GRAS Notice (GRN) No. 700
https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm

GRN 000700

GRAS Notice for Astaxanthin-Rich Carotenoid Extracts from *Paracoccus carotinifaciens*

Prepared for:

Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD
20740

Submitted by:

JX Nippon Oil & Energy Corporation
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Japan

April 10, 2017

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FOOD ADDITIVE SAFETY

GRAS Notice for Astaxanthin-Rich Carotenoid Extracts from *Paracoccus carotinifaciens*

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Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, JX Nippon Oil & Energy Corporation (JX Nippon) hereby informs the U.S. Food and Drug Administration (FDA) of the view that its astaxanthin-rich carotenoid extracts derived from *Paracoccus carotinifaciens* are not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on JX Nippon's conclusion that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Part 1.3 below. In addition, as a responsible official of JX Nippon, hereby certifies that all data and information presented in this notice constitutes a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to JX Nippon and pertinent to the evaluation of the safety and GRAS status of its astaxanthin-rich extracts as ingredients for addition to food, as described herein.

Signed,

(b) (6)

April 18, 2017

Takashi Ishibashi
Chief Specialist
ishibashi.takashi@jxgr.com

Date

1.1 Name and Address of Notifier

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1.2 Common Name of Notified Substance

Asataxanthin-rich carotenoid extracts

1.3 Conditions of Use

JX Nippon's asataxanthin-rich carotenoid extracts are intended to be used as sources of dietary astaxanthin in baked goods, baking mixes, beverages, beverage bases, energy, sports, isotonic drinks, non-milk based meal replacements, cereals, cereal products, chewing gum, coffee and tea, dairy product analogs, frozen dairy desserts and mixes, hard candy, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and soft candy, at levels providing 0.15 mg astaxanthin/serving. These intended uses are identical to those described by INNOBIO Ltd. (INNOBIO) in a prior GRAS notice filed by FDA as GRN No. 580 (INNOBIO Limited, 2015; U.S. FDA, 2015). These uses exclude USDA regulated products and infant formula.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b) of the *Code of Federal Regulations* (CFR), astaxanthin-rich carotenoid extracts manufactured by JX Nippon has been concluded to have GRAS status for use as an ingredient for addition to specified conventional foods as described in Part 1.3, on the basis of scientific procedures (U.S. FDA, 2016).

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the FDA for review and copying upon request during business hours at the offices of:

JX Nippon Oil & Energy Corporation
1-2,Otemachi 1-chrome,
Chiyoda-ku, Tokyo 100-8162,
Japan

In addition, should the FDA have any questions or additional information requests regarding this notification during or after the Agency`s review of the notice, Summit Life Sciences will supply these data and information.

1.6 Freedom of Information Act, 5 U.S.C. Section 552

It is JX Nippon's view that all data and information presented in parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information that is privileged or

confidential, and therefore all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. Section 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

JX Nippon's astaxanthin-rich carotendoid extracts are derived from the gram-negative proteobacteria *Paracoccus carotinifaciens* (*P. carotinifaciens*). The extracts contain a blend of naturally occurring carotenoids, including astaxanthin, adonirubin, adonixanthin, canthaxanthin, β-carotene, echinenone, asteroidenone, and 3-hydroxyechinenone, among other carotenoids.

JX Nippon manufactures three versions of the extract, astaxanthin-rich carotenoid extracts derived from *P. carotinifaciens* (ARE-C), ARE-1P, and ARE-10P. ARE-C is the unprocessed and undiluted form of the extract. Through slight modifications to the manufacturing process and the addition of approved processing aids and food additives, ARE-1P exhibits improved dispersion in water while ARE-10P features a lower degree of crystallization.

2.2 Common or Usual Name

Astaxanthin-rich carotenoid extracts

2.3 Chemical Name

The chemical name for astaxanthin

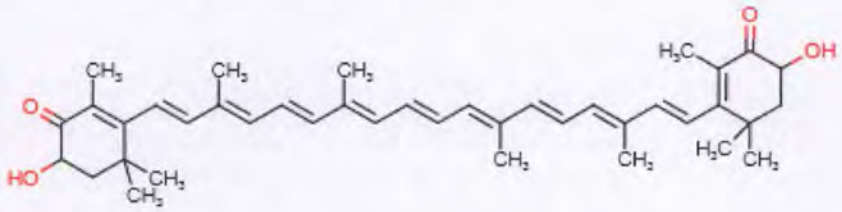
2.4 Chemical Abstract Service (CAS) Number of Astaxanthin

472-61-7

2.5 Molecular Weight of Astaxanthin

596.8

2.6 Molecular Structure of Astaxanthin



2.7 Method of Manufacturing

2.7.1 Production Organism

P. carotinifaciens, the source of ARE-C, is a gram-negative, aerobic, rod-shaped bacterium. *P. carotinifaciens* was isolated, characterized, and proposed as a new species of *Paracoccus* during a search of soil isolates for chromogenic, astaxanthin-producing bacteria (Tsubokura *et al.*, 1999). The isolate, strain E-396, proved phylogenetically related to α-3 Proteobacteria using 16S rRNA homology and formed a cluster with species belonging to the genus *Paracoccus.* The E-396 strain of *P. carotinifaciens* isolated and identified by Tsubokura *et al.* (1999) has been cataloged with GENBANK (ID AB006899). The species name (*i.e.*, carotinifaciens) for the bacterial strain was assigned due to the increased production of carotenoids. The carotenoids produced by the bacterial cells contribute to the orange bacterial colonies that result when grown on nutrient agar. The orange pigment-producing bacterial strain contains approximately 4% carotenoids, with astaxanthin accounting for the majority of the carotenoid content. Other carotenoids identified in dehydrated *Paracoccus carotinifaciens* (DPC) include canthaxanthin, adonirubin, β carotene, echinenone, adonixanthin, 3-hydroxyechinenone and 3'-hydroxyechinenone (*i.e.*, asteroidenone).

JX Nippon utilizes a mutant strain, NOC-18, of *P. carotinifaciens* in nutrient-rich medium. This mutant strain was developed using classical mutation and selection techniques and has not been subjected to genetic engineering. The organic compound, *N*-methyl-*N'*-nitro-*N*-nitrosoguanide, or ultraviolet light was used to mutate the wild-type strain (E-396) of *P. carotinifaciens.* JX Nippon selected mutants that had an increase in carotenoid production, especially astaxanthin. The mutants were initially selected based on an increase in orange pigmentation of colonies on agar. The mutants were then tested for concentration of astaxanthin by High Performance Liquid Chromatography (HPLC). JX Nippon selected the mutant strain, NOC-18, for use in the manufacturing of ARE-C, based on its ability to produce a large quantity of astaxanthin during the fermentation process. This strain has been deposited with NBRC (National Institute of Technology and Evaluation, Biological Resource Center) in Japan (NITE Safety Deposit Number: 00017). As shown in Table 2.7.1-1, the characteristics of strain NOC-18 are the same as for the wild strain, E-396, except for astaxanthin production.

Table 2.7.1-1 Characteristics of Strain E-396 and NOC-18		
Character	**Strain E-396**	**Strain NOC-18**
Morphology	Rod	Rod
Spore Formation	None	None
Gram Stain	Negative	Negative
Behavior toward oxygen	Aerobic	Aerobic
Quinone system	Q-10	Q-10
Production of Urease	Negative	Negative
Production of Oxidase	Positive	Positive
Production of Catalase	Positive	Positive
Production of Indole	Negative	Negative
Utilization of D-Glucose	Positive	Positive
Utilization of Sucrose	Positive	Positive
Utilization of n-Capric acid	Negative	Negative
Utilization of Adipic acid	Negative	Negative
Utilization of Phenyl acetate	Negative	Negative
Hydrolysis of Gelatin	Negative	Negative

2.7.2 Manufacturing Process

The manufacturing processes of ARE-C, ARE-1P, and ARE-10P are in compliance with current Good Manufacturing Practice (cGMP) guidelines. During the fermentation process, *P. carotinifaciens* is cultured in liquid medium containing glucose, ammonia, phosphate, minerals, vitamins, and an anti-foaming agent in an oxygen-controlled environment.

