

DESCRIPTION

Title of Invention: FAT OR OIL COMPOSITION EXHIBITING ANTI-
OXIDATION PROPERTIES

5 Technical Field

[0001]

The present invention relates to an antioxidant oil and
fat composition with high antioxidative activity comprising food
10 or food additives, and to the production method thereof.

Background Art

[0002]

The addition of a water-soluble antioxidant to oil and
15 fat has been known to achieve an oxidation-preventing effect
(Patent Literature (PTL) 1).

PTL 2 discloses oil and fat comprising water-soluble
tea polyphenol and an emulsifier, and having oxidative stability.

PTL 3 discloses that the antioxidative activity of
20 chlorogenic acids with a specific composition is improved with a
further addition of a sugar.

PTL 4 discloses a tea extract-containing oily
composition for deodorization and anti-oxidization comprising a
solid phase containing a tea extract and an oil phase containing
25 an oil component and an emulsifier with an HLB of 10 or less, the
solid phase being dispersed in the oil phase, wherein the tea
extract-containing solid phase comprises fine particles having an
average particle size of 5 μm or less, and wherein the content of
water or alcoholic aqueous solution in the solid phase is 30 wt%
30 or less.

[0003]

PTL 5 discloses an oil-soluble antioxidant comprising a
water-soluble antioxidant component derived from a natural
product and having an average particle size of 40 to 120 nm, the
35 oil-soluble antioxidant being transparently dispersible in oil

and fat.

PTL 6 discloses a lipophilic antioxidant obtained by emulsifying gallic acid, a water-soluble antioxidant, and an oil-soluble antioxidant into a water-in-oil type emulsion with a
5 lipophilic emulsifying agent.

PTL 7 discloses an oil and fat composition comprising (A) 0.0001 to 1 part by weight of ascorbic acid and/or a salt thereof and (B) 0.0001 to 1 part by weight of at least one member selected from citric acid, citric acid salts, malic acid, and
10 malic acid salts, per 100 parts by weight of oil and fat.
[0004]

TBHQ (tertiary butylhydroquinone) has been reported to serve as a strong synthetic antioxidant (Non-patent Literature (NPL) 1), and can be used as an index in screening for a novel
15 antioxidant (NPL 2).

Citation List

Patent Literature

[0005]

20 PTL 1: WO2001/096506
PTL 2: WO2013/172348
PTL 3: JPH09-143465A
PTL 4: JP2000-229118A
PTL 5: JP2013-159730A
25 PTL 6: JP2002-142673A
PTL 7: JPH09-235584A

Non-patent Literature

[0006]

30 NPL 1: J Am Oil Chem Soc, Vol. 58, No. 3, pp. 275-278 (1981, 03)
NPL 2: LWT Food Sci Technol, Vol. 41, No. 5, pp. 816-825 (2008, 06)

Summary of Invention

35 Technical Problem

[0007]

An object of the present invention is to provide, using a simple method, an antioxidant oil and fat composition with unprecedentedly strong oxidative stability. This antioxidant oil and fat composition desirably maintains its effect for a long period of time.

Solution to Problem

[0008]

10 The use of the technique disclosed in PTL 1 or PTL 2 makes it possible to obtain oil and fat with oxidative stability that has been improved to some extent.

 However, PTL 1 basically discloses oil and fat for frying, and a certain amount or more of water is not assumed to be contained in the oil and fat. In fact, PTL 1 nowhere suggests an effect on polyunsaturated fatty acid-containing oil and fat, which are far more susceptible to oxidative deterioration than oil and fat for frying.

 Similar to PTL 1, PTL 2 also discloses oil and fat that are preferably used for cooking with heat, e.g., for frying, and a certain amount or more of water is not assumed to be contained in the oil and fat. Further, PTL 2 nowhere suggests an effect on polyunsaturated fatty acid-containing oil and fat.

[0009]

25 In terms of PTL 3, since chlorogenic acids with a specific composition must be used, the versatility was low. Further, the antioxidant of PTL 3 is basically water-soluble; therefore, the use thereof as an agent for preventing oxidation of oil and fat is difficult. PTL 3 nowhere suggests achieving extremely strong antioxidative activity in oils and fats, which is an object of the present invention.

 In PTL 4, dehydration is substantially performed to obtain a tea extract as a solid, which is dispersed in oil. PTL 4 nowhere suggests an oxidation-preventing effect that is strong enough to prevent oxidation of PUFA oils.

Examples in PTL 5 show many restrictions such that the antioxidant is substantially limited to catechins, and that it is necessary to use a water-soluble emulsifier. Moreover, the antioxidative activity achieved is less than 1.5 times that of the control oil (corn oil); thus, it could hardly be believed possible to impart oxidative stability to polyunsaturated fatty acid-containing oil and fat, which have much lower oxidative stability than that of corn oil.

[0010]

In PTL 6, the use of gallic acid and an oil-soluble antioxidant is essential, which makes the preparation complicated. Further, the evaluation performed in PTL 6 is only with respect to lard. Thus, the effect on polyunsaturated fatty acid-containing oil and fat, which have much lower oxidative stability than that of lard, cannot be expected.

Some of the Examples in PTL 6 use glucose. According to PTL 6, however, the use of glucose is only for expectation of obtaining the effect of a sugar-amino reaction product; PTL 6 nowhere suggests that the presence of a sugar itself improves the effect of water-soluble antioxidant.

In PTL 7, it is essential to use at least one of citric acid, citrates, malic acid, or malates; and this may adversely affect the flavor, depending on the amount used. Although the specification discloses using a sugar alcohol or liquid sugar as a solvent for emulsification to obtain a water-in-oil product, the sugar alcohol or liquid sugar is nowhere used in the Examples, and the effects thereof are nowhere disclosed.

[0011]

Although TBHQ is known to serve as a strong antioxidant, the use thereof for food is not currently approved in Japan. It is presumed that a substance having antioxidative activity greater than that of TBHQ is actually required to further improve the oxidative stability of oil and fat that contain a large amount of polyunsaturated fatty acids. As a matter of course, such a substance must be able to be used as food.

[0012]

In view of the related art, although some antioxidative activity was declared, only limited antioxidative activity was actually demonstrated, and no substance existed that showed
5 antioxidative activity high enough to impart oxidative stability to oils with low stability, such as polyunsaturated fatty acid-containing oils and fats. Thus, it appeared impossible to further improve the antioxidative activity in this field.

Nevertheless, the present inventors further conducted
10 extensive research to consequently find that an antioxidant oil and fat composition shows an extremely strong oxidation stabilizing effect, the antioxidant oil and fat composition comprising an aqueous phase containing a water-soluble antioxidant and a carbohydrate, and having a certain degree or
15 more of water-soluble solids content, the aqueous phase being dispersed in an oil phase, wherein the antioxidant oil and fat composition contains a certain amount of water. The present invention has thus been accomplished.

[0013]

20 More specifically, the present invention relates to the following:

(1) An antioxidant oil and fat composition with a water content of 0.5 to 18 wt% comprising

an aqueous phase containing a water-soluble antioxidant
25 in an amount of 2.5 to 65 wt%, and further containing a carbohydrate in an amount such that the aqueous phase has a total water-soluble solids content of 18 to 79 wt%,

wherein 1 to 38 wt% of the aqueous phase is dispersed in an oil phase.

30 (2) The antioxidant oil and fat composition according to Item (1), wherein the oil phase contains at least one oil-soluble emulsifier selected from polyglycerol condensed ricinoleates, sugar esters, glycerol fatty acid esters, or lecithins, in an amount that is 0.7 to 9 times the weight amount
35 of the water.

(3) The antioxidant oil and fat composition according to Item (1), wherein the water-soluble antioxidant is at least one member selected from vitamin C, an amino acid, or catechin.

5 (4) The antioxidant oil and fat composition according to Item (2), wherein the water-soluble antioxidant is at least one member selected from vitamin C, an amino acid, or catechin.

(5) The antioxidant oil and fat composition according to Item (3), wherein the amino acid is at least one member selected from glycine, glutamic acid, alanine, proline, lysine, 10 tryptophan, methionine, valine, serine, histidine, isoleucine, leucine, phenylalanine, arginine, threonine, cysteine, aspartic acid, glutamine, or asparagine.

(6) The antioxidant oil and fat composition according to Item (4), wherein the amino acid is at least one member 15 selected from glycine, glutamic acid, alanine, proline, lysine, tryptophan, methionine, valine, serine, histidine, isoleucine, leucine, phenylalanine, arginine, threonine, cysteine, aspartic acid, glutamine, or asparagine.

(7) The antioxidant oil and fat composition according to Item (6), wherein the carbohydrate is at least one member 20 selected from sugar alcohols, glucose, sucrose, dextrans, maltose, or oligosaccharides.

(8) The antioxidant oil and fat composition according to any one of Items (1) to (5), wherein the carbohydrate is at 25 least one member selected from sugar alcohols, glucose, sucrose, dextrans, maltose, or oligosaccharides.

(9) A method for producing an antioxidant oil and fat composition, the method comprising the following steps of:

1: preparing an aqueous phase containing a water- 30 soluble antioxidant in an amount of 2.5 to 65 wt%, and further containing a carbohydrate in an amount such that the aqueous phase has a total water-soluble solids content of 18 to 79 wt%; and

2: dispersing the aqueous phase in an oil phase such 35 that the percentage of the aqueous phase becomes 1 to 38 wt% to

obtain an antioxidant oil and fat composition with a water content of 0.5 to 18 wt%.

(10) The production method according to Item (9), the method comprising the step of adding, to the oil phase, at least
5 one oil-soluble emulsifier selected from polyglycerol condensed ricinoleates, sugar esters, glycerol fatty acid esters, or lecithins, in an amount that is 0.7 to 9 times the weight amount of the water contained in the antioxidant oil and fat composition.

(11) The production method according to Item (9),
10 wherein the water-soluble antioxidant is at least one member selected from vitamin C, an amino acid, or catechin.

(12) The production method according to Item (10), wherein the water-soluble antioxidant is at least one member selected from vitamin C, an amino acid, or catechin.

(13) The production method according to Item (11),
15 wherein the amino acid is at least one member selected from glycine, glutamic acid, alanine, proline, lysine, tryptophan, methionine, valine, serine, histidine, isoleucine, leucine, phenylalanine, arginine, threonine, cysteine, aspartic acid,
20 glutamine, or asparagine.

(14) The production method according to Item (12), wherein the amino acid is at least one member selected from glycine, glutamic acid, alanine, proline, lysine, tryptophan, methionine, valine, serine, histidine, isoleucine, leucine,
25 phenylalanine, arginine, threonine, cysteine, aspartic acid, glutamine, or asparagine.

(15) The production method according to Item (9), wherein the carbohydrate is at least one member selected from sugar alcohols, glucose, sucrose, dextrans, maltose, or
30 oligosaccharides.

(16) The production method according to Item (10), wherein the carbohydrate is at least one member selected from sugar alcohols, glucose, sucrose, dextrans, maltose, or oligosaccharides.

35 (17) The production method according to Item (11),

wherein the carbohydrate is at least one member selected from sugar alcohols, glucose, sucrose, dextrans, maltose, or oligosaccharides.

(18) The production method according to Item (12),
5 wherein the carbohydrate is at least one member selected from sugar alcohols, glucose, sucrose, dextrans, maltose, or oligosaccharides.

(19) The production method according to Item (13),
wherein the carbohydrate is at least one member selected from
10 sugar alcohols, glucose, sucrose, dextrans, maltose, or oligosaccharides.

(20) The production method according to Item (14),
wherein the carbohydrate is at least one member selected from
sugar alcohols, glucose, sucrose, dextrans, maltose, or
15 oligosaccharides.

(21) A method for producing an antioxidant oil and fat composition, the method comprising the step of dehydrating 12 to 76 wt% of water from the antioxidant oil and fat composition obtained by the production method of any one of Items (9) to (20)
20 to obtain an antioxidant oil and fat composition with a water content of 0.5 to 18 wt%.

(22) An unsaturated fatty acid-containing oil and fat composition comprising 0.1 to 100 wt% of the antioxidant oil and fat composition according to any one of Items (1) to (7).

25 (23) An unsaturated fatty acid-containing oil and fat composition comprising 0.1 to 100 wt% of the antioxidant oil and fat composition according to Item (8).

(24) The unsaturated fatty acid-containing oil and fat composition according to Item (22), comprising DHA and EPA in a
30 total amount of 0.1 to 60 wt%.

(25) The unsaturated fatty acid-containing oil and fat composition according to Item (23), comprising DHA and EPA in a total amount of 0.1 to 60 wt%.

[0014]

35 In other words, the invention includes the following.

(31) An antioxidant oil and fat composition with a water content of 0.5 to 18 wt% comprising

an aqueous phase containing a water-soluble antioxidant in an amount of 2.5 to 65 wt%, and further containing a carbohydrate in an amount such that the aqueous phase has a total water-soluble solids content of 18 to 79 wt%, wherein 1 to 38 wt% of the aqueous phase is dispersed in an oil phase.

(32) The antioxidant oil and fat composition according to Item (31), wherein the oil phase contains at least one oil-soluble emulsifier selected from polyglycerol condensed ricinoleates, sugar esters, glycerol fatty acid esters, or lecithins, in an amount that is 0.7 to 6.8 times the weight amount of the water.

(33) The antioxidant oil and fat composition according to Item (31) or (32), wherein the water-soluble antioxidant is at least one member selected from vitamin C, an amino acid, or catechin.

(34) The antioxidant oil and fat composition according to Item (33), wherein the amino acid is at least one member selected from glycine, alanine, valine, threonine, serine, proline, or phenylalanine.

(35) The antioxidant oil and fat composition according to any one of Items (31) to (34), wherein the carbohydrate is at least one member selected from sugar alcohols, glucose, sucrose, dextrans, maltose, or oligosaccharides.

(36) A method for producing an antioxidant oil and fat composition, the method comprising the following steps of:

1: preparing an aqueous phase containing a water-soluble antioxidant in an amount of 2.5 to 65 wt%, and further containing a carbohydrate in an amount such that the aqueous phase has a total water-soluble solids content of 18 to 79 wt%; and

2: dispersing the aqueous phase in an oil phase such that the percentage of the aqueous phase becomes 1 to 38 wt% to

obtain an antioxidant oil and fat composition with a water content of 0.5 to 18 wt%.

(37) The production method according to Item (36), the method comprising the step of adding, to the oil phase, at least
5 one oil-soluble emulsifier selected from polyglycerol condensed ricinoleates, sugar esters, glycerol fatty acid esters, or lecithins, in an amount that is 0.7 to 6.8 times the weight amount of the water contained in the antioxidant oil and fat composition.

