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(54) **OIL FROM SEEDS WITH A MODIFIED FATTY ACID COMPOSITION**

AUS SAATEN GEWONNENES ÖL MIT MODIFIZIERTER FETTSÄUREZUSAMMENSETZUNG

HUILE PROVENANT DE GRAINES A TENEUR EN ACIDES GRAS MODIFIEE

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(73) Proprietor: **Consejo Superior de Investigaciones Cientificas**
41012 Sevilla (ES)

(72) Inventors:
- **MARTINEZ-FORCE, Enrique**
  E-41008 Sevilla (ES)
- **PEREZ-VICH, Begona**
  E-41926 Mairena del Aljarafe (ES)
- **FERNANDEZ-MARTINEZ, José Maria**
  E-14012 Córdoba (ES)
- **GARCES, Rafael**
  E-41907 Valencia de la Concepción (ES)

(74) Representative:
Van Someren, Petronella F. H. M. et al
Arnold & Siedsma,
Advocaten en Octrooigemachtigden,
Sweelinckplein 1
2517 GK Den Haag (NL)

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- **DATABASE FSTA [Online] INTERNATIONAL FOOD INFORMATION SERVICE (IFIS), FRANFURT/MAIN, DE; FERNANDEZ-MARTINEZ J M ET AL: "Sunflower mutant containing high levels of palmitic acid in high oleic background." Database accession no. 1998-03-n0113 XP002150056 & EUPHYTICA 1997 INST. DE AGRIC. SOSTENIBLE, CSIC, APDO. 4084, 14080 CORDOBA, SPAIN, vol. 97, no. 1, pages 113-116,**

Description

[0001] The present invention relates to oil from seeds having a palmitic acid content of more than 20% and less than 40% by weight based upon the total fatty acid content, while the palmitoleic acid content is less than 4% based upon the total fatty acid content and the asclepic (n-7 isomer of oleic acid) acid content is less than 4%.

[0002] In particular, the invention relates to sunflower oil comprising an oleic acid content of more than 5% and less than 65%, by weight based upon the total fatty acid content, a linoleic acid content less than 65% by weight based upon the total fatty acid content, a palmitic acid content of more than 20% and less than 40% by weight based upon the total fatty acid content, a stearic acid content of more than 3% and less than 15% based upon the total fatty acid content, while the palmitoleic acid content is less than 4% upon the total fatty acid content and the asclepic acid content is less than 4% based upon the total fatty acid content.

[0003] The oil of the invention may be used for applications in the food industry which require high thermostability or plastic fats.

BACKGROUND OF THE INVENTION

[0004] Sunflower is generally cultivated for obtaining oil which has saturated fatty acids (palmitic and stearic) and unsaturated fatty acids (oleic and linoleic), the stearic acid content is always less than 10% (Gunstone, F.D. et al. "The lipid handbook"; Chapman and Hall, 1986), normally comprised between 3% and 7%. In relation with the unsaturated fatty acids there are two different kinds of sunflower seeds: the normal sunflower which has a linoleic acid content between 50% and 70% (Knowles, P.F. "Recent advances in oil crops breeding"; AOCS Proceedings, 1988) and the high oleic sunflower which has 2-10% of linoleic acid and 75-90% of oleic acid (Soldatov, K.I. "Chemical mutagenesis in sunflower breeding"; Int. Proc. 7th Intern. Sunflower Conference, 352-357, 1976). Another high oleic sunflower line has been referred by Fick (US-B1-4,627,192), with an oleic acid content of approximately 80% or greater.

[0005] Referring to saturated fatty acids, high stearic sunflower lines are disclosed in WO 95/20313. Further references to sunflower lines with high palmitic acid content are WO 96/39804 (corresponding to US-A-5 710 366) and Retske et al. "Triacylglycerol composition and structure in genetically modified sunflower and soybean oils"; JAOCS 74, 989-998 (1997), European Patent Appl. 98201871.5 and Nikolova et al. "Gametocidal effect of gibberellic acid (GA_3) on biochemical characteristics of sunflower seeds", Helia 15, Nr. 17, 45-50, (1992). In all these lines, the increase in palmitic acid implies higher palmitoleic acid content, always over 4%, and where the two "cis" isomers n-7 octadecenoic acid (asclepic) and n-9 octadecenoic (oleic) acid have been analyzed, an increase in the n-7 (asclepic acid) isomer has been observed.

[0006] Table 1 shows the fatty acid composition for all this indicated sunflower oil varieties.

