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(54) **Title**: MODIFICATION OF PLANT LIPIDS CONTAINING PUFAS

(57) **Abstract**: The present invention generally is concerned with the modification of plant lipids containing PUFAs. In this context, the invention is particularly concerned with plants and plant materials for such modifications, wherein the plants preferably are oil-seed plants. Regarding plant parts, the invention is particularly concerned with seeds of such plants and preferably seeds of oilseed plants. The invention is also concerned with plant positions obtainable or obtained by the modification method of the invention, and with full stuff of feedstuff comprising such liquid compositions.

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Modification of plant lipids containing PUFAs

This application claims priority to U.S. Provisional Patent Application Serial Number 62/079622 application number filed November 14, 2014 and to U.S. Provisional Patent Application Serial Number 62/234373 filed September 29, 2015, which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present invention generally is concerned with the modification of plant lipids containing PUFAs. In this context, the invention is particularly concerned with plants and plant materials for such modifications, wherein the plants preferably are oilseed plants. Regarding plant parts, the invention is particularly concerned with seeds of such plants and preferably seeds of oilseed plants. The invention is also concerned with plant compositions obtainable or obtained by the modification method of the invention, and with foodstuff of feedstuff comprising such liquid compositions.

BACKGROUND OF THE INVENTION

It is generally recognised that polyunsaturated fatty acids ("PUFAs") convey health benefits. In this context, EPA and DHA are particularly coveted; they are used as dietary supplements for example to alleviate cardiovascular or neurological pathological conditions or ailments. Polyunsaturated fatty acids are currently predominantly obtained from fish oils, because wild-type plants lack the required enzymes to produce polyunsaturated fatty acids, particularly EPA and DHA, in sufficient quantities. Efforts have been made to produce polyunsaturated fatty acids in plants and particularly in oilseed plants.

The production of EPA and DHA is a metabolic pathway wherein fatty acids are treated by desaturases and elongases to produce ever longer and more unsaturated fatty acids. A depiction of the pathway can be found in WO 2006/012325, figure 9, and WO 2005/083093, figure 1. The desaturases and elongases involved in the pathway generally react both on omega-3 and omega-6 polyunsaturated fatty acids. One intermediate in the production of EPA and DHA generally is arachidonic acid. This polyunsaturated fatty acid is generally undesirable in dietary compositions, foodstuff and feedstuff due to its involvement in inflammatory processes. Thus, it is generally desired to obtain compositions with a high content of EPA and/or DHA and a low content of arachidonic acid. However, as arachidonic acid is a metabolite in the production of DHA and because arachidonic acid can be converted by omega-3 desaturases to and from EPA, it is generally not possible to avoid concomitant production of arachidonic acid in transgenic plant metabolism.

The present invention thus attempts to provide materials and methods for reducing the content of arachidonic acid in lipid compositions containing EPA and/or DHA. In particular, it is desirable that the invention to provide materials and methods for reducing the content of arachidonic acid in plant lipid compositions, preferably in lipid compositions obtainable or obtained from oilseed plants.

It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

BRIEF SUMMARY OF THE INVENTION

In a first aspect of the invention, provided is an extracted *Brassica napus* plant lipid composition comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

In a second aspect of the invention, provided is a transgenic *Brassica napus* plant or part thereof, comprising

a) lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), and one or both of:

b) a Delta-5 elongase gene under the control of a FAE promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development,

c) a Delta-5 desaturase gene under the control of a promoter such that expression of the Delta-5 desaturase gene is reduced or prevented in late stage seed development,

wherein, when the transgenic *Brassica napus* plant or part thereof is grown, the content of ARA decreases at least 0.5% by weight of total lipids while preferably the content of EPA and/or DHA increases.

Also described is extracted plant lipid compositions comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

Also described are plants or parts thereof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or
b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or
c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

Also, described are plants or parts thereof, comprising lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein, when the plant or part thereof is grown, the content of ARA decreases while preferably the content of EPA and/or DHA increases.

According to the invention is also provided a plant comprising a nucleic acid comprising

a) a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development, and/or
b) a Delta-5 desaturase gene under the control of a promoter such that expression of the Delta-5 desaturase gene is reduced or prevented in late stage seed development.

Seeds of a plant of the present disclosure are also described.

Further, described are plant lipid compositions obtainable or obtained by a process comprising the steps of

a) growing a plant of the present invention at least until the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased, and
b) harvesting the plant or a part thereof and
c) extracting lipids composition from the harvested material to obtain said lipid composition.

The disclosure also provides foodstuff or feedstuff comprising a lipid composition.

Furthermore, described are methods of altering plant lipids composition, comprising the step of growing a plant of the invention to produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased while preferably the content of EPA and/or DHA has increased.

And the disclosure provides methods of producing a plant lipid composition, comprising the steps of

a) growing plants of the invention,
b) harvesting the plants or a part thereof when the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides extracted plant lipid compositions. The lipid compositions comprise EPA and DHA. The extracted plant lipid compositions will generally also comprise ARA, even though this compound is generally not desired as a component but is normally unavoidable due to its function as intermediate metabolite in the production of EPA and/or DHA.

According to the invention, the content of EPA is at least 5% higher than the content of ARA. Unless indicated otherwise, in the context of the present invention a comparison of content numbers is to mean the difference between the respective percentage numbers; such difference is sometimes also called a difference in points percentage. Thus, when the content of EPA in an exemplary composition is for example 7 wt.-%, the content of ARA in the composition is at most 2 wt.-% of total fatty acids of the lipids composition.