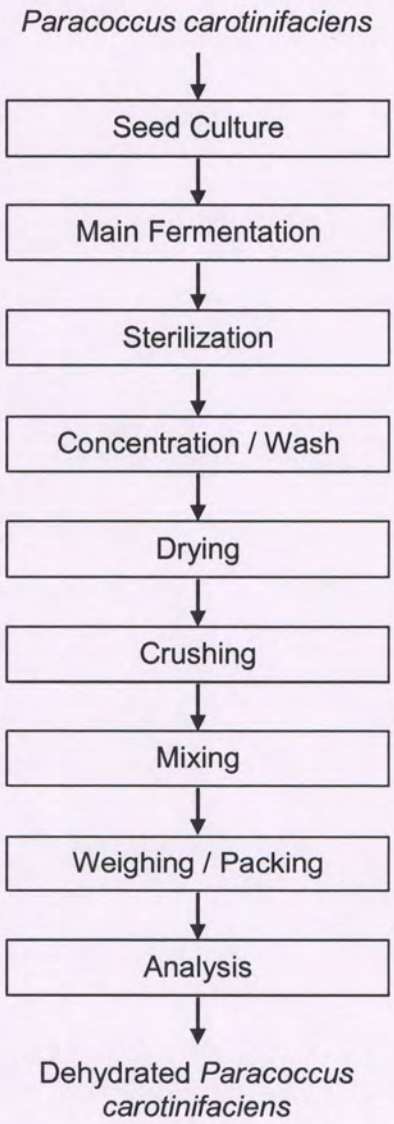
After fermentation is complete, the mixture is heat-treated to kill the *P. carotinifaciens* cells and inactivate endogenous enzymes. The mixture of cells and fermentation broth is filtered, washed, and concentrated. *P. carotinifaciens* cells are then dried, milled, mixed, weighed, and packaged into aluminum bags. The resulting product consists of small, dark-red granules containing approximately 2% astaxanthin. Potential microbial contamination is monitored throughout the fermentation process. A check of the microbial colony profile is completed for the cell bank used in each preparation lot, the final broth in each seed culture, and the final broth in the production culture.

Stabilizers, diluting agents, food additives and medicinal products are not added to the resulting product; it is 100% DPC. The resulting product is analyzed for quality control specifications, labeled, and stored.

A flow diagram of the manufacturing process for DPC is shown in Figure 2.7.2-1. The carotenoid content of DPC is evaluated using solvent extraction, separation by HPLC, and detection by absorbance at 470 nm. The HPLC method was fully validated, following OECD

procedures, and Good Laboratory Practices (GLP) conditions. The proposed shelf life of DPC is 24 months.

Figure 2.7.2-1 Schematic Overview of the Manufacturing Process for DPC

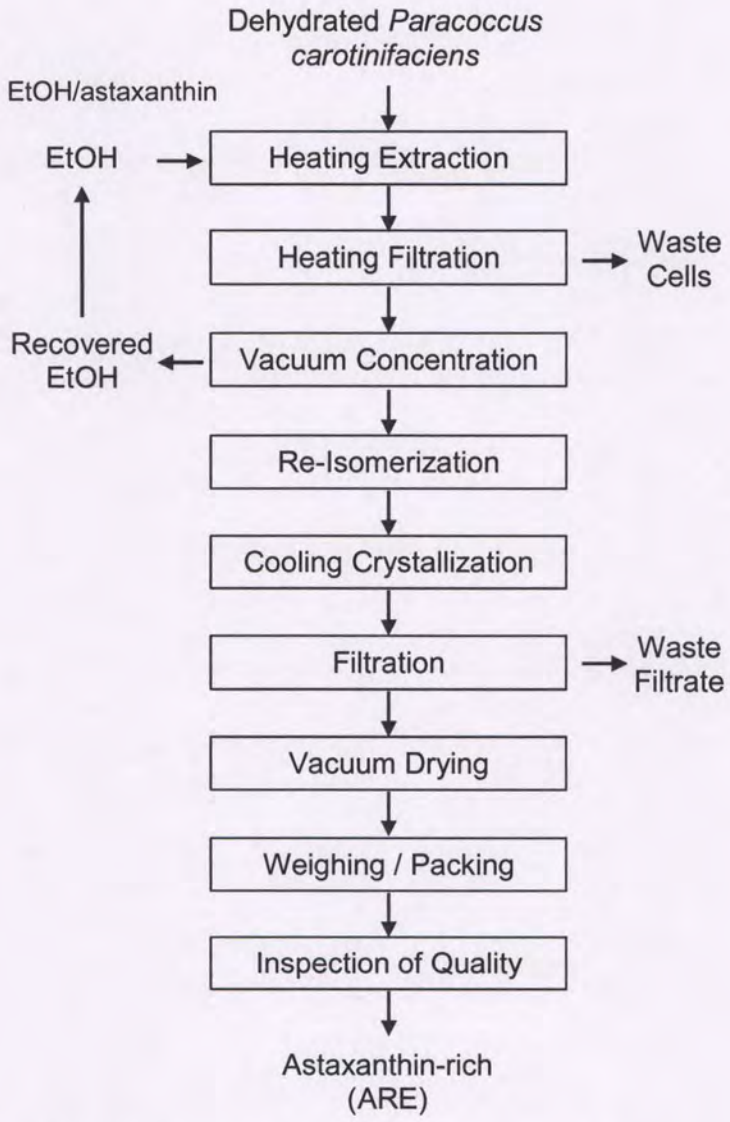


DPC = dehydrated *Paracoccus carotinifaciens*

To produce ARE-C, carotenoids are extracted from the DPC powder with ethyl alcohol and deaerated nitrogen gas, and heat treatment. Residual biomass of DPC is removed by filtration. The filtrate is concentrated under reduced pressure, adjusted to the weight of the original filtrate by adding ethyl alcohol, and re-isomerized to improve the recovery rate of carotenoids. The filtrate is crystallized and vacuum dried to remove ethyl alcohol. The resulting product consists of small black-purple powder crystal containing more than 85% carotenoids and approximately

60% of the total carotenoids are astaxanthin. A flow diagram of the complete manufacturing process for ARE-C is shown in Figure 2.7.2-2.

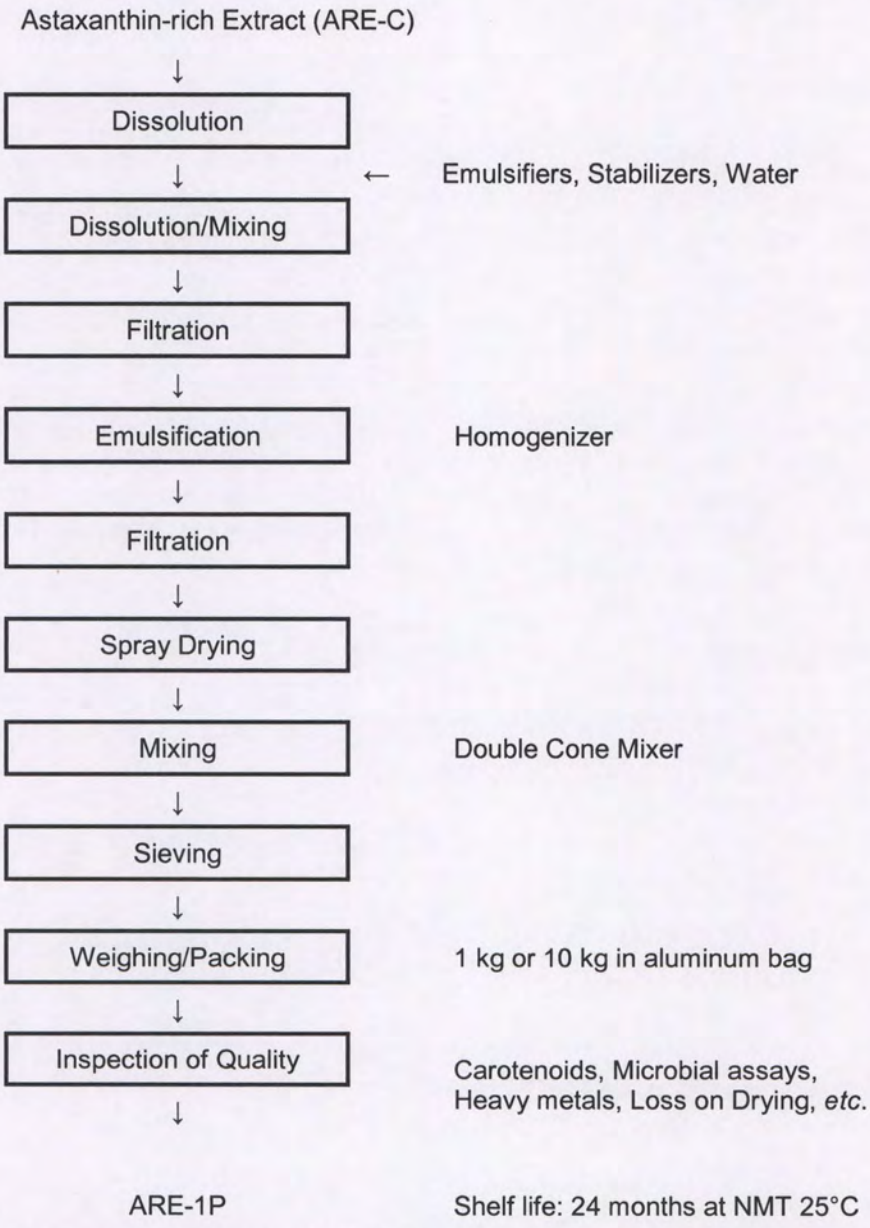
Figure 2.7.2-2 Schematic Overview of the Manufacturing Process for ARE-C