Table 1

Reference	Line	Fatty acids composition (%)								
		16:0	16:1	16:2	18:0	18:1	18:1A	18:2	20:0	22:0
Gunstone	Normal	6	-	-	5	18	-	68	-	1
	Oleic	5	-	-	4	88	-	2	-	1
Nikolova, 1992		29	5	*	2	9	*	55	*	*
Reske, 1997		25	6	*	3	60	*	4	-	2
		27	4	*	3	17	*	47	-	1
	CAS-5	31	5	1	3	5	6	47	-	1
	CAS-12	30	7	-	2	52	4	3	-	2

* These fatty acids were not determined in those papers.

- = Traces.

[0007] The saturated fatty acid content of an oil is directly related with the physical and chemical characteristics thereof. In case that said content is sufficiently high, the oil can be a solid at room temperature like some animal fats. Normal sunflower oil is always a liquid under said conditions. In the food industry like for the production of confectionery or margarine, animal fats or hydrogenated vegetable fats are usually used because a solid or semisolid product is required. By means of hydrogenation unsaturated fatty acids are converted into saturated fatty acids. Animal fats as well as hydrogenated fats are not very recommendable from a nutritional point of view (Chow, C.K. "Fatty acids in food and their health implications", Dekker, N.Y., 1992). Animal fats have a relatively high cholesterol content. Too much

cholesterol in the diet may be detrimental to the health. Therefore animal fats have been substituted in the last years by hydrogenated vegetable fats which do not contain cholesterol.

[0008] However, said hydrogenated fats present another problem derived from the hydrogenation process. In said process positional isomerization (shift of double bonds) and stereo-chemical transformations (formation of "trans" isomers) take place. Isomers are produced in an amount of up to 30%-50% of the total fatty acids amount. These isomers are not very healthy from a nutritional point of view (Wood, R. "Biological effects of geometrical and positional isomers of monounsaturated fatty acids in humans", Dekker, N.Y. 1990; Willet, W.C. & Ascherio, A., "Trans Fatty Acids: Are the effects only marginal?", American Journal of Public Health, Vol. 84, 5, 1994). Therefore, the use of hydrogenated fats in the food industry should be avoided.

[0009] As previously referred, the increase in palmitic acid implies higher palmitoleic acid contents, always over 4% (see WO 96/39804). These oils are useful for food industry which requires high thermostability, but the presence of the indicated palmitoleic acid contents is still undesirable. Studies carried out on macadamia oil, which has 4% palmitoleic acid content indicate a negative effect on plasmatic cholesterol when compared with palmitic and oleic (Nestel et al., "Effects of increasing dietary palmitoleic acid compared with palmitic and oleic acids on plasma lipids of hypercholesterolemic men", Journal Lipid Research, vol. 35, pp. 656-662, 1994). This oil has also higher asclepic acid contents (n-7 isomer of octadecanoic acid) than other normal sunflower oil that have 0.6% or other vegetable oils, like soybean or rape which have 0.8 and 0.9% respectively (Mukherjee K.D. and Kiewitt I., "Formation of (n-9) and (n-7) cis-monounsaturated fatty acids in seeds of higher plants", Planta, vol. 149, pp. 461-463.

[0010] It can be concluded that an oil having higher palmitic and stearic acid contents than normal sunflower oil, but maintaining reduced levels of palmitoleic and asclepic acids would meet all the requirements for food industry implying high thermostability and plasticity to be spread.

BRIEF DESCRIPTION OF THE INVENTION

[0011] The present invention relates to oil from seeds comprising an cleic acid content of more than 5% and less than 65% by weight based upon the total fatty acid content, a linoleic acid content of more than 1% and less than 65% by weight based upon the total fatty acid content, a palmitic acid content of more than 20% and less than 40% by weight based upon the total fatty acid content, a stearic acid content of more than 3% and less than 15% based upon the total fatty acid content, while the palmitoleic acid content is less than 4% based upon the total fatty acid content and the asclepic acid content is less than 4% based upon the total fatty acid content. Preferably, the palmitoleic acid content is less than 3% based upon the total fatty acid content and the asclepic acid content is less than 3% based upon the total fatty acid content.

[0012] The oil from seeds according to the present invention has an oleic acid content which is at least 40% by weight based upon the total fatty acid content and a linoleic acid content which is less than 18%. The total level of saturated fatty acids in the oil is at least 26%, preferably higher than 35%, most preferably higher than 45% based upon the total fatty acids content.

[0013] In reference to the position of the fatty acid groups in the triacylglycerols (TAG), the oil of the invention has less than 10%, preferably less than 5% by weight of the saturated fatty acid groups in the 2 position of the TAGs.