It is a particular advantage of the present invention to provide means for the production of lipid compositions exhibiting such strong difference in contents between EPA and ARA. It was particularly surprising that such a marked difference could be maintained in plant lipid compositions, that is in lipid compositions produced in plant material or extracted therefrom as described herein, because both ARA and EPA are produced by the same class of enzymes, that is Delta-5 desaturases, and typically a Delta-5 desaturase producing EPA will also produce ARA. Also, ARA and EPA are converted into each other by action of omega 3 desaturases naturally present in material or introduced into the plant genome for the purposes of polyunsaturated fatty acid production. It was therefore expected that the composition of plant lipids could not be tilted in favour of high EPA contents without also increasing ARA content. However, the inventors have surprisingly found and provide herein a way not only to increase the EPA content without correspondingly also increasing the content of unwanted ARA; instead, the invention surprisingly provides means for actually decreasing the lipid content of ARA during lipid production already in the plant. Such decrease in ARA content at a time of continuing synthesis of EPA had not been observed or been expected to be possible at all before.

The invention therefore also advantageously provides extracted plant lipid compositions wherein the sum of contents of EPA plus DHA is at least 7% higher than the content of ARA. Providing such a marked difference in contents is even more surprising as EPA is converted into DHA by the action of Delta-5 elongase and Delta 4 desaturase. Thus, EPA is effectively consumed in the production of DHA; it is therefore a particular advantage of the present invention to maintain a high difference in the contents of EPA, DHA and ARA. As described hereinafter, achieving such high difference in contents is possible by the unexpected depletion of ARA in plant lipids during ongoing synthesis of EPA and DHA.

The invention also provides extracted plant lipid compositions wherein the content of ARA is less than 4% and the content of EPA is more than 7%. Even more preferably, the invention provides extracted plant lipid compositions wherein the content of ARA is less than 4%, the content of EPA is more than 7% and the content of DHA is more than 2%. Such compositions are particularly advantageous results of the unexpected mechanism of plant lipid production provided by the invention and simultaneously attain a high EPA/DHA content and a low ARA content.

Polyunsaturated fatty acids (PUFAs) are generally known to the skilled person, important polyunsaturated fatty acids are categorised into an omega-3, omega-6 and omega-9 series, without any limitation intended. Polyunsaturated fatty acids of the omega-6 series include, for example, and without limitation, linoleic acid (18:2 n-6; LA), gamma-linolenic acid (18:3 n-6; GLA), di-homo-gamma-linolenic acid (C20:3 n-6; DGLA), arachidonic acid (C20:4 n-6; ARA), adrenic acid (also called docosatetraenoic acid or DTA; C22:4 n-6) and docosapentaenoic acid (C22:5 n-6). Polyunsaturated fatty acids of the omega-3 series include, for example and without limitation, alpha-linolenic acid (18:3 n-3, ALA), stearidonic acid (18:4 n-3; STA or SDA), eicosatrienoic acid (C20:3 n-3; ETA), eicosatetraenoic acid (C20:4 n-3; ETA), eicosapentaenoic acid (C20:5 n-3; EPA), docosapentaenoic acid (C22:5 n-3; DPA) and docosahexaenoic acid (C22:6 n-3; DHA). Polyunsaturated fatty acids also include fatty acids with greater than 22 carbons and 4 or more double bonds, for example and without limitation, C28:8 (n-3). Polynsaturated fatty acids of the omega-9 series include, for example, and without limitation, mead acid (20:3 n-9; 5,8,11-

eicosatrienoic acid), erucic acid (22:1 n-9; 13-docosenoic acid) and nervonic acid (24:1 n-9; 15-tetracosenoic acid). Further polyunsaturated fatty acids are eicosadienoic acid (C20:2d11,14; EDA) and eicosatrienoic acid (20:3d11,14,17; ETrA).

Within the context of the present invention, lipids content is expressed as weight percentage of a specific fatty acid relative to total fatty acids determined in the respective lipids composition. Preferably, the total fatty acids tested for are: 14:0, 16:0, 16:1n-7, 16:1n-9, 16:3n-3, 17:0, 18:0, 18:1n-7, 18:1n-9, 18:2n-6 (LA), 18:2n-9, 18:3n-3 (ALA), 18:3n-6 (GLA), 18:4n-3 (SDA), 20:0, 20:1n-9, 20:2n-6, 20:2n-9, 20:3n-3, 20:3n-6 (DGLA), 20:3n-9, 20:4n-3 (ETA), 20:4n-6 (ARA), 20:5n-3 (EPA), 22:0, 22:1n-9, 22:2n-6, 22:4n-3, 22:4n-6, 22:5n-3 (DPA), 22:5n-6, 22:6n-3 (DHA), 24:0 and 24:1n-9.

It is a particular advantage of the present invention that the lipids contents described herein, unless explicitly noted otherwise, are determined without artificial enrichment or depletion of one or more fatty acids; the lipid content of a fatty acid is thus substantially the same as in the plant or part thereof prior to extraction.

The extracted lipid preferably is in the form of an oil, wherein at least 90%, more preferably least 95% and even more preferably at least about 98%, or between 95% and 98%, by weight of the oil is the lipid. Such oils can be obtained from plant material by methods known to the skilled person and/or as described herein.

According to the invention, the extracted plant lipid composition is a composition produced by a plant or plant material - preferred ways of producing such lipid compositions in plants and plant materials are also described herein-, and extracted from such lipids and optionally purified. Preferably, the extracted plant lipid composition is a composition to which no additional fatty acids have been added. It is a particular advantage of the present invention that the high difference between the contents of EPA and ARA can be achieved without adding "foreign" EPA to the composition, that is without addition of EPA that has not been produced by the plant or plant material the extract is obtained from. In particular, the contents of EPA and DHA can be achieved according to the invention without addition of fish oil or of corresponding polyunsaturated fatty acids obtained from fish oil.

Within the context of the present invention, reference is made to plants and to corresponding plant material. The plants (and correspondingly the plant material) refer to preferably is of family Brassicaceae. It is a particular advantage of the present invention that the lipid compositions of the present invention can be produced in and extracted from plants of this family, because such plants allow for the production of high amounts of fatty acids particularly in their seed oil. Also, many species belonging to this family have a long tradition as crop plants, the contents of their oil is thus generally considered useful for consumption and/or easy to obtain and purify for technical purposes or for purposes of consumption.