ARE-C = Astaxanthin-rich extract derived from *Paracoccus carotinifaciens*

To produce ARE-1P, ARE-C is dissolved in gum arabic, maltodextrin, *dl*-α-tocopherol, medium chain triglycerides, and L-ascorbic acid in water to produce ARE-1P. Then the mixture is filtered and homogenized to further emulsify ARE-C. The emulsified filtrate is again filtered and then spray dried. Once dry, the product is further mixed in a double cone mixer and filtered for the last time. A flow diagram of the complete manufacturing process for ARE-1P is shown in Figure 2.7.2-3.

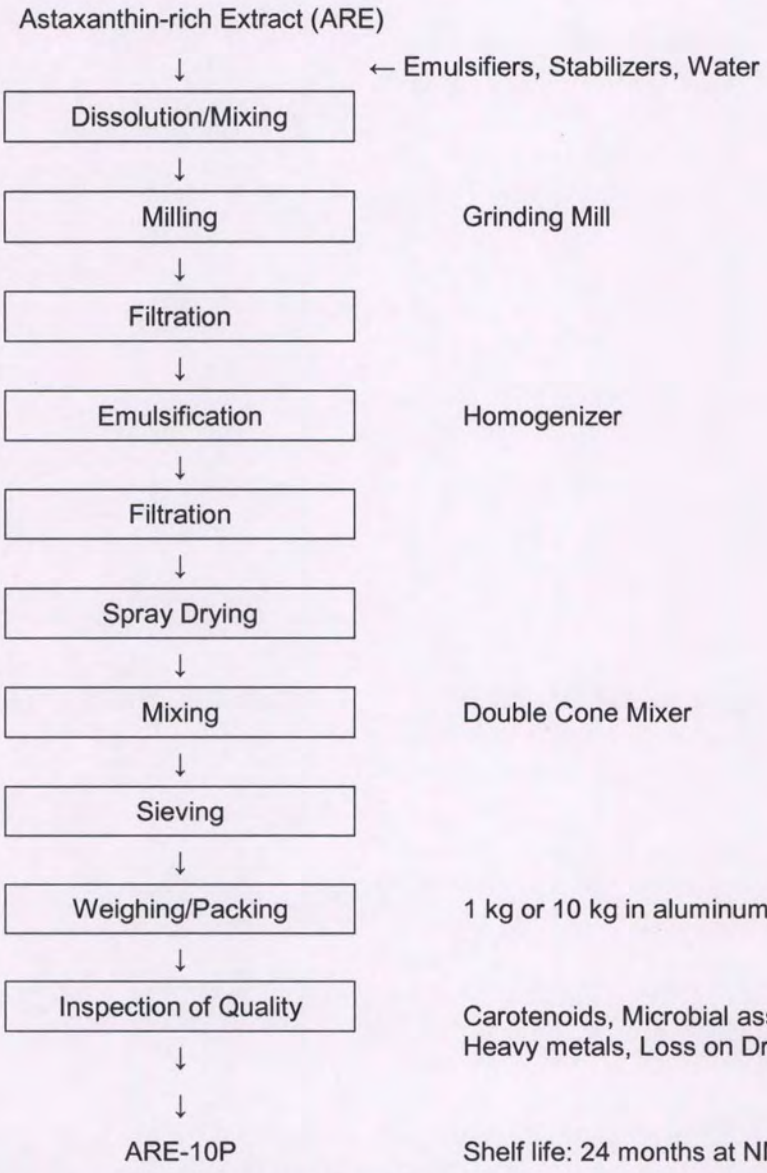
Figure 2.7.2-3 Schematic Overview of the Manufacturing Process for ARE-1P



ARE-1P = 1% Astaxanthin-rich extract derived from *Paracoccus carotinifaciens*; NMT = not more than

To produce ARE-10P, ARE0C is dissolved in maltodextrin, starch sodium octenyl succinate, L-ascorbic acid and water. Then the mixture milled and passed through a mesh filter and homogenized to further emulsify ARE. The emulsified filtrate is again filtered and spray dried. Once dry, the product is further mixed in a double corn mixer and filtered for the last time. A flow diagram of the complete manufacturing process for ARE-10P is shown in Figure 2.7.2-4.

Figure 2.7.2-4 Schematic Overview of the Manufacturing Process for ARE-10P



ARE-10P = 10% Astaxanthin-rich extract derived from *Paracoccus carotinifaciens*; NMT = not more than

2.7.3 Quality Control

ARE-C, ARE-1P, and ARE-10P are manufactured in compliance with cGMP.

2.7.4 Emulsifiers and Stabilizers

The emulsifiers and stabilizers used in the manufacture of diluted astaxanthin-rich carotenoid extracts (ARE-1P and ARE-10P) are listed in Table 2.7.4-1. All raw materials and processing aids are food-grade quality and are safe and suitable for use in the manufacture of food ingredients consistent with appropriate U.S. federal regulations, or have previously been determined to be GRAS.

Table 2.7.4-1 Regulatory Status of Emulsifiers and Stabilizers for ARE-1P and ARE-10P	
Ingredient & Regulation	**Conditions/Limitations**
Ascorbic Acid PART 182—SUBSTANCES GENERALLY RECOGNIZED AS SAFE Subpart D—Chemical Preservatives §182.3013—Ascorbic Acid Subpart I—Nutrients §182.8013--Ascorbic Acid	This substance is generally recognized as safe when used in accordance with good manufacturing practice.
Maltodextrin PART 184—DIRECT FOOD SUBSTANCES GENERALLY RECOGNIZED AS SAFE Subpart B—Listing of Specific Substances Affirmed as GRAS §184.1444—Maltodextrin	This substance is generally recognized as safe when used in accordance with good manufacturing practice.
Fatty Acids (Medium Chain Triglycerides) *PART 172 – FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION* *Subpart I – Mulitpurpose Food Additives* *§172.860*	The food additive fatty acids may be safely used in food and in the manufacture of food components in accordance with the following prescribed conditions: (a) The food additive consists of one or any mixture of the following straight-chain monobasic carboxylic acids and their associated fatty acids manufactured from fats and oils derived from edible sources: Capric acid, caprylic acid, lauric acid, myristic acid, oleic acid, palmitic acid, and stearic acid. (b) The food additive meets the following specifications: (1) Unsaponifiable matter does not exceed 2 percent. (2) It is free of chick-edema factor: (d) It is used or intended for use as follows: (1) In foods as a lubricant, binder, and as a defoaming agent in accordance with good manufacturing practice. (2) As a component in the manufacture of other food-grade additives. (e) To assure safe use of the additive, the label and labeling of the additive and any premix thereof shall bear, in addition to the other information required by the act, the following: (1) The common or usual name of the acid or acids contained therein. (2) The words "food grade," in juxtaposition with and equally as prominent as the name of the acid.

