

Specification

Title of Invention:

FAT COMPOSITION FOR USE IN INFANT FORMULA

5 Technical Field

The present invention relates to a fat and/or oil composition for use in infant formula. More specifically, the present invention relates to a fat and/or oil composition meeting a nutritional balance suitable as an infant formula and having sufficiently low contents of 3-MCPD, glycidol, and fatty acid esters thereof as a fat
10 and/or oil composition for use in infant formula.

Background Art

When 3-MCPD (3-monochloropropane-1,2-diol) fatty acid esters contained in edible fats and/or oils are metabolized *in vivo* to thereby form 3-MCPD, this product is
15 suspected to be carcinogenic and toxic.

Meanwhile, it has been reported that fats and/or oils containing DAG (diacylglycerol) at a high concentration contain glycidol fatty acid esters. When such glycidol fatty acid esters are metabolized *in vivo* to thereby form glycidol, the carcinogenicity and toxicity are concerned. Thus, it is desired that edible fats and/or
20 oils have low contents of 3-MCPD, glycidol, and fatty acid esters thereof.

Patent Literature 1 states that the contents of 3-MCPD, glycidol, and fatty acid esters thereof can be reduced by transesterifying fats and/or oils, followed by a treatment with acidic activated clay, and deodorization at low temperatures. In addition, Patent Literature 1 states as follows. According to the announcement by
25 WHO/FAO, the tolerable daily intake (TDI) of 3-MCPD is 2 µg/kg (body weight). Given that the average daily intake of fats and/or oils is 12.5 g for an adult having a body weight of 60 kg, the tolerable concentration of 3-MCPD in fats and/or oils is 9.6 ppm. Likewise, according to the data of the German Federal Institute for Risk Assessment (BfR), the estimated benchmark dose lower confidence limit (BMDL10) of
30 glycidol is 4.06 mg/kg (body weight), and the tolerable concentration of glycidol in fats

and/or oils is 2.0 ppm calculated on the basis that the margin of exposure used as an indicator by the Food Safety Commission is 10000 or more and that the average daily intake of fats and/or oils is 12.5 g for an adult having a body weight of 60 kg.

5 On the other hand, processed powdered milk for raising infant or child as a substitute food for mother's milk is required to nutritionally meet a certain standard in Japan and internationally. Human milk fats contain certain amounts of essential fatty acids necessary for the growth of infants, such as linoleic acid, α -linolenic acid, arachidonic acid, and docosahexaenoic acid. For this reason, in the guideline issued
10 by the Consumer Affairs Agency (Japan) (Consumer Affairs Agency, Food Labeling Notification No. 277 (June 23, 2011)), the approval standard for the labeling as processed powdered milk for infant instructs that a composition per 100 kcal should contain 4.4 to 6.0 g of lipids, 0.3 to 1.4 g of linoleic acid, and 0.05 g or more of α -linolenic acid.

15 Patent Literature 2 discloses that a fat and/or oil composition having a particular fatty acid composition was prepared as a human-milk analogous fat and/or oil composition.

 Moreover, Patent Literature 3 discloses a fat and/or oil composition containing a combination of randomized palm oil or randomized palm olein oil with other fats
20 and/or oils, and states that the fat and/or oil composition is usable as a fat and/or oil composition for infant nutrition.

Citation List

Patent Literatures

- 25 Patent Literature 1: Japanese Patent Application Publication No. 2012-136655
Patent Literature 2: Japanese Patent Application Publication No. Hei 7-107904
Patent Literature 3: Japanese Patent Application Publication No. Hei 2-231037

Summary of Invention

30 **Problem to be solved by the invention**

As described in Patent Literature 1, given that the average daily intake of fats and/or oils is 12.5 g for an adult having a body weight of 60 kg, the tolerable concentration of 3-MCPD derived from fats and/or oils is 9.6 ppm, and the tolerable concentration of glycidol is 2.0 ppm. Meanwhile, infants and so forth are different in the average daily intake of fats and/or oils and the body weight. For example, given that the average daily intake of fats and/or oils is 36.0 g for an infant and so forth having a body weight of 6 kg, the tolerable concentration of 3-MCPD in a fat and/or oil composition is 0.33 ppm, and the tolerable concentration of glycidol is 0.07 ppm. Accordingly, 3-MCPD and glycidol are desirably at these tolerable concentrations or lower.

Performing transesterification, treatment with acidic activated clay, and low-temperature deodorization as described in Patent Literature 1 can reduce the contents of 3-MCPD, glycidol, and fatty acid esters thereof. However, transesterifying palm oil increases the melting point of the fat and/or oil composition, and crystals are formed at low temperatures, so that the distribution at high temperatures is required.

As described above, certain standards are established for linoleic acid and α -linolenic acid in fat and/or oil compositions for use in infant formula. Moreover, as a substitute food for mother's milk, such compositions need to have a particular fatty acid composition as described in Patent Literature 2. Highly unsaturated fatty acids such as linoleic acid and α -linolenic acid are generally poor in oxidation stability, and the flavor deteriorates over time through the distribution at high temperatures. For this reason, fat and/or oil compositions of these are distributed (tank storage, truck transportation) at low temperatures. However, crystals formed during the distribution at low temperatures clog a strainer when the fat and/or oil composition is transferred (truck $\Leftrightarrow$ tank), resulting in a problem of hindering the transfer. Fat and/or oil compositions for use in infant formula need to have such storage stability that no crystals are formed during the distribution at low temperatures.