Plants according to the invention and corresponding plant material preferably belong to the tribus Aethionemeae, Alysseae, Alyssopsideae, Anastaticeae, Anchonieae, Aphragmeae, Arabideae, Asteae, Biscutelleae, Bivonaeeae, Boechereae, Brassiceae, Buniadeae, Calepineae, Camelineae, Cardamineae, Chorisporeae, Cochlearieae, Coluteocarpeae, Conringieae, Cremolobeae, Crucihimalayeae, Descurainieae, Dontostemoneae, Erysimeae, Euclidieae, Eudemeae, Eutremeae, Halimolobeae, Heliophileae, Hesperideae, Iberideae, Isatideae, Kernereae, Lepidieae, Malcolmieae, Megacarpaeeae, Microlepidieae, Noccaeeae, Notothlaspideae, Oreophytoneae, Physarieae, Schizopetaleae, Scoliaxoneae, Sisymbrieae, Smelowskieae, Stevenieae, Thelypodieae, Thlaspideae, Turritideae or Yinshanieae, and even more preferably belong genus Ammosperma, Brassica, Brassica x Raphanus, Cakile, Carrichtera, Ceratocnemum, Coincya, Cordylocarpus, Crambe, Crambella, Didesmus, Diplotaxis, Douepea, Enarthrocarpus, Eremophyton, Eruca, Erucaria, Erucastrum, Euzomodendron, Fezia, Foleyola, Fortuynia, Guiraoa, Hemicrambe, Henophyton, Hirschfeldia, Kremeriella, Moricandia, Morisia, Muricaria, Nasturtiopsis, Orychophragmus, Otocarpus, Physorhynchus, Pseuderucaria, Psychine, Raffenaldia, Raphanus, Rapistrum, Rytidocarpus, Savignya, Schouwia, Sinapidendron, Sinapis, Succowia, Trachystoma, Vella or Zilla. Plants of the aforementioned taxa belong to the family of Brassicaceae and thus can allow for the easy manifestation of the advantages described above in view of said taxonomic family.

Even more preferably the plant or plant material according to the invention belongs to a crop plant of genus Camelina or Brassica. Plants of these genera have traditionally been used in agriculture, their oils have been used for human or animal consumption for a long time. Also, agricultural practices in view of these genera have long been established, for example materials and methods for defense against fungi, insects and weeds. Thus, the production of plant lipids according to the invention in such genera is made particularly easy for the person skilled in agriculture.

Even more preferably a plant and correspondingly plant material according to the invention belongs to any of the species Camelina sativa, Brassica aucheri, Brassica balearica, Brassica barrelieri, Brassica carinata, Brassica carinata x Brassica napus, Brassica carinata x Brassica rapa, Brassica cretica, Brassica deflexa, Brassica desnottesii, Brassica drepanensis, Brassica elongata, Brassica fruticulosa, Brassica gravinae, Brassica hilarionis, Brassica hybrid cultivar, Brassica incana, Brassica insularis, Brassica juncea, Brassica macrocarpa, Brassica maurorum, Brassica montana, Brassica napus (rape, canola), Brassica napus x Brassica rapa, Brassica nigra, Brassica oleracea, Brassica oleracea x Brassica rapa subsp. pekinensis, Brassica oxyrrhina, Brassica procumbens, Brassica rapa, Brassica rapa x Brassica nigra, Brassica repanda, Brassica rupestris, Brassica ruvo, Brassica souliei, Brassica spinescens, Brassica tournefortii or Brassica villosa, even more preferably to any of the species Brassica carinata, Brassica carinata x Brassica napus or Brassica napus, most preferably of species Brassica napus. Plants of genus Brassica napus are also known as rape seed or canola and have a long tradition as a cheap and readily available source of plant oils and lipids fit for human or animal consumption.

Particularly preferred plants and plant materials are derived from transgenic Brassica event LBFLFK deposited as ATCC Designation “PTA-121703” as described herein, Brassica event LBFLFK contains two insertions of the binary T-plasmid VC-LTM593-1qcz rc as described in the examples section, or from transgenic Brassica event LBFDAU deposited as ATCC Designation “PTA-122340” as also described herein. For these events, particularly high contents of EPA and DHA can be achieved together with low contents of ARA.

Plants and plant materials also preferred according to the invention can be obtained by propagation of these events into other germplasms of plants of genus Camelina and even more preferably of genus Brassica. It is particularly preferred to use as plants and plant materials according to the invention plants resulting from a crossing of a transgenic event according to the invention, particularly of the event LBLFK, with plants of the species to Brassica carinata, even more preferably after backcrossing into Brassica napus. For such plants particularly high contents of EPA and/or DHA and low contents of ARA in the plant lipids according to the invention can be achieved.

According to the invention, the content of ARA preferably decreases by at least .5% during growth of the plant or plant material, preferably during seed development. Thus, by analysing the composition of plant lipids in said plant or plant material, a peak of ARA content can be observed. For example, when a peak content of ARA of 4% is observed, the plant or plant material is harvested only after the content of ARA has decreased to at most 3.5%. It is an advantage of the present invention that a reduction in lipids content of ARA by 0.5 percentage points can be achieved without compromising total lipids production and particularly without compromising the amount and content of EPA and/or DHA obtainable from such plant or plant material.

Preferably, when the plant or plant material of the invention is grown, the lipids content of EPA is maintained even during the reduction of ARA content. Even more preferably the lipids content of EPA increases by at least 1% during the period in which the content of ARA is reduced. Thus, for example the lipids content of EPA in plant seeds increases from 6% to 7% while simultaneously the lipids content of ARA in said plant material decreases from 4% to at most 3.5%. Even more preferably, the lipids content of EPA and DHA increase during the period of reduction of ARA lipids content when the plant or plant material of the present invention is grown. As noted herein before, it is a particular advantage that the present invention allows for such ongoing synthesis of EPA and DHA even though the content of the metabolic intermediate ARA is reduced.