10 (38) The production method according to Item (36) or (37), wherein the water-soluble antioxidant is at least one member selected from vitamin C, an amino acid, or catechin.

(39) The production method according to Item (38), wherein the amino acid is at least one member selected from
15 glycine, alanine, valine, threonine, serine, proline, or phenylalanine.

(40) The production method according to any one of Items (36) to (39), wherein the carbohydrate is at least one member selected from sugar alcohols, glucose, sucrose, dextrins,
20 maltose, or oligosaccharides.

(41) An unsaturated fatty acid-containing oil and fat composition comprising 0.1 to 100 wt% of the antioxidant oil and fat composition according to any one of Items (31) to (35).

(42) The unsaturated fatty acid-containing oil and fat
25 composition according to Item (41), comprising DHA and EPA in a total amount of 0.1 to 40 wt%.

(43) A method for producing an unsaturated fatty acid-containing oil and fat composition, the method comprising adding the oil and fat composition according to any one of Items (31) to
30 (35) to unsaturated fatty acid-containing oil and fat to obtain an unsaturated fatty acid-containing oil and fat composition comprising the oil and fat composition in an amount of 0.1 to 99 wt%.

(44) A method for preventing oxidation of unsaturated
35 fatty acid-containing oil and fat, the method comprising adding

the oil and fat composition according to any one of Items (31) to (35) to unsaturated fatty acid-containing oil and fat to obtain an unsaturated fatty acid-containing oil and fat composition comprising the oil and fat composition in an amount of 0.1 to 99 wt%.

[0015]

The invention also includes the following.

(51) An oil and fat composition with a water content of 1.9 to 18 wt% satisfying the following requirements:

1. 3 to 65 wt% of a water-soluble antioxidant is contained in an aqueous phase;

2. the aqueous phase optionally contains a carbohydrate and has a total water-soluble solids content of 18 to 75 wt%; and

3. the aqueous phase in an amount of 4.3 to 38 wt% with a particle size of 300 nm or less is dispersed in an oil phase.

(52) The oil and fat composition according to Item (51), wherein the aqueous phase contains at least one carbohydrate selected from sugar alcohols, glucose, sucrose, or dextrans.

(53) The oil and fat composition according to Item (51), wherein the oil phase contains at least one oil-soluble emulsifier selected from polyglycerol condensed ricinoleates, sugar esters, or glycerol fatty acid esters, in an amount that is 0.7 to 6.8 times the weight amount of the water.

(54) The oil and fat composition according to Item (52), wherein the oil phase contains at least one oil-soluble emulsifier selected from polyglycerol condensed ricinoleates, sugar esters, or glycerol fatty acid esters, in an amount that is 0.7 to 6.8 times the weight amount of the water.

(55) The oil and fat composition according to Item (52), wherein the water-soluble antioxidant is at least one member selected from vitamin C, an amino acid, or catechin.

(56) The oil and fat composition according to Item (53), wherein the water-soluble antioxidant is at least one member selected from vitamin C, an amino acid, or catechin.

(57) The oil and fat composition according to Item (54),

wherein the water-soluble antioxidant is at least one member selected from vitamin C, an amino acid, or catechin.

(58) The oil and fat composition according to Item (56), wherein the amino acid is at least one member selected from
5 glycine, proline, or serine.

(59) The oil and fat composition according to Item (57), wherein the amino acid is at least one member selected from glycine, proline, or serine.

(60) A method for producing an oil and fat composition,
10 comprising the following steps:

1. preparing an aqueous phase containing a water-soluble antioxidant in an amount of 3 to 65 wt%, optionally containing a carbohydrate, and having a total water-soluble solids content of 18 to 75 wt%; and

15 2. adding the aqueous phase to an oil phase to obtain a water-in-oil oil and fat composition comprising the aqueous phase in an amount of 4.3 to 38 wt% with a particle size of 300 nm or less, and having a water content of 1.9 to 18 wt%.

(61) The production method according to Item (60),
20 wherein the carbohydrate is at least one member selected from sugar alcohols, glucose, sucrose, or dextrins.

(62) The production method according to Item (61), wherein the oil phase contains at least one oil-soluble emulsifier selected from polyglycerol condensed ricinoleates,
25 sugar esters, or glycerol fatty acid esters, in an amount that is 0.7 to 6.8 times the weight amount of the water in the oil and fat composition.

(63) The production method according to Item (62), wherein the water-soluble antioxidant is at least one member
30 selected from vitamin C, an amino acid, or catechin.

(64) The production method according to Item (63), wherein the amino acid is at least one member selected from glycine, proline, or serine.

(65) The production method according to Item (63),
35 wherein the oil and fat composition is subjected to partial

dehydration to dehydrate 12 to 76% of water contained in the oil and fat composition so that the oil and fat composition has a water content of 1.9 to 18 wt%.

(66) The production method according to Item (61),
5 wherein the water-soluble antioxidant is at least one member selected from vitamin C, glycine, proline, serine, or catechin, and wherein the oil and fat composition is subjected to partial dehydration to dehydrate 12 to 76% of water contained in the oil and fat composition so that the oil and fat composition has a
10 water content of 1.9 to 18 wt%.

(67) The production method according to Item (64),
wherein the oil and fat composition is subjected to partial dehydration to dehydrate 12 to 76% of water contained in the oil and fat composition so that the oil and fat composition has a
15 water content of 1.9 to 18 wt%.

(68) The production method according to Item (60),
wherein the oil phase contains at least one oil-soluble emulsifier selected from polyglycerol condensed ricinoleates, sugar esters, or glycerol fatty acid esters, in an amount that is
20 0.7 to 6.8 times the weight amount of the water in the oil and fat composition, wherein the water-soluble antioxidant is at least one member selected from vitamin C, glycine, proline, serine, or catechin, and wherein the oil and fat composition is subjected to partial dehydration to dehydrate 12 to 76% of water
25 contained in the oil and fat composition so that the oil and fat composition has a water content of 1.9 to 18 wt%.

(69) A method for producing unsaturated fatty acid-containing oil and fat, the method comprising adding the oil and fat composition according to Item (57) to unsaturated fatty acid-
30 containing oil and fat to obtain unsaturated fatty acid-containing oil and fat comprising the oil and fat composition in an amount of 0.1 to 40 wt%.

(70) A method for preventing oxidation of unsaturated fatty acid-containing oil and fat, the method comprising adding
35 the oil and fat composition according to Item (57) to unsaturated

fatty acid-containing oil and fat to obtain unsaturated fatty acid-containing oil and fat comprising the oil and fat composition in an amount of 0.1 to 40 wt%.

5 Advantageous Effects of Invention

[0016]

The present invention provides an antioxidant oil and fat composition with extremely high antioxidative activity using general-purpose edible starting materials. The invention also
10 provides a polyunsaturated fatty acid-containing oil and fat etc. with high oxidative stability using the antioxidant oil and fat composition.

Description of Embodiments

15 [0017]

The antioxidant oil and fat composition according to the present invention has high antioxidative activity. The antioxidant oil and fat composition according to the present invention can be used by only mixing with a target to which
20 antioxidative activity is intended to be imparted. The antioxidant oil and fat composition according to the present invention is an oil and fat composition in the form of water-in-oil emulsion. Thus, for use with oils and fats, the antioxidant oil and fat composition according to the present invention can be
25 easily dispersed in the oils and fats.

The target oils and fats to be used may be any oils and fats, as long as they contain unsaturated fatty acids. The antioxidant oil and fat composition according to the present invention can thus also be used to improve the stability of
30 commonly used oils and fats, such as soybean oil and rapeseed oil. Additionally, when the antioxidant oil and fat composition according to the present invention is used with oil and fat containing fatty acids with multiple unsaturated bonds, i.e., PUFA, the antioxidant oil and fat composition according to the
35 present invention demonstrates remarkable performance, leading to

preferable results. In the present invention, docosahexaenoic acid may be sometimes abbreviated as "DHA," and eicosapentaenoic acid may be sometimes abbreviated as "EPA." Further, in the present invention, oil and fat containing large amounts of DHA and EPA may be sometimes simply referred to as "PUFA oils."

5 [0018]

The effect in terms of the oxidative stability of the antioxidant oil and fat composition according to the present invention is evaluated by adding a predetermined amount of the antioxidant oil and fat composition to predetermined oil and fat, storing the resulting mixture at a constant temperature, and measuring its peroxide value (POV). Suppression of the increase in the POV for a longer period of time represents higher oxidative stability. A specific measurement method is described in the Examples; the test is an accelerated test, which is performed at a temperature higher than the temperature in expected use, and so stirring is required.

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The POV is denoted in "meq/kg." In the present invention, when the term "POV" is simply stated, the unit thereof is meq/kg.

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A CDM test, which involves a simple measurement method, can also provide a certain evaluation index. The term "CDM" (a Conductometric Determination Method) as used herein refers to a value (time) that represents the oxidative stability of oil and fat, and the value can serve as an index for evaluation of oxidative stability. When a higher CDM value (time) is measured, the oxidative stability is more excellent.

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The CDM test can be performed using a dedicated test device "Rancimat." In terms of this method as well, a specific measurement method is described in the Examples. This test is an accelerated test, which is performed at a temperature higher than the temperature in expected use, and involves a measurement method comprising bubbling air; thus, the results are presented under stirring conditions. The antioxidant oil and fat composition according to the present invention, which contains a

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certain degree of water, is measured at 96°C, rather than 120°C, which is a commonly applied measurement temperature.

The numerical values obtained as a result of the CDM test are denoted as time (hr). In the present invention, the unit
5 of a simple expression such as "a CDM value" is also time (hr).
[0019]

The water-soluble antioxidant as used herein is an antioxidant that is soluble in water. Specific examples include vitamin C (in this specification, sometimes abbreviated as "VC"),
10 catechin, tea extracts, amino acids, and *Myrica rubra* extracts; with vitamin C, tea extracts, and amino acids being preferable; and vitamin C and amino acids being more preferable.

Of amino acids, it is preferable to use at least one amino acid selected from glycine, glutamic acid, alanine, proline,
15 lysine, tryptophan, methionine, valine, serine, histidine, isoleucine, leucine, phenylalanine, arginine, threonine, cysteine, aspartic acid, glutamine, or asparagine. It is more preferable to use at least one amino acid selected from glycine, glutamic acid, alanine, proline, lysine, tryptophan, methionine, valine, serine,
20 histidine, isoleucine, leucine, or phenylalanine; and it is even more preferable to use at least one amino acid selected from glycine, glutamic acid, alanine, proline, lysine, tryptophan, or methionine.

It is also possible to add the water-soluble
25 antioxidant as a salt. For example, sodium glutamate may be added instead of glutamic acid, and sodium aspartate may be added instead of aspartic acid. In a similar manner, sodium ascorbate may be used instead of vitamin C.

In the present invention, even when the term "water-soluble antioxidant" or "amino acid" is simply used, the scope of
30 these terms also encompass salts thereof.
[0020]

In the present invention, these water-soluble antioxidants may be used alone or in combination by suitably
35 selecting from the above. With the use of an appropriate water-

soluble antioxidant, an antioxidant oil and fat composition that has strong antioxidative activity can be obtained.

In tea extracts, catechin is said to be the main cause of this activity. Thus, even when a tea extract is used, the tea
5 extract may be referred to as "catechin." Catechin and tea extracts may also be collectively referred to as "polyphenols."

Needless to say, the term "water-soluble antioxidant" as used herein is not limited to encompass those that have been known as water-soluble antioxidants, but also encompasses water-
10 soluble components that were newly found to have antioxidative activity. For amino acids, in particular, those that were newly found to have antioxidative activity by this study are also encompassed.

[0021]

15 The present invention is characterized by achieving antioxidative activity with the use of vitamin C, which is approved for use in many countries and whose safety have been confirmed through long eating experience, the antioxidative activity being greater than that achieved with the use of
20 synthetic antioxidants.

[0022]

In the present invention, the amount of the water-soluble antioxidant contained in the aqueous phase is 2.5 to 65 wt%, preferably 10 to 63 wt%, and more preferably 13 to 60 wt%.
25 The use of an appropriate water-soluble antioxidant in an appropriate amount makes it possible to obtain an antioxidant oil and fat composition having strong antioxidative activity.

[0023]

The aqueous phase in the present invention is a mixture
30 of water and starting materials that are water-soluble from among the starting materials of the antioxidant oil and fat composition.

The amount of the aqueous phase in the antioxidant oil and fat composition according to the present invention must be within a range of 1 to 38 wt%, preferably 3 to 34 wt%, and more
35 preferably 5 to 30 wt%. With an appropriate amount of the aqueous

phase, the antioxidant oil and fat composition according to the present invention exerts strong antioxidative activity.

[0024]

In the present invention, the aqueous phase with a
5 higher water-soluble solids content achieves more preferred
antioxidative activity. In the present invention, the water-
soluble solids content in the aqueous phase is 18 to 79 wt%,
preferably 30 to 79 wt%, and more preferably 35 to 79 wt%. The
water-soluble solids content here includes the amount of the
10 water-soluble antioxidant.

[0025]

Various materials that can be used as food or food
additives may be used as water-soluble solids, in addition to the
water-soluble antioxidants. In particular, carbohydrates, which
15 are inexpensive and have an effect of further increasing
antioxidative activity, are preferable. The carbohydrate is
preferably at least one member selected from sugar alcohols,
oligosaccharides, various monosaccharides, such as glucose,
various disaccharides, such as sucrose and maltose, or dextrins;
20 more preferably a sugar alcohol, glucose, sucrose, dextrins, or
maltose; and even more preferably sucrose or a sugar alcohol.

Of sugar alcohols, preferred are glycerol, maltitol,
sorbitol, erythritol, reduced palatinose, lactitol, xylitol, and
mannitol, more preferred are glycerol, maltitol, sorbitol, and
25 erythritol, and even more preferred are sorbitol and erythritol.

Adjusting the water-soluble solids content in the
aqueous phase to an appropriate value by using an appropriate
carbohydrate or, as necessary, two or more appropriate
carbohydrates makes it possible to prevent the occurrence of
30 precipitation, and obtain an antioxidant oil and fat composition
having strong antioxidative activity.

[0026]

The oil phase as used in the present invention is
obtained by dissolving in oil one or more oil-soluble components
35 from among the starting materials of the antioxidant oil and fat

composition according to the present invention. Here, examples of usable oils include soybean oil, rapeseed oil, fractionated palm oil, polyunsaturated fatty acid-containing oils and fats, and other various edible oils and fats. From the viewpoint of the production process, however, it is more advantageous to use soybean oil or the like as the oil and fat used at this stage, and use the prepared antioxidant oil and fat composition by mixing with polyunsaturated fatty acid-containing oil and fat. If the starting materials include no oil-soluble components other than oil, then the oil itself is referred to as an oil phase.