Table 2.7.4-1 Regulatory Status of Emulsifiers and Stabilizers for ARE-1P and ARE-10P

<table>
<tr><th>Ingredient & Regulation</th><th>Conditions/Limitations</th></tr>
<tr><td>dl-α-Tocopherol

PART 184—DIRECT FOOD SUBSTANCES GENERALLY RECOGNIZED AS SAFE
Subpart B—Listing of Specific Substances Affirmed as GRAS
§184.1890—dl-α-Tocopherol</td><td>(a) The [alpha]- tocopherols that are the subject of this GRAS affirmation regulation are limited to the following:
(2) dl -[alpha]- tocopherol (CAS Reg. No. 10191-41-0) is a mixture of stereoisomers of 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyl-tridecyl)-6-chromanol. It is chemically synthesized by condensing racemic isophytol with trimethyl hydroquinone. It is a pale yellow viscous oil at room temperature.

(b) The ingredients meet the specifications of the Food Chemicals Codex, 5th (2003), pp. 478-479, which is incorporated by reference (FCC, 2003). Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, or available for inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html.</td></tr>
<tr><td>Starch Sodium Octenyl Succinate

PART 172 -- FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION

Subpart I--Multipurpose Additives
§ 172.892 Food starch-modified.</td><td>Food starch-modified as described in this section may be safely used in food. The quantity of any substance employed to effect such modification shall not exceed the amount reasonably required to accomplish the intended physical or technical effect, nor exceed any limitation prescribed. To insure safe use of the food starch-modified, the label of the food additive container shall bear the name of the additive "food starch-modified" in addition to other information required by the Act. Food starch may be modified by treatment prescribed as follows:
(d) Food starch may be esterified by treatment with one of the following:
<table>
<tr><th></th><th>Limitation</th></tr>
<tr><td>1-Octenyl succinic anhydride, not to exceed 3 percent</td><td></td></tr>
<tr><td>1-Octenyl succinic anhydride, not to exceed 2 percent, and aluminum sulfate, not to exceed 2 percent</td><td></td></tr>
<tr><td>1-Octenyl succinic anhydride, not to exceed 3 percent, followed by treatment with a beta-amylase enzyme that is either an approved food additive of is generally recognized as safe</td><td>Limited to use as a stabilizer or emulsifier in beverages and beverage bases as defined in 170.3(n)(3) of this chapter.</td></tr>
<tr><td>Succinic anhydride, not to exceed 4 percent</td><td></td></tr>
</table>
</td></tr>
</table>

Table 2.7.4-1 Regulatory Status of Emulsifiers and Stabilizers for ARE-1P and ARE-10P

Ingredient & Regulation	Conditions/Limitations
Gum Arabic PART 184—DIRECT FOOD SUBSTANCES GENERALLY RECOGNIZED AS SAFE Subpart B—Listing of Specific Substances Affirmed as GRAS §184.1330—*Gum Arabic*	May be safely used in designated foods for the purposes and in accordance with the maximum usage levels permitted, as follows:

Food (as served)	%	Function
Beverages and beverage bases, §170.3(n)(3)	2.0	Emulsifier and emulsifier salt, §170.3(o)(8) of this chapter; flavoring agent and adjuvant, §170.3(o)(12) of this chapter; formulation aid, §170.3(o)(14) of this chapter; stabilizer and thickener, §170.3(o)(28) of this chapter.
Chewing gum, §170.3(n)(6)	5.6	Flavoring agent and adjuvant, §170.3(o)(12) of this chapter; formulation aid, §170.3(o)(14) of this chapter; humectant, §170.3(o)(16) of this chapter; surface-finishing agent, §170.3(o)(30) of this chapter.
Confections and frostings, §170.3(n)(9)	12.4	Formulation aid, §170.3(o)(14) of this chapter; stabilizer and thickener, §170.3(o)(28) of this chapter; surface-finishing agent, §170.3(o)(30) of this chapter.
Dairy product analogs, §170.3(n)(10)	1.3	Formulation aid, §170.3(o)(14) of this chapter; stabilizer and thickener, §170.3(o)(28) of this chapter.
Fats and oils, §170.3(n)(12)	1.5	Formulation aid, §170.3(o)(14) of this chapter; stabilizer and thickener, §170.3(o)(28) of this chapter.
Gelatins, puddings, and fillings, §170.3(n)(22)	2.5	Emulsifier and emulsifier salt, §170.3(o)(8) of this chapter; formulation aid, §170.3(o)(14) of this chapter.; stabilizer and thickener, §170.3(o)(28) of this chapter.
Hard candy and cough drops, §170.3(n)(25)	46.5	Flavoring agent and adjuvant, §170.3(o)(12) of this chapter; formulation aid, §170.3(o)(14) of this chapter.
Nuts and nut products, §170.3(n)(32)	8.3	Formulation aid, §170.3(o)(14) of this chapter; surface-finishing agent, §170.3(o)(30) of this chapter.
Quiescently frozen confection products	6.0	Formulation aid, §170.3(o)(14) of this chapter; stabilizer and thickener, §170.3(o)(28) of this chapter.
Snack foods, §170.3(n)(37)	4.0	Emulsifier and emulsifier salt, §170.3(o)(8) of this chapter; formulation aid, §170.3(o)(14) of this chapter.
Soft candy, §170.3(n)(38)	85.0	Emulsifier and emulsifier salt, §170.3(o)(8) of this chapter; firming agent, §170.3(o)(10) of this chapter; flavoring agent and adjuvant, §170.3(o)(12) of this chapter; formulation aid, §170.3(o)(14) of this chapter, humectant, §170.3(o)(16) of this chapter; stabilizer and thickener, §170.3(o)(28) of this chapter; surface-finishing agent, §170.3(o)(30) of this chapter.
All other food categories	1.0	Emulsifier and emulsifier salt, §170.3(o)(8) of this chapter; flavoring agent and adjuvant, §170.3(o)(12) of this chapter; formulation aid, §170.3(o)(14) of this chapter; processing aid, §170.3(o)(24) of this chapter; stabilizer and thickener, §170.3(o)(28) of this chapter; surface-finishing agent, §170.3(o)(30) of this chapter; texturizer, §170.3(o)(32) of this chapter.

2.8 Product Specifications and Batch Analyses

2.8.1 Proposed Product Specifications

The product specifications for JX Nippon's ARE-C, ARE-1P, and ARE-10P are detailed in Table 2.8.1-1, 2.8.1-2, and 2.8.1-3, respectively. All methods of analyses are nationally or internationally recognized (*e.g.*, Japan Food Research Laboratories, JP = The Japanese Pharmacopoeia) or have been validated by JX Nippon . Appropriate limits for heavy metals, microbial impurities, and ethanol, the only solvent used in manufacturing, have been established. However, due to the pure culture conditions and sourcing of ingredients, heavy metal residues are not expected. Preliminary analyses confirm absence of detectable amounts and thus data are not presented herein.

Table 2.8.1-1 Product Specifications and Analytical Methods for ARE-C

Parameter	Acceptance Criteria	Typical Value	Assay Method
Appearance	Black-purple powder	Complies	Visual test
Odor	Characteristic faint odor	Complies	Organoleptic examination
Loose Bulk density	0.2-0.4 g/mL	Complies	JP 3.01
Loss on Drying	≤1%, w/w	0.1%	JP 2.41
Residual Ethanol	≤0.2%	≤0.1%	JP 2.46
Carotenoids assay			
Astaxanthin	≥55%, w/w	65%	HPLC [a]
Total carotenoids	≥85%, w/w	90%	HPLC [a]
Microbiological Assays			
Aerobic plate count	≤1,000 cfu/g	300/g	JFRL (Standard Agar plating method)
Coliform bacteria	Negative	Negative/2.22 g	JFRL (BGLB broth inoculating method)
Viable molds count	≤100 cfu/g	Negative/0.1 g	JFRL (Potato Dextrose [10%] Agar plating method)
Viable yeasts count	≤100 cfu/g	Negative/0.1 g	JFRL (Potato Dextrose [10%] Agar plating method)
Coagulase positive *Staphylococci*	Negative	Negative/0.01 g	JFRL (Surface spread plating method)
Salmonella	Negative	Negative/25 g	JFRL (Enrichment culture method)

BGBL = brilliant green lactose broth; cfu = colony forming units; HPLC = high performance liquid chromatography; JFRL = Japan Food Research Laboratories; JP = The Japanese Pharmacopoeia; ND = Not detected; QL= Quantitation Limit
[a] JXE method