As described above, the plants and plant material of the present invention preferably are oilseed plants. When the plants of the present invention are grown, it is preferred that they reach their maximum ARA lipids content before late stage seed development. Thus, sufficient time remains for the plant of the present invention to produce in its seed the desired quantities and contents of EPA and/or DHA while reducing the lipids content of ARA. According to the invention, the maximum of ARA lipids content is preferably reached in the developing seeds within 25 to 35 days after flowering where the plants of the present invention belong to species Brassica napus. Correspondingly late stage seed development preferably starts 38 days after flowering in Brassica napus, even more preferably 36 days and even more preferably 35 days after flowering. The skilled person understands that oilseed plants develop many flowers and that individual flowers start to bloom at different days. Thus, the term "days after flowering" refers to the days after flowering of the individual flower and not to the first flower detected on for example a field of plants of the present invention.

A plant or plant material according to the present invention preferably comprises a nucleic acid comprising

a) a Delta-5 elongase gene under the control of a promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development, and/or
b) a Delta-5 desaturase gene under the control of a promoter such that expression of the Delta-5 desaturase gene is reduced or prevented in late stage seed development.

The inventors have found that by carefully regulating the expression particularly of Delta-5 elongase activity it is possible to achieve the desired reduction in ARA lipids content while maintaining ongoing synthesis of EPA and/or DHA.

The promoters according to the present invention preferably are seed specific promoters. Gene expression can be regulated by any means available to the skilled person. For example, gene expression can be achieved by creating the appropriate construct topology such that transformed nucleic acids (also called "T-DNA" in the art) will, by their very own arrangement of promoters, genes and terminators (collectively also called "expression cassette") achieve the desired regulation pattern. For example, an expression cassette comprising a promoter and operably linked thereto a Delta-5 elongase gene located in the vicinity of another promoter exhibiting strong late stage seed development gene expression can allow for maintained or increased expression of the Delta-5 elongase gene in late stage seed development. This is particularly so where the expression cassette comprising the Delta-5 elongase gene is separated from one border of integrated T-DNA by at most one expression cassette and from the other border of the T-DNA by at least 5 expression cassettes. This way the T-DNA is long enough to effectively insulate the expression cassettes of the T-DNA from, teen effects of the plant chromosome the T-DNA has integrated into. Preferably, the expression cassette comprising the Delta- 5 the gene is separated from one border of the T-DNA by at most 3, more preferably 1 or 2 and even more preferably by 1 other expression cassette. For the purposes of the present invention, the expression cassette is preferred to in this paragraph contain genes required for the synthesis of polyunsaturated fatty acids and particularly genes coding for desaturases and elongases.

Increased Delta-5 elongase gene expression can also be achieved by the action of an inductor, such that at least one Delta-5 elongase gene is under the control of an inducible promoter; increase can also be achieved by removal of a repressor, such that the repressor is only being produced during early stages of seed development. Preferably, at least one Delta-5 elongase gene is additionally present and expressed under the control of a constitutively and strongly active promotor to achieve a high Delta-5 elongase gene expression also in early and mid seed development stages.

Decreased Delta-5 desaturase expression can be correspondingly achieved by T-DNA topology and/or by placing a Delta-5 desaturase gene under the control of an inducible promoter, wherein the inductor is not or to a lesser extent produced during late seed development, and/or by placing a Delta-5 desaturase gene under the control of a repressible promoter wherein the repressor is produced predominantly or only during late stage seed development.

Examples of corresponding promoters, inductors and repressors and their interaction are described in Hull et al., Plant Science 114, 181-192, Fujiwara et al., Plant Molecular Biology 20, 1059-1069 and Vilardell et al., Plant Molecular Biology 24, 561-569, all incorporated herein by reference.

The invention also provides seeds of a plant of the present invention. Such seeds are useful for planting of new plants of the present invention to produce polyunsaturated fatty acids. Seeds of the present invention are also useful for extraction purposes to obtain and extracted plant lipid composition of the present invention. In each case, the benefits described above can be achieved by the seeds of the present invention.

The invention also provides a plant lipid composition obtainable or obtained by a process comprising the steps of

a) growing a plant of the present invention at least until the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased, and
b) harvesting the plant or a part thereof and
c) extracting lipids composition from the harvested material to obtain said lipid composition.

In such process, the beneficial reduction of ARA content in plant lipids provided for by the present invention can be achieved and the corresponding benefits for plant lipid compositions can be materialised.

The process optionally also comprises the step of storing of harvested material, preferably of plant seeds. It is a particular advantage of the present invention that the plant seeds can be stored without compromising the amount and composition of plant seed oils and lipids. This was particularly surprising because polyunsaturated fatty acids are particularly prone to oxidation. Thus, it is advantageous that the plant seeds according to the present invention obtained as harvested material in said process can be stored for example for a month or at least for 7 days at ambient temperatures without loss of seed oil content and particularly without decrease of EPA and/or DHA in seed lipids and seed oil.

The process preferably also comprises the steps of threshing and collecting of seats. Particularly for plants of genus Brassica that a seeds are produced in house gutter and thus need to be separated from unwanted plant material. It is an advantage it is an advantage of the present invention that the seeds can be separated from unwanted plant material for example by threshing without compromising polyunsaturated fatty acid amount and composition in seed lipids and seed oil.

In the process, extraction preferably is performed using pressure and most preferably under an atmosphere with reduced oxygen content compared to ambient temperature; preferably, extraction is performed in the absence of oxygen, for example under a protective atmosphere. Corresponding extraction procedures are known to the skilled person, some extraction procedures are also described herein.

In the process harvesting of plant materials and preferably harvesting of seeds is preferably effected on ripe seeds, that is in late stage seed development. In ripe seeds the lipids content of ARA has had enough time to decrease and the contents of EPA and/or DHA could be increased. When such process is applied on plants of the invention of genus Brassica, harvesting is done preferably after 30 days after first flowering, preferably after 35 days, even more preferably after 40 days, even more preferably after 42 days, even more preferably on or after 43 days and even more preferably after or on 44 days and even more preferably on or after 45 and even more preferably on or after 46 days after first flowering of the plants.