[0014] The invention in particular relates to sunflower oil, which is extracted from sunflower seeds obtained by crossing sunflower seeds of the mutant sunflower line IG-1297M deposited on 20 January 1998 with ATCC under deposit accession number ATCC-209591 with the mutant sunflower line CAS-3, deposited on 14 December 1994 with the ATCC under deposit accession number ATCC-75968.

[0015] The invention further relates to sunflower seeds comprising a sunflower oil with a fatty acid composition as referred herein above and to a method for preparing sunflower seeds, comprising the steps of:

a) crossing sunflower seeds of the mutant sunflower line IG-1297M deposited on 20 January 1998 with ATCC under deposit accession number ATCC-209591 with the mutant sunflower line CAS-3, deposited on 14 December 1994 with the ATCC under deposit accession number ATCC-75968;
b) self-pollinating F1 progeny plants of step a) for at least two generations to produce inbred plants;
c) selecting from the progeny of step b) plants with seeds containing an oil having a palmitic acid higher than 20%, palmitoleic acid content of less than 4% and an asclepic acid content of less than 3%;
d) collecting progeny seeds from step c) and optionally;
e) repeating the cycle of culturing, selection and collection of seeds.

[0016] The invention further relates to plants producing the seeds of the invention and to plants obtainable by growing the seeds of the invention and their progeny.

[0017] The sunflower oil, prepared by extracting said sunflower seeds may be used in roasting, cooking, frying, baking and in general at high temperature conditions which constitute heating by any means at temperatures of at

least 70°C. Said oil may also be used in the production of edible fats or fat mixtures, such as margarine, shortening or vegetable-dairy as well as in confectionery and bakery.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The method for preparing seeds with a modified fatty acid composition comprises mutagenesis of seeds with a suitable mutagenic agent. The mutagenesis will produce inheritable genetic changes in the DNA of the seeds. According to the invention it was possible after several different experiments to select some treatments that produce a high number of genetic modifications in the genes that control the seed fatty acid biosynthesis. These treatments comprise the use of sodium azide or an alkylating agent, like ethyl methane sulfonate. Of course any other mutagenic agent having the same or similar effects may also be used.

[0019] Then, the next seed generation was analyzed with a new methodology described in Garcés, R. and Mancha, M. "One step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues"; Analytical Biochemistry, 211:139-143, 1993. This allowed for the detection of seeds with modifications in the composition of any fatty acid. Selected seeds showing a desirable fatty acid composition have been cultivated to the fifth generation showing that this new genetic trait is inheritable and stable and independent of growth conditions. In the method of the invention the parent seeds are for example treated during 2 hours at room temperature with a solution of 70 mM ethyl methane sulfonate in water, or during 2 hours at room temperature with a solution of 2 mM sodium azide in water. Further, the mutation and selection steps may be followed by conventional plant improvement techniques thus leading to seeds having a desirable fatty acids content.

[0020] The seeds of the invention may be subjected to one or more further mutation treatments. Another way of obtaining mutagenized seeds consists of submitting the seeds to X-rays action, growing the treated seeds, self-pollination and further analysis of the fatty acid content. Further growing and selection steps will lead to plants with the desired new character fixed.

[0021] Sunflower oil having the desirable fatty acid composition may be prepared by extraction from sunflower seeds of the invention in any manner known to the person skilled in the art. Such extraction methods are well known and for example described in "Bailey's industrial oil and fat products", Vol.2, Chapter 3; 4th Edition, John Wiley and Sons, New York (1982).

[0022] By the referred methods seeds and oil having high stearic acid and high palmitic acid content can be obtained. High palmitic acid content normally implies high palmitoleic acid content which is not desirable from a nutritional point of view, as previously indicated. However, biochemical research on sunflower mutant lines indicates that the high stearic mutant has less stearoyl desaturase activity over palmitoyl-ACP than other sunflower mutant lines. Crossing a high stearic line (CAS-3) with a high palmitic line (CAS-12) and selecting in search of different fatty acid compositions, it turned out that in certain F2 generations that amounts of palmitoleic and asclepic acid decreased. Thus, the desaturation of palmitic into palmitoleic in the high palmitic acid mutants could be reduced introducing the stearoyl desaturase enzimatic activity of the high stearic mutant lines.

[0023] The invention is further illustrated by means of the following examples.