[0027]

An oil-soluble emulsifier is optionally used as an oil-soluble component as a starting material in the present invention. In the present invention, emulsifiers having an HLB of 7 or less and lecithins are defined as oil-soluble emulsifiers. The oil-soluble emulsifier is preferably at least one member selected from polyglycerol esters, sugar esters, sorbitan esters, monoglycerol fatty acid esters, or lecithins. The oil-soluble emulsifier is more preferably a polyglycerol ester, a sugar ester, a distilled monoglyceride, or a lecithin, particularly preferably a polyglycerol ester, and most preferably a polyglycerol condensed ricinoleic acid ester (polyglycerol ester of polyricinoleic acid). The term "polyglycerol condensed ricinoleate (polyglycerol polyricinoleate)" may be abbreviated as "PGPR."

The amount of the oil-soluble emulsifier contained in the antioxidant oil and fat composition is preferably 0.7 to 9 times, more preferably 0.7 to 6.8 times, and even more preferably 0.7 to 5 times the weight amount of the water. The use of an appropriate oil-soluble emulsifier in an appropriate amount as required can provide an antioxidant oil and fat composition having strong antioxidative activity.

[0028]

In the antioxidant oil and fat composition according to the present invention, the aqueous phase with a particle size of

300 nm or less is dispersed in the oil phase. The particle size is more preferably 160 nm or less, and even more preferably 130 nm or less.

Adjusting the particle size to an appropriate value makes it possible to stably achieve strong antioxidative activity. The occurrence of precipitation indicates that the particle size is greater than 300 nm. This means that precipitation should not be visually observed. When no precipitation occurs, it is confirmed that the particle size is 300 nm or less. Therefore, in the Examples in which precipitation does not occur, the particle size is shown as 300 nm or less.

[0029]

In the present invention, the particle size is measured using the following device under the following conditions.

Device name: Zetasizer Nano S; Manufacturer: Malvern

The measurement is performed by diluting 10 μ l of an oil and fat composition to be measured in 2 ml of hexane.

(The measurement results of the sample on the first day after preparation are used for evaluation.)

Temperature: 20.0°C

Equilibrium time: 240 seconds

Cell: Glass cell

Measurement angle: 173°

Positioning method: Optimum position selected

Selection of automatic attenuation: Yes

[0030]

The antioxidant oil and fat composition according to the present invention has a water content of 0.5 to 18 wt%. The water content is more preferably 1 to 15 wt%, and even more preferably 1.5 to 13 wt%.

An appropriate amount of water prevents precipitation from occurring in the antioxidant oil and fat composition, and enables an achievement of high antioxidative activity. Needless to say, the water refers to water that is incorporated from the aqueous phase.

[0031]

In the present invention, the antioxidant oil and fat composition described above is mixed with unsaturated fatty acid-containing oil and fat to give an unsaturated fatty acid-

5 containing oil and fat composition having antioxidative activity.

The amount of antioxidant oil and fat composition contained in the unsaturated fatty acid-containing oil and fat composition is preferably 0.1 to 100 wt%, more preferably 3 to 95 wt%, and even more preferably 5 to 91 wt%. Incorporation of an
10 appropriate amount of the antioxidant oil and fat composition can achieve preferable antioxidative activity.

The oil and fat used in the preparation of the antioxidant oil and fat composition may be unsaturated fatty acid-containing oil and fat, and in this case, the antioxidant
15 oil and fat composition is equal to the unsaturated fatty acid-containing oil and fat composition. That is, the amount of the antioxidant oil and fat composition contained in the unsaturated fatty acid-containing oil and fat composition is 100 wt%.

[0032]

20 In the present invention, the antioxidant oil and fat composition according to the present invention containing the water-soluble antioxidant at a higher concentration tends to achieve greater antioxidative activity. Specifically, in systems in which the same amount of water-soluble antioxidant is used in
25 PUFA oil, the antioxidative activity tends to become higher with a small amount of the antioxidant oil and fat composition that contains a large amount of water-soluble antioxidant, rather than with a large amount of the antioxidant oil and fat composition that contains a small amount of water-soluble antioxidant.

30 The antioxidant oil and fat composition according to the present invention contains the water-soluble antioxidant at a concentration of preferably 1 to 25 wt%, more preferably 2 to 15 wt%, and most preferably 3.5 to 7 wt%. When the concentration is adjusted to be within the preferred range, preferable
35 antioxidative activity can be obtained.

[0033]

In the present invention, the amount of DHA and EPA in the unsaturated fatty acid-containing oil and fat composition is preferably 0.1 to 60 wt%, more preferably 1 to 50 wt%, and even
5 more preferably 5 to 40 wt%.

Incorporation of appropriate amounts of DHA and EPA makes it possible to obtain an unsaturated fatty acid-containing oil and fat composition with promising physiological effects etc.

[0034]

10 Hereafter, examples of the method for preparing an antioxidant oil and fat composition according to the present invention are described.

In the present invention, a water-soluble antioxidant and a carbohydrate are dissolved in water to prepare an aqueous
15 phase. Here, these components must be contained in the aqueous phase in a substantially dissolved state. The determination of whether these components are substantially dissolved is made by introducing 5 ml of the aqueous phase into a 20-ml centrifugal tube, followed by centrifugal separation (3000 x g, 1 minute) at
20 20°C. If no precipitate is visually observed even after this process, the components are determined as being substantially dissolved. In the present invention, the expression such as "contained in the aqueous phase" means "being present in the aqueous phase in the dissolved state."

25 If precipitate is observed in the aqueous phase, it is possible that the antioxidant oil and fat composition does not have high antioxidative activity.

[0035]

The oil phase is prepared by dissolving one or more
30 oil-soluble components in oil and fat. In the present invention, an oil-soluble emulsifier is optionally used as an oil-soluble component.

Subsequently, the oil phase and the aqueous phase are mixed and emulsified to give an oil and fat composition in the
35 form of water-in-oil emulsion. To perform emulsification here, a

commonly used emulsifying device is used to thus easily obtain a preferable antioxidant oil and fat composition. Specific examples of usable emulsifying device include a high-pressure homogenizer, an ultrasonic emulsifying device, and a two-component

5 impingement-type emulsifying device, which is also called a wet-jet mill. The use of an appropriate emulsifying device makes it possible to obtain a predetermined antioxidant oil and fat composition. Emulsification with the use of a high-pressure homogenizer is commonly performed at 30 to 40 MPa and 10 to 30
10 passes.

The use of a commonly used emulsifying device can easily reduce the particle size; this possibly relates to the fact that the aqueous phase contains a high degree of water-soluble solids in the dissolved state.

15 [0036]

In the present invention, emulsification is preferably performed using an appropriate emulsifying device so that the particle size becomes 300 nm or less. The particle size is more preferably 160 nm or less, and even more preferably 130 nm or
20 less. Adjusting the particle size to below a certain degree makes it possible to obtain a preferable antioxidant oil and fat composition, in terms of the antioxidative activity and its stability.

[0037]

25 In the present invention, it is preferable to dehydrate a portion of water after emulsification. The method for dehydration is not limited, and dehydration may be performed by bubbling gas or under reduced pressure. For dehydration, partial dehydration of 12 to 76 wt%, and more preferably 20 to 67 wt%, of
30 water contained in the prepared emulsified product is preferred. By performing appropriate partial dehydration, an antioxidant oil and fat composition having strong antioxidative activity is obtained. Further, by performing appropriate partial dehydration, the occurrence of precipitation can be delayed, whereby the
35 commodity value is improved.

The precipitation is believed to be caused by the primary action of the antioxidant oil and fat composition; thus, when precipitation occurs, it is believed to be impossible to achieve antioxidative activity without stirring. If continuous stirring is required to achieve high antioxidative activity, the use thereof is very difficult. Thus, no precipitation preferably occurs in the antioxidant oil and fat composition according to the present invention.

[0038]

Even after partial dehydration is performed, the antioxidant oil and fat composition according to the present invention contains a certain amount of water. Therefore, it could be difficult to use oil and fat containing the antioxidant oil and fat composition as oil and fat for cooking with heat, such as for frying.

More specifically, the antioxidant oil and fat composition according to the present invention is particularly suitably used for, for example, stabilizing polyunsaturated fatty acid-containing oil and fat, such as PUFA oils.

The Examples are described below.

For the "sugar powder" in the formulations, pulverized sugar was used.

Examples

[0039]

Study 1

Samples of formulations shown in Table 1-1 were prepared in accordance with the "Method for preparing an antioxidant oil and fat composition (study 1)."

The obtained samples were evaluated by the "Method of evaluation of oxidative stability based on CDM (study 1)." Table 1-2 shows the results.

In this study, although an actual measurement of the particle size with the use of a device was not performed after the emulsifying operation, because it was visually confirmed that

precipitation etc. did not occur, the particle sizes were all considered to be 300 nm or less.

[0040]

Table 1-1: Formulations

Antioxidant oil and fat composition		Comp. Ex. 1-1	Comp. Ex. 1-2	Ex. 1-1	Ex. 1-2	Ex. 1-3	Ex. 1-4
		-	-	0.95	0.89	0.54	0.58
Aqueous phase	Vitamin C	-	-	0.29	0.54	0.32	0.35
	Catechin	-	-	0.29	0.32	0.19	1.98
	Sugar powder	-	-	3.47	3.25	1.95	2.10
	Water	-	-	95.00	95.00	97.00	94.99
Oil phase	Soybean oil	100.00	99.98	-	-	-	-
	TBHQ	-	0.02	-	-	-	-
Total		100.00	100.00	100.00	100.00	100.00	100.00
Water-soluble solids content in aqueous phase (wt%)		-	-	30.6%	35.0%	35.0%	58.1%
Amount of water-soluble antioxidant in aqueous phase (wt%)		-	-	24.8%	28.6%	28.6%	18.6%
Amount of aqueous phase (wt%)		-	-	5.0%	5.0%	3.0%	5.0%
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)		0	0	1.24	1.43	0.86	0.93
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)		-	-	300 or less	300 or less	300 or less	300 or less

(The formulations are in percent by weight.)

- For the catechin, "Sunphenon 90S" produced by Taiyo Kagaku Co., Ltd. was used.

- For the soybean oil, soybean *sirasimeyu* (refined soybean oil) produced by Fuji Oil Co., Ltd. was used.

5 - For TBHQ, a product of Amalfi (Product Number 3015200) was used.

- Precipitation was not observed in all of the aqueous phase prepared in accordance with the formulations.

[0041]

Method for preparing an antioxidant oil and fat composition

10 (study 1)

1. In accordance with the formulations, the aqueous phase components were dissolved in water.

2. In accordance with the formulations, the oil phase components were dissolved in oil.

15 3. The aqueous phase was introduced into the oil phase, and the mixture was mixed using a homomixer.

4. Further, the resulting mixture was emulsified using a high-pressure homogenizer (37 MPa, 20 passes) to obtain a water-in-oil emulsified product as a sample.

20 [0042]

Method of evaluation of oxidative stability based on CDM (study 1)

1. Each sample was added to fish oil containing DHA (20.0%) and EPA (5.7%) such that the ratio of sample:fish oil = 1:9, followed
25 by stirring.

2. The oxidative stability of the liquid mixture obtained in 1 above was evaluated using a CDM measurement device ("Rancimat" produced by Metrohm) at 96°C, at an air-blowing rate of 20.0 L/h with a sample-introducing amount of 3.0 g.

30 3. The samples that had a CDM value equal to or greater than that of Comparative Example 1-2 were evaluated as pass.

[0043]

Table 1-2: Results

	Comp. Ex. 1-1	Comp. Ex. 1-2	Ex. 1-1	Ex. 1-2	Ex. 1-3	Ex. 1-4
CDM value (hr)	5.0	8.8	9.7	10.6	10.5	11.8
Pass/Fail	Fail	-	Pass	Pass	Pass	Pass

[0044]

5 Discussion

The results revealed that the addition of the prepared antioxidant oil and fat compositions of predetermined formulations greatly improved the oxidative stability of fish oil. The improving effect was greater than that of TBHQ.

10 [0045]

Study 2(1): Type of oil-soluble emulsifier

Samples of formulations shown in Table 2-1 were prepared in accordance with the "Method for preparing an oil and fat composition (study 2)."

15 The obtained samples were evaluated by the "Method of evaluation of oxidative stability based on POV (study 2)." Table 2-2 shows the results.

In this study as well, an actual measurement of the particle size with the use of a device was not performed after
20 the emulsifying operation; however, because it was visually confirmed that precipitation etc. did not occur in the samples of the Examples, the particle sizes in the Examples were all considered to be 300 nm or less.

25

	Ex. 2-8	Ex. 2-9	Ex. 2-10	Ex. 2-11	Ex. 2-12	Ex. 2-13	Ex. 2-14	Ex. 2-15
Oil-soluble emulsifier	Sunsoft A-172E	Sugar ester O-170	ER-190	Sunsoft A-173E	-	Emulsy OL-100H	Emulsy MU	-
Water-soluble emulsifier	-	-	-	-	Sunsoft Q-17S	-	-	Tween80
Emulsifier composition	Polyoxyethylene sorbitan monolaurate	Sucrose oleic acid ester	Sucrose erucic acid ester	Pentaglycerol trioleate	Decaglycerol monooleate	Distilled monoglyceride	Distilled monoglyceride	Polyoxyethylene sorbitan oleate
Emulsifier HLB	-	1	2	7	12	4.3	4.2	15
Emulsifier manufacturer	Taiyo Kagaku	Mitsubishi-Chemical Foods	Mitsubishi-Chemical Foods	Taiyo Kagaku	Taiyo Kagaku	Riken Vitamin	Riken Vitamin	Kao
Antioxidant oil and fat composition	Vitamin C	1.1	1.1	1.1	1.1	1.1	1.1	1.1
	Catechin	0.7	0.7	0.7	0.7	0.7	0.7	0.7
	Sugar powder	4	4	4	4	4	4	4
	Water-soluble emulsifier	-	-	-	10	-	-	10
	Water	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Oil phase	Soybean oil	80	80	80	80	80	80	80
	Oil-soluble emulsifier	10	10	10	10	10	10	-
	TBHQ	-	-	-	-	-	-	-
Total	100	100	100	100	100	100	100	100
Water-soluble solids content in aqueous phase (wt%)	58%	58%	58%	58%	79%	58%	58%	79%
Amount of water-soluble antioxidant in aqueous phase (wt%)	18%	18%	18%	18%	9%	18%	18%	9%
Amount of aqueous phase (wt%)	10.0%	10.0%	10.0%	10.0%	20.0%	10.0%	10.0%	20.0%
Oil-soluble emulsifier/water	2.4	2.4	2.4	2.4	-	2.4	2.4	-
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)	300 or less	300 or less	300 or less	300 or less	300 or less	300 or less	300 or less	300 or less

(The formulations are in percent by weight.)