The process preferably further comprises degumming, deodorising, bleaching, decolourising, drying, winterizing and/or fractionating of the extracted lipids to obtain said lipid composition. This way unwanted impurities of the lipids and/or oil can be removed. Corresponding processes and techniques are known to the skilled person.

The invention also provides foodstuff or feedstuff comprising a lipid composition of the invention. Such food- and feedstuff benefit from the high EPA and/or DHA lipids content and low ARA lipids content achieved by the present invention.

Correspondingly, the invention also provides a method of altering plant lipids composition, comprising the step of growing a plant pf the present invention to produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased while preferably the content of EPA and/or DHA has increased. As described above, the content of ARA is preferably decreased by at least 0.5% and preferably is finally at most 4%, preferably is at most 3% and even more preferably at most 2.6% by weight of total lipids. Also as described above, the content of EPA is preferably increased by at least 1% and preferably is finally at least 7%, even more preferably at least 7.5% of total lipids.

The invention also provides a method of producing a plant lipid composition, comprising the steps of

a) growing plants of the present invention,
b) harvesting the plants or a part thereof when the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased.

The method allows to materialize the advantages and benefits described herein.

Preferably, Brassica plants of the present invention are grown on a field of commercial scale, preferably at least one acre of size. After 25 days after first appearance of flowers, samples of developing seeds and their lipids are analysed as described herein. Over the next 15 days, preferably over the next 10 days, at least two additional samples of developing seeds are taken and their lipids are also analysed. This way the peak of ARA lipids content can be detected and harvesting can be appropriately delayed to allow the plants of the invention to decrease ARA content and increase EPA and/or DHA content in the lipids of developing seeds.

Also, the invention provides a method of producing seed, comprising the steps of

a) growing plants of the present invention, and
b) harvesting seeds of the plants when the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased.

The method allows to materialize the advantages and benefits described herein.

The invention is hereinafter further described by way of examples; the examples are provided for illustrative purposes only and are not intended to limit the invention or the scope of the claims.

EXAMPLES

Example 1: Plant Growth and Sampling

Homozygous T3 plants of event LBFLFK (containing a two copies VC-LTM593-1qcz rc), homozygous T3 plants of event LBFGKN (containing one copy of VC-LTM593-1qcz rc), homozygous T4 plants of event LANPMZ (containing one copy each of VC-LJB2197-1qcz and VC-LLM337-1qcz rc) and homozygous T4 plants of event LAODDN (containing one copy each of VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc) were sown in the field. Plants of the events were obtained and propagated as described in the examples of the priority documents; these are included herein by reference. All events comprise one gene coding for a Delta-5 elongase based on that obtained from Ostreococcus tauri (“d5Elo OT_GA3”). All events further contain one gene coding for a Delta-5 desaturase based on that obtained from Thraustochytrium sp. (“d5Des Tc_GA2”). Events LBFLFK and LBFGKN contain a further copy of the Delta-5 desaturase gene under the control of another promoter (SETL instead of Conlinin). In the week following the date of first flower, individual racemes were visibly marked on the stem just above the most recently opened flower. For every raceme, the three pods immediately below the mark were considered to be the same age (i.e. flowered or were pollinated on the same day). Starting at 14 days after marking and until 46 days after marking, the three pods below the mark on each raceme were collected at various time points. At each time point, approximately 150 pods from 50 individual plants were sampled. Each individual plant was sampled only once in its lifespan. Immature seeds were dissected from the pods immediately after removal from the raceme and were promptly frozen on dry ice. The age of the seeds was determined by the age of the mark on the raceme, meaning that the three pods (and the seeds inside) taken from immediately below a 15 day-old mark were assumed to be 15 days after flowering. For each event, at each time point, seeds from about 150 pods were pooled into a single sample. For analysis, each seed sample was pulverized to powder while still frozen, and the powder was dispensed into aliquot amounts to be used as technical replicates for lipid analysis and gene expression analysis by quantitative real time PCR.

Example 2: Lipid extraction and lipid analysis of plant oils

Lipids were extracted as described in the standard literature including Ullman, Encyclopedia of Industrial Chemistry, Bd. A2, S. 89-90 und S. 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Bd. 17; Rehm et al. (1993) Biotechnology, Bd. 3, Kapitel III: "Product recovery and purification", S. 469-714, VCH: Weinheim; Belter, P.A., et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., und Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., und Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Bd. B3; Kapitel 11, S. 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications.

It is acknowledged that extraction of lipids and fatty acids can be carried out using other protocols than those cited above, such as described in Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940, and Browse et al. (1986) Analytic Biochemistry 152:141-145. The protocols used for quantitative and qualitative analysis of lipids or fatty acids are described in Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 S. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) u.d.T.: Progress in the Chemistry of Fats and Other Lipids CODEN.

The fatty acids analysed were: 14:0, 16:0, 16:1n-7, 16:1n-9, 16:3n-3, 17:0, 18:0, 18:1n-7, 18:1n-9, 18:2n-6 (LA), 18:2n-9, 18:3n-3 (ALA), 18:3n-6 (GLA), 18:4n-3 (SDA), 20:0, 20:1n-9, 20:2n-6, 20:2n-9, 20:3n-3, 20:3n-6 (DGLA), 20:3n-9, 20:4n-3 (ETA), 20:4n-6 (ARA), 20:5n-3 (EPA), 22:0, 22:1n-9, 22:2n-6, 22:4n-3, 22:4n-6, 22:5n-3 (DPA), 22:5n-6, 22:6n-3 (DHA), 24:0, 24:1n-9.

The content (levels) of fatty acids is expressed throughout the present invention as percentage (weight of a particular fatty acid) of the (total weight of all fatty acids).

Example 3: Quantitative real time PCR protocol

RNA was extracted according to the protocol "SG-MA_0007-2009 RNA isolation" using Spectrum Plant Total RNA-KIT part number STRN50 (SIGMA-ALDRICH GmbH, Munich, Germany). In average the concentration of total RNA was about 450ng/µl. The 260/280 ratio was at 2.2 and the 260/230 ratio at 2.3.