The term storage stability herein means having a favorable flavor without forming crystals during the distribution at low temperatures.

Accordingly, an object of the present invention is to provide a fat and/or oil

composition for use in infant formula, the composition having sufficiently low concentrations of 3-MCPD, glycidol, and fatty acid esters thereof, further being excellent in storage stability, and nutritionally meeting a certain standard.

5 Means to solve the Problem

The above-described object of the present invention is achieved by a fat and/or oil composition containing linoleic acid and α -linolenic acid, and having a solid fat content at 35°C of 0.5% or less, a 3-MCPD concentration of 0.33 ppm or less, and a glycidol concentration of 0.07 ppm or less. Hence, a fat and/or oil composition for use in infant formula excellent in nutrition, safety, and storage stability is provided.

Other aspects of the present invention may be as follows.

[1] A fat and/or oil composition for use in infant formula, comprising linoleic acid and α -linolenic acid, wherein the fat and/or oil composition has a solid fat content at 35°C of 0.5% or less, a 3-monochloropropane-1,2-diol (3-MCPD) concentration of 0.33 ppm or less, and a glycidol concentration of 0.07 ppm or less.

[2] The fat and/or oil composition according to [1], comprising a transesterified fat and/or oil of a fat and/or oil mixture containing a first fat and/or oil selected from palm oil and fractionated oils thereof, and a second fat and/or oil selected from palm kernel oil and fractionated oils thereof.

[3] The fat and/or oil composition according to [1] or [2], wherein the fat and/or oil mixture for producing the transesterified fat and/or oil contains the first fat and/or oil in an amount of less than 50% by mass relative to a total mass of the fat and/or oil composition.

[4] The fat and/or oil composition according to any one of [1] to [3], comprising linoleic acid in an amount of 5.0 to 32.0% by mass and α -linolenic acid in an amount of 0.8% by mass or more relative to the total mass of the fat and/or oil composition.

[5] The fat and/or oil composition according to any one of [1] to [4], wherein the fat and/or oil mixture for producing the transesterified fat and/or oil contains one or more fats and/or oils other than the first fat and/or oil and the second fat and/or oil.

[6] The fat and/or oil composition according to any one of [1] to [5], comprising one or more other fats and/or oils than the transesterified fat and/or oil.

[7] The fat and/or oil composition according to any one of [1] to [6], wherein the fat and/or oil composition is deodorized at 200°C or more to 230°C or less.

5 [8] An infant formula comprising the fat and/or oil composition according to any one of [1] to [7].

[9] A method for producing the fat and/or oil composition according to any one of [1] to [7], comprising the following steps:

(i) mixing a first fat and/or oil selected from palm oil and fractionated oils
10 thereof with a second fat and/or oil selected from palm kernel oil and fractionated oils thereof and a third fat and/or oil, which is optionally contained, other than the first fat and/or oil and the second fat and/or oil to prepare a fat and/or oil mixture, and transesterifying the fat and/or oil mixture to prepare a transesterified fat and/or oil;

(ii) decolorizing the transesterified fat and/or oil or a fat and/or oil mixture
15 prepared by incorporating the transesterified fat and/or oil with another optional fat and/or oil; and

(iii) deodorizing the fat and/or oil or the mixture at 200°C or more to 230°C or less.

20 **Advantageous Effects of Invention**

The fat and/or oil composition of the present invention makes it possible to produce an infant formula having a nutritional balance suitable as an infant formula, particularly certain amounts of linoleic acid and α -linolenic acid, and sufficiently low concentrations of 3-MCPD, glycidol, and fatty acid esters thereof whose intakes are
25 desirably low from the viewpoint of safety.

Further, the use of the transesterified fat and/or oil from the fat and/or oil mixture makes excellent storage stability as a fat and/or oil composition for infant formula.

30 **Description of Embodiments**

<Fat and/or Oil Composition for Use in Infant Formula>

A fat and/or oil composition of the present invention is used in an infant formula.

The term infant formula refers to powdered milk used as a substitute for mother's milk, and infant formulas have ingredients as similar as possible to those in mother's milk in many cases. Main ingredients of human milk are proteins, fats, carbohydrates, and so forth. As the fats, human milk contains certain amounts of essential fatty acids necessary for the growth of infants, such as linoleic acid, α -linolenic acid, arachidonic acid, and docosahexaenoic acid.

In the Consumer Affairs Agency, the approval standard for the labeling as processed powdered milk for infant instructs that a composition per 100 kcal should contain 4.4 to 6.0 g of lipids, 0.3 to 1.4 g of linoleic acid, and 0.05 g or more of α -linolenic acid. In the present invention, the term "fat and/or oil composition for use in infant formula" means a fat and/or oil composition for meeting such a nutritional standard.

An infant formula using the fat and/or oil composition of the present invention contains linoleic acid and α -linolenic acid, and more preferably contains lipids in an amount of 4.4 to 6.0 g, linoleic acid in an amount of 0.3 to 1.4 g, and α -linolenic acid in an amount of 0.05 g or more, per 100 kcal of the composition. In other words, the infant formula preferably contains, as the fats and/or oils, 5.0 to 32.0% by mass of linoleic acid and 0.8% by mass or more of α -linolenic acid.