EXAMPLE 1

[0024] Sunflower seeds RDF-1-532 (Sunflower Collection of Instituto de Agricultura Sostenible, CSIC, Córdoba, Spain), which have 4% to 7% stearic acid content were mutagenized with a solution of 70 mM of ethyl methane sulfonate (EMS) in water. The treatment was performed at room temperature during 2 hours while shaking (60 rpm). After mutagenesis the EMS solution was discarded and seeds were washed during 16 hours under tap water.

[0025] Treated seeds were germinated in the field and plants were self-pollinated. The seeds collected from these plants were used to select new sunflower lines with modifications in the fatty acid composition. By using the method of Garcés, R. and Mancha, M, referred to herein above, the seed fatty acid composition was determined by gas-liquid chromatography, after converting the fatty acids into their corresponding methyl esters.

[0026] A first plant with 9 to 17% stearic acid content in the oil was selected. The progeny was cultivated for five generations wherein the stearic acid content increased and the new genetic trait became stably fixed in the genetic material of the seed. This line is called CAS-3. A selected sample of this line was analyzed resulting in a stearic acid content of 26% (Table 2). The minimum and the maximum stearic acid content of the line were 19 and 35%, respectively. The stearic acid content of oil extracted from seeds from this cell line may thus lie between 19 and 35%.

EXAMPLE 2

[0027] Sunflower seeds RDF-1-532 were mutagenized with sodium azide, at a concentration of 2 mM in water. The treatment was performed at room temperature during two hours while shaking. Then the mutagenesis solution was

discarded and seeds were washed during 16 hours with tap water.

[0028] Seeds were planted in the field and plants were self-pollinated. Seeds from these plants were collected, and the fatty acid composition was determined by gas-liquid chromatography, after converting the fatty acids into their corresponding methyl esters using the method described in Example 1. Seeds from a plant having around 10% stearic acid in the oil were selected and cultivated for five generations. During this procedure the stearic acid content was increased and the new genetic trait fixed. This line is called CAS-4. A selected sample of this line was analyzed resulting in a stearic acid content of 16.1%. The minimum and the maximum values were 12 and 19% respectively.

EXAMPLE 3

[0029] 5000 dry sunflower seeds were mutagenized by treatment with X-rays 300 cGy/min, beam 200 kV, 18 mA^{-1} and dose 160 Gy with a Siemens Stabilipan (Erlangen, Germany), seeds were grown in spring in the field. Self-pollinated plants were collected individually and seeds analyzed for their fatty acid content. Seeds with at least three times more saturated fatty acid content than the standard deviation for the specific fatty acid were selected and successively grown until the new character was fixed. Several putative new mutant lines were selected by this method. After further selection for triacylglycerol composition line IG-1297M was selected.

EXAMPLE 4

[0030] Sunflower plants were grown from the sunflower seeds of CAS-3 according to Example 1. Sunflower plants were grown from the sunflower seeds of IG-1297M according to Example 3.

[0031] The lines were crossed. The plants were assisted by artificial pollination in order to ensure adequate seed production occurred. The F1 was produced on the IG-1297M and harvested. F2 IG-1297M parent seeds with a high oleic acid background having more than 20% palmitic acid and less than 4% of both palmitoleic and asclepic acid were selected.

[0032] Although the oil produced by these selected lines is the oil of the present invention, the level of production is limited, therefore fixed inbred lines evidencing seeds with these oil profiles are desirable. These homozygous fixed high oleic, high palmitic, low palmitoleic, low asclepic inbred lines can then be crossed to form hybrid seed, which will produce F2 seed evidencing the desired oil traits of the present invention.

[0033] Toward this end the F1 seeds were planted and produced plants were selfed in isolated conditions and F2 seed was produced. The F2 seed, called QQ-3598-M was tested for the four traits: high palmitic, high oleic, low palmitoleic, and low asclepic. The remaining portion of the seeds evidencing these traits was employed to grow plants to form F3 seed. The selfing and screening and selection process is repeated to develop the fixed homozygous QQ-3598-M line, having the following fatty acid profile: C16:0 30,5%; C18:0 9,6%; C18:1 47,2%; C18:2 6,7%; C16:1 2,1%; C18:1A 1,1%, and less than 1% of other minor fatty acids.

[0034] Once the trait is fixed, similar QQ-3598-M lines can cross to form hybrid seed having the desired traits. This characteristic fatty acid profile is an inheritable trait and is fairly independent from the growing conditions.