- For the catechin, "Sunphenon 90S" produced by Taiyo Kagaku Co., Ltd. was used.

- For the soybean oil, soybean *sirasimeyu* (refined soybean oil) produced by Fuji Oil Co., Ltd. was used.

5 - The "oil-soluble emulsifier/water" refers to the amount of oil-soluble emulsifier in each antioxidant oil and fat composition, relative to water.

- For TBHQ, a product of Amalfi (Product Number 3015200) was used.

10 - Precipitation was not observed in all of the aqueous phase prepared in accordance with the formulations.

[0047]

Method for preparing an oil and fat composition (study 2)

1. The aqueous phase components and the oil phase components were separately dissolved by mixing. TBHQ was dissolved in the oil
15 phase.

2. The aqueous phase and the oil phase were mixed, and the mixture was roughly emulsified to obtain a water-in-oil emulsified liquid.

3. The emulsified liquid obtained in 2 above was emulsified using
20 a high-pressure homogenizer (37 MPa, 20 passes) to obtain a water-in-oil emulsified product.

[0048]

Method of evaluation of oxidative stability based on POV (study 2)

25 1. Each sample (7.5 g) was diluted 4-fold with oil and fat containing EPA (19.8%) and DHA (45.4%).

2. The oil and fat obtained in 1 above after dilution was placed into a 50-ml glass bottle, and the bottle was covered with a lid, which was then placed into an incubator at 60°C, followed by
30 stirring at 80 rpm.

3. Samples were taken on day 5, and the peroxide value (POV) was measured. The POV was measured in accordance with the Standard methods for the analysis of fats, oils and related materials.

35 4. The samples with a POV of 14 or less on day 5 were evaluated as pass.

[0049]

Table 2-2: Results

	Comp. Ex. 2-1	Comp. Ex. 2-2	Ex. 2-1	Ex. 2-2	Ex. 2-3	Ex. 2-4	Ex. 2-5	Ex. 2-6	Ex. 2-7
POV (Day 5)	53.1	15.2	7.3	0	0	0	3.3	0	2.8

	Ex. 2-8	Ex. 2-9	Ex. 2-10	Ex. 2-11	Ex. 2-12	Ex. 2-13	Ex. 2-14	Ex. 2-15
POV (Day 5)	0	3	0	0	12.3	0	0	13.8

[0050]

5 Discussion 1

- When TBHQ, which is a strong antioxidant, was used, the POV was reduced to 15.2, although the POV of the control (Comparative Example 2-1) was 53.1. The results also confirmed that the antioxidant oil and fat composition according to the present invention further prevented the increase in the POV; when the oil-soluble emulsifier was used in combination, this effect was further enhanced.
- In the present invention, the oil-soluble emulsifier used was preferably a polyglycerol ester, a sugar ester, or a distilled monoglyceride, particularly preferably a polyglycerol ester, and most preferably a polyglycerol condensed ricinoleic acid ester.
- The use of an emulsifier with a high HLB did not sufficiently prevent the increase in the POV.

[0051]

20 Study 2(2): Type of oil-soluble emulsifier

Samples of formulations shown in Table 2-3 were prepared in accordance with the "Method for preparing an oil and fat composition (study 2)."

The obtained samples were evaluated by the "Method of evaluation of oxidative stability based on CDM (study 2-2)."

Table 2-4 shows the results.

In this study as well, an actual measurement of the particle size with the use of a device was not performed after the emulsifying operation; however, because it was visually

confirmed that precipitation etc. did not occur in the samples of the Examples, the particle sizes in the Examples were all considered to be 300 nm or less.

[0052]

Table 2-3: Formulations

Antioxidant oil and fat composition	Aqueous phase	Oil phase	Comp. Ex. 2-3	Comp. Ex. 2-4	Comp. Ex. 2-5	Comp. Ex. 2-6	Comp. Ex. 2-7	Ex. 2-16	Ex. 2-17
			-	-	0.9	-	0.8	0.8	0.8
	Vitamin C		-	-	-	-	-	0.8	0.8
	Sugar powder		-	-	-	-	-	6.2	6.2
	Water		-	-	3.3	3.3	3.0	3.0	3.0
	Soybean oil	100	99.98	76.6	77.4	77.4	91.2	86.0	86.0
	Lecithin	-	-	19.2	19.3	19.3	5.0	4.0	-
	Vitamin E	-	-	-	-	-	-	-	-
	Emulsifier 1	-	-	-	-	-	-	-	4.0
	TBHQ	-	200 ppm	-	-	-	-	-	-
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Water-soluble solids content in aqueous phase (wt%)			-	-	21.4%	-	21.6%	70.0%	70.0%
Amount of water-soluble antioxidant in aqueous phase (wt%)			-	-	21.4%	-	21.6%	8.2%	8.2%
Amount of aqueous phase (wt%)			-	-	4.2%	3.3%	3.8%	10.0%	10.0%
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)			0.0	0.0	0.9	0.0	0.8	0.8	0.8
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)			-	-	over 300 (precipitation)	over 300 (precipitation)	over 300 (precipitation)	300 or less	300 or less

Antioxidant oil and fat composition		Ex. 2-18	Ex. 2-19	Ex. 2-20	Ex. 2-21	Ex. 2-22	Comp. Ex. 2-8	Comp. Ex. 2-9
		0.8	0.8	0.8	0.8	0.8	0.8	0.8
Aqueous phase	Vitamin C	6.3	6.2	6.2	6.2	3.7	-	-
	Sugar powder	3.0	3.0	3.0	3.0	3.0	3.0	3.0
	Water	86.9	87.0	86.0	85.0	88.5	95.2	93.2
	Soybean oil	3.0	3.0	3.0	3.0	3.0	1.0	1.0
Oil phase	Lecithin	-	-	1.0	2.0	1.0	-	2.0
	Vitamin E	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Total		70.3%	70.0%	70.0%	70.0%	60.0%	21.6%	21.6%
Water-soluble solids content in aqueous phase (wt%)		7.9%	8.2%	8.2%	8.2%	11.0%	21.6%	21.6%
Amount of water-soluble antioxidant in aqueous phase (wt%)		10.1%	10.0%	10.0%	10.0%	7.5%	3.8%	3.8%
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)		0.8	0.8	0.8	0.8	0.8	0.8	0.8
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)		300 or less	300 or less	300 or less	300 or less	300 or less	300 or less	300 or less

(The formulations are in percent by weight.)

- For the soybean oil, soybean *sirasimeyu* (refined soybean oil) produced by Fuji Oil Co., Ltd. was used.

- For the vitamin E, "E-Mix 70L" produced by Eisai Food & Chemical Co., Ltd. was used.

5 - For the lecithin, soybean lecithin was used.

- For emulsifier 1, a polyglycerol condensed ricinoleic acid ester "CRS-75" produced by Sakamoto Yakuhin Kogyo Co., Ltd. was used.

- Precipitation was not observed in all of the aqueous phase prepared in accordance with the formulations.

[0053]

Method of evaluation of oxidative stability based on CDM (study 2-2)

1. Each sample was added to PUFA oil containing DHA (37%) and EPA (1.9%) such that the ratio of sample:PUFA oil = 2 parts:8 parts, followed by stirring.

2. The oxidative stability of the liquid mixture obtained in 1 above was evaluated using a CDM measurement device ("Rancimat" produced by Metrohm) at 96°C, at an air-blowing rate of 20.0 L/h with a sample-introducing amount of 3.0 g.

3. The samples that had a CDM value equal to or greater than that of Comparative Example 2-4 were evaluated as pass.

[0054]

Table 2-4: Results

	Comp. Ex. 2-3	Comp. Ex. 2-4	Comp. Ex. 2-5	Comp. Ex. 2-6	Comp. Ex. 2-7	Ex. 2-16	Ex. 2-17
CDM value	6.95	11	-	-	-	16.08	24.21
Pass/Fail	-	-	-	-	-	Pass	Pass

	Ex. -18	Ex. 2-19	Ex. 2-20	Ex. 2-21	Ex. -22	Comp. Ex. 2-8	Comp. Ex. 2-9
CDM value	14.67	15.75	14.31	14.95	13.93	9.7	10.3
Pass/Fail	Pass	Pass	Pass	Pass	Pass	Fail	Fail

25

- In Comparative Examples 2-5, 2-6, and 2-7, precipitation was observed in the prepared antioxidant oil and fat composition; thus, the subsequent antioxidative activity evaluation was not

performed.

[0055]

Discussion 2

- As stated above, even when a lecithin was used, excellent
5 antioxidative activity was achieved.

- On a trial basis, vitamin E, which is an oil-soluble
antioxidant, was added; however, the effect thereof could not be
observed.

[0056]

10 Study 3: Study of each water-soluble solids content

Samples of formulations shown in Table 3-1 were
prepared in accordance with the "Method for preparing an oil and
fat composition (study 3)." The obtained samples were evaluated
by the "Method of evaluation of oxidative stability based on CDM
15 (study 3)." Table 3-2 shows the results.

In Example 3-15 and Comparative Example 3-9, evaluation
was performed in accordance with the "Method of evaluation of
oxidative stability based on POV (study 3)." Table 3-3 shows the
results.

20

[0057]

Table 3-1: Formulations

Antioxidant oil and fat composition	Aqueous phase	Oil phase	Total	Comp. Ex. 3-1	Ex. 3-1	Ex. 3-2	Ex. 3-3	Ex. 3-4	Ex. 3-5	Ex. 3-6	Ex. 3-7
				-	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Antioxidant oil and fat composition	Aqueous phase	Oil phase	Total	Vitamin C	4.0	-	-	-	-	-	-
				Sugar	-	4.0	-	-	-	-	-
				Dextrin 1	-	-	4.0	-	-	-	-
				Erythritol	-	-	-	-	-	-	-
				Maltitol	-	-	-	-	-	-	4.0
				Fructooligo saccharide	-	-	-	4.0	-	-	-
				Maltodextrin	-	-	-	-	4.0	-	-
				Polydextrin	-	-	-	-	-	4.0	-
				Water	8.0	8.0	8.0	8.0	8.0	8.0	8.0
				Emulsifier	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Antioxidant oil and fat composition	Aqueous phase	Oil phase	Total	Soybean oil	74.0	74.0	74.0	74.0	74.0	74.0	74.0
				Water-soluble solids content in aqueous phase (wt%)	0.0%	42.9%	42.9%	42.9%	42.9%	42.9%	42.9%
				Amount of water-soluble antioxidant in aqueous phase (wt%)	0.0%	14.3%	14.3%	14.3%	14.3%	14.3%	14.3%
				Amount of aqueous phase (wt%)	8.0%	14.0%	14.0%	14.0%	14.0%	14.0%	14.0%
				Emulsifier/water	1.5	1.5	1.5	1.5	1.5	1.5	1.5
				Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)	0.0	2.0	2.0	2.0	2.0	2.0	2.0
				Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)	over 300	64.45	62.71	W	No precipitation	46.74	50.66

		Ex. 3-8	Comp. Ex. 3-2	Ex. 3-9	Ex. 3-10	Comp. Ex. 3-3	Comp. Ex. 3-4	Ex. 3-11	Comp. Ex. 3-5
Antioxidant oil and fat composition	Aqueous phase	Vitamin C	2	1.11	1.11	0.8	0.8	0.8	1.11
		Sugar	-		1.39	3.18	-	0.97	-
		Sorbitol	4	-	-	-	-	-	-
		Water	8	10	10	10	10	10	10
	Oil phase	Emulsifier	12	12	12	12	12	12	12
Soybean oil		74	76.89	75.5	73.71	77.2	76.23	73.71	76.89
Total		100	100	100	100	100	100	100	100
Water-soluble solids content in aqueous phase (wt%)		42.9%	10.0%	20.0%	30.0%	7.4%	15.0%	30.0%	10.0%
Amount of water-soluble antioxidant in aqueous phase (wt%)		14.3%	10.0%	8.9%	7.8%	7.4%	6.8%	5.6%	10.0%
Amount of aqueous phase (wt%)		14.0%	11.1%	12.5%	14.3%	10.8%	11.8%	14.3%	11.1%
Emulsifier/water		1.5	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)		2	1.11	1.11	1.11	0.8	0.8	0.8	1.11
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)		149.3	Precipitation	61.3	62.06	Precipitation	Precipitation	65.55	Precipitation

Antioxidant oil and fat composition	Aqueous phase	Oil phase	Total	Ex. 3-12	Ex. 3-13	Comp. Ex. 3-6	Comp. Ex. 3-7	Ex. 3-14	Comp. Ex. 3-8	Ex. 3-15	Comp. Ex. 3-9
				1.11	1.11	0.8	0.8	0.8	2	1	1
	Vitamin C			1.39	3.18	-	0.97	3.49	-	-	-
	Sugar			-	-	-	-	-	-	3.1	-
	Glycerol			10	10	10	10	10	8	3.3	3.3
	Water			12	12	12	12	12	12	5	5
	Emulsifier			75.5	73.71	77.2	76.23	73.71	78	87.6	90.7
	Soybean oil			100	100	100	100	100	100	100	100
Water-soluble solids content in aqueous phase (wt%)				20.0%	30.0%	7.4%	15.0%	30.0%	20.0%	55.4%	23.3%
Amount of water-soluble antioxidant in aqueous phase (wt%)				8.9%	7.8%	7.4%	6.8%	5.6%	14.3%	13.5%	23.3%
Amount of aqueous phase (wt%)				12.5%	14.3%	10.8%	11.8%	14.3%	14.0%	7.4%	4.3%
Emulsifier/water				1.2	1.2	1.2	1.2	1.2	1.5	1.5	1.5
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)				1.11	1.11	0.8	0.8	0.8	2	1	1
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)				61.3	62.06	Precipitation	Precipitation	65.55	Precipitation	No precipitation	Precipitation

(The formulations are in percent by weight.)

- For dextrin 1, "Sandec 150" produced by Sanwa Starch Co., Ltd. was used.

- For the fructooligosaccharide, fructooligosaccharide produced by Wako Pure Chemical Industries, Ltd. was used.