There are various methods for measuring the amounts of linoleic acid and α -linolenic acid in the fats and/or oils. In this Description, used are values which are measured according to a standard method for the analysis of fats and/or oils (2.4.2.2 - 2013, Fatty Acid Composition, FID temperature-programmed gas chromatography).

In the fat and/or oil composition, the ratio between linoleic acid and α -linolenic acid (linoleic acid/ α -linolenic acid) is preferably within a range of 5 to 15 from the nutritional viewpoint.

The fat and/or oil composition for use in infant formula of the present invention has a solid fat content at 35°C of 0.5% or less. Hence, no crystals are formed at 35°C for 3 weeks. Because of this property, the fat and/or oil composition is excellent in storage stability at 35°C. More preferably, the fat and/or oil composition of the present invention has a solid fat content at 35°C of 0.0%.

The fat and/or oil composition for use in infant formula of the present invention has a 3-MCPD concentration of 0.33 ppm or less and a glycidol concentration of 0.07 ppm or less. More preferably, the 3-MCPD concentration is 0.25 ppm or less, and the glycidol concentration is 0.06 ppm or less.

Raw material fats and/or oils of the fat and/or oil composition of the present invention can be selected for use such that the produced fat and/or oil composition meets the above-described properties. Various fats and/or oils are preferably used in combination such that particularly linoleic acid and α -linolenic acid are incorporated in accordance with to the above-described approval standard. Examples of such fats and/or oils include palm oil and fractionated oils of palm oil such as palm olein, double fractionated palm olein, palm stearin, and palm mid fraction; palm kernel oil and fractionated oils of palm kernel oil such as palm kernel olein and palm kernel stearin; vegetable oils such as coconut oil, soybean oil, rapeseed oil, corn oil, sunflower oil, safflower oil, perilla oil, flaxseed oil, high oleic sunflower oil, high oleic rapeseed oil, cottonseed oil, rice bran oil, sesame oil, olive oil, peanut oil, and cacao oil; animal oils such as fish oil, beef tallow, and pig fat; and the like. Moreover, it is also possible to use fractionated oils of the latter fats and/or oils.

A preferable combination is, for example, a combination of palm oil or a fractionated oil thereof with palm kernel oil or a fractionated oil thereof, and further another fat and/or oil may be combined therewith.

The fat and/or oil composition of the present invention preferably contains a transesterified fat and/or oil of a fat and/or oil mixture containing a first fat and/or oil

selected from palm oil and fractionated oils thereof, and a second fat and/or oil selected from palm kernel oil and fractionated oils thereof.

The fractionated oils of palm oil mean fats and/or oils obtained by fractionating palm oil, and include palm olein, double fractionated palm olein, palm stearin, palm mid
5 fraction, and the like. Preferable are palm olein and double fractionated palm olein.

The fractionated oils of palm kernel oil mean fats and/or oils obtained by fractionating palm kernel oil, and include palm kernel olein, palm kernel stearin, and the like. Preferable is palm kernel olein.

In the present invention, in a case of using a fat and/or oil composition
10 containing a transesterified fat and/or oil of only a fat and/or oil selected from palm oil and fractionated oils thereof, the 3-MCPD concentration can be sufficiently reduced, but the storage stability is poor. For this reason, a transesterified fat and/or oil is prepared from a fat and/or oil mixture containing a fat and/or oil selected from palm oil and
15 fractionated oils thereof as well as a fat and/or oil selected from palm kernel oil and fractionated oils thereof. This makes it possible to sufficiently reduce the 3-MCPD concentration to a safe level as infant formulas, and to obtain a fat and/or oil excellent in storage stability, too.

In the case where the fat and/or oil composition of the present invention
20 contains the aforementioned transesterified fat and/or oil, the fat and/or oil mixture for producing the transesterified fat and/or oil contains the first fat and/or oil (fat and/or oil selected from palm oil and fractionated oils thereof) preferably in an amount of less than 50% by mass relative to the total mass of the fat and/or oil composition. This is because the fat and/or oil composition of the present invention has an excellent storage
25 stability with the first fat and/or oil within this range. The amount of the first fat and/or oil is further preferably 40% by mass or less relative to the total mass of the fat and/or oil composition. The first fat and/or oil is preferably incorporated in an amount of 20% by mass or more relative to the total mass of the fat and/or oil composition.

The ratio of the first fat and/or oil to the second fat and/or oil is preferably
30 20/80 to 80/20, further preferably 30/70 to 70/30, and furthermore preferably 40/60 to

60/40.

The fat and/or oil mixture for producing the transesterified fat and/or oil may contain one or more third fats and/or oils other than the first fat and/or oil and the
5 second fat and/or oil.

The third fats and/or oils are not limited, as long as linoleic acid and α -linolenic acid are within the ranges of the approval standard. Examples of the third fats and/or oils include liquid oils such as soybean oil, rapeseed oil, corn oil, sunflower oil, safflower oil, perilla oil, flaxseed oil, high oleic sunflower oil, and high oleic rapeseed
10 oil. Preferable are soybean oil and rapeseed oil.

