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(71) Applicant *(for all designated States except US)*: **BF PHARMA SOCIETA' PER AZIONI** [IT/IT]; Via Monfalcone, 1, I-12045 Fossano (IT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **CRAVOTTO, Giancarlo** [IT/IT]; Via Buenos Aires, 106, I-10137 Torino (IT). **AVOGADRO, Milvio** [IT/IT]; Via Nizza, 6/B, I-16145 Genova (IT).

(74) Agents: **JORIO, Paolo** et al.; STUDIO TORTA S.r.l., Via Viotti, 9, I-10121 Torino (IT).

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(54) **Title:** USE OF SOYBEAN GERM OIL, PROCEDURE FOR ITS PREPARATION AND HIGH-PURITY SOYBEAN GERM OIL

(57) **Abstract:** The present invention relates to the use of soybean germ oil as a preservative and against oxidation by degradation, preferably of unsaturated molecules or of products containing such molecules, and to procedures for its production and to a high-purity soybean germ oil.

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USE OF SOYBEAN GERM OIL, PROCEDURE FOR ITS PREPARATION AND HIGH-PURITY SOYBEAN GERM OIL

TECHNICAL FIELD

The present invention relates to the use of soybean germ oil as a preservative and against oxidation by degradation, preferably of unsaturated molecules or of products containing such molecules, and to procedures for its production and to a high-purity soybean germ oil.

BACKGROUND ART

In particular, it is known to use α-tocopherol as such, commonly known as vitamin E, as an antioxidant additive both in cosmetic and pharmaceutical preparations and in food products rich in fats and oils.

Vitamin E though is a not very heat-stable molecule which therefore does not allow for prolonged preservation of products rich in unsaturated molecules, particularly if exposed to high temperatures. There is moreover epidemiological evidence published by the American Heart Association (2004) indicating an increase in mortality associated with Vitamin E intake in large doses (above 400 U.I. a day).

Therefore, in the food, pharmaceutical and cosmetic fields the need is felt for a preservative,

particularly with an antioxidant activity, that is able to protect from degradation molecules containing unsaturations even at high temperatures, that is natural, devoid of side effects and therefore usable even in high concentrations.

DISCLOSURE OF INVENTION

According to the present invention there is provided the use of a soybean germ oil according to claims 1, 2 and 3, a combination of soybean germ oil and fish oil according to claim 16 and its use according to claim 19, a process for the preparation of soybean germ oil according to claim 20 and a soybean germ oil according to claims 31, 32 and 33.

Soybean germ, i.e. the hypocotyle of the seed of soy (*Glycine max* L.), of which it represents only 2-3% wt., is rich in natural substances with a high nutritional and therapeutic value.

These substances are also present in the rest of the cotyledon, commonly called bean, but at a concentration 3 to 10 times lower.

The substances mainly contained in the soybean germ are isoflavones, such as for instance genisteine, gliciteine and daidzeine, both omega-3 and omega-6 polyunsaturated fatty acids, such as linoleic acid in an amount of 55÷57%, linolenic acid in an amount of 14-16% and oleic acid in an amount of 10-12%). This

content is much higher compared to the rest of the plant. Furthermore the soybean germ contains lipids present in an amount of approximately 12% wt., but also important vitamins such as niacin (25-30 mg/kg), pantotenic acid (11-15 mg/kg), riboflavin (5mg/kg) and further thiamin, pyridoxin, folates, vitamin B12 and vitamin K.

Also the soybean germ unsaponifiable lipidic fraction is a concentrate of bioactive molecules comprising for example saponines and particularly phytosterols (approximately 43-46 g/kg).

Finally, the soybean germ also contains high concentrations of tocopherols as shown in Table 1.

TABLE 1

Tocopherols	mg/kg
α-tocopherol	1980
β- and γ-tocopherols	2170
δ-tocopherol	250
Total tocopherols	4400

The high content in unsaponifiable fraction supplies an important amount of liposoluble vitamins, and at the same time it protects polyunsaturated fatty acids from their easy oxidation by air and light.

In particular, the present invention provides the use of soybean germ oil as a preservative.

Furthermore there is provided the use of soybean

germ oil against chemical and biological oxidation and against the degradation of unsaturated molecules.

Preferably soybean germ oil has a purity higher than 80%, more preferably higher than 95%.

Further, preferably soybean germ oil is obtained from soybean germ powder with a content in germ higher than 80%, more preferable from soybean germ powder with a content in germ of 95%.