Table 2.8.1-2 Product Specifications and Analytical Methods for ARE-1P			
Parameter	**Acceptance Criteria**	**Typical Value**	**Assay Method**
Appearance	A deep to vivid yellowish red powder	Complies	Visual test
Odor	Characteristic faint odor	Complies	Organoleptic examination
Loose Bulk Density	0.2-0.4 g/mL	0.3 g/mL	JP 3.01
Loss on drying	≦10.0%, w/w	2.2%	JSSFA (105°C, 5hr)
Residual Ethanol	≦0.1%	ND (QL: 50 ppm)[a]	JFRL
Carotenoids assay			
Astaxanthin	≧1.0%, w/w	1.2%	HPLC[b]
Total carotenoids	≧1.3%, w/w	1.7%	HPLC[b]
Microbiological Assays			
Aerobic plate count	≦1,000 cfu/g	20/g	JFSR (Standard Method Agar Medium)
Coliform bacteria	Negative	Negative	JFSR (Desoxycholate Agar Medium)
Viable molds count	≦100 cfu/g	50/g	JFSR (Potato Dextrose Agar Medium with Antibiotics)
Viable yeasts count	≦100 cfu/g	0/g	JFSR (Potato Dextrose Agar Medium with Antibiotics)
Staphylococcus aureus	Negative	Negative	JFSR (Staphylococcus Agar Medium with Egg Yolk)
Salmonella	Negative	Negative/25g	JFRL (Enrichment culture method)

ARE-1P = 1% Astaxanthin-rich extract derived from *Paracoccus carotinifaciens;* cfu = colony forming units; HPLC = high performance liquid chromatography; JFRL = Japan Food Research Laboratories; JP = The Japanese Pharmacopoeia; JSSFA = Japan's Specifications and Standards for Food Additives; ND = Not detected; QL = Quantitation Limit

[a] 50 ppm = 0.005%

[b] JXE method

Table 2.8.1-3 Product Specifications and Analytical Methods for ARE-10P			
Parameter	**Acceptance Criteria**	**Typical Value**	**Assay Method**
Appearance	A very red powder	Complies	Visual test
Odor	Characteristic faint odor	Complies	Organoleptic examination
Loose Bulk Density	0.3-0.5 g/mL	0.4 g/mL	JP 3.01
Loss on drying	≦10.0%, w/w	2.6%	JSSFA (105°C, 5 hr)
Residual Ethanol	≦2%	1.5%	JFRL
Carotenoids assay			
Astaxanthin	≧10%, w/w	12%	HPLC
Total carotenoids	≧13%, w/w	17%	HPLC
Metals			
Heavy metals(as Pb)	≦10 ppm	ND (QL: 5 ppm)	JSSFA (Sodium Sulfide Method)
Arsenic (as As_2O_3)	≦2 ppm	ND (QL: 0.1 ppm)	JSSFA (Atomic absorption spectrometry)
Lead	≦1 ppm	ND (QL: 0.05 ppm)	JFRL (Atomic absorption spectrometry)
Mercury	≦1 ppm	ND (QL: 0.01 ppm)	JFRL (Cold vapor Atomic absorption spectrometry)

Table 2.8.1-3 Product Specifications and Analytical Methods for ARE-10P			
Parameter	**Acceptance Criteria**	**Typical Value**	**Assay Method**
Cadmium	≦1 ppm	ND (QL: 0.01 ppm)	JFRL (Atomic absorption spectrometry)
Microbiological Assays			
Aerobic plate count	≦1,000 cfu/g	10/g	JFSR (Standard Method Agar Medium)
Coliform bacteria	Negative	Negative	JFSR (Desoxycholate Agar Medium)
Viable molds count	≦100 cfu/g	0/g	JFSR (Potato Dextrose Agar Medium with Antibiotics)
Viable yeasts count	≦100 cfu/g	0/g	JFSR (Potato Dextrose Agar Medium with Antibiotics)
Staphylococcus aureus	Negative	Negative	JFSR (Staphylococcus Agar Medium with Egg Yolk)
Salmonella	Negative	Negative/25g	JFRL (Enrichment culture method)

ARE-10P = Astaxanthin-rich extract derived from *Paracoccus carotinifaciens;* cfu = colony forming units; HPLC = high performance liquid chromatography; JFRL = Japan Food Research Laboratories; JP = The Japanese Pharmacopoeia; JSSFA = Japan's Specifications and Standards for Food Additives; ND = Not detected; QL = Quantitation Limit

2.8.2 Batch Analyses

Five (5) non-consecutive batches each of ARE-C, ARE-1P, and ARE-10P were analyzed to verify that the manufacturing process produces a consistent product that meets the proposed product specifications. The results of the batch analyses are provided in Table 2.8.2-1, 2.8.2-2, and 2.8.2-3, respectively.

Table 2.8.2-1 Batch Analyses Data for ARE-C

Test/Standard	Criteria	Batch Number (b) (4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted] (b) (4)	[redacted]	[redacted]
Appearance	BPP	BPP	BPP	BPP	BPP	BPP	BPP	BPP	BPP	BPP
Odor	CFO	CFO	CFO	CFO	CFO	CFO	CFO	CFO	CFO	CFO
Loose Bulk Density (g/mL)	0.2-0.4	0.23	0.28	0.27	0.25	0.25	0.24	0.26	0.23	0.26
Loss on Drying (%)	≤1	0.10	0.08	0.08	0.04	0.10	0.08	0.23	0.17	0.08
Ethanol (%)	≤0.2%	0.110	0.088	0.126	0.111	0.172	0.076	0.090	0.076	0.031
Carotenoids assay										
Astaxanthin (%) (w/w)	≥55	60.2	59.2	59.4	59.3	59.6	60.0	59.6	66.5	66.2
Total carotenoids (%) (w/w)	≥85	90.9	90.2	90.2	89.6	90.2	91.8	90.5	92.7	91.4
Metals										
Heavy metals as Pb (ppm)	≤10	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)
Arsenic as As_2O_3 (ppm)	≤2	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)
Lead (ppm)	≤1	ND (QL: 0.05)	ND (QL: 0.05)	ND (QL: 0.05)	ND (QL: 0.05)	ND (QL: 0.05)	ND (QL: 0.05)	ND (QL: 0.05)	ND (QL: 0.05)	ND (QL: 0.05)
Mercury (ppm)	≤1	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)
Cadmium (ppm)	≤1	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)
Microbiological Assays										
Aerobic plate count (cfu/g)	≤1000	≤300	≤300	≤300	≤300	≤300	≤300	≤300	≤300	≤300
Viable mold count (cfu/g)	≤100	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Viable yeast count (cfu/g)	≤100	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Coliform bacteria	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Coagulase positive Staphylococci	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Salmonella	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

BPP = Black-purple powder; CFO = Characteristic faint odor; ND = Not detected; QL = Quantitation Limit

(b) (4) [redacted].

Table 2.8.2-2 Batch Analyses Data for ARE-1P						
	Batch Number					
Test/Standard	Criteria	(b) (4) [redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Appearance	A deep to vivid yellowish red powder	Deep to vivid yellowish red powder	Deep to vivid yellowish red powder	Deep to vivid yellowish red powder	Deep to vivid yellowish red powder	Deep to vivid yellowish red powder
Odor	Characteristic faint odor	Characteristic faint odor	Characteristic faint odor	Characteristic faint odor	Characteristic faint odor	Characteristic faint odor
Loose Bulk Density (g/mL)	0.2-0.4	0.31	0.32	0.33	0.32	0.33
Loss on Drying (%)(w/w)	≦10.0	2.9	3.3	2.8	3.1	3.2
Residual Ethanol (%)	≦0.1	ND (QL: 50 ppm)[a]	ND (QL: 50 ppm)	ND (QL: 50 ppm)	ND (QL: 50 ppm)	ND (QL: 50 ppm)
Carotenoids assay						
Astaxanthin (%) (w/w)	≧1.0	1.23	1.19	1.18	1.21	1.19
Total carotenoids (%) (w/w)	≧1.3	1.62	1.52	1.53	1.55	1.52
Metals						
Heavy metals as Pb (ppm)	≦10	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)
Arsenic as As_2O_3 (ppm)	≦2	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)
Lead (ppm)	≦1	0.55	ND (QL: 0.05)	0.08	0.07	0.06
Mercury (ppm)	≦1	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)
Cadmium (ppm)	≦1	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)
Microbiological Assays						
Aerobic plate count (cfu/g)	≦1,000	≤300	≤300	≤300	≤300	≤300
Coliform bacteria	Negative	Neg.	Neg.	Neg.	Neg.	Neg.
Viable molds count (cfu/g)	≦100	40	Neg.	Neg.	Neg.	Neg.
Viable yeasts count (cfu/g)	≦100	Neg.	Neg.	Neg.	Neg.	Neg.
Coagulase positive Staphylococci	Negative	Neg.	Neg.	Neg.	Neg.	Neg.
Salmonella	Negative	Neg.	Neg.	Neg.	Neg.	Neg.