For cDNA synthesis for qPCR 1µg of total RNA was treated with DNAseI (DEOXYRIBUNUCLEASE I (AMP-D1, Amplification Grade from SIGMA-Aldrich, GmbH) according to the supplier's protocol. After DNAseI treatment, the reverse transcription reaction was performed with the SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR

(Invitrogen, Cat. No. 11752-250) and with a combination of oligo dT and random hexamers to ensure thorough and even representation of all transcripts, regardless of length.

Transcript measurement by quantitative real time PCR was carried out using procedures considered standard to those skilled in the art; see Livak and Schmittgen (2001). The qPCR reactions were done as simplex TaqMan reactions. The endogenous reference gene was isolated in house and used due to predicted stability of the transcript based on the observed stability of the transcript corresponding to the orthologue in *Arabidopsis thaliana* during development. The brassica napus ortholog was isolated and the gene, SEQ ID, was part of the glycosyl-phosphatidylinositol aminotransferase pathway (GPI). The cDNA reactions, described above, were diluted 1:4. 2µl cDNA, which corresponded to 25ng of total RNA, was used per 10µl qPCR reaction with JumpStart TAQ ReadyMix (P2893-400RXN Sigma-Aldrich, GmbH). Primer/probe concentrations were 900 nmol for forward and reverse primer and 100nmol TaqMan probe. The TaqMan probes for targets of interest were labeled with FAM/BHQ1, and the reference gene was labeled with Yakima Yellow/BHQ1.

Each qPCR assay included a 1:1 dilution curve (5 dilution steps) with cDNA from the pool VC-RTP10690-1qcz_F, a no template control, three -RT controls (VC-RTP10690-1qcz_F, VC-LTM593-1qcz rc (~4w) and co-transformation VC-LJB2197-1qcz + VC-LLM337-1qcz rc). From each pool three independent aliquots of cDNA were measured as technical repeats. The ABI PRISM® 7900 sequence detection system (Applied Biosystem) was used with the following PCR Conditions:

Initial denaturation	95°C for 300 seconds	1 cycle
Amplification	95°C for 15 seconds/60°C for 60 seconds	repete for 40 cycles

The raw data were the Ct values for the target and the endogenous reference gene, respectively. The dCt values were calculated by subtraction: Ct(GOI)-Ct(Ref). The Reference dCt value was set to equal zero, which was interpreted as meaning that if there was no difference between GPI and the gene of interest (dCt = 0) the expression was = 1. The fold expression was equal to 2^{-dCt} (where the dCt = (Ct(GOI)-Ct(Ref)-0)). Three samples from each pool were taken and the geometric mean was calculated. The slopes of dilution curves were calculated for each gene of interest and the endogenous reference gene (GPI) as a measure for the amplification efficiency. Table PCR1, Table PCR2 and Table PCR3 indicate the probes and primers used to amplify the genes for qPCR assays.

Table PCR1: Probes used in the qPCR reactions

Target of Interest	Probe Name	Probe Oligo

D12Des(PS-GA)	D12DESPS-138Fam	TGCCTGGATACCTCTTCTTCAACGCTACTG
d6-Des(Otfebit)	D6DES-653FAM	ACTCCATGCACAACAAGCACCACGC
d6Elo (Pp GA)	D6Elo-296-FAM	TGTGCGTGGGTATCGCTTACCAAGC
d6Elo(Tp GA)	D6Elo-280-FAM	AGGAACGGATACACCGTTATGCCATGC
d5DES(Tc_GA)	D5DES-652-FAM	TTGGAGCACGATGTGGATTTGA
d5DES(Tc_GA)3’	D5DES-1147-Fam	CAACCGCTCCACAATTCAGGTTCAAGG
o3DES(Pi_GA2)	o3DES-594FAM	CGCTCACTTCTTCGTTGCTGGACTCTC
o3DES(PIR_GA)	o3DESPIR-198FAM	ATCATCTCTCTCGGAGTTC
d5Elo(Ot_GA3)	E011	TGACAAACAAGCCACCAAGCCCAA
d4DES(TC_GA)	D4DES-Tc-FAM	TGCTTCCCCAATGTACGTTGCTAGGTTCT
d4Des(Eg_GA)	D4DES-Eg-FAM	AAGGCACATCCTCC
d4Des(Pl_GA2)	D4DES-Pl-770FAM	AGCTTCTTTTCTTGGACGCCCTTGAGC
GPI	Exp3-78-YAK	GGATTCGACATTCCATCGGCTTTGA

Table PCR2: Forward primers used in qPCR

Target of Interest	Forward Primer Name	Forward Primer Oligo
D12Des(PS-GA)	D12DESPS-112F	CGTGTACATGTTGGTTGTTGGAT
d6-Des(Otfebit)	D6DES-629F	TGGCTGGATCTGGAGATATGTG
d6Elo (Pp GA)	D6Elo-271F	TTCTGCTTCGCTTTGTCTCTTTAC
d6Elo(Tp GA)	D6Elo-259F	GAGGCTGGATTCCTCGCTTA
d5DES(Tc_GA)	D5DES-631Fa	CACCACGCTGCTCCAAACAG
d5DES(Tc_GA)3’	D5DES-1120F	ACTTCCAAATCGAGCACCACTT
o3DES(Pi_GA2)	o3DES-572F	CCGCTGTGGTTATCTCTTTGC
o3DES(PIR_GA)	o3DESPIR-160F	CTTGGGAGGCTATGTATGTTAGAAGA
d5Elo(Ot_GA3)	MA54	GCAATCGTTGGTAGCCATGA
d4DES(TC_GA)	D4DES-Tc-F	CAAATCGATGCTGAGTGCAGAT
d4Des(Eg_GA)	D4DES-EG-F	TGACAAGTAAGCCATCCGTCAGT
d4Des(Pl_GA2)	D4DES-Pl-746-F	CTGGTGAGGCTATGTACGCTTTT
GPI	Exp 3-52F	GATGAATATCCTCCTGATGCTAACC