Preferably unsaturated molecules are selected from the group comprising polyunsaturated acids, esters of polyunsaturated acids, polyunsaturated alcohols, terpenoids, olefines and their derivatives such as salts, amids, aldehydes and ethers.

More preferably polyunsaturated acids belong to the omega-3 or omega-6 series or, alternatively, they are selected from the group comprising eicosapentaenoc acid, docosahesaenoic acid, linoleic acid, linolenic acid, gamma-linolenic acid, arachidonic acid, eleostearic acid and their derivatives, such as for example isomers like cis-linolenic acid or cis-linoleic acid).

Preferably the esters of polyunsaturated acids are selected from the group comprising esters of sterols, stanols, lecithins, polyalcohols, more preferably glycerol.

Preferably, terpenoids are carotenoids, more

preferably selected from the group comprising Vitamin A, lycopene, Beta-Carotene.

Alternatively, in a preferred embodiment of the present invention, soybean germ oil can be advantageously used against the degradation of products containing unsaturated molecules.

Preferably, these products are selected from the group comprising fish oil, rapeseed oil, seed oils, flax oil, hemp oil, chia oil, kiwi oil, musqueta rose oil, pumpkin oil, walnut oil, oil extracted from seaweeds, oil extracted from mushrooms, oil extracted from monomolecular biomasses and their mixtures, butter, margarine and food creams.

It has advantageously been found how the addition of soybean germ oil to unsaturated molecules or to products containing these molecules remarkably increases their resistance to oxidation with respect to antioxidants normally employed in industry. In particular, this increased resistance to oxidation is due to a synergic action of components present in the soybean germ oil itself.

According to a preferred embodiment, the present invention provides the use of a combination of soybean germ oil and fish oil, preferably comprising soybean germ oil at a concentration up to 30%, more preferably from 10 to 20%.

According to the present invention there is also provided the use of a combination of soybean germ oil and fish oil for the preparation of a medicament for the treatment of dyslipidemias.

Further, the present invention provides a new process for the preparation of soybean germ oil.

According to this process soy seeds, after a first cleaning step, are hulled superficially.

Advantageously, thanks to this step, it is possible to separate from the rest of the seed the cuticle covering the seed, to which the germ is bound.

The decortication phase is performed by friction hulling, wherein friction determines the mechanical detachment of the cuticle and the germ.

Alternatively, the decortication phase is performed by steam humidification hulling which is follwed by a toasting phase. The swelling caused by water allows the cuticle and germ to be removed, thus leaving the seed intact.

A phase of separating the cuticle from the soybean germ follows, which is perfoermed by sifting, for example a pneumatic sifting, followed by aspiration. Optionally it is possible to perform a further phase of gravimetric separation.

From the processing of the soybean germ thus obtained a high purity oil with marked preservative

properties is extracted.

The phase of soybean germ oil production follows.

According to a first preferred embodiment of the invention, the soybean oil production is carried out through extraction with an organic solvent. Preferably the organic solvent is selected from the grouo comprising hexane, pentane, petrolether and cyclohexane,.

After the extraction phase with the solvent, the phase of evaporating the organic solvent from said soybean germ oil can be carried out.

According to a second embodiment of the invention, the soybean germ oil production is carried out through a phase of pressing the soy germs followed by a filtration phase. This process advantageously allows one to avoid the solvent evaporation phase.

According to a third embodiment of the invention, the soybean germ oil production is carried out by means of an extraction phase with supercritical fluids extraction, preferably with carbon dioxide.

Advatangeously the supercritical fluid is an excellent solvent for unsaturated molecules. Furthermore, upon completion of the extraction phase, carbon dioxide can be separated in the gas phase with no solvent residues remaining in the final product.

The oil obtained according to the processes

described above can be used in different forms, e.g. raw, semirefined, refined both by chemical methods through reaction with alkaline substances and by physical distillation. Through the processes described above a soybean germ oil is yielded with a degree of purity exceeding 80%, more preferably exceeding 95%.

Further characteristics of the present invention will appear in the following description of merely illustrative and not limiting examples.

Example 1

Procedure for the preparation of soybean germ oil.

Initially soy seeds, after the harvest, and possibly drying, are subjected to a first sifting phase e.g. through vibrating screens. Thus soy seeds are separated from portions of the plant having a larger diameter such as for instance leaves, portions of the pod, wood and from those having a smaller diameter such as broken seeds, powder, small stones.