Claims

1. Sunflower seeds that contain an oil having an oleic acid content of more than 5% and less than 65% by weight based upon the total fatty acid content, a linoleic acid content of more than 1% and less than 65% by weight based upon the total fatty acid content, a palmitic acid content of more than 20% and less than 40% by weight based upon the total fatty acid content, a stearic acid content of more than 3% and less than 15% based upon the total fatty acid content, **characterized in that**

   - the palmitoleic acid content is less than 4% based upon the total fatty acid content; and
   - the asclepic acid content is less than 4% based upon the total fatty acid content.

2. Seeds according to claim 1, **characterized in that** the palmitoleic acid content in the oil is less than 3% based upon the total fatty acid content.

3. Seeds according to claim 1 or 2, **characterized in that** the asclepic acid content in the oil is less than 2% based upon the total fatty acid content.

4. Seeds according to claims 1-3, **characterized in that** the oleic acid content in the oil is at least 40% by weight based upon the total fatty acid content.

5. Seeds according to claims 1-4, **characterized in that** the total level of saturated fatty acids in the oil is at least 24% by weight based upon the total fatty acid content.

6. Seeds according to claim 5, **characterized in that** the total level of saturated fatty acids in the oil is at least 35% by weight based upon the total fatty acids content.

7. Seeds according to claim 6, **characterized in that** the total level of saturated fatty acids in the oil is at least 45% by weight based upon the total fatty acids content.

8. Seeds according to claims 1-7, **characterized in that** the linoleic acid content in the oil is less than 18% by weight based upon the total fatty acids content.

9. Seeds according to claims 1-8, **characterized in that** the oil has less than 10% by weight of the saturated fatty acid groups in the 2 position of the triacylglycerol molecules of the oil.

10. Seeds according to claim 9, **characterized in that** the oil has a maximum of 5% of the saturated fatty acid groups in the 2 position of the triacylglycerol molecules of the oil.

11. Seeds according to any one of the claims 1-10, obtainable by crossing sunflower seeds of the mutant sunflower line IG-1297M deposited on 20 January 1998 with ATCC under deposit accession number ATCC-209591 with the mutant sunflower line CAS-3, deposited on 14 December 1994 with the ATCC under deposit accession number ATCC-75968.

12. Sunflower oil having an oleic acid content of more than 5% and less than 65% by weight based upon the total fatty acid content, a linoleic acid content of more than 1% and less than 65% by weight based upon the total fatty acid content, a palmitic acid content of more than 20% and less than 40% by weight based upon the total fatty acid content, a stearic acid content of more than 3% and less than 15% based upon the total fatty acid content, **characterized in that** the palmitoleic acid content is less than 4% based upon the total fatty acid content, and; and the asclepic acid content is less than 4% based upon the total fatty acid content.

13. Oil according to claim 12, obtainable by extracting seeds as claimed in claims 1-11.

14. Sunflower plants producing the seeds as claimed in claims 1-11.

15. Plants, obtainable by growing seeds as claimed in claims 1-12.

16. Progeny of the plants according to claims 14-15.

17. Method for preparing sunflower seeds as claimed in 11, comprising the steps of:

a) crossing sunflower seeds of the mutant sunflower line IG-1297M deposited on 20 January 1998 with ATCC under deposit accession number ATCC-209591 with the mutant sunflower line CAS-3, deposited on 14 December 1994 with the ATCC under deposit accession number ATCC-75968;
b) self-pollinating F1 progeny plants of step a) for at least two generations to produce inbred plants.
c) selecting from the progeny of step b) plants with seeds containing an oil having a palmitic acid content of more than 20%, a palmitoleic acid content of less than 4% and an asclepic acid content of less than 3%.
d) collecting progeny seeds from step c) and optionally
e) repeating the cycle of self-pollination, selection and collection of seeds.

18. Method for preparing an oil according to claim 12-13, by extracting seeds as claimed in claims 1-11.

19. Use of oil according to claims 12-13 at high temperature conditions.

20. Use of oil as claimed in claim 19, wherein the high temperature conditions constitute baking.

21. Use of oil as claimed in claim 19, wherein the high temperature conditions constitute cooking.

22. Use of oil as claimed in claim 19, wherein the high temperature conditions constitute roasting.

23. Use of oil as claimed in claim 19, wherein the high temperature conditions constitute heating by any means at temperatures of at least 70°C.

24. Use of the oil according to claims 12-13 in the production of edible fats or fat mixtures, such as margarine or vegetable-dairy.