5 - For the maltodextrin, "Pinedex #4" produced by Matsutani Chemical Industry Co., Ltd. was used.

- For the polydextrin, "Litesse Ultra" produced by DuPont Danisco was used.

10 - For the emulsifier, a polyglycerol condensed ricinoleic acid ester "CRS-75" produced by Sakamoto Yakuhin Kogyo Co., Ltd. was used.

- For the soybean oil, soybean *sirasimeyu* (refined soybean oil) produced by Fuji Oil Co., Ltd. was used.

15 - For the glycerol, a food additive, glycerol, produced by Kishida Chemical Co., Ltd. was used.

- The row titled ". . . particle size . . . (Day 1) (nm)" shows the particle sizes measured on day 1 after the emulsification step. Here, "W" means that a double peak was present, although no precipitation was observed. The term "No precipitation" means
20 that it was visually confirmed that precipitation did not occur, although the particle size was not measured.

- Precipitation was not observed in all of the aqueous phase prepared in accordance with the formulations.

[0058]

25 Method for preparing an oil and fat composition (study 3)

1. The aqueous phase components and the oil phase components were separately dissolved by mixing.

2. The aqueous phase and the oil phase were mixed, and the mixture was roughly emulsified to obtain a water-in-oil
30 emulsified liquid.

3. The emulsified liquid obtained in 2 above was emulsified using a high-pressure homogenizer (37 MPa, 20 passes) to obtain a water-in-oil emulsified product.

[0059]

35 Method of evaluation of oxidative stability based on CDM (study

3)

1. Each sample was diluted 4-fold with PUFA oil (containing EPA: 19.8% and DHA: 45.4%).

2. The oxidative stability of each diluted sample (3.0 g)

5 obtained in 1 above was evaluated using a CDM measurement device ("Rancimat" produced by Metrohm) at 96°C, at an air-blowing rate of 20.0 L/h with a sample-introducing amount of 3.0 g (amount of water: 70 ml). Additionally, soybean oil was diluted instead of the samples 4-fold with PUFA oil, and measurement was performed
10 in a manner similar to the above. The measured values are shown in the "CDM of control oil and fat."

3. The CDM value of each diluted sample obtained in 2 above was divided by the "CDM of control oil and fat," and the resulting values of 2.9 or greater were evaluated as pass.

15 For the samples in which precipitation was observed at the time of the measurement, basically, the CDM value was not measured.

(The DCM value of 3.0 g of soybean oil measured under the conditions of 2 above was 20 hrs.)

20 [0060]

Method of evaluation of oxidative stability based on POV (study

3)

1. Four parts by weight of tuna oil ("DHA22K" produced by Maruha Nichiro Foods, Inc.) was mixed per 1 part by weight of the sample
25 of Example 3-15 or Comparative Example 3-9.

2. Fifty milliliters of the obtained mixed oil was introduced into a 100-ml tall beaker, and the resulting product was stirred at 80 rpm in an incubator at 60°C in an open system while shielded from light with aluminum foil.

30 3. Samples were taken over time, and the peroxide value (POV) was measured. The POV measurement was performed in accordance with an iodometric titration method.

4. The samples with a POV of 5.0 or less on day 10 were evaluated as pass, while the samples with a POV of 1 or greater on day 3
35 were evaluated as fail.

As a control, a product obtained by mixing 4 parts by weight of the same tuna oil per 1 part by weight of soybean oil was used.

[0061]

5 Table 3-2: Results 1

	Comp. Ex. 3-1	Ex. 3-1	Ex. 3-2	Ex. 3-3	Ex. 3-4	Ex. 3-5	Ex. 3-6	Ex. 3-7
CDM of control oil and fat	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
CDM of sample	2.9	21.1	17.2	22.7	16.5	17.2	17.5	21.4
Sample/control	0.5	3.8	3.1	4.1	3.0	3.1	3.2	3.9

	Ex. 3-8	Comp. Ex. 3-2	Ex. 3-9	Ex. 3-10	Comp. Ex. 3-3	Comp. Ex. 3-4	Ex. 3-11	Comp. Ex. 3-5
CDM of control oil and fat	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
CDM of sample	26.9	-	24.46	29.66	-	-	24.88	-
Sample/control	4.9	-	4.4	5.4	-	-	4.5	-

	Ex. 3-12	Ex. 3-13	Comp. Ex. 3-6	Comp. Ex. 3-7	Ex. 3-14	Comp. Ex. 3-8
CDM of control oil and fat	5.5	5.5	5.5	5.5	5.5	5.5
CDM of sample	24.5	29.7	-	-	24.9	(15.5)
Sample/control	4.5	5.4	-	-	4.5	(2.8)

[0062]

10 Table 3-3: Results 2

	Control	Ex. 3-15	Comp. Ex. 3-9
Day 1	7.7	-	-
Day 2	27.1	0	0
Day 3	123.1	-	-
Day 4	-	-	-
Day 5	-	-	0
Day 6	-	0	0
Day 10	-	0	27.5
Day 13	-	0	-

[0063]

Discussion

- The use of various carbohydrates as water-soluble solids resulted in the prevention of the occurrence of precipitation.
- In Comparative Example 3-8, precipitation was observed on day 1.

When precipitation was observed, the CDM value was basically not measured. On a trial basis, however, the CDM value was measured. The results confirmed that both Example 3-1, which used a sugar, and Example 3-8, which used sorbitol, achieved a higher CDM value
5 than Comparative Example 3-8. These results confirmed that the use of a carbohydrate, which is believed to not have antioxidative activity in itself, improved not only emulsification stability but also antioxidative activity.

- The CDM value is a numerical value obtained by a method for
10 measuring antioxidative activity under stirring, the method comprising bubbling air. Thus, in the application to food products, which are basically stored while left to stand, the antioxidative activity was presumed to be further reduced due to the occurrence of precipitation.

15 [0064]

Study 3-2: Study of each water-soluble solids content

Samples of formulations shown in Table 3-4 were prepared in accordance with the "Method for preparing an oil and fat composition (study 3-2)." The obtained samples were evaluated
20 by the "Method of evaluation of oxidative stability based on CDM (study 3-2)." Table 3-5 shows the results.

25

30

35

[0065]

Table 3-4: Formulations 2

			Comp. Ex. 3-10	Comp. Ex. 3-11	Ex. 3-16
Antioxidant oil and fat composition	Aqueous phase	Vitamin C	-	-	4.1
		Catechin	-	-	9.9
		Sorbitol	-	-	4.3
		Water	-	-	15.0
	Oil phase	Emulsifier	-	-	12.0
		TBHQ	-	0.02	-
		Soybean oil	100.0	99.98	54.7
	Total		100.0	100.0	100.0
Water-soluble solids content in aqueous phase (wt%)			0.0%	0.0%	55.0%
Amount of water-soluble antioxidant in aqueous phase (wt%)			0.0%	0.0%	42.0%
Amount of aqueous phase (wt%)			0.0%	0.0%	14.0%
Emulsifier/water			0	0	0.8
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)			0.0	0.0	14.0
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)			-	-	124.3

(The formulations are in percent by weight.)

- 5 - For the catechin, "Sunphenon 90S" produced by Taiyo Kagaku Co., Ltd. was used.
- For the soybean oil, soybean *sirasimeyu* (refined soybean oil) produced by Fuji Oil Co., Ltd. was used.
- For TBHQ, a product of Amalfi (Product Number 3015200) was used.
- 10 - Precipitation was not observed in all of the aqueous phase prepared in accordance with the formulations.

[0066]

Method for preparing an oil and fat composition (study 3-2)

1. In Example 3-16, the aqueous phase components and the oil
15 phase components were separately dissolved by mixing. In Comparative Examples 3-10 and 3-11, only the oil phase was prepared to use it as a sample.
2. In Example 3-16, the aqueous phase and the oil phase were
mixed, and the mixture was roughly emulsified to obtain a water-
20 in-oil emulsified liquid.

3. The emulsified liquid obtained in 2 above was emulsified using a high-pressure homogenizer (37 MPa, 20 passes) to obtain a water-in-oil emulsified product.

[0067]

5 Method of evaluation of oxidative stability based on CDM (study 3-2)

1. Each sample was added to PUFA oil containing EPA (19.8%) and DHA (45.4%) such that the ratio of each sample:PUFA oil = 1 part by weight:9 parts by weight, followed by dilution.

10 2. The oxidative stability of each diluted sample (3.0 g) obtained in 1 above was evaluated using a CDM measurement device ("Rancimat" produced by Metrohm) at 96°C, at an air-blowing rate of 20.0 L/h with a sample-introducing amount of 3.0 g (amount of water: 70 ml).

15 3. The values obtained by using the samples of Comparative Example 3-11 and Example 3-16 were separately divided by the value obtained by using the sample of Comparative Example 3-10. When the resulting value was 2 or greater, the sample was evaluated as pass.

20 [0068]

Table 3-5: Results 2

	Comp. Ex. 3-10	Comp. Ex. 3-11	Ex. 3-16
CDM value of Comp. Ex. 3-10 (hrs) (control)	5.6	5.6	5.6
CDM value of sample (hrs)	5.6	8.6	40.4
Sample/control	-	1.5	7.2

[0069]

Discussion

25 - Even in evaluation systems that contain a large amount of PUFA oil, the antioxidant oil and fat composition according to the present invention had antioxidative activity higher than that of TBHQ. In the evaluation systems, the total amount of DHA and EPA was 58.7 wt%. This suggests that high stability would also be
30 achieved in the unsaturated fatty acid-containing oil and fat composition that contains DHA and EPA in a total amount of about

60 wt%.

[0070]

Study 4: Study of amino acids as an antioxidant

5 Samples of formulations shown in Table 4-1 were
prepared in accordance with the "Method for preparing an
antioxidant oil and fat composition (study 4)." The obtained
samples were evaluated in accordance with the "Method of
evaluation of oxidative stability based on CDM (study 4)." Table
4-2 shows the results.

10

[0071]

Table 4-1: Formulations

Antioxidant oil and fat composition	Ex. 4-1	Ex. 4-2	Ex. 4-3	Ex. 4-4	Ex. 4-5	Ex. 4-6	Ex. 4-7	Ex. 4-8	Ex. 4-9	Comp. Ex. 4-1
Aqueous phase	Glycine	2.00					2.00		4.00	
	Proline							2.00		
	Alanine	1.14								
	Phenylalanine		0.34							
	Threonine			0.57						
	Serine				3.43					
	Valine					0.57				
	Sugar powder						4.00	4.00		
	Maltitol	4.00	4.00	4.00	4.00	4.00			4.00	4.00
	Water	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Oil phase	Emulsifier	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
	Soybean oil	74.00	74.86	75.66	75.43	75.43	74.00	74.00	72.00	76.00
Total		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Water-soluble solids content in aqueous phase (wt%)		42.9%	35.2%	36.4%	48.1%	36.4%	42.9%	42.9%	50.0%	33.3%
Amount of water-soluble antioxidant in aqueous phase (wt%)		14.3%	2.8%	4.5%	22.2%	4.5%	14.3%	14.3%	25.0%	0.0%
Amount of aqueous phase (wt%)		14.0%	12.3%	12.6%	15.4%	12.6%	14.0%	14.0%	16.0%	12.0%
Emulsifier/water		1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)		2.0	0.3	0.6	3.4	0.6	2.0	2.0	4.0	0.0
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)		64.82	No precipitation	No precipitation	No precipitation	No precipitation	No precipitation	67.82	No precipitation	No precipitation

(The formulations are in percent by weight.)

- For the soybean oil, soybean *sirasimeyu* (refined soybean oil) produced by Fuji Oil Co., Ltd. was used.

- For the emulsifier, a polyglycerol condensed ricinoleic acid ester "CRS-75" produced by Sakamoto Yakuhin Kogyo Co., Ltd. was used.

- For the glycine and serine, food additive grade glycine and serine produced by Kyowa Hakko Kogyo Co., Ltd. were used. For the other amino acids, those of food additive grades produced by Nippon Rika Co., Ltd. were used.

- Precipitation was not observed in all of the aqueous phase prepared in accordance with the formulations.

[0072]

Method for preparing an oil and fat composition (study 4)

1. The aqueous phase components and the oil phase components were separately dissolved by mixing.

2. The aqueous phase and the oil phase were mixed, and the mixture was roughly emulsified to obtain a water-in-oil emulsified liquid.

3. The emulsified liquid obtained in 2 above was emulsified using a high-pressure homogenizer (37 MPa, 20 passes) to obtain a water-in-oil emulsified product.

[0073]

Method of evaluation of oxidative stability based on CDM (study 4)

1. Each sample was added to PUFA oil containing EPA (19.1%) and DHA (37.3%) such that the ratio of sample:PUFA oil = 1:9, followed by stirring.

2. The oxidative stability of the liquid mixture obtained in 1 above was evaluated using a CDM measurement device ("Rancimat" produced by Metrohm) at 96°C, at an air-blowing rate of 20.0 L/h with a sample-introducing amount of 3.0 g.

3. Those with a CDM value 1.2 times or more greater than that of the control obtained by using soybean oil instead of the samples used in 1 above were evaluated as pass.

[0074]

Table 4-2: Results

	Ex. 4-1	Ex. 4-2	Ex. 4-3	Ex. 4-4	Ex. 4-5	Ex. -6	Ex. 4-7	Ex. 4-8	Ex. 4-9	Comp. Ex. 4-1
CDM of control oil and fat (hr)	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
CDM value of sample (hr)	13.36	13.34	7.59	9.88	14.24	8.4	18.3	19.41	31.36	6.4
Sample/control	2.30	2.30	1.31	1.70	2.46	1.45	3.16	3.35	5.41	1.10

[0075]

Discussion

5 Amino acids have different solubility according to
their type; amino acids of low solubility dissolved less in an
aqueous phase, and accordingly, tended to achieve less
antioxidative activity. Nevertheless, it was confirmed that the
use of all of the amino acids had a tendency of achieving
10 increased antioxidative activity.

[0076]

Study 4-2: Study of amino acids as an antioxidant (2)

 To compare the antioxidative activity of each amino
acid, comparative analysis was performed by adjusting the
15 concentration of each amino acid in the aqueous phase to 144 mM
or 25 mM.

 Samples of formulations shown in Table 4-3 were
prepared in accordance with the "Method for preparing an oil and
fat composition (study 4)." The obtained samples were evaluated
20 in accordance with the "Method of evaluation of oxidative
stability based on CDM (study 4-2)." Table 4-4 shows the results.