Table PCR3: Reverse primers used for qPCR

Target of Interest	Reverse Primer Name	Reverse Primer Oligo
D12Des(PS-GA)	D12DESPS-201R	TGAGACCTAGACTTTCCCCAGTACTT
d6-Des(Otfebit)	D6DES-706R	CCATATCGTGCCTCACTTTTTG
d6Elo (Pp GA)	D6Elo-345R	CCACAAGGAATATCTCCAGGTGAT
d6Elo(Tp GA)	D6Elo-330R	TGGATCGTTCACGTTGAAGTG
d5DES(Tc_GA)	D5DES-695R	AAAGCAACGAGTGGCAAGGT
d5DES(Tc_GA)3’	D5DES-1200R	AGAGAGCCTCAACTCTTGGAGAGA
o3DES(Pi_GA2)	o3DES-652R	TCTTAAGTCCCAACTGGAGAGACA
o3DES(PIR_GA)	o3DESPIR-262R	AAACCAAGGAGCGTCAAGTCTAGA
d5Elo(Ot_GA3)	MA55	CGTGTACCACCACGCTTTGT
d4DES(TC_GA)	D4DES-Tc-988R	AACACGGTCAAAGCCTTCATAATC
d4Des(Eg_GA)	D4DES-Eg-R	ACTTTTCACCACCGACGAAGTT
d4Des(Pl_GA2)	D4DES-Pl-817R	CCTCCCACCTCCAAGCAA
GPI	Exp 3-128R	CTTGCATGATGATCAGGAAAGC

Example 4:

According to the procedures in example 3 mRNA concentrations in seed were determined for each event at various times after flowering. Tables QPCR1 and QPCR2 describe the amounts of mRNA coding for Delta-5 elongase and Delta-5 desaturase genes, respectively. Missing values indicated that no measurements were taken at the respective day for the plants of the respective event. The mRNA concentrations are given in arbitrary units; within each table QPCR1 and QPCR2 the values are thus commensurate; absolute values cannot be compared within tables but comparisons can be validly made for tendencies and trends.

Table QPCR1 shows that expression of the only Delta-5 elongase gene of the events LBFGKN and LBFLFK continued even after 30 days after flowering, whereas expression of the Delta-5 elongase gene of the events LANPMZ and LAODDN was severely reduced or only marginally detectable after 30 days of flowering. Table QPCR2 shows that for all events clearly detectable Delta-5 desaturase mRNA could be detected at all assay dates.

Table QPCR1: Total Delta-5 elongase (d5Elo(Ot_GA3)) mRNA quantity, assay-specific units

	event			
Days after flowering	LANPMZ	LAODDN	LBFGKN	LBFLFK
14	13,3	14,5	11,0	20,7
17		55,7	10,1	6,0
18	15,7			

21	38,8	66,8	29,4	55,5
24		53,6	9,5	40,1
25	19,7			
28	15,7	26,6	10,0	45,1
31		10,6	13,4	23,6
32	0,9			
35	0,9	0,8	10,5	17,9
38		9,0	10,2	13,3
39	0,5			
42	1,3	1,7	19,1	
45		0,9	10,4	30,8
46	1,5			35,7

Table QPCR2: Total Delta-5 desaturase mRNA quantity, assay-specific units

	Event			
Days after flowering	LANPMZ	LAODDN	LBFGKN	LBFLFK
14	55,0	72,7	80,9	124,4
17		168,0	98,1	45,0
18	70,5			
21	199,2	364,7	302,5	292,6
24		308,6	453,4	722,4
25	388,1			
28	615,8	864,2	440,8	1767,1
31		2072,5	763,8	1076,8
32	996,8			
35	452,9		578,6	558,3
38		2987,3	391,5	302,6
39	369,1			
42	497,4	914,4	602,8	
45		679,0	472,5	762,9
46	385,7			1396,4

Example 5: Lipids composition data

The composition of seed lipids of the events was analysed as described above in example 2. As can be seen in Table FA1, the content of ARA in total extracted seed lipids of events LANPMZ and LAODDN did not significantly decrease over time, whereas the content of ARA ARA in total extracted seed lipids of events LBFGKN and LBFLFK decreases by 0.53 % and 0.72%, respectively. Table FA2 shows that EPA content continued to increase in total extracted seed lipids for all events; Table FA3 shows that also DHA content increased in total extracted seed lipids for all events.

Table FA4 summarizes the seed lipids compositions in the last extracts obtained for each event. The table shows that only for events LBFGKN and LBFLFK a difference in EPA and ARA content of more than 5% could be achieved and a difference in (EPA+DHA) and ARA content of more than 7% could be achieved.

Table FA1: ARA content of seed lipids

	event			
Days after flowering	LANPMZ	LAODDN	LBFGKN	LBFLFK
14	0,1	0,1	0,2	0,2
17		0,3	0,3	0,6
18	0,9			
21	2,0	0,9	1,2	1,9
24		1,2	1,8	2,5
25	2,8			
28	3,1	1,5	2,6	3,0
31		1,5	3,0	3,3
32	3,3			
35	3,6	1,48	2,8	3,1
38		1,4	2,6	2,8
39	3,6			
42	3,6	1,4	2,6	
45		1,3	2,5	2,6
46	3,6			2,5

Table FA2: EPA contents of seed lipids

	event			
Days after flowering	LANPMZ	LAODDN	LBFGKN	LBFLFK
14	0,1	0,2	0,1	0,0
17		0,4	0,4	1,0

18	0,7			
21	1,7	1,6	2,0	3,3
24		2,4	3,2	4,8
25	3,2			
28	3,9	3,2	4,7	6,2
31		4,0	6,1	7,5
32	4,7			
35	5,2	4,53	6,7	7,8
38		4,6	6,8	8,1
39	5,3			
42	5,5	4,9	7,3	
45		4,5	7,6	8,3
46	5,6			8,6