In the case of containing the aforementioned transesterified fat and/or oil, the fat and/or oil composition of the present invention may contain one or more other fats and/or oils than the transesterified fat and/or oil. Such fats and/or oils are not limited,
15 as long as linoleic acid and α -linolenic acid are within the target ranges. Examples of the fats and/or oils include liquid oils such as soybean oil, rapeseed oil, corn oil, sunflower oil, safflower oil, perilla oil, flaxseed oil, high oleic sunflower oil, and high oleic rapeseed oil. Preferable are soybean oil and rapeseed oil.

The transesterified fat and/or oil is preferably incorporated in an amount of 50
20 to 100% by mass, further preferably incorporated in an amount of 60 to 100% by mass, relative to the total mass of the fat and/or oil composition of the present invention.

<Method for Producing Fat and/or Oil Composition>

In the embodiment in which the fat and/or oil composition of the present
25 invention contains the above-described transesterified fat and/or oil, a method for producing the fat and/or oil composition is a method including the following steps:

(i) mixing a first fat and/or oil selected from palm oil and fractionated oils thereof with a second fat and/or oil selected from palm kernel oil and fractionated oils thereof and a third fat and/or oil, which is optionally contained, to prepare a fat and/or
30 oil mixture, and transesterifying the fat and/or oil mixture to prepare a transesterified fat

and/or oil;

(ii) decolorizing the transesterified fat and/or oil or a fat and/or oil mixture prepared by incorporating the transesterified fat and/or oil with another optional fat and/or oil; and

5 (iii) deodorizing the decolorized fat and/or oil or the mixture at 200°C or more to 230°C or less.

As the transesterification method in the production method of the present invention, any method known to those skilled in the art may be performed. Nevertheless, a chemical transesterification method using a metal catalyst is preferably
10 performed.

A more preferable embodiment of the production method of the present invention is characterized in that at least the following steps (1) to (3) are performed. The steps are: transesterification step (1) using a metal catalyst, decolorization step (2),
15 and deodorization step (3).

<Transesterification Step (1) Using Metal Catalyst>

First, according to a conventional method, a chemical transesterification is performed in the presence of a metal catalyst, preferably in the presence of sodium
20 methyle, in such a manner that the transesterification percentage is 90% or more. The transesterification is performed under reduced pressure by stirring under heating at a temperature of approximately 50 to 120°C. Moreover, the reaction time of the transesterification is normally 10 minutes to 60 minutes. Nevertheless, in the present invention, it is necessary to perform the transesterification for 10 minutes or more,
25 preferably approximately 20 to 60 minutes, in such a manner that the transesterification percentage is 90% or more. Then, water is added to stop the reaction, and an aqueous solution of citric acid may also be added.

The catalyst may be used, for example, within a range of approximately 0.1 to 2% by mass relative to the mass of the raw material fats and/or oils to be transesterified.

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<Decolorization Step (2)>

The transesterified fat and/or oil obtained in step (1) is decolorized according to a conventional method. As an adsorbent used in the decolorization, acidic activated clay is preferably used, and more preferably activated carbon is used in combination.

- 5 In order to perform the decolorization efficiently in a shorter time, it is suitable to perform mixing and stirring under a reduced pressure (generally 6.7 kPa or less) while heating to a temperature of approximately 90 to 120°C. The mixing and stirring should be performed generally for approximately 30 minutes, although the time varies depending on the treatment amount and so on.

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<Deodorization Step (3)>

The deodorization step is not particularly limited, except for the temperature condition (200 to 230°C). This step may be a deodorization method by steam distillation under reduced pressure as normally employed in deodorizing edible fats and/or oils.

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In the present invention, the fat and/or oil composition having a glycidol concentration of 0.07 ppm or less can be obtained through the deodorization step performed at a temperature lower than a normal refining temperature of fats and/or oils, that is, preferably at 200°C or more to 230°C or less, more preferably 210°C or more to 220°C or less. If the deodorization temperature is less than 200°C, the content of glycidol can be further reduced, but odorous ingredients cannot be sufficiently removed from the fat and/or oil, so that the quality as a product deteriorates. Hence, the deodorization temperature of less than 200°C is not preferable.

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On the other hand, if the deodorization temperature exceeds 230°C, the content of glycidol increases. Hence, such temperatures are not preferable. Especially, at 260°C or more, the content of such ingredients remarkably increases. Hence, such temperatures are not particularly preferable.

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The fat and/or oil composition obtained by the above-described production method has low concentrations of 3-MCPD, glycidol, and fatty acid esters thereof even

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after the deodorization step. The 3-MCPD concentration is reduced to 0.33 ppm or less, and the glycidol concentration is reduced to 0.07 ppm or less. Further, the flavor is also favorable.

It should be noted that, in the fat and/or oil composition, 3-MCPD and glycidol are present in the forms of 3-MCPD fatty acid ester and glycidol fatty acid ester, respectively. Nonetheless, this Description expresses these by the "3-MCPD concentration" and the "glycidol concentration" in the fat and/or oil composition by using values measured as 3-MCPD and glycidol.

10 <Infant Formula>

An infant formula of the present invention contains the above-described fat and/or oil composition of the present invention, and further contains an optional ingredient incorporated in infant formulas.