[0077]

Table 4-3: Formulations

	Study Ex.4-1	Study Ex.4-2	Study Ex.4-3	Study Ex.4-4	Study Ex.4-5	Study Ex.4-6	Study Ex.4-7	Study Ex. 4-8	Study Ex.4-9	Study Ex.4-10
Aqueous phase	Glycine	0.086								
	Leucine		0.151							
	Isoleucine			0.151						
	Arginine				0.200					
	Asparagine monohydrate					0.173				
	Glutamine						0.168			
	Methionine							0.172		
	Threonine								0.137	
	Histidine									0.178
	Sugar	8.00	7.91	7.85	7.80	7.85	7.83	7.83	7.86	7.82
Oil phase	Water	8	8	8	8	8	8	8	8	8
	Emulsifier	12	12	12	12	12	12	12	12	12
	Soybean oil	72.0	72.004	71.999	72.000	71.997	72.002	71.998	72.003	72.002
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Water-soluble solids content in aqueous phase (wt%)	50	50	50	50	50	50	50	50	50	50
Amount of water-soluble antioxidant in aqueous phase (wt%)		0.54	0.94	0.94	1.25	1.08	1.05	1.07	0.86	1.11
Amino acid concentration (mM) in aqueous phase	-	144	144	144	144	144	144	144	144	144
Particle size (µm) 5°C after 1 day	48.94	46.85	45.91	45.92	46.41	46.34	52.15	53.2	52.55	51.9

	Study Ex.4-11	Study Ex.4-12	Study Ex.4-13	Study Ex.4-14	Study Ex.4-15	Study Ex.4-16	Study Ex.4-17	Study Ex.4-18	Study Ex.4-19	Study Ex.4-20
Alanine	0.102									
Lysine hydrochloride		0.21								
Proline			0.132							
Serine				0.121						
Valine					0.135					
Sodium glutamate						0.194				
Sodium aspartate							0.204			
Cysteine								0.024		
Phenylalanine									0.033	
Tryptophan										0.041
Sugar	7.9	7.79	7.87	7.88	7.87	7.81	7.98	7.8	7.97	7.96
Water	8	8	8	8	8	8	8	8	8	8
Oil	12	12	12	12	12	12	12	12	12	12
Emulsifier										
Soybean oil	71.998	72	71.998	71.999	71.995	71.996	71.816	72.176	71.997	71.999
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Water-soluble solids content in aqueous phase (wt%)	50	50	50	50	50	50	50	50	50	50
Amount of water-soluble antioxidant in aqueous phase (wt%)	0.64	1.31	0.82	0.76	0.84	1.21	1.26	0.15	0.21	0.26
Amino acid concentration (mM) in aqueous phase	144	144	144	144	144	144	144	25	25	25
Particle size (µm)	52.04	53.20	52.50	50.08	49.72	50.64	54.13	52.07	48.00	49.82

(The formulations are in percent by weight.)

- For the soybean oil, soybean *sirasimeyu* (refined soybean oil) produced by Fuji Oil Co., Ltd. was used.

- For the emulsifier, a polyglycerol condensed ricinoleic acid ester "CRS-75" produced by Sakamoto Yakuhin Kogyo Co., Ltd. was used.

- Precipitation was not observed in all of the aqueous phase prepared in accordance with the formulations of the Examples.

[0078]

Method of evaluation of oxidative stability (study 4-2)

1. Each sample was added to fish oil containing DHA (20.0%) and EPA (5.7%) such that the ratio of sample:fish oil = 1:9, followed by stirring.

2. The oxidative stability of the liquid mixture obtained in 1 above was evaluated using a CDM measurement device ("Rancimat" produced by Metrohm) at 96°C, at an air-blowing rate of 20.0 L/h with a sample-introducing amount of 3.0 g.

3. The samples that had a CDM value equal to or greater than that of Study Example 4-1 were evaluated as pass.

[0079]

Table 4-4: Results

	Study Ex.4-1	Study Ex.4-2	Study Ex.4-3	Study Ex.4-4	Study Ex.4-5	Study Ex.4-6	Study Ex.4-7	Study Ex.4-8	Study Ex.4-9	Study Ex.4-10
CDM value (hrs)	6.15	8.45	8.97	8.9	8.21	8.04	7.62	10.08	8.61	8.21

	Study Ex.4-11	Study Ex.4-12	Study Ex.4-13	Study Ex.4-14	Study Ex.4-15	Study Ex.4-16	Study Ex.4-17	Study Ex.4-18	Study Ex.4-19	Study Ex.4-20
CDM value (hrs)	8.72	8.87	10.09	8.27	8.91	8.02	8.28	7.32	7.28	8.13

[0080]

Discussion

- To compare the antioxidative activity per molar concentration of each amino acid, a test was performed by adjusting the concentrations to be equal to each other such that each target amino acid for comparison could be easily dissolved.

- As shown in Table 4-4, all of the tested amino acids had a CDM value equal to or greater than that of Study Example 4-1, and were evaluated as pass.

5 - According to the above results, it is presumed that the oil and fat composition prepared with the concentration predetermined according to the present invention would achieve sufficient antioxidative activity.

[0081]

10 Study 5: Study of the solids content, the amount of antioxidants, etc.

Samples of formulations shown in Table 5-1 were prepared in accordance with the "Method for preparing an oil and fat composition (study 5)." The obtained samples were evaluated in accordance with the "Method of evaluation of oxidative
15 stability based on CDM (study 5)." Table 5-2 shows the results.

[0082]

Table 5-1: Formulations

				Ex. 5-1	Ex. 5-2	Ex. 5-3	Ex. 5-4	Ex. 5-5	Ex. 5-6	Ex. 5-7	Ex. 5-8
Antioxidant oil and fat composition	Aqueous phase	Vitamin C		2.20	2.20	2.25	2.20	2.20	2.20	2.25	2.25
		Catechin		-	-	-	-	-	-	-	1.35
		Sugar		16.50	12.60	7.55	3.13	2.10	1.23	3.08	7.55
		Water		8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
	Oil phase	Soybean oil		61.30	65.20	70.20	74.67	75.70	76.57	80.27	68.85
Emulsifier		12.00	12.00	12.00	12.00	12.00	12.00	12.00	6.40	12.00	
Total				100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Water-soluble solids content in aqueous phase (wt%)											
Amount of water-soluble antioxidant in aqueous phase (wt%)				8.2%	9.6%	12.6%	16.5%	17.9%	19.2%	16.9%	18.8%
Amount of aqueous phase (wt%)				26.7%	22.8%	17.8%	13.3%	12.3%	11.4%	13.3%	19.2%
Emulsifier/water ratio				1.5	1.5	1.5	1.5	1.5	1.5	0.8	1.5
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)				2.2	2.2	2.3	2.2	2.2	2.2	2.3	3.6
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)				79.3	60.42	68.13	56.5	58.8	64.0	63.94	61

Antioxidant oil and fat composition	Aqueous phase	Oil phase	Total	Ex. 5-9	Comp. Ex. 5-1	Ex. 5-10	Ex. 5-11	Comp. Ex. 5-2	Comp. Ex. 5-3	Ex. 5-12
				Vitamin C	1.11	1.11	1.11	0.80	0.80	0.80
				Catechin	-	-	-	-	-	-
				Sugar	-	1.39	3.18	-	0.97	3.49
				Water	10.00	10.00	10.00	10.00	10.00	10.00
				Soybean oil	76.89	75.50	73.71	77.20	76.23	73.71
				Emulsifier	12.00	12.00	12.00	12.00	12.00	12.00
					100.00	100.00	100.00	100.00	100.00	100.00
Water-soluble solids content in aqueous phase (wt%)				58.2%	10.0%	20.0%	30.0%	7.4%	15.0%	30.0%
Amount of water-soluble antioxidant in aqueous phase (wt%)				18.8%	10.0%	8.9%	7.8%	7.4%	6.8%	5.6%
Amount of aqueous phase (wt%)				19.2%	11.1%	12.5%	14.3%	10.8%	11.8%	14.3%
Emulsifier/water ratio				1	1.2	1.2	1.2	1.2	1.2	1.2
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)				3.6	1.11	1.11	1.11	0.8	0.8	0.8
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)				60	Precipitation	61.3	62.06	Precipitation	Precipitation	65.55

(The formulations are in percent by weight.)

- For the catechin, "Sunphenon 90S" produced by Taiyo Kagaku Co., Ltd. was used.

- For the soybean oil, soybean *sirasimeyu* (refined soybean oil) produced by Fuji Oil Co., Ltd. was used.

5 - For the emulsifier, a polyglycerol condensed ricinoleic acid ester "CRS-75" produced by Sakamoto Yakuhin Kogyo Co., Ltd. was used.

- Precipitation was not observed in all of the aqueous phase prepared in accordance with the formulations of the Examples.

10 [0083]

Method for preparing an oil and fat composition (study 5)

1. The aqueous phase components shown in the formulations were dissolved by mixing to obtain an aqueous phase.

2. As shown in the formulations, the emulsifier was dissolved in
15 oil and fat to obtain an oil phase.

3. The aqueous phase was introduced into the oil phase, and the mixture was mixed using a homomixer to obtain an emulsified liquid.

4. Further, emulsification was performed using a high-pressure
20 homogenizer (37 MPa, 20 passes) to obtain a water-in-oil emulsified product.

[0084]

Method of evaluation of oxidative stability based on CDM (study 5)

25 1. Each sample was added to PUFA oil containing DHA (37%) and EPA (1.9%) such that the ratio of sample:PUFA oil = 2 parts:8 parts, followed by stirring.

2. The oxidative stability of the liquid mixture obtained in 1 above was evaluated using a CDM measurement device ("Rancimat"
30 produced by Metrohm) at 96°C, at an air-blowing rate of 20.0 L/h with a sample-introducing amount of 3.0 g.

3. The samples with a CDM value 1.2 times or more greater than that of the control obtained by using soybean oil instead of the samples used in 1 above were evaluated as pass.

35

[0085]

Table 5-2: Results

	Ex. 5-1	Ex. 5-2	Ex. 5-3	Ex. 5-4	Ex. 5-5	Ex. 5-6	Ex. 5-7	Ex. 5-8
CDM value of control oil and fat	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
CDM value of sample	16.43	16.48	26.83	18.3	18.3	16.2	20.2	19.1
Sample/control	3.0	3.0	4.9	3.3	3.3	2.9	3.7	3.5

	Ex. 5-9	Comp. Ex. 5-1	Ex. 5-10	Ex. 5-11	Comp. Ex. 5-2	Comp. Ex. 5-3	Ex. 5-12
CDM value of control oil and fat	5.5	5.5	5.5	5.5	5.5	5.5	5.5
CDM value of sample	17.7	-	24.46	29.66	-	-	24.88
Sample/control	3.2	-	4.4	5.4	-	-	4.5

5 [0086]

Discussion

- Adjusting the water-soluble solids content and the amount of water-soluble antioxidant to predetermined values made it possible to obtain oil and fat compositions that had high
10 antioxidative activity.

[0087]

Study 6: Study of the solids content, the amount of antioxidant, etc. 2

Samples of formulations shown in Table 6-1 were
15 prepared in accordance with the "Method for preparing an oil and fat composition (study 6)." The obtained samples were evaluated by the "Method of evaluation of oxidative stability based on CDM (study 6)." Table 6-2 shows the results.

[0088]

Table 6-1

Antioxidant oil and fat composition		Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.
		6-1	6-2	6-3	6-4	6-5	6-6	6-7	6-8	6-9	6-10	6-11	6-12
Aqueous phase	Vitamin C	2.25	2.25	2.25	2.81	3.52	4.23	2.25	2.25	2.25	2.25	2.25	2.25
	Catechin	-	-	1.36	1.70	2.13	2.54	1.35	1.35	1.35	1.35	1.35	1.35
	Sugar	7.55	7.55	7.44	9.30	11.63	13.95	1.30	2.20	3.21	4.40	5.78	7.55
	Water	8.00	8.00	8.00	10.00	12.50	15.00	8.00	8.00	8.00	8.00	8.00	8.00
	Soybean oil	75.80	74.20	74.55	68.19	60.22	52.28	75.10	74.20	73.19	72.00	70.62	68.85
Oil phase	Emulsifier	6.40	8.00	6.40	8.00	10.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Total		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Water-soluble solids content in aqueous phase (wt%)		55.1%	55.1%	58.0%	58.0%	58.0%	58.0%	38.0%	42.0%	46.0%	50.0%	54.0%	58.2%
Amount of water-soluble antioxidant in aqueous phase (wt%)		12.6%	12.6%	19.0%	18.9%	19.0%	19.0%	27.9%	26.1%	24.3%	22.5%	20.7%	18.8%
Amount of aqueous phase (wt%)		17.8	17.8	19.05	23.81	29.78	35.72	12.9	13.8	14.81	16	17.38	19.15
Emulsifier/water		0.8	1.0	0.8	0.8	0.8	0.8	1.5	1.5	1.5	1.5	1.5	1.5
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)		2.3	2.3	3.6	4.5	5.7	6.8	3.6	3.6	3.6	3.6	3.6	3.6
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)		66	80	-	-	-	-	81	74	72	69	67	68

(The formulations are in percent by weight.)

Note: In the particle size row, the symbol “-” indicates that although no measurement was performed using a device, it was visually confirmed that precipitation did not occur.

- For the catechin, “Sunphenon 90S” produced by Taiyo Kagaku Co., Ltd. was used.

- For the soybean oil, soybean *sirasimeyu* (refined soybean oil) produced by Fuji Oil Co., Ltd. was used.

- For the emulsifier, a polyglycerol condensed ricinoleic acid ester “CRS-75” produced by Sakamoto Yakuhin Kogyo Co., Ltd. was used.

- Precipitation was not observed in all of the aqueous phase prepared in accordance with the formulations.

[0089]

Method for preparing an oil and fat composition (study 6)

1. The aqueous phase components shown in the formulations were dissolved by mixing to obtain an aqueous phase.

2. As shown in the formulations, the emulsifier was dissolved in oil and fat to obtain an oil phase.

3. The aqueous phase was introduced into the oil phase, and the mixture was mixed using a homomixer to obtain an emulsified liquid.

4. Further, emulsification was performed using a high-pressure homogenizer (37 MPa, 20 passes) to obtain a water-in-oil emulsified product.

[0090]

Method of evaluation of oxidative stability based on CDM (study 6)

1. Each sample was added to PUFA oil containing DHA (37%) and EPA (1.9%) such that the ratio of sample:PUFA oil = 2 parts:8 parts, followed by stirring.