The so obtained seeds are subjected to a hulling phase through cuticle friction. The friction induces the detachment of the cuticle from the germ, the formation of dust and yields a seed without cuticle. Then an aspiration phase is carried out that separates the light parts, i.e. the cuticle, the germ and the dust, from the seed.

The Products obtained during the aspiration phase

are submitted to a second sifting, carried out for example by means of a three-way vibrating screen.

Thus it is possible to separate the soybean germ from both the cuticle, discarded by the large-mesh sifter, and from dust, discarded by the small-mesh sifter.

Finally in order to further separate the soybean germ from the remaining cuticle fragments, a gravimetric separation follows which is carried out by means of vibrating gravimetric tables with turbulent air flow. This phase that takes advantage of the different weight of the soybean germ with respect to the remaining cuticle fragments is carried out by means of a fluid bed system that enables soybean germ selection based on density. Thus a soybean germ is produced with a degree of purity exceeding 95%.

The thus obtained soybean germ can be used as such or it can be processed to yield soybean germ oil.

The soybean germ oil production is carried out by means of an extraction phase with hexane at 60°C. The solvent evaporation phase at its boiling point and drying of the oil in a nitrogen stream follow. The final yield in soybean germ oil is about 10-12% by weight with respect to the germ.

Example 2

Preservation tests, through Rancimat, were carried

out on fish oil added with 5000 ppm of Vitamin E and different amounts of soybean germ oil respectively.

Table 2 shows the results obtained after heating at 100°C.

TABLE 2

Oil	Resistance to oxidation (hours)
Fish Oil	0.17
Fish oil + 5,000 ppm Vitamin E	1.06
Fish oil + 5% of soybean germ oil	1.99
Fish oil + 10% of soybean germ oil	2.06
Fish oil + 15% of soybean germ oil	2.18
Fish oil + 20% of soybean germ oil	2.26

As can be seen from the results of resistance to oxidation obtained, a mixture of fish oil added with 10% soybean germ oil shows a resistance to oxidation twelve times greater than pure fish oil. Furthermore a mixture of fish oil added with 15% soybean germ oil was shows a doubled resistance to oxidation with respect to a mixture of fish oil and vitamin E.

Example 3

Preservation tests, through Rancimat, were carried out on fish oil added with 5000 ppm of Vitamin E and different amounts of soybean germ oil respectively.

Table 3 shows the results obtained after heating at 110°C.

TABLE 3

Oil	Resistance to oxidation (hours)
Fish oil	0.16
Fish oil + 5,000 ppm Vitamin E	0.13
Fish oil + 5% of soybean germ oil	0.40
Fish oil + 15% of soybean germ oil	0.59
Fish oil + 20% of soybean germ oil	0.64

As can be seen from the results of resistance to oxidation obtained, a mixture of fish oil added with 15% soybean germ oil shows a resistance to oxidation nearly 5 times greater than that of a mixture of fish oil and vitamin E.

Example 4

Preservation tests were carried out, through Rancimat, that would compare soybean germ oil with 95% purity according to the present invention and oil from

soybean enriched at 37% with soybean germ known from the literature.

Table 4 shows the results obtained after heating at 90°C

TABLE 4

Oil	Resistance to oxidation (hours)
Soybean oil enriched at 37 % with soybean germ	1.10
Soybean germ oil, 95%	2.06

As can be seen from the results of resistance to oxidation obtained, soybean germ oil with 95% purity according to the present invention shows a resistance to oxidation nearly twice as large as a Soybean oil enriched at 37 % with soybean germ available on the market.

Example 5

Measurements were carried out to determine the total cholesterol, triglycerides and polyunsaturated fatty acids (PUFA) levels in the blood LDL of 4 groups of "Wistar 66" rats about 7 days old fed for 6 weeks with food containing 15% purity oil.

The four groups were respectively treated with: soybean germ oil according to the present invention (OGS), fish oil (OP), fish oil added with soybean germ oil according to the present invention at 15% (OP +

OGS) and a placebo composition. Measured values are shown in table 5.

TABLE 5

	OGS	OP	OP+OGS	Placebo
Total Cholesterol (mg /dL)	60.1	49.9	43.2	73.4
Triglycerides (mg/dL)	48.6	37.8	32.9	79.6
LDL-PUFA (%)	41.0	48.2	57.3	32.0

Advantageously, data reported in table 5 show a reduction in cholesterol and triglyceride levels in the blood of rats treated with fish oil added with soybean germ oil according to the present invention at 15% with respect to groups treated with fish oil and soybean germ oil only.