Examples of the optional ingredient include protein sources, carbohydrate sources, vitamins, minerals, other nutritional elements, and the like.

The infant formula contains the fat and/or oil composition of the present invention in an amount of, for example, 10 to 30% by mass, but the amount is not limited thereto.

20 [Examples]

(Production Example 1)

First, 20 kg of palm olein was dehydrated under a reduced pressure of 4 kPa at 90°C for 30 minutes. To 100 parts by mass of the palm olein, 0.12 parts by mass of sodium methylate (product name: Sodium Methylate, manufactured by Nippon Soda Co., Ltd.) was added and subjected to a chemical transesterification under a reduced pressure of 4 kPa at 90°C for 30 minutes. To 100 parts by mass of the palm olein, 50 parts by mass of water was added and gently mixed to stop the chemical transesterification. The resultant was left standing at 70°C for 30 minutes, and the soap content was removed, followed by the dehydration under a reduced pressure of 4 kPa at 90°C for 30 minutes. In the manner described above, a transesterified fat and/or

oil of Production Example 1 was prepared.

(Production Example 2)

5 A transesterified fat and/or oil of Production Example 2 was prepared by transesterifying fats and/or oils according to a composition shown in Table 1 under the same conditions as those in Production Example 1.

(Production Example 3)

10 A transesterified fat and/or oil of Production Example 3 was prepared by transesterifying fats and/or oils according to a composition shown in Table 1 under the same conditions as those in Production Example 1.

(Production Example 4)

15 A transesterified fat and/or oil of Production Example 4 was prepared by transesterifying fats and/or oils according to a composition shown in Table 1 under the same conditions as those in Production Example 1.

(Example 1)

20 The transesterified fat and/or oil of Production Example 2 in an amount of 67 parts by mass was mixed with 21 parts by mass of soybean oil and 12 parts by mass of rapeseed oil. To 100 parts of the fats and/or oils, 2.0 parts by mass of acidic activated clay (product name: Galleon Earth V2, manufactured by Mizusawa Industrial Chemicals, Ltd.) was added for the decolorization at normal pressure at 90°C for 60 minutes. The acidic activated clay was removed by filtration under reduced pressure
25 using a filter paper (product name: Toyo Filter Paper No. 2, manufactured by Advantec) and a filter aid (product name: Silica #600H, manufactured by Chuo Silika Co., Ltd.). Thereby, a decolorized oil was obtained. This decolorized oil in an amount of 15 kg was deodorized under conditions of 210°C, 75 minutes, a degree of vacuum of 0.4 kPa or less, and the amount of steam introduced: 3.0% (relative to the oil in terms of weight).
30 Thus, a deodorized oil was obtained.

(Examples 2, 3, 4, 5, 6 and Comparative Examples 1, 2)

Fat and/or oil compositions were produced by decolorizing and deodorizing fats and/or oils according to compositions shown in Table 2 under the same conditions
5 as those in Example 1.

The fats and/or oils obtained in Production Examples, Examples, and Comparative Examples were measured for the 3-MCPD and glycidol contents by analysis methods described below. Moreover, the flavor, melting point, storage test,
10 solid fat content, and fatty acid composition analyses were also conducted. Table 3 shows the analysis results.

(Analysis Method for 3-MCPD etc.)

The measurements were performed according to "DFG Standard Methods,
15 Section C-Fats C-IV 18 (10)."

1. Standard Solutions

For all of the following standard stock solutions and standard solutions, toluene was used as a solvent.

1) 3-MCPD-1,2-palmitoyl ester (manufactured by Wako Pure Chemical Industries,
20 Ltd., $\geq 98\%$)

3-MCPD-1,2-palmitoyl ester standard stock solution (approximately 1000 ppm)

3-MCPD-1,2-palmitoyl ester standard solution (approximately 40 ppm)

2) d5-3-MCPD-1,2-palmitoyl ester (manufactured by Wako Pure Chemical
25 Industries, Ltd., $\geq 98\%$)

* used as a surrogate

d5-3-MCPD-1,2-palmitoyl ester standard stock solution (approximately 1000 ppm)

d5-3-MCPD-1,2-palmitoyl ester standard solution (approximately 40 ppm)

30 3) Glycidyl stearate (manufactured by TCI, $96\% \geq$)

glycidyl stearate standard stock solution (approximately 1000 ppm)

glycidyl stearate standard solution (approximately 40 ppm)