2. The oxidative stability of the liquid mixture obtained in 1 above was evaluated using a CDM measurement device (“Rancimat” produced by Metrohm) at 96°C, at an air-blowing rate of 20.0 L/h with a sample-introducing amount of 3.0 g.

3. The samples with a CDM value 1.2 times or more greater than

that of the control obtained by using soybean oil instead of the samples used in 1 above were evaluated as pass.

[0091]

Table 6-2: Results

	Ex. 6-1	Ex. 6-2	Ex. 6-3	Ex. 6-4	Ex. 6-5	Ex. 6-6	Ex. 6-7	Ex. 6-8	Ex. 6-9	Ex. 6-10	Ex. 6-11	Ex. 6-12
CDM value of control oil and fat	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
CDM value of sample	16.7	22.5	16.9	18.5	20.3	22.2	21	23.3	24.1	24.8	23.5	23.7
Sample/control	3.0	4.1	3.1	3.4	3.7	4.0	3.8	4.2	4.4	4.5	4.3	4.3

5

[0092]

Discussion

- The results confirmed the following: irrespective of the difference in the formulations, if the water-soluble solids content etc. were within the predetermined ranges, high
10 antioxidative activity would be achieved.

[0093]

Study 7: Study of production method

The difference in the antioxidative activity as a
15 result of the difference in the production method was analyzed.

Samples of formulations shown in Table 7-1 were prepared in accordance with the "Method for preparing an oil and fat composition (study 7)." As shown in Table 7-2, samples for measurement of antioxidative activity were prepared using the
20 obtained samples, and were subjected to Rancimat to measure the CDM value. The measurement was performed at 96°C, at an air-blowing rate of 20.0 L/h with a sample-introducing amount of 3.0 g (amount of water: 70 ml). The "Samples for measurement of antioxidative activity" were prepared by mixing the components in
25 accordance with the formulations, and lightly stirring the mixture for homogenization.

Table 7-3 shows the CDM values. The samples with a CDM value 1.5 times or more greater than that of Comparative Example 7-1 were evaluated as pass.

30

[0094]

Table 7-1: Formulations

			Ex. 7-1	Ex. 7-2
Antioxidant oil and fat composition	Aqueous phase	Vitamin C	0.33	3
		Catechin	0.17	1.5
		Sugar powder	1.13	10.18
		Water	1.33	12
	Oil phase	Emulsifier	2	18
		Soybean oil	95.04	55.32
		Total	100	100
Water-soluble solids content in aqueous phase (wt%)			55.1%	55.0%
Amount of water-soluble antioxidant in aqueous phase (wt%)			16.9%	16.9%
Amount of aqueous phase (wt%)			3.0%	26.7%
Emulsifier/water			1.50	1.50
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)			0.50	4.50
Aqueous phase particle size of antioxidant oil and fat composition (Day 1) (nm)			No precipitation	No precipitation

(The formulations are in percent by weight.)

- 5 - For the catechin, "Sunphenon 90S" produced by Taiyo Kagaku Co., Ltd. was used.
- For the soybean oil, soybean *sirasimeyu* (refined soybean oil) produced by Fuji Oil Co., Ltd. was used.
- For the emulsifier, a polyglycerol condensed ricinoleic acid
- 10 ester "CRS-75" produced by Sakamoto Yakuhin Kogyo Co., Ltd. was used.

15

20

[0095]

Table 7-2

		Comp. Ex. 7-1	Ex. 7-3	Ex. 7-4
Samples for measurement of antioxidative activity	Antioxidant oil and fat composition of Ex. 7-1	-	90	-
	Antioxidant oil and fat composition of Ex. 7-2	-	-	10
	Soybean oil	90		80
	PUFA oil	10	10	10
	Total	100	100	100
Composition at the time of CDM measurement (calculated value)	Vitamin C	-	0.3	0.3
	Catechin	-	0.15	0.15
	Sugar powder	-	1.02	1.02
	Water	-	1.2	1.2
	Emulsifier	-	1.8	1.8
	Soybean oil	90	85.53	85.53
	PUFA oil	10	10	10
	Total	100	100	100

(The formulations are in percent by weight.)

- 5 - For PUFA oil, oil and fat containing DHA and EPA in a total amount of 56.4 wt% was used.

[0096]

Method for preparing an oil and fat composition (study 7)

1. The aqueous phase components shown in the formulations were
10 dissolved by mixing to obtain an aqueous phase.
2. As shown in the formulations, the emulsifier was dissolved in
oil and fat to obtain an oil phase.
3. The aqueous phase was introduced into the oil phase, and the
mixture was mixed using a homomixer to obtain an emulsified
15 liquid.
4. Further, emulsification was performed using a high-pressure
homogenizer (37 MPa, 20 passes) to obtain a water-in-oil
emulsified product.

[0097]

20 Table 7-3: Results

	Comp. Ex. 7-1	Ex. 7-3	Ex. 7-4
CDM value	17.2	86.0	122.0
Each sample/Comp. Ex. 7-1	1.0	5.0	7.1

[0098]

Discussion

The antioxidant oil and fat compositions were prepared by varying the concentration of the antioxidant such that the amounts of the antioxidant in the compositions were consequently equal to each other, and the CDM value was measured.

Table 7-3 indicates that the antioxidant oil and fat composition containing the antioxidant at a predetermined concentration had high antioxidative activity.

It was also confirmed that the antioxidant oil and fat composition of Example 7-4, which contained the antioxidant at a higher concentration, achieved higher antioxidative activity.

[0099]

Study 8: Analysis of partial dehydration

Oil and fat compositions of formulations "before" the dehydration step of tests A to X shown in Table 8-1 were prepared in accordance with the "Method for preparing an oil and fat composition (study 8)." Subsequently, partial dehydration was performed with nitrogen aeration. The formulations "after" the dehydration step shown in Table 8-1 refer to the compositions after dehydration according to calculations, considering the mass reduced as a result of the dehydration operation as dehydrated water.

Table 8-1 shows the particle size of each sample as required.

The obtained samples were evaluated in accordance with the "Method of evaluation of oxidative stability based on CDM (study 3)."

Table 8-2 shows the results.

[0100]

Table 8-1: Formulations

	Test A		Test B		Test C		Test D		Test E	
	Ex. 8-1	Ex. 8-2	Ex. 8-3	Ex. 8-4	Ex. 8-5	Ex. 8-6	Ex. 8-7	Ex. 8-8	Ex. 8-9	Ex. 8-10
Dehydration step	before	after	before	after	before	after	before	after	before	after
	2.20	2.22	2.20	2.33	2.25	2.31	2.25	2.33	2.25	2.34
	16.50	16.68	12.60	12.79	11.00	11.29	8.80	9.10	7.55	7.84
Antioxidant oil and fat composition	8.00	6.90	8.00	6.51	8.00	5.55	8.00	4.87	8.00	4.49
	61.30	62.07	65.20	66.19	66.75	68.53	68.95	71.29	70.20	72.87
	12.00	12.13	12.00	12.18	12.00	12.32	12.00	12.41	12.00	12.46
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Water-soluble solids content in aqueous phase (wt%)	70.0%	73.3%	64.9%	69.9%	62.4%	71.0%	58.0%	70.1%	55.1%	69.4%
Amount of water-soluble antioxidant in aqueous phase (wt%)	8.2%	8.6%	9.6%	10.8%	10.6%	12.1%	11.8%	14.3%	12.6%	15.9%
Amount of aqueous phase (wt%)	26.7%	25.8%	22.8%	21.6%	21.3%	19.2%	19.1%	16.3%	17.8%	14.7%
Emulsifier/water ratio	1.5	1.8	1.5	1.9	1.5	2.2	1.5	2.5	1.5	2.8
Dehydration percentage	-	13.8%	-	18.6%	-	30.6%	-	39.2%	-	43.9%
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)	2.2	2.22	2.2	2.33	2.25	2.31	2.25	2.33	2.25	2.34
Particle size on Day 1 (nm)	79.3	78.2	60.42	60.97	No precipitation	63.87	No precipitation	65	68.13	65.66
Particle size on Day 9 (nm)	W	W	W	No precipitation	No precipitation	No precipitation	No precipitation	107.4	Precipitation	No precipitation

		Test F		Test G		Test H		Test I		Test J		Comp. Ex. 8-1											
		Ex. 8-11	Ex. 8-12	Ex. 8-13	Ex. 8-14	Ex. 8-15	Ex. 8-16	Ex. 8-17	Ex. 8-18	Ex. 8-19	Ex. 8-20												
Antioxidant oil and fat composition		Dehydration step		before	after	before	after	before	after	before	after	after											
				2.25	2.34	2.25	2.36	2.25	2.37	2.20	2.32	2.25	2.40										
				6.60	6.86	4.40	4.62	3.08	3.25	2.10	2.22	-	-										
		Aqueous phase		8.00	4.37	8.00	3.47	8.00	3.02	8.00	2.79	8.00	1.93	1.82									
Oil phase		Soybean oil		71.15	73.96	73.35	76.96	74.67	78.71	75.70	80.01	77.75	82.88	82.97									
		Emulsifier		12.00	12.47	12.00	12.59	12.00	12.65	12.00	12.66	12.00	12.79	12.81									
Total		100.00		100.00		100.00		100.00		100.00		100.00											
Water-soluble solids content in aqueous phase (wt%)		52.5%		67.8%		45.4%		66.8%		40.0%		65.1%		35.0%		61.9%		22.0%		55.5%		56.9%	
Amount of water-soluble antioxidant in aqueous phase (wt%)		13.4%		17.2%		15.4%		22.6%		16.9%		27.5%		17.9%		31.7%		22.0%		55.5%		56.9%	
Amount of aqueous phase (wt%)		16.9%		13.6%		14.7%		10.4%		13.3%		8.6%		12.3%		7.3%		10.3%		4.3%		4.2%	
Emulsifier/water ratio		1.5		2.9		1.5		3.6		1.5		4.2		1.5		4.5		1.5		6.6		7.0	
Dehydration percentage		-		45.4%		-		56.7%		-		62.3%		-		65.1%		-		75.9%		77.2%	
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)		2.25		2.34		2.25		2.36		2.25		2.37		2.2		2.32		2.25		2.40		2.40	
Particle size on Day 1 (nm)		No precipitation		61.36		No precipitation		63.91		62.94		57.68		58.8		53.7		No precipitation		97.62		Precipitation	
Particle size on Day 9 (nm)		No precipitation		106.7		No precipitation		103.8		No precipitation		No precipitation		94.0		83.2		No precipitation		Precipitation		—	

	Test K		Test L		Test M		Test N		Test O	
	Ex. 8-21	Ex. 8-22	Ex. 8-23	Ex. 8-24	Ex. 8-25	Ex. 8-26	Ex. -27	Ex. 8-28	Ex. 8-29	Ex. 8-30
Antioxidant oil and fat composition	Dehydration step									
	before	after	before	after	before	after	before	after	before	after
	2.25	2.38	2.25	2.35	2.25	2.33	3.52	3.65	4.23	4.42
	-	-	-	-	-	-	2.13	2.21	2.54	2.65
	VC									
	Catechin									
	Sugar									
Antioxidant oil phase	Water									
	8.00	2.76	8.00	3.86	8.00	4.72	12.50	9.08	15.00	11.19
	Soybean oil									
	80.27									
	Emulsifier									
Total	6.40									
	100.00									
Water-soluble solids content in aqueous phase (wt%)	40.0%	67.1%	55.1%	72.6%	55.1%	68.3%	58.0%	66.4%	58.0%	65.9%
Amount of water-soluble antioxidant in aqueous phase (wt%)	16.9%	28.3%	12.6%	16.7%	12.6%	15.7%	19.0%	21.7%	19.0%	21.5%
Amount of aqueous phase (wt%)	13.3%	8.4%	17.8	14.1	17.8	14.87	29.78	27.02	35.72	32.83
Emulsifier/water ratio	0.8	2.4	0.8	1.7	1.0	1.8	0.8	1.1	0.8	1.1
Dehydration percentage	-	65.5%	-	51.8%	-	41.0%	-	27.4%	-	25.4%
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)	2.3	2.38	2.25	2.35	2.25	2.33	5.65	5.86	6.77	7.07
Particle size on Day 1 (nm)	63.94	57.63	66	58	80	78	-	69	-	73
Particle size on Day 9 (nm)	No precipitation	No precipitation	-	-	-	-	-	-	-	-

		Test P		Test Q			Test R		Test S		Test T	
		Ex. 8-31	Ex. 8-32	Ex. 8-33	Ex. 8-34	Ex. 8-35	Ex. 8-36	Ex. 8-37	Ex. 8-38	Ex. 8-39	Ex. 8-40	Ex. 8-41
Dehydration step		before	after	before	after	after	before	after	before	after	before	after
		2.30	2.30	2.30	2.30	2.40	2.25	2.38	2.25	2.35	2.25	2.35
Antioxidant oil and fat composition		-	-	-	-	-	1.35	1.43	1.35	1.41	1.35	1.41
		8.80	9.10	8.80	9.10	9.20	-	-	1.30	1.36	2.20	2.30
		8.00	5.30	8.00	4.80	4.00	8.00	2.65	8.00	3.97	8.00	3.77
		Soybean oil	72.90	75.10	74.50	77.20	77.70	76.40	80.84	75.10	78.38	74.20
Oil phase		8.00	8.20	6.40	6.60	6.70	12.00	12.70	12.00	12.53	12.00	12.55
Total		100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Water-soluble solids content in aqueous phase (wt%)		58.1%	68.3%	58.1%	70.4%	74.4%	31.0%	59.0%	38.0%	56.3%	42.0%	61.6%
Amount of water-soluble antioxidant in aqueous phase (wt%)		12.0%	13.8%	12.0%	14.2%	15.4%	31.0%	59.0%	27.9%	41.4%	26.1%	38.3%
Amount of aqueous phase (wt%)		19.1	16.7	19.1	16.2	15.6	11.6	6.46	12.9	9.09	13.8	9.83
Emulsifier/water ratio		1.0	1.5	0.8	1.4	1.7	1.5	4.8	1.5	3.2	1.5	3.3
Dehydration percentage		-	33.8%	-	40.0%	50.0%	-	66.9%	-	50.4%	-	52.9%
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)		2.3	2.30	2.30	2.30	2.40	3.60	3.81	3.60	3.76	3.60	3.76
Particle size on Day 1 (nm)		-	61	-	63.1	63.9	124	105	81	71	74	66
Particle size on Day 9 (nm)		-	-	-	-	-	-	-	-	-	-	-

	Test Z			Test Z2		
	Ex. 8-52	Ex. 8-53	Comp. Ex. 8-3	Ex. 8-54	Ex. 8-55	Comp. Ex. 8-4
Dehydration step	before	after	after	before	after	after
	0.28	0.28	0.28	0.36	0.36	0.36
	0.16	0.16	0.16	0.21	0.21	0.21
	0.94	0.94	0.95	0.30	0.30	0.30
	1.00	0.50	0.20	1.30	0.80	0.40
Antioxidant oil and fat composition	Aqueous phase	VC				
		Catechin				
	Sorbitol					
Oil phase	Water					
	Soybean oil					
Total	Emulsifier					
	100.0		100.0	100.0	100.0	100.0
Water-soluble solids content in aqueous phase (wt%)	57.9%	73.4%	87.4%	40.1%	52.1%	68.5%
Amount of water-soluble antioxidant in aqueous phase (wt%)	11.6%	14.9%	17.6%	16.5%	21.6%	28.3%
Amount of aqueous phase (wt%)	2.4%	1.9%	1.6%	2.2%	1.7%	1.3%
Emulsifier/water ratio	4.0	8.0	20.2	3.1	5.0	10.1
Dehydration percentage	-	50.0%	80.0%	-	38.5%	69.2%
Concentration of water-soluble antioxidant in antioxidant oil and fat composition (wt%)	0.44	0.44	0.44	0.57	0.57	0.57
Particle size on Day 1 (nm)	61.7	58.1	Precipitation	54.6	45.8	Precipitation
Particle size on Day 9 (nm)						

(The formulations are in percent by weight.)