ARE-1P = 1% Astaxanthin-rich extract derived from *Paracoccus carotinifaciens;* CFO = Characteristic faint odor; Neg. = Negative; ND =Not detected; QL = Quantitation Limit

[a] 50 ppm = 0.005%

Table 2.8.2-3 Batch Analyses Data for ARE-10P						
	Batch Number					
Test/Standard	Criteria	[redacted]	[redacted]	[redacted]	[redacted]	(b) (4) [redacted]
Appearance	A very red powder	A very red powder	A Very red powder	A Very red powder	A Very red powder	A Very red powder
Odor	Characteristic faint odor	Characteristic faint odor	Characteristic faint odor	Characteristic faint odor	Characteristic faint odor	Characteristic faint odor
Loose Bulk Density (g/mL)	0.3-0.5	0.42	0.39	0.39	0.41	0.40
Loss on Drying (%)(w/w)	≦10.0%	3.0	2.8	2.7	2.4	2.6
Residual Ethanol (%)	≦2	0.75	0.94	0.93	1.0	1.01
Carotenoids assay						
Astaxanthin (%) (w/w)	≧10	12.3	11.8	12.2	12.2	12.7
Total carotenoids (%) (w/w)	≧13	17.8	16.7	17.2	17.1	17.8
Metals						
Heavy metals as Pb (ppm)	≦10	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)	ND (QL: 5)
Arsenic as As_2O_3 (ppm)	≦2	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)	ND (QL: 0.1)
Lead (ppm)	≦1	ND (QL: 0.05)	ND (QL: 0.05)	ND (QL: 0.05)	ND (QL: 0.05)	ND (QL: 0.05)
Mercury (ppm)	≦1	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)
Cadmium (ppm)	≦1	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)	ND (QL: 0.01)
Microbiological Assays						
Aerobic plate count (cfu/g)	≦1,000	≤300	≤300	≤300	≤300	≤300
Coliform bacteria	Negative	Neg.	Neg.	Neg.	Neg.	Neg.
Viable molds count (cfu/g)	≦100	Neg.	Neg.	Neg.	Neg.	Neg.
Viable yeasts count (cfu/g)	≦100	Neg.	Neg.	Neg.	Neg.	Neg.
Coagulase positive Staphylococci	Negative	Neg.	Neg.	Neg.	Neg.	Neg.
Salmonella	Negative	Neg.	Neg.	Neg.	Neg.	Neg.

ARE-10P = 10% Astaxanthin-rich extract derived from *Paracoccus carotinifaciens;* CFO = Characteristic faint odor; cfu = colony forming units; Neg. = Negative; ND = Not detected; QL = Quantitation Limit

2.9 Carotenoid Content

JX Nippon has developed and validated methods for determination of carotenoids (*e.g.*, β-carotene, echinenone, 3-hydroxyechinenone, canthaxanthin, adonirubin, asteroidenone, astaxanthin, and adonixanthin) ARE, ARE-1P, and ARE-10P using HPLC. These methods have been validated for specificity, linearity, content, and stability in auto sampler, in compliance with the GLP (OECD 1998a) using appropriate reference standards.

Table 2.9-1 shows the carotenoid content (mg/g) of representative batches of ARE-C, ARE-1P, and ARE-10P (see Appendices 1, 2, 3 for certificates of analyses.).

Table 2.9-1 Carotenoid Content (mg/g) of ARE-C, ARE-1P, and ARE-10P

LOT #	(b)(4)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
ARE-C										
CK-001	602.0	15.0	8.7	47.8	177.1	8.5	3.2	45.8	1.3	909.4
CK-002	591.8	16.3	9.0	48.3	173.7	9.5	4.0	46.6	3.1	902.3
CK-003	593.8	16.1	9.1	48.1	174.5	9.7	3.5	46.5	0.8	902.2
CK-004	593.2	15.5	8.6	46.9	171.8	9.5	3.2	46.6	1.0	896.3
CK-005	596.2	15.7	8.9	47.2	174.1	8.8	3.3	46.7	1.6	902.3
CK-006	600.2	16.3	10.1	49.0	177.2	11.5	3.8	47.8	2.5	918.3
CK-007	596.0	15.8	8.9	48.1	175.4	8.6	3.3	47.2	1.6	904.8
DK-001	664.9	13.0	8.5	28.6	116.5	11.8	3.6	76.1	3.9	926.8
EK-001	661.6	12.9	8.7	23.6	85.7	13.5	3.5	97.7	6.5	913.7
ARE-1P										
ARE-1P-SA001	12.3	0.2	0.2	0.4	1.6	< 0.1	0.1	1.4	< 0.1	16.2
ARE-1P-SA003	11.9	0.2	0.1	0.4	1.6	< 0.1	0.1	0.9	< 0.1	15.2
ARE-1P-SA005	11.8	0.2	0.1	0.4	1.8	< 0.1	0.1	0.9	< 0.1	15.3
ARE-1P-SA007	12.1	0.2	0.1	0.4	1.8	< 0.1	0.1	0.8	< 0.1	15.5
ARE-1P-SA009	11.9	0.2	0.1	0.4	1.7	< 0.1	0.1	.08	< 0.1	15.2
ARE-10P										
ARE-10P-SA002	123.0	2.0	1.0	4.0	17.0	1.0	1.0	23.0	< 0.1	178.0
ARE-10P-SA004	118.0	2.0	1.0	5.0	21.0	1.0	1.0	17.0	< 0.1	167.0
ARE-10P-SA006	122.0	2.0	1.0	5.0	22.0	1.0	1.0	17.0	< 0.1	172.0
ARE-10P-SA008	122.0	2.0	1.0	5.0	22.0	1.0	1.0	17.0	< 0.1	171.0
ARE-10P-SA0010	127.0	2.0	1.0	5.0	22.0	1.0	1.0	18.0	< 0.1	178.0

3-ECH = 3-Hydroxyechinenone; ADR = Adonirubin; ADX = Adonixanthin; ATRD = Asteroidenone; ATX = Astaxanthin; β-Car = β-carotene; CTX = Canthaxanthin; ECH = Echinenone; TC = Total Carotenoids

The astaxanthin products contained in INNOBIO's GRAS products contain much lower levels of carotenoids (up to 10%) than ARE-C. Astaxanthin levels are comparable to ARE-1P and ARE-10P. As use levels for JX Nippon's products are proposed on the basis of astaxanthin content, differences in the astaxanthin content between ARE-C and INNOBIO's products are not anticipated to make a difference with respect to safety.

INNOBIO's GRAS Notified products contain only minor levels of other carotenoids present in JX Nippon's products. ARE-C, ARE-1P, and ARE-10P also contain canthaxanthin, adonirubin, β-carotene, echinenone, adonixanthin, 3-hydroxyechinenone and asteroidenone. The safety of these other carotenoids will be discussed in Part 6.

2.10 Stability

2.10.1 Stability of ARE-C

The stability of 3 lots (b) (4) [redacted]) of ARE-C was tested in aluminum film bags at both 4 ± 3°C and 25 ± 1°C, as well as under accelerated storage conditions (40 ± 1°C, 75 ± 3% relative humidity). All samples were observed for odor and appearance and the carotenoid content was measured by HPLC at 0, 1, 3, 6, and 12 months while samples stored in aluminum film bags at both 4 ± 3°C and 25 ± 1°C were tested up to 36 months. At each time point, the remaining percentages of astaxanthin and total carotenoids in the 3 lots under all conditions were around 100%, and appearance and odor were unchanged. Based on these results, ARE-C can be considered stable under the proposed storage conditions of 36 months at not more than 25°C.