Furthermore, in rats treated with fish oil added with soybean germ oil according to the present invention at 15% a remarkable increase in PUFA-LDL was observed, thus determining a marked reduction in the risk of atherosclerotic plaque formation.

CLAIMS

1. Use of soybean germ oil as a preservative.

2. Use of soybean germ oil against oxidative degradation.

3. Use of soybean germ oil against the degradation of unsaturated molecules or of products containing said molecules.

4. Use according to claims 1 to 3, characterized in that said soybean germ oil is obtained from soybean germ powder with a germ content of 80%.

5. Use according to claim 4, characterized in that said soybean germ oil is obtained from soybean germ powder with a germ content of 95%.

6. Use according to claims claims 1 to 3, characterized in that said soybean germ oil has a purity exceeding 80%.

7. Use according to claims claims 1 to 3, characterized in that said soybean germ oil has a purity exceeding 95%.

8. Use according to any of claims 3 to 7, characterized in that said unsaturated molecules are selected from the group consisting of polyunsaturated fatty acids, esters of polyunsaturated acids, polyunsaturated alcohols, terpenoids, olefins and their derivatives.

9. Use according to claim 8, characterized in

that said polyunsaturated acids belong to the omega-3 or omega-6 series.

10. Use according to claim 8, characterized in that said polyunsaturated fatty acids are selected from the group consisting of eicosapentaenoic acid, docosahesaenoic acid, linoleic acid, linolenic acid, gamma-linolenic acid, arachidonic acid, eleostearic acid and their derivatives.

11. Use according claim 8, characterized in that said esters of polyunsaturated acids are selected from the group consisting of sterols, stanols, lecithins, polyalcohols.

12. Use according to claim 11, characterized in that said polyalcohols are glycerol.

13. Use according to claim 8, characterized in that said terpenoids are carotenoids.

14. Use according claim 13, characterized in that said carotenoids are selected from the group consisting of vitamin A, beta-carotene, lycopene.

15. Use according to any of claims 3 to 14, characterized in that said products containing said molecules are selected from the group consisting of fish oil, rapeseed oil, seed oil, flax oil, hemp oil, chia oil, kiwi oil, musqueta rose oil, pumpkin oil, walnut oil, oil extracted from seaweeds, oil extracted from mushrooms, oil extracted from biomasses and their

mixtures, butter, margarine and food creams.

16. Use of a combination of soybean germ oil and fish oil.

17. Use according to claim 16, characterized in that soybean germ oil is comprised up to a concentration of 30%.

18. Use according to claim 16 or 17, characterized in that soybean germ oil is comprised in a concentration between 10 and 20%.

19. Use of a combination of soybean germ oil and fish oil for the preparation of a medicament for the treatment of dyslipidemic diseases.

20. Process for the preparation of soybean germ oil, characterized in that it comprises a phase of hulling superficially the soy seeds.

21. Process according to claim 20, characterized in that said hulling phase is a phase of hulling by friction.

22. Process according to claim 20, characterized in that said hulling phase is a phase of hulling by humidification.

23. Process according to claim 20, characterized in that it comprises a phase of roasting.

24. Process claims from 20-23 characterized in that it comprises a phase of extracting said soybean germ oil with an organic solvent.

25. Process according to claim 24, characterized in that it comprises a phase of evaporating said organic solvent from said soybean germ oil.

26. Process according to 24 or 25, characterized in that said organic solvent is selected from the group comprising hexane, pentane, petrolether and cyclohexane,.

27. Process according to claims 20-23, characterized in that it comprises a phase of pressing the soybean germs to obtain said soybean germ oil.

28. Process according to claim 27, characterized in that it comprises a phase of filtering said soybean germ oilby means of earths.

29. Process according to claim 20-23, characterized in that it comprises a phase of extraction by means of a supercritical fluid.

30. Process according to claim 29, characterized in that said supercritical fluid is carbon dioxide.

31. Soybean germ oil obtained through a procedure according to any of claims 20-30.

32. Soybean germ oil with a purity degree exceeding 80%

33. Soybean germ oil with a purity degree exceeding 95%.

INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. C11B5/00 A23D9/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C11B A23D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA, BIOSIS, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Saettel, Damien

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INTERNATIONAL SEARCH REPORT

International application No
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Information on patent family members

International application No
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