2. Reagents

- 5 1) Ultrapure water
- 2) Toluene (manufactured by Kanto Chemical Co., Inc., for pesticide residue analysis, 5000-fold concentrated)
- 3) t-Butyl methyl ether (t-BME) (manufactured by Kanto Chemical Co., Inc., for pesticide residue analysis, 5000-fold concentrated)
- 10 4) Methanol (manufactured by Kanto Chemical Co., Inc., for pesticide residue analysis, 5000-fold concentrated)
- 5) Hexane (manufactured by Kanto Chemical Co., Inc., for pesticide residue analysis, 5000-fold concentrated)
- 6) Ethyl acetate (manufactured by Kanto Chemical Co., Inc., for pesticide residue analysis, 5000-fold concentrated)
- 15 7) Diethyl ether (manufactured by Kanto Chemical Co., Inc., for pesticide residue analysis, 5000-fold concentrated)
- 8) Isooctane
- 9) Sodium methoxide
- 20 10) Sodium methoxide-methanol solution (25 g/L): the volume was set such that 0.25 g of sodium methoxide was dissolved per 10 mL using methanol <*prepared when used (the long-term storage is impossible because sodium methoxide is easily broken down by water)>
- 11) Sodium chloride (manufactured by Kanto Chemical Co., Inc., special grade)
- 25 12) Sodium chloride solution (NaCl 200 g/L solution): 50 g of sodium chloride was dissolved in ultrapure water to make the volume 250 mL
- 13) Sodium bromide (manufactured by Kanto Chemical Co., Inc., special grade)
- 14) Aqueous solution of sodium bromide (NaBr 600 g/L solution)
- 15) Sulfuric acid (25%, 6N): sulfuric acid (96%, 36N) was 6-fold diluted
- 30 ex) after 10 mL of sulfuric acid (96%, 36N) was added to 50 mL of ultrapure

water, the volume was made to 60 mL

16) Acidic aqueous solution of sodium chloride (200 g/L): 35 mL of sulfuric acid (25%) was added to 1 L of an aqueous solution of sodium chloride

5 ex) 20 mL of an aqueous solution of sodium chloride + 700 μ L of sulfuric acid (25%)

17) Acidic aqueous solution of sodium bromide (salt water containing no chloride): 35 mL of sulfuric acid (25%) was added to 1 L of an aqueous solution of sodium bromide (600 g/L)

10 ex) 20 mL of an aqueous solution of sodium bromide + 700 μ L of sulfuric acid (25%)

18) Phenylboronic acid (PBA)

19) Derivatization reagent: phenylboronic acid was dissolved in diethyl ether to reach a saturated state with some precipitation <*prepared when used>

15 3. Equipment

1) Pasteur pipettes

2) Graduated cylinders: 25 mL, 50 mL, 100 mL

3) Volumetric flasks: 5 mL, 10 mL

4) Pipetmans: P-5000, P-1000, P-200

20 5) Screw vials (glass made, capacity: 1.5 mL)

6) Syringes

7) Filters (hydrophobic)

4. Analysis method

25 1) A sample in an amount of 100 mg ($\pm$ 0.5 mg) was collected into a 1.5-mL screw vial.

2) As a surrogate, 100 μ L of a d5-3-MCPD-1,2-palmitoyl ester standard solution (approximately 50 ppm) and 100 μ L of t-butyl methyl ether were added thereto and stirred.

30 ※ To check contamination, a sample (ex. extra-virgin olive oil) from which 3-MCPD and so forth were not detected was analyzed. Moreover, to a spiked sample,

100 µL of a 3-MCPD-1,2-palmitoyl ester standard solution (approximately 40 ppm) was added together with the surrogate, and the recovery rate was checked.

3) After 200 µL of a sodium methoxide-methanol solution was added and stirred, the mixture was allowed to react at normal temperature for 3.5 to 5.5 minutes.

5 4) To stop the reaction, 600 µL of an acidic aqueous solution of sodium chloride was added in the analysis [A], and 600 µL of an acidic aqueous solution of sodium bromide was added in the analysis [B].

5) After 600 µL of hexane was added and stirred, the mixture was left standing for 5 minutes or more, and the hexane layer was removed. Again, 600 µL of hexane was
10 added, and the same operation was repeated.

6) After 600 µL of a mixture solution of diethyl ether/ethyl acetate (6:4) was added and stirred, the solvent layer was recovered in a vial containing sodium sulfate (anhydrous). The same operation was further repeated twice.

7) After 100 µL of a PBA solution was added for the derivatization, the resultant was
15 solidified to dryness by blowing a nitrogen gas.

8) The solid was dissolved again in 1 mL of isooctane, filtered with a filter, and then measured by GC/MS.

5. System and Analysis Conditions

20 System: GC/MS (5975C/7890A manufactured by Agilent Technologies)

Column: DB-17MS having an inner diameter of 0.25 mm × 30 m and a film thickness of 0.25 µm

Injected amount: 2 µL

Injection inlet temperature: 240°C

25 Splitless time: 1.5 min

Split flow rate: 20 mL/min

Carrier gas: helium, 1.2 mL/min, constant flow rate

Oven temperature: 85°C (0.5 min) → 6°C/min → 150°C → 12°C/min → 180°C →
25°C/min → 280°C (7 min)

30 Ionization: EI (positive)

Measured ions: 3-MCPD m/z = 147 (for quantification); 146, 196, and 198 (for confirmation)

3-MCPD-d5 m/z = 150 (surrogate); 149, 201, and 203 (for confirmation)

- 5 Ion source temperature: 250°C
Quadrupole: 150°C

6. Quantification Method

[Internal Standard Method]

- 10 The d5-3-MCPD-1,2-palmitoyl ester added as the surrogate was converted as the d5-3-MCPD concentration, and an area ratio obtained by dividing the value of the 3-MCPD area by the value of the surrogate area was multiplied by the surrogate concentration to calculate the 3-MCPD concentration.