2.10.2 Stability of ARE-1P and ARE-10P

2.10.2.1 ARE-1P

The stability of 3 lots of ARE-1P (b) (4) [redacted]) was tested in aluminum film bags at 4 ± 3°C and 25 ± 1°C, as well as under accelerated storage conditions (40 ± 1°C, 75 ± 3% relative humidity). Samples were observed for odor and appearance and the carotenoid content was measured by HPLC at 0, 1, 3, and 6 months. No degradation was observed when samples were stored at 4°C for 6 months, based on the percent recovery (~100%) of astaxanthin and total carotenoids compared to baseline, and appearance and odor were unchanged. Samples stored at 25 and 40°C for 6 months were reported to be outside the range of analytical error. Based on these results, ARE-1P is considered stable at 4°C for 6 months. Testing will continue for an additional 24 months to determine whether the current shelf life can be extended.

2.10.2.2 ARE-10P

The stability of 3 lots of ARE-10P ((b) (4) [redacted]) was tested in aluminum film bags at 4 ± 3°C and 25 ± 1°C, as well as under accelerated storage conditions (40 ± 1°C, 75 ± 3% relative humidity). Samples were observed for odor and appearance and the carotenoid content was measured by HPLC at 0, 1, 3, and 6 months. No degradation was observed when samples were stored at 4°C, 25°C, or 40°C (75% relative humidity) for 6 months, based on the percent recovery (~100%) of astaxanthin and total carotenoids

compared to baseline, and appearance and odor were consistently unchanged. Based on these results, ARE-10P can be considered stable at 4°C, 25°C, or 40°C (75% relative humidity) for up to 6 months. Testing will continue for an additional 24 months to determine whether the current shelf life can be extended.

Part 3. §170.235 Dietary Exposure

3.1 Probable Consumption

Dietary Intake in General U.S. Population from all Proposed Food Uses

ARE-C, ARE-1P, and ARE-10P are intended to be used as sources of dietary astaxanthin in baked goods, baking mixes, beverages, beverage bases, energy, sports, isotonic drinks, non-milk based meal replacements, cereals, cereal products, chewing gum, coffee and tea, dairy product analogs, frozen dairy desserts and mixes, hard candy, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and soft candy, at levels providing 0.15 mg astaxanthin/serving. These intended uses are identical to those described by INNOBIO in a prior GRAS notice filed by FDA as GRN No. 580 (INNOBIO Limited, 2015; U.S. FDA, 2015). INNOBIO calculated the mean and 90th percentile, users-only, dietary exposures to astaxanthin by the general population to be 0.96 mg/person/day (16 µg/kg body weight/day) and 1.62 mg/p/day (28 µg/kg body weight/day), respectively. In response, FDA indicated it had no questions regarding INNOBIO's conclusion that the use of *Haematococcus pluvialis* (*H. pluvialis*) extract containing astaxanthin esters as described is GRAS through scientific procedures.

Because JX Nippon's ARE ingredients are intended as alternative sources to Fuji's and INNOBIO's GRAS astaxanthin ingredient, and the conditions of use are identical, no impact on the intake of astaxanthin in the overall diet of the public would be expected. The present case simply constitutes an introduction into the market by another supplier who will have to compete in the same markets and foods.

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with ARE-C, ARE-1P, and ARE-10P.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. §170.250 Narrative

6.1 History of Safe Use

Astaxanthin as a naturally-occurring substance already present in the human diet. The safety of astaxanthin has also previously been considered in GRAS notices GRN 294 and GRN 580 (Fuji Chemical Industry Co., Ltd., 2009; U.S. FDA, 2010, 2015; INNOBIO Limited, 2015). In 2009, Fuji notified the FDA (with no questions) that the use of *H. pluvialis* extract containing astaxanthin esters is GRAS, through scientific procedures, for use as an ingredient in baked goods, baking mixes, beverages, beverage bases, energy, sports, isotonic drinks, non-milk based meal replacements, cereals, cereal products, chewing gum, coffee and tea, dairy product analogs, frozen dairy desserts and mixes, hard candy, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and soft candy at levels providing 0.1 mg astaxanthin/serving. Subsequently, in 2015, INNOBIO notified FDA (with no questions) that the use of *H. pluvialis* extract containing astaxanthin esters is GRAS, through scientific procedures, for use as an ingredient in these same applications at levels providing 0.15 mg astaxanthin/serving is GRAS (GRN 580 – INNOBIO Limited, 2015; U.S. FDA, 2015).

In addition to astaxanthin, JX Nippon's ARE-C, ARE-1P, and ARE-10P contain several other carotenoids. Canthaxanthin is a naturally-occurring carotenoid found in wild salmonids. Based on consideration of reports of crystalline deposits in the human retina following oral intake of canthaxanthin for skin tanning, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established in 1996 an acceptable daily intake (ADI) level of 0.03 mg/kg body weight/day (1.8 mg/day in a 60-kg person) (JECFA, 1996). The ADI represents an estimate of the amount of a substance in food or drinking water, expressed on a body weight basis (standard human = 60 kg), that can be ingested daily over a lifetime without appreciable risk (JECFA, 2009). The ADI was derived by applying a factor of 10 to a no-observable-effect level of 0.25 mg/kg body weight/day from a study of 27 human subjects with porphyria that were treated with canthaxanthin at levels ranging from 15 mg/day (0.25 mg/kg body weight/day) to 120 mg/day (2 mg/kg body weight/day) from 5 weeks to several months. European Food Safety Authority (EFSA) independently evaluated the use of canthaxanthin as a food additive and derived an ADI that was in line with JECFA (EFSA, 2010).

Adonirubin is a naturally-occurring intermediate in the biosynthesis of astaxanthin. In 2007, the EFSA stated in its opinion about the use of a carotenoid-rich *P. carotinifaciens* feed additive for salmon and trout, that adonirubin should be considered along with canthaxanthin in exposure assessments, as a worst-case scenario (EFSA, 2007). The reasons were 2-fold: (1) structural similarity to canthaxanthin, a substance associated with ocular effects (crystalline deposits in retina); and (2) the absence of compound-specific safety information. EFSA suggests that the JECFA ADI of 0.03 mg/kg body weight/day be applied to the combination of canthaxanthin and adonirubin.

The minor carotenoids (*i.e.*, adonixanthin, β-carotene, echinenone, asteroidenone, and 3-hydroxyechinenone) are naturally occurring metabolites in the production of astaxanthin and thus, would be present in the human food chain as a component of seafood. As such, there presence in ARE-C, ARE-1P, and ARE-10P are expected to be safe under the proposed conditions of use.

6.2 Pivotal Safety Data

The conclusion that ARE-C, ARE-1P, ARE-10P, as described herein, are GRAS under the conditions of their intended use is based on scientific procedures using generally available data from the 90-day safety study on ARE-C itself, as well as other published data related to the safety of astaxanthin, including data related to the absorption, distribution, metabolism, and excretion (ADME) and safety of astaxanthin that has been previously reviewed (see GRAS notices 294 and GRN 580) (U.S. FDA, 2010, 2015). Supporting safety studies include an unpublished acute oral toxicity study, a 14-day dose-range finding study, and unpublished genotoxicity studies with ARE-C and a series of toxicity studies performed on the production organism, and DPC, the dried biomass of *P. carotinifaciens* from which ARE-C is extracted. These data were not considered critical to the review since a 90-day study on the material itself was available. A summary of the main findings is provided below.

- In a GLP-compliant acute oral toxicity study, the LD_{50} value of ARE-C by a single oral administration to rats was reported to be higher than 2,000 mg/kg, the highest dose tested (Bozo Research Center, 2013a).

- A 14-day dose-range finding oral toxicity study, no test substance-related changes were observed in body weight, food consumption, hematological parameters, blood chemistry or organ weights. No gross or microscopic changes were seen at necropsy following the administration of 40, 250, 500, and 1,000 mg/kg/day of ARE-C by oral gavage (Bozo Research Center, 2013b).

- The pivotal 90-day, repeated-dose, oral toxicity study of ARE-C was reported by (Katsumata *et al.*, 2014). This study was conducted in accordance with guidelines established by OECD, including GLP and Guidelines for the Testing of Chemicals relating to animal welfare (OECD, 1998a,b). Ten 6-week-old Sprague-Dawley strain SPF rats/sex/group were administered 0 (olive oil), 250, 500, and 1,000 mg/kg/day of ARE-C by oral gavage using stomach tubes. Dark red feces were observed in all male and female rats administered ARE-C, due to the excretion of the test material. No deaths occurred. No test material-related changes were observed in clinical observations, manipulative test, grip strength, motor activity, body weight, food consumption, ophthalmological examination, urinalysis (including water intake), hematological parameters, blood chemistry, organ weights, necropsy, or histopathological examination. On this basis, the no-observed-adverse-effect level

(NOAEL) of ARE-C was established at 1,000 mg/kg/day under the conditions of the study. Given that the astaxanthin content of batch (b) (4) is 596 mg/g, a NOAEL 1,000 mg/kg/day of ARE-C is equivalent to a 596 mg astaxanthin/kg/day.