- For the VC, ascorbic acid was used.
- For the catechin, "Sunphenon 90S" produced by Taiyo Kagaku Co., Ltd. was used.
- For the soybean oil, soybean *sirasimeyu* (refined soybean oil) produced by Fuji Oil Co., Ltd. was used.
- For the emulsifier, a polyglycerol condensed ricinoleic acid ester "CRS-75" produced by Sakamoto Yakuhin Kogyo Co., Ltd. was used.
- Precipitation was not observed in all of the aqueous phase prepared in accordance with the formulations.

[0101]

Method for preparing an oil and fat composition (study 8)

1. The aqueous phase components shown in the formulations were dissolved by mixing to obtain an aqueous phase.
2. As shown in the formulations, the emulsifier was dissolved in oil and fat to obtain an oil phase.
3. The aqueous phase was introduced into the oil phase, and the mixture was mixed using a homomixer to obtain an emulsified liquid.
4. Further, emulsification was performed using a high-pressure homogenizer (37 MPa, 20 passes) to obtain a water-in-oil emulsified product.
5. Partial dehydration was performed with nitrogen aeration.

[0102]

Table 8-2: Results

	Test A		Test B		Test C		Test D		Test E	
	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.
	8-1	8-2	8-3	8-4	8-5	8-6	8-7	8-8	8-9	8-10
CDM value of control oil and fat	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
CDM value of sample	16.4	16.4	16.48	16.78	-	18.85	-	18.97	26.83	27.4
Sample/control	3.0	3.0	3.0	3.1	-	3.4	-	3.4	4.9	5.0

	Test F		Test G		Test H		Test I		Test J		
	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Comp.
	8-11	8-12	8-13	8-14	8-15	8-16	8-17	8-18	8-19	8-20	Ex. 8-1
CDM value of control oil and fat	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
CDM value of sample	-	20.26	-	19.08	26.6	27.6	18.3	18.6	-	14.2	-
Sample/control	-	3.7	-	3.5	4.8	5.0	3.3	3.4	-	2.6	-

	Test K		Test L		Test M		Test N		Test O	
	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.
	8-21	8-22	8-23	8-24	8-25	8-26	8-27	8-28	8-29	8-30
CDM value of control oil and fat	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
CDM value of sample	20.2	21.6	16.7	20	22.5	22.9	20.3	20.5	22.2	22.3
Sample/control	3.7	3.9	3.0	3.6	4.1	4.2	3.7	3.7	4.0	4.1

	Test P		Test Q				Test R		Test S		Test T	
	Ex. 8-31	Ex. 8-32	Ex. 8-33	Ex. 8-34	Ex. 8-35	Ex. 8-36	Ex. 8-37	Ex. 8-38	Ex. 8-39	Ex. 8-40	Ex. 8-41	
CDM value of control oil and fat	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	
CDM value of sample	-	17	-	16.3	17	18.1	20.2	21	19.1	23.3	23	
Sample/control	-	3.1	-	3.0	3.1	3.3	3.7	3.8	3.5	4.2	4.2	

	Test U		Test V		Test W		Test X		Test Y		
	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Ex.	Comp.
	8-42	8-43	8-44	8-45	8-46	8-47	8-48	8-49	8-50	8-51	Ex. 8-2
CDM value of control oil and fat	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.9	5.9	5.9
CDM value of sample	24.1	24.2	24.8	24.5	23.5	24.3	23.7	25.1	16.8	18.4	—
Sample/control	4.4	4.4	4.5	4.5	4.3	4.4	4.3	4.6	2.9	3.1	—

	Test Z			Test Z2		
	Ex.	8-52	Comp.	Ex.	8-53	Comp.
CDM value of control oil and fat	5.9	5.9	5.9	5.9	5.9	5.9
CDM value of sample	22.0	23.2	—	22.7	22.9	—
Sample/control	3.8	4.0	—	3.9	3.9	—

[0103]

Discussion

- This study confirmed that appropriate partial dehydration achieved effects, such that the particle size was reduced, and the occurrence of precipitation was prevented with time. Moreover, there were cases in which antioxidative activity was improved as a result of dehydration.

- However, when dehydration was overly performed, an adverse effect could arise in terms of the occurrence of precipitation.

For example, it was inappropriate to perform dehydration until the water in the antioxidant oil and fat composition became less than 0.5 wt%, as in Comparative Examples 8-2, 8-3, and 8-4, since precipitation occurred.

[0104]

Study 9: Confirmation of effects

The oil and fat compositions obtained in the present invention were compared in terms of antioxidative activity.

Table 9-1 shows the samples. The effect was confirmed based on the measurement of POV (peroxide value). The details of the evaluation were described below in the "Method of evaluation of oxidative stability based on POV (study 9)." Fig. 1 shows the results.

[0105]

Table 9-1: Sample details

	Comp. Ex. 9-1	Comp. Ex. 9-2	Comp. Ex. 9-3	Ex. 9-1
Soybean oil	100	10	10	-
PUFA oil	-	90	89.98	90
Sample of Ex. 5-10	-	-	-	10
TBHQ	-	-	0.02	-
Total	100	100	100	100

(The formulations are in percent by weight.)

- For the PUFA oil, oil and fat containing EPA (19.8%) and DHA (45.4%) was used.

[0106]

Method of evaluation of oxidative stability based on POV (study

9)

1. Each of the oil and fat of the formulations shown in Table 9-1 was placed into a 50-ml glass bottle, and the bottle was covered with a lid, which was then placed into an incubator at 60°C, followed by stirring at 80 rpm.

2. Samples were taken at an appropriate time, and the peroxide value (POV) was measured. The POV was measured in accordance with the Standard methods for the analysis of fats, oils and related materials.

[0107]

Discussion

As shown in Fig. 1, when the oil and fat composition according to the present invention was used, the PUFA oil achieved a higher value in terms of the oxidative stability than that of soybean oil, and its antioxidative activity was more excellent than that of TBHQ, which is a synthetic antioxidant.

Due to its low oxidative stability, the use of PUFA oil was limited in many cases. However, it is presumed that the use of the oil and fat composition according to the present invention has made it possible to use PUFA oil in a manner similar to that of general-purpose oils and fats, such as soybean oil.

In this study, a sensory evaluation was also performed by a method in which an unpleasant odor was confirmed. Here as well, the occurrence of an unpleasant odor such as a fishy odor was greatly prevented in PUFA oil with which the oil and fat composition according to the present invention was used, indicating that general-purpose use was possible. Heretofore, fish oil etc. could have an unpleasant odor before the peroxide value actually increases, and for this reason, it was presumed that the occurrence of an unpleasant odor could be caused by a factor other than oxidation. However, the use of the oil and fat composition according to the present invention having overwhelmingly strong antioxidative activity prevented the occurrence of an unpleasant odor, which suggested that oxidation was the main cause of the occurrence of an unpleasant odor such

as a fishy odor.

Brief Description of Drawings

[0108]

- 5 Fig. 1 is a graph showing the oxidation-preventing effect of the oil and fat composition according to the present invention.

CLAIMS

[Claim 1]

An antioxidant oil and fat composition with a water
5 content of 0.5 to 18 wt% comprising
an aqueous phase containing a water-soluble antioxidant
in an amount of 2.5 to 65 wt%, and further containing a
carbohydrate in an amount such that the aqueous phase has a total
water-soluble solids content of 18 to 79 wt%,
10 wherein the amount of the aqueous phase in the
antioxidant oil and fat composition is within a range of 1 to 38
wt%, and the aqueous phase with a particle size of 300 nm or less
is dispersed in an oil phase, and
wherein the oil phase contains PUFA oil and/or fish oil.

15

[Claim 2]

The antioxidant oil and fat composition according to
claim 1,
wherein the oil phase contains at least one oil-soluble
20 emulsifier selected from polyglycerol condensed ricinoleates,
sugar esters, glycerol fatty acid esters, or lecithins, in an
amount that is 0.7 to 9 times the weight amount of the water.

[Claim 3]

25 The antioxidant oil and fat composition according to
claim 1,
wherein the water-soluble antioxidant is at least one
member selected from vitamin C, an amino acid, or catechin.

[Claim 4]

30 The antioxidant oil and fat composition according to
claim 2,
wherein the water-soluble antioxidant is at least one
member selected from vitamin C, an amino acid, or catechin.

35

[Claim 5]

The antioxidant oil and fat composition according to claim 3,

wherein the amino acid is at least one member selected
5 from glycine, glutamic acid, alanine, proline, lysine, tryptophan, methionine, valine, serine, histidine, isoleucine, leucine, phenylalanine, arginine, threonine, cysteine, aspartic acid, glutamine, or asparagine.

10 [Claim 6]

The antioxidant oil and fat composition according to claim 4,

wherein the amino acid is at least one member selected
from glycine, glutamic acid, alanine, proline, lysine, tryptophan,
15 methionine, valine, serine, histidine, isoleucine, leucine, phenylalanine, arginine, threonine, cysteine, aspartic acid, glutamine, or asparagine.

[Claim 7]

20 The antioxidant oil and fat composition according to claim 6,

wherein the carbohydrate is at least one member
selected from sugar alcohols, glucose, sucrose, dextrans, maltose,
or oligosaccharides.

25

[Claim 8]

The antioxidant oil and fat composition according to any one of claims 1 to 5,

wherein the carbohydrate is at least one member
30 selected from sugar alcohols, glucose, sucrose, dextrans, maltose, or oligosaccharides.

[Claim 9]

A method for producing an antioxidant oil and fat
35 composition, the method comprising the following steps of:

1: preparing an aqueous phase containing a water-soluble antioxidant in an amount of 2.5 to 65 wt%, and further containing a carbohydrate in an amount such that the aqueous phase has a total water-soluble solids content of 18 to 79 wt%;

5 and

2: dispersing the aqueous phase in an oil phase such that the percentage of the aqueous phase becomes 1 to 38 wt% with a particle size of 300 nm or less to obtain an antioxidant oil and fat composition with a water content of 0.5 to 18 wt%,

10 wherein the oil phase contains PUFA oil and/or fish oil.

[Claim 10]

The production method according to claim 9,
the method comprising the step of adding, to the oil
15 phase, at least one oil-soluble emulsifier selected from polyglycerol condensed ricinoleates, sugar esters, glycerol fatty acid esters, or lecithins, in an amount that is 0.7 to 9 times the weight amount of the water contained in the antioxidant oil and fat composition.

20

[Claim 11]

The production method according to claim 9,
wherein the water-soluble antioxidant is at least one member selected from vitamin C, an amino acid, or catechin.

25

[Claim 12]

The production method according to claim 10,
wherein the water-soluble antioxidant is at least one member selected from vitamin C, an amino acid, or catechin.

30

[Claim 13]

The production method according to claim 11,
wherein the amino acid is at least one member selected from glycine, glutamic acid, alanine, proline, lysine, tryptophan,
35 methionine, valine, serine, histidine, isoleucine, leucine,

phenylalanine, arginine, threonine, cysteine, aspartic acid, glutamine, or asparagine.

[Claim 14]

5 The production method according to claim 12,
 wherein the amino acid is at least one member selected
 from glycine, glutamic acid, alanine, proline, lysine, tryptophan,
 methionine, valine, serine, histidine, isoleucine, leucine,
 phenylalanine, arginine, threonine, cysteine, aspartic acid,
10 glutamine, or asparagine.

[Claim 15]

 The production method according to claim 9,
 wherein the carbohydrate is at least one member
15 selected from sugar alcohols, glucose, sucrose, dextrins, maltose,
 or oligosaccharides.

[Claim 16]

 The production method according to claim 10,
20 wherein the carbohydrate is at least one member
 selected from sugar alcohols, glucose, sucrose, dextrins, maltose,
 or oligosaccharides.

[Claim 17]

25 The production method according to claim 11,
 wherein the carbohydrate is at least one member
 selected from sugar alcohols, glucose, sucrose, dextrins, maltose,
 or oligosaccharides.

[Claim 18]

30 The production method according to claim 12,
 wherein the carbohydrate is at least one member
 selected from sugar alcohols, glucose, sucrose, dextrins, maltose,
 or oligosaccharides.

35

[Claim 19]

The production method according to claim 13,
wherein the carbohydrate is at least one member
selected from sugar alcohols, glucose, sucrose, dextrans, maltose,
5 or oligosaccharides.

[Claim 20]

The production method according to claim 14,
wherein the carbohydrate is at least one member
10 selected from sugar alcohols, glucose, sucrose, dextrans, maltose,
or oligosaccharides.

[Claim 21]

A method for producing an antioxidant oil and fat
15 composition,
the method comprising the step of dehydrating 12 to 76
wt% of water from the antioxidant oil and fat composition
obtained by the production method of any one of claims 9 to 20 to
obtain an antioxidant oil and fat composition with a water
20 content of 0.5 to 18 wt%.

[Claim 22]

The antioxidant oil and fat composition according to
any one of claims 1 to 8, wherein the antioxidant oil and fat
25 composition is an unsaturated fatty acid-containing oil and fat
composition.

[Claim 23]

The antioxidant oil and fat composition according to
30 any one of claims 1 to 8 and 22, comprising DHA and EPA in a
total amount of 0.1 to 60 wt%.