- 15 Quantified value = surrogate concentration × value of 3-MCPD area / value of surrogate area

The quantified value obtained above was divided by the amount of the sample collected to determine the concentration in the sample.

- 3-MCPD amount [ppm] = quantified value [μg/L] / amount of sample collected
20 [mg]

※ The 3-MCPD—FS amount was determined in the analysis [A], while the 3-MCPD amount from which glycidol was removed was determined in the analysis [B].

- From the measurement results in the analyses [A] and [B], the 3-MCPD
25 amount was subtracted from the 3-MCPD—FS amount, and the difference was multiplied by the glycidol conversion coefficient to calculate the glycidol amount.

Glycidol amount [ppm] = (3-MCPD—FS amount [A] - 3-MCPD amount [B])
× glycidol conversion coefficient

- 30 <Calculation of Glycidol Conversion Coefficient>

The conversion coefficient from glycidol to 3-MCPD was calculated by creating a calibration curve in [A] in the presence of a chloride. Glycidol (as glycidol ester) at multiple concentrations was added to a fat and/or oil sample, which was not contaminated, and treated according to the analysis [A]. As a result, a calibration
5 curve $y = mx + n$ was obtained. The reciprocal of the slope is equal to the glycidol conversion coefficient t .

Glycidol conversion coefficient $t = 1/m$

(Storage Test (Crystal and Flavor) Method)

- 10 1. Into a 500-mL glass beaker, 450 g of the fat and/or oil composition was introduced and hermetically sealed (wrapped).
2. The fat and/or oil composition introduced in the 500-mL glass beaker was stored in a thermostatic chamber set to one of temperature ranges (30°C, 35°C).
3. The presence or absence of crystals was visually checked 3 days, 1 week, 2 weeks,
15 and 3 weeks after the storage was started.

In Table 2, "○" means that no crystals were observed at all. "×" means that crystals were visually observed.

4. The flavor was checked 3 days, 1 week, 2 weeks, and 3 weeks after the storage was started.

- 20 In Table 2, "Y" means that no change occurred from the initial flavor. "×" means that a change occurred from the initial flavor.

(Analysis Method for Fatty Acid Composition)

- The fatty acid compositions were analyzed by a standard method for the
25 analysis of fats and/or oils (2.4.2.2 - 2013, Fatty Acid Composition, FID temperature-programmed gas chromatography).

(Analysis Method for Melting Point)

- The melting points were analyzed by a standard method for the analysis of fats
30 and/or oils (3.2.2.2 – 2013, melting point (increase melting point)).

(Method for Analyzing Solid Fat Content)

The solid fat contents were analyzed by a standard method for the analysis of fats and/or oils (2.2.9 – 2013, solid fat content (NMR method)).

5

(Results)

Each of the fats and/or oils of Examples 1 to 6 had a solid fat content at 35°C of 0.5% or less and 3-MCPD and glycidol concentrations of 0.33 ppm or less and 0.07 ppm or less, respectively. In these fat and/or oil compositions, no crystals were formed at 30°C for 3 days and at 35°C for 3 weeks or more. The storage stabilities were excellent.

In contrast, the fat and/or oil composition of Comparative Example 2 having the same raw material fat and/or oil composition as Example 1 but not transesterified had a solid content at 35°C of 0.5% or less, no crystals were formed at 30°C and 35°C for 3 weeks, and the storage stability was excellent. However, the 3-MCPD level greatly exceeded the tolerable concentration. Meanwhile, the fat and/or oil composition of Comparative Example 1 had the same raw material fat and/or oil composition as Example 1, but only the palm olein oil was transesterified. The 3-MCPD and glycidol concentrations were 0.33 ppm or less and 0.07 ppm or less, respectively. This fat and/or oil composition had a solid fat content at 35°C of 0.6%, crystals were formed at 35°C in 1 week, and the storage stability was not excellent.

[Table 1]

Raw material fats/oils	Production Examples of transesterified fats/oils			
	1	2	3	4
Palm oil	-	-	-	-
Palm olein	100	55	42	37
Palm kernel olein	-	45	34	30
Soybean oil	-	-	24	21

Rapeseed oil	-	-	-	12
Total	100	100	100	100
Amount of catalyst added (%)	0.12	0.12	0.12	0.12
Catalytic reaction temperature (°C)	90	90	90	90

[Table 2]

Raw material fats/oils	Example						Comparative Example	
	1	2	3	4	5	6	1	2
Palm oil	-	-	-	-	-	-	-	-
Palm olein	-	-	-	-	-	-	-	37
Palm kernel olein	-	-	11	-	15	-	30	30
Soybean oil	21	-	22.8	-	18	-	21	21
Rapeseed oil	12	12	-	-	3	-	12	12
High oleic sunflower oil	-	-	-	-	-	15	-	-
Safflower oil	-	-	-	-	-	12	-	-
Perilla oil	-	-	-	-	0.5	5.2	-	-
Production Example 1	-	-	-	-	-	-	37	-
Production Example 2	67	-	66.2	-	63.5	67.8	-	-
Production Example 3	-	88	-	-	-	-	-	-
Production Example 4	-	-	-	100	-	-	-	-
Total	100	100	100	100	100	100	100	100
Whether transesterified oil was used or not	used	used	used	used	used	used	used	not used
Decolorization: amount of clay added	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Deodorization: deodorization temperature (°C)	210	210	210	210	210	210	210	210
C12-0	12.2	12.7	16.8	12.4	17.7	12.5	12.4	12.3
C16-0	20.4	19.9	21.0	20.4	20.3	19.2	20.4	20.6
cis C18-1	35.9	35.9	31.0	36.3	32.3	38.9	35.7	35.9
cis C18-2	18.3	17.9	17.1	17.9	15.0	15.8	18.3	18.2

cis C18-3	2.6	2.5	1.5	2.3	1.6	3.1	2.5	2.5
cis C18-2/cis C18-3	7.0	7.2	11.4	7.8	9.1	5.2	7.3	7.4

