palm oil, red palm oil, red palm olein, red palm fiber oil or any crude oil having free fatty acid of more than 3.50% by weight of total composition.

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