25. Use of the oil according to claims 12-13 in confectionery or bakery.

Patentansprüche

1. Sonnenblumensamen, die ein Öl mit einem Ölsäuregehalt von mehr als 5 Gew.-% und weniger als 65 Gew.-% auf Basis des Gesamtfettsäuregehaltes, einem Linolsäuregehalt von mehr als 1 Gew.-% und weniger als 65 Gew.-% auf Basis des Gesamtfettsäuregehaltes, einem Palmitinsäuregehalt von mehr als 20 Gew.-% und weniger als 40 Gew.-% auf Basis des Gesamtfettsäuregehaltes, einem Sterinsäureanteil von mehr als 3 Gew.-% und weniger als 15 Gew.-% auf Basis des Gesamtfettsäuregehaltes enthält, **dadurch gekennzeichnet, dass**

- der Palmitolsäuregehalt weniger als 4% auf Basis des Gesamtfettsäuregehaltes ist; und
- der Asclepialsäuregehalt weniger als 4% auf Basis des Gesamtfettsäuregehaltes ist.

2. Samen gemäß Anspruch 1, **dadurch gekennzeichnet, dass** der Palmitolsäuregehalt des Öls weniger als 3% auf Basis des Gesamtfettsäuregehaltes ist.

3. Samen gemäß Anspruch 1 oder 2, **dadurch gekennzeichnet, dass** der Asclepialsäuregehalt des Öls weniger als 2% auf Basis des Gesamtfettsäuregehaltes ist.

4. Samen gemäß den Ansprüchen 1-3, **dadurch gekennzeichnet, dass** der Ölsäuregehalt des Öls wenigstens 40 Gew.-% auf Basis des Gesamtfettsäuregehaltes ist.

5. Samen gemäß den Ansprüchen 1-4, **dadurch gekennzeichnet, dass** das Gesamtniveau der gesättigten Fettsäuren des Öls wenigstens 24 Gew.-% auf Basis des Gesamtfettsäuregehaltes ist.

6. Samen gemäß Anspruch 5, **dadurch gekennzeichnet, dass** das Gesamtniveau der gesättigten Fettsäuren des Öls wenigstens 35 Gew.-% auf Basis des Gesamtfettsäuregehaltes ist.

7. Samen gemäß Anspruch 6, **dadurch gekennzeichnet, dass** das Gesamtniveau der gesättigten Fettsäuren des Öls wenigstens 45 Gew.-% auf Basis des Gesamtfettsäuregehaltes ist.

8. Samen gemäß den Ansprüchen 1-7, **dadurch gekennzeichnet, dass** der Linolsäuregehalt des Öls weniger als 18 Gew.-% auf Basis des Gesamtfettsäuregehaltes ist.

9. Samen gemäß den Ansprüchen 1-8, **dadurch gekennzeichnet, dass** das Öl weniger als 10 Gew.-% der gesättigten Fettsäuregruppen in der 2-Position der Triacylglycerolmoleküle des Öls hat.

10. Samen gemäß Anspruch 9, **dadurch gekennzeichnet, dass** das Öl maximal 5% der gesättigten Fettsäuregruppen in der 2-Position der Triacylglycerolmoleküle des Öls hat.

11. Samen gemäß einem der Ansprüche 1-10, der durch Kreuzen von Sonnenblumensamen der mutanten Sonnenblumenlinie IG-1297M, die am 20. Januar 1998 bei der ATCC unter der Hinterlegungs-Akzessionsnummer ATCC-209591 hinterlegt wurde, mit der mutanten Sonnenblumenlinie CAS-3, die am 14. Dezember 1994 bei der ATCC unter der Hinterlegungs-Akzessionsnummer ATCC-75968 hinterlegt wurde.

12. Sonnenblumenöl mit einem Ölsäuregehalt von mehr als 5 Gew.-% und weniger als 65 Gew.-% auf Basis des Gesamtfettsäuregehaltes, einem Linolsäuregehalt von mehr als 1 Gew.-% und weniger als 65 Gew.-% auf Basis des Gesamtfettsäuregehaltes, einem Palmitinsäuregehalt von mehr als 20 Gew.-% und weniger als 40 Gew.-% auf Basis des Gesamtfettsäurgehalt, einem Sterinsäuregehalt von mehr als 3 Gew.-% und weniger als 15 Gew.-% auf Basis des Gesamtfettsäuregehaltes, **dadurch gekennzeichnet, dass** der Palmitolsäuregehalt weniger als 4% auf Basis des Gesamtfettsäuregehaltes ist und der Asclepialsäuregehalt weniger als 4% auf Basis des Ge-

samtfettsäuregehaltes ist.

13. Öl gemäß Anspruch 12, das durch Extrahieren der Samen wie in den Ansprüchen 1-11 beansprucht erhältlich ist.