[Table 3]

Raw material fats/oils	Example												Comparative Example			
	1		2		3		4		5		6		1		2	
Initial flavor and melting point analyses																
Flavor	favorable		favorable		favorable		favorable		favorable		favorable		favorable		favorable	
Melting point (°C)	25.8		24.3		25.3		25.4		25.8		26.1		27.1		16.2	
3-MCPD and glycidol analyses																
3-MCPD (ppm)	0.13		0.21		0.14		0.21		0.24		0.20		0.24		1.00	
glycidol (ppm)	0.01		0.03		0.05		0.01		0.03		0.03		0.04		0.04	
Storage test (crystal, flavor)	crystal	flavor	crystal	flavor	crystal	flavor	crystal	flavor	crystal	flavor	crystal	flavor	crystal	flavor	crystal	flavor
30°C storage																
3 days	○	○	○	○	○	○	○	○	○	○	○	○	×	○	○	○
1 weeks	×	○	×	○	×	○	×	○	×	○	×	○	×	○	○	○
2 weeks	×	○	×	○	×	○	×	○	×	○	×	○	×	○	○	○
3 weeks	×	○	×	○	×	○	×	○	×	○	×	○	×	○	○	○
35°C storage																
3 days	○	○	○	○	○	○	○	○	○	○	○	○	×	○	○	○
1 weeks	○	○	○	○	○	○	○	○	○	○	○	○	×	○	○	○
2 weeks	○	○	○	○	○	○	○	○	○	○	○	○	×	○	○	○
3 weeks	○	○	○	○	○	○	○	○	○	○	○	○	×	○	○	○
Solid fat content (%)																
25°C	3.5		2.1		3.8		1.9		3.6		4.0		3.8		0.0	
30°C	0.2		0.0		0.0		0.0		0.3		1.0		1.7		0.0	
35°C	0.0		0.0		0.0		0.0		0.0		0.0		0.6		0.0	
45°C	0.0		0.0		0.0		0.0		0.0		0.0		0.2		0.0	

Claims

1. A fat and/or oil composition for use in infant formula, comprising linoleic acid and α -linolenic acid, wherein the fat and/or oil composition has a solid fat content at
5 35°C of 0.5% or less, a 3-monochloropropane-1,2-diol (3-MCPD) concentration of 0.33 ppm or less, and a glycidol concentration of 0.07 ppm or less.
2. The fat and/or oil composition according to claim 1, comprising a transesterified fat and/or oil of a fat and/or oil mixture containing a first fat and/or oil
10 selected from palm oil and fractionated oils thereof, and a second fat and/or oil selected from palm kernel oil and fractionated oils thereof.
3. The fat and/or oil composition according to claim 1 or 2, wherein the fat and/or oil mixture for producing the transesterified fat and/or oil contains the first fat and/or oil
15 in an amount of less than 50% by mass relative to a total mass of the fat and/or oil composition.
4. The fat and/or oil composition according to any one of 1 to 3, comprising linoleic acid in an amount of 5.0 to 32.0% by mass and α -linolenic acid in an amount of
20 0.8% by mass or more relative to the total mass of the fat and/or oil composition.
5. The fat and/or oil composition according to any one of claims 1 to 4, wherein the fat and/or oil mixture for producing the transesterified fat and/or oil contains one or more third fats and/or oils other than the first fat and/or oil and the second fat and/or oil.
25
6. The fat and/or oil composition according to any one of claims 1 to 5, comprising one or more other fats and/or oils than the transesterified fat and/or oil.
7. The fat and/or oil composition according to any one of claims 1 to 6, wherein
30 the fat and/or oil composition is deodorized at 200°C or more to 230°C or less.

8. An infant formula comprising the fat and/or oil composition according to any one of claims 1 to 7.

5 9. A method for producing the fat and/or oil composition according to any one of claims 1 to 7, comprising the following steps:

(i) mixing a first fat and/or oil selected from palm oil and fractionated oils thereof with a second fat and/or oil selected from palm kernel oil and fractionated oils thereof and a third fat and/or oil, which is optionally contained, other than the first fat
10 and/or oil and the second fat and/or oil to prepare a fat and/or oil mixture, and transesterifying the fat and/or oil mixture to prepare a transesterified fat and/or oil;

(ii) decolorizing the transesterified fat and/or oil or a fat and/or oil mixture prepared by incorporating the transesterified fat and/or oil with another optional fat and/or oil; and

15 (iii) deodorizing the fat and/or oil or the mixture at 200°C or more to 230°C or less.