Table FA3: DHA seeds lipid content

	event			
Days after flowering	LANPMZ	LAODDN	LBFGKN	LBFLFK
14	0,0	0,0	0,0	0,0
17		0,0	0,1	0,2
18	0,1			
21	0,3	0,2	0,4	0,4
24		0,3	0,5	0,6
25	0,5			
28	0,7	0,5	0,8	0,8
31		0,7	1,1	1,0
32	0,9			
35	1,1	0,87	1,4	1,1
38		1,0	1,4	1,2
39	1,2			
42	1,3	1,0	1,6	
45		0,9	1,7	1,3
46	1,3			1,4

Table FA4: composition of last lipids extract obtained for each event

	Event			
Content of:	LANPMZ	LAODDN	LFGKN	LBLFK

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EPA	5,55	4,54	7,64	8,57
DHA	3	2,46	2,39	3,61
ARA	3,59	1,26	2,47	2,54
EPA-ARA	1,96	3,28	5,17	6,03
(EPA+DHA)-ARA	4,96	5,74	7,56	9,64

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word “comprise” or variations such as “comprises” or “comprising” is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

0-1	**Form PCT/RO/134 Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared Using	PCT Online Filing Version 3.5.000.244e MT/FOP 20141031/0.20.5.20
0-2	**International Application No.**	PCT/EP2015/076630
0-3	**Applicant's or agent's file reference**	BPS150220PC

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	**page**	7
1-2	**line**	10
1-3	**Identification of deposit**	
1-3-1	Name of depositary institution	ATCC American Type Culture Collection (ATCC)
1-3-2	Address of depositary institution	10801 University Boulevard, Manassas, Virginia 20110-2209, United States of America
1-3-3	Date of deposit	04 November 2014 (04.11.2014)
1-3-4	Accession Number	ATCC PTA-121703
1-5	**Designated States for Which Indications are Made**	All designations
2	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
2-1	**page**	7
2-2	**line**	13
2-3	**Identification of deposit**	
2-3-1	Name of depositary institution	ATCC American Type Culture Collection (ATCC)
2-3-2	Address of depositary institution	10801 University Boulevard, Manassas, Virginia 20110-2209, United States of America
2-3-3	Date of deposit	31 July 2015 (31.07.2015)
2-3-4	Accession Number	ATCC PTA-122340
2-5	**Designated States for Which Indications are Made**	All designations

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0-4	**This form was received with the international application:** (yes or no)	YES
0-4-1	Authorized officer	Wilson, Patrick

Claims

1. Transgenic *Brassica napus* plant or part thereof, comprising
   a) lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), and one or both of:
   b) a Delta-5 elongase gene under the control of a FAE promoter such that expression of the Delta-5 elongase gene is maintained or increased in late stage seed development,
   c) a Delta-5 desaturase gene under the control of a promoter such that expression of the Delta-5 desaturase gene is reduced or prevented in late stage seed development,

   wherein, when the transgenic *Brassica napus* plant or part thereof is grown, the content of ARA decreases at least 0.5% by weight of total lipids while preferably the content of EPA and/or DHA increases.

2. Transgenic *Brassica napus* plant or part thereof according to claim 1, wherein the content of EPA increases at least 1.0% by weight of total lipids.

3. Transgenic *Brassica napus* plant or part thereof according to claim 1 or 2, wherein when the transgenic *Brassica napus* plant or part thereof is grown,
   a) the content of EPA is at least 5% higher than of ARA, and/or
   b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or
   c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%.

4. Seed of a transgenic *Brassica napus* plant according to any of claims 1 to 3.

5. *Brassica napus* plant lipid composition obtained by a process comprising the steps of
   a) growing a transgenic *Brassica napus* plant according to any of claims 1 to 3 at least until the lipids content of ARA has decreased and preferably the lipids content of EPA and/or DHA has increased, and
   b) harvesting the plant or a part thereof and

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c) extracting lipids composition from the harvested material to obtain said lipid composition.

6. *Brassica napus* plant lipid composition according to claim 5, wherein the process further comprises degumming, deodorising, bleaching, decolourising, drying, winterizing and/or fractionating of the extracted lipids to obtain said lipid composition.

7. Extracted *Brassica napus* plant lipid composition comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and optionally arachidonic acid (ARA), wherein

a) the content of EPA is at least 5% higher than of ARA, and/or

b) the sum of contents of EPA+DHA is at least 7% higher than ARA and/or

c) the content of ARA is less than 4% and the content of EPA is more than 7% and the content of DHA is more than 2%,

wherein the lipid composition is extracted from the *Brassica napus* plant of any one of claims 1 to 3 or the seed of claim 4, or is obtained according to the process of claim 5 or 6.

8. Foodstuff or feedstuff comprising a *Brassica napus* plant lipid composition according to any of claims 5 to 7.

9. Method of altering plant lipids composition, comprising the step of growing a transgenic *Brassica napus* plant according to any of claims 2 to 4 to produce lipids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and arachidonic acid (ARA), wherein the step of growing and lipids production is continued until the content of ARA has decreased at least 0.5% by weight of total lipids during seed development while the content of EPA and/or DHA has increased and the content of EPA has increased at least 1.0% by weight of total lipids.

10. Method of producing a *Brassica napus* plant lipid composition, comprising the steps of

a) growing transgenic *Brassica napus* plants according to any of claims 2 to 4,

b) harvesting the plants or a part thereof when the lipids content of ARA has decreased at least 0.5% by weight of total lipids during seed development while the lipids content of EPA and/or DHA has increased and the content of EPA has increased at least 1.0% by weight of total lipids.

11. Method according to claim 10, further comprising the steps of

i) detecting the peak of ARA content in developing seeds prior to harvesting,

ii) extracting the lipid composition from said seeds following harvesting,

wherein late stage seed development starts 38 days after flowering.

12. Method according to any one of claims 9 to 11, wherein ARA decreases by from at most 4 wt.-% to at most 3.5 wt.-%, while EPA increases by from at least 6 wt.-% to at least 7 wt.-%.