14. Sonnenblumenpflanzen, die die Samen wie in den Ansprüchen 1-11 beansprucht produzieren.

15. Pflanzen, die durch Züchten von Samen wie in den Ansprüchen 1-12 beansprucht erhältlich sind.

16. Nachkommenschaft der Pflanzen gemäß den Ansprüchen 14-15.

17. Verfahren zur Herstellung von Sonnenblumensamen, wie in Anspruch 11 beansprucht, das die Schritte umfasst:

a) Kreuzen von Sonnenblumensamen der mutanten Sonnenblumenlinie IG-1297M, die am 20. Januar 1998 bei der ATCC unter der Hinterlegungs-Akzessionsnummer ATCC-209591 hinterlegt wurde, mit der mutanten Sonnenblumenlinie CAS-3, die am 14. Dezember 1994 bei der ATCC unter der Hinterlegungs-Akzessionsnummer ATCC-75968 hinterlegt wurde;
b) Selbstbefruchten von F1-Pflanzen der Nachkommenschaft nach Schritt a) für wenigstens zwei Generationen, um Inzuchtpflanzen herzustellen;
c) Auswählen von Pflanzen aus der Nachkommenschaft aus Schritt b) mit Samen die ein Öl mit einem Palmitinsäuregehalt von mehr als 20%, einem Palmitolsäuregehalt von weniger als 4% und einem Asclepiolsäuregehalt von weniger als 3% enthalten;
d) Sammeln der Samen der Nachkommenschaft aus Schritt c) und optional
e) Wiederholen des Zyklusses der Selbstbefruchtung, Auswahl und Sammlung der Samen.

18. Verfahren zur Herstellung eines Öls gemäß Anspruch 12-13 durch Extrahieren von Samen, wie in den Ansprüchen 1-11 beansprucht.

19. Verwendung eines Öls gemäß den Ansprüchen 12-13 unter Hochtemperaturbedingungen.

20. Verwendung eines Öls wie in Anspruch 19 beansprucht, wobei die Hochtemperaturbedingungen Backen darstellen.

21. Verwendung eines Öls wie in Anspruch 19 beansprucht, wobei die Hochtemperaturbedingungen Kochen darstellen.

22. Verwendung eines Öls wie in Anspruch 19 beansprucht, wobei die Hochtemperaturbedingungen Rösten darstellen

23. Verwendung eines Öls wie in Anspruch 19 beansprucht, wobei die Hochtemperaturbedingungen Erhitzen mit irgendwelchen Mitteln bei Temperaturen von wenigstens 70°C darstellen.

24. Verwendung des Öls gemäß den Ansprüchen 12-13 in der Herstellung von essbaren Fetten oder Fettmischungen wie z.B. Margarine oder pflanzlichen Milchprodukten.

25. Verwendung des Öls gemäß den Ansprüchen 12-13 in der Feinbäckerei oder Bäckerei.

Revendications

1. Graines de tournesol qui contiennent une huile à teneur en acide oléique supérieure à 5 % et inférieure à 65 % en poids par rapport à la teneur totale en acides gras, une teneur en acide linoléique supérieure à 1 % et inférieure à 65 % en poids par rapport à la teneur totale en acides gras, une teneur en acide palmitique supérieure à 20 % et inférieure à 40 % en poids par rapport à la teneur totale en acides gras, une teneur en acide stéarique supérieure à 3 % et inférieure à 15 % par rapport à la teneur totale en acides gras, **caractérisées en ce que** :

- la teneur en acide palmitoléique est inférieure à 4 % par rapport à la teneur totale en acides gras ; et
- la teneur en acide asclépique est inférieure à 4 % par rapport à la teneur totale en acides gras.

2. Graines selon la revendication 1, **caractérisées en ce que** la teneur en acide palmitoléique dans l'huile est infé-

rieure à 3 % par rapport à la teneur totale en acides gras.

3. Graines selon la revendication 1 ou 2, **caractérisées en ce que** la teneur en acide asclépique dans l'huile est inférieure à 2 % par rapport à la teneur totale en acides gras.

4. Graines selon les revendications 1 à 3, **caractérisées en ce que** la teneur en acide oléique dans l'huile atteint au moins 40 % en poids par rapport à la teneur totale en acides gras.

5. Graines selon les revendications 1 à 4, **caractérisées en ce que** le taux total en acides gras saturés dans l'huile atteint au moins 24 % en poids par rapport à la teneur totale en acides gras.

6. Graines selon la revendication 5, **caractérisées en ce que** le taux total en acides gras saturés dans l'huile atteint au moins 35 % en poids par rapport à la teneur totale en acides gras.

7. Graines selon la revendication 6, **caractérisées en ce que** le taux total en acides gras saturés dans l'huile atteint au moins 45 % en poids par rapport à la teneur totale en acides gras.

8. Graines selon les revendications 1 à 7, **caractérisées en ce que** la teneur en acide linoléique dans l'huile est inférieure à 18 % en poids par rapport à la teneur totale en acides gras.

9. Graines selon les revendications 1 à 8, **caractérisées en ce que** l'huile contient moins de 10 % en poids de groupes d'acides gras saturés en position 2 des molécules de triacylglycérol de l'huile.

10. Graines selon la revendication 9, **caractérisées en ce que** l'huile a une teneur maximale de 5 % de groupes d'acides gras saturés en position 2 des molécules de triacylglycérol de l'huile.

11. Graines selon l'une quelconque des revendications 1 à 10, pouvant être obtenues par croisement de graines de tournesol de la lignée mutante de tournesol IG-1279M déposées le 20 janvier 1998 avec ATCC sous le numéro d'accès ATCC-209591 avec la lignée mutante de tournesol CAS-3, déposée le 14 décembre 1994 avec ATCC sous le numéro d'accès ATCC-75968.

12. Huile de tournesol à teneur en acide oléique supérieure à 5 % et inférieure à 65 % en poids par rapport à la teneur totale en acides gras, à teneur en acide linoléique supérieure à 1 % et inférieure à 65 % en poids par rapport à la teneur totale en acides gras, à teneur en acide palmitique supérieure à 20 % et inférieure à 40 % en poids par rapport à la teneur totale en acides gras, à teneur en acide stéarique supérieure à 3 % et inférieure à 15 % par rapport à la teneur totale en acides gras, **caractérisée en ce que** la teneur en acide palmitoléique est inférieure à 4 % par rapport à la teneur totale en acides gras, et la teneur en acide asclépique est inférieure à 4 % par rapport à la teneur totale en acides gras.

13. Huile selon la revendication 12, pouvant être obtenue par extraction des graines comme revendiquées selon les revendications 1 à 11.

14. Plants de tournesol produisant les graines comme revendiquées selon les revendications 1 à 11.

15. Plants pouvant être obtenue en cultivant les graines comme revendiquées selon les revendications 1 à 12.

16. Descendants des plants selon les revendications 14 et 15.

17. Procédé de préparation des graines de tournesol selon la revendication 11, comprenant les étapes consistant à :

a) croiser les graines de tournesol de la lignée mutante de tournesol IG-1297M déposée le 20 janvier 1998 avec ATCC sous le numéro d'accès ATCC-209591 avec la lignée mutante de tournesol CAS-3, déposée le 14 décembre 1994 avec ATCC sous le numéro d'accès ATCC-75968 ;
b) auto-polliniser les plants des descendants F1 de l'étape a) pendant au moins deux générations pour produire des plants consanguins ;
c) sélectionner à partir des descendants de l'étape b) les plants dont les graines contiennent une huile dont la teneur en acide palmitique est supérieure à 20 %, la teneur en acide palmitoléique est inférieure à 4 % et la teneur en acide asclépique est inférieure à 3 % ;

d) recueillir les graines des descendants de l'étape c) et éventuellement ;
e) répéter le cycle d'auto-pollinisation, de sélection et de recueil des graines.

18. Procédé de préparation d'une huile selon les revendications 12 et 13, en extrayant les graines comme revendiquées selon les revendications 1 à 11.

19. Utilisation de l'huile selon les revendications 12 et 13 à des conditions de températures élevées.

20. Utilisation de l'huile comme revendiquée selon la revendication 19, dans laquelle la pâtisserie constitue les conditions de températures élevées.

21. Utilisation de l'huile comme revendiquée selon la revendication 19, dans laquelle la cuisson constitue les conditions de températures élevées.

22. Utilisation de l'huile comme revendiquée selon la revendication 19, dans laquelle la grillade constitue les conditions de températures élevées.

23. Utilisation de l'huile comme revendiquée selon la revendication 19, dans laquelle le chauffage par l'un quelconque des moyens à des températures atteignant au moins 70°C constitue les conditions de températures élevées.

24. Utilisation de l'huile selon les revendications 12 et 13 dans la production de matières grasses ou mélanges de matières grasses alimentaires, comme les margarines ou les produits laitiers végétaux.

25. Utilisation de l'huile selon les revendications 12 et 13 en confiserie ou en boulangerie-pâtisserie.