- ARE-C was negative in a reverse mutation assay in *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535, and *Escherichia coli* strain WP2 *uvrA* with and without metabolic activation at concentrations of 1.22, 2.44, 4.88, 9.77, and 19.5 µg/plate. No growth inhibition was observed in any strain with or without metabolic activation (Bozo Research Center, 2013c).

- Likewise the potential for ARE-C to induce chromosomal aberrations was evaluated in an *in vitro* cytogenetics assay, using Chinese hamster lung fibroblast cell in the absence or presence of rat liver post-mitochondrial fraction S-9. In the short-term method of the chromosome aberration test, cells were exposed to 0, 78.1, 156, 313, and 625 µg/mL of ARE-C in the absence of S9 and 0, 625, 1,250, 2,500, and 5,000 µg/mL of ARE-C in the presence of S9. In the continuous method of the chromosome aberration test, cells were exposed to 0, 39.1, 78.1, 156, and 313 µg/mL of ARE-C in the absence of S9. The incidence of cells treated with ARE-C with structural aberrations excluding gaps and the incidence of the occurrence of polyploidy fell within the results expected for the negative control. Thus it was concluded that under the parameters of this study, ARE-C does not induce chromosome aberrations (Bozo Research Center, 2013d).

- *P. carotinifaciens* is a non-pathogenic and non-toxigenic organism. It does not possess antimicrobial activity. Safety studies on DPC demonstrated no evidence of mutagenicity or genotoxicity. Additionally, no toxicity or pathological effects were noted when DPC was administered to rats at concentrations up to 5% in the diet for 90 days (Inveresk Research, 2004; SafePharm Laboratories, 2004a,b, 2005, 2006).

- Data related to the safety of the individual carotenoids present in ARE-C are considered supportive of the safety of GRAS substances. Astaxanthin, the major carotenoid component of ARE-C has been the subject of a number of preclinical and clinical investigations, all of which support the safety of 3 ARE products under the proposed conditions of use. These studies have been reviewed previously in GRAS Notices 246 and 548

- Canthaxanthin, another carotenoid present in ARE-C, has been associated with the deposition of crystals in the retina following prolonged oral intake at high-dose levels through its use as an oral bronzing agent. As a result, JECFA has established an ADI of 0 to 0.03 mg/kg body weight. Intake of ARE ingredients in accordance with recommended use instructions would result in an estimated maximum daily intakes far below the JECFA ADI.

- Metabolism and pharmacokinetic studies of astaxanthin in humans (Østerlie *et al.*, 2000; Coral-Hinostroza *et al.,* 2004). Elmadfa and Majchrzak (1999) suggest that absorption of astaxanthin from JX Nippon's products, which is present in the unesterified form, would be comparable to or less than the amount likely to be absorbed from the available astaxanthin from *Haematococcus* algae, which is present as esterified astaxanthin.

- The FDA's Office of Dietary Supplement Programs, Center for Food Safety and Applied Nutrition responded with no objections or questions related to safety to JX Nippon's "NDI 961: Astaxanthin-Rich Carotenoid extract derived from *Paracoccus carotinifaciens* (under trade name of ARE-1P and ARE-10P" that included recommended intakes of 12 mg astaxanthin/day.

6.4 Expert Panel Evaluation

JX Nippon has concluded that ARE-C, ARE-1P, and ARE-1P, extracted from the dehydrated biomass of *P. carotinifaciens*, manufactured consistent with cGMP and meeting appropriate food grade specifications, is GRAS for use as an ingredient in select food categories, as described in Part 1.3, on the basis of scientific procedures.

JX Nippon's conclusion on the GRAS status of ARE-C, ARE-1P, and ARE10P under the conditions of their intended use is based on data from traditional toxicology studies generally available in the public domain.

A Panel of Experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients unanimously concluded on the GRAS status of the ARE-C, ARE-1P, and ARE-10P under conditions of its intended use. The Expert Panel consisted of the following qualified scientific experts: Dr. John Thomas (Adjunct Professor, Indiana University School of Medicine), Dr. Robert Nicolosi (Professor Emeritus, University of Massachusetts Lowell) and Dr. David Bechtel (Bechtel Consulting, Inc.).[1]

The Expert Panel, convened by JX Nippon, independently and critically evaluated all data and information presented herein and concluded that ARE-C, ARE-1P, and ARE-10P, meeting appropriate food-grade specifications and manufactured consistent with cGMP, is safe and suitable for use as an ingredient in specified food categories, as described in Part 1.3, and is GRAS based on scientific procedures. A summary of data and information reviewed by the Expert Panel is presented in Appendix 4

[1] The panelists participated in their individual capacities. Institutional affiliations are provided for identification purposes only.

6.5 Conclusion

Based on the above data and information presented herein, JX Nippon has concluded that the intended uses of ARE-C, ARE-1P, and ARE-10P in specified food and beverage products, as described in Part 1.3, are GRAS based on scientific procedures. The GRAS status of ARE-C, ARE-1P, and ARE-10P is further supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training to evaluate the safety of food ingredients, who concluded that the intended use of ARE-C, ARE-1P, and ARE-10P as sources of dietary astaxanthin in baked goods, baking mixes, beverages, beverage bases, energy, sports, isotonic drinks, non-milk based meal replacements, cereals, cereal products, chewing gum, coffee and tea, dairy product analogs, frozen dairy desserts and mixes, hard candy, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and soft candy, at levels providing 0.15 mg astaxanthin/serving. The mean and 90th percentile, users-only, dietary exposures to astaxanthin by the general population to be 0.96 mg/person/day (16 µg/kg body weight/day) and 1.62 mg/p/day (28 µg/kg body weight/day), respectively.

ARE-C, ARE-1P, and ARE-1P, therefore. may be marketed and sold for their intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

Part 7. §170.255 List of Supporting Data and Information

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Table of CFR Sections Referenced (Title 21—Food and Drugs)		
Part	**Section §**	**Section Title**
170—Food additives	170.30	Eligibility for classification as generally recognized as safe (GRAS)
172—Food additives permitted for direct addition to food for human consumption	172.860	Fatty acids
	172.892	Food starch-modified
182—Substances generally recognized as safe	182.8013	Ascorbic acid
184—Direct food substances affirmed as generally recognized as safe	184.1330	Acacia (gum arabic)
	184.1444	Maltodextrin
	184.1890	α-Tocopherols

Appendix 1

Certificate of Analyses for ARE-C

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

Lot No. : [redacted]

Date of Manufacturing : November 29, 2012

Test item	Results	Method
Appearance	Black-purple powder crystal	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	0.10 %	JP *2.41 (0.5g, 25°C, in vacuum, silica gel, 4hours)
Ethanol	0.110 %	JP* 2.46 (Gas chromatography)
Loose Bulk Density	0.23 g/ml	JP *3.01 (20ml cylinder)

* JP: The Japanese Pharmacopoeia 16th Edition

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 13, 2013

Kazuaki Hirasawa
Senior Chemist
Advanced Materials R&D Group
Central Technical Research Laboratory

Date: September 13, 2013

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 13049097002-02 1/1
June 11, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda Saitama 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot (b) (4)

Received date: May 31, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Arsenic (as As_2O_3)	Not detected	0.1 ppm		1
Heavy metals (as Pb)	Not detected	5 ppm		2
Lead	Not detected	0.05 ppm		1
Mercury	Not detected	0.01 ppm		3
Aerobic plate count	Not more than 300/g	----		4
Coliform bacteria	Negative/2.22g	----		5
Residue on ignition	0.4 %	----	1	

QL: Quantitation limit N: Notes M: Method

Notes

1:"Residue on Ignition Test (Sample 1 g, 600 °C, Constant Weight)", General Tests, The Japanese Pharmacopoeia, Sixteenth Edition.

Method

1:Atomic absorption spectrometry
2:Sodium sulfide colorimetric method
3:Cold vapor atomic absorption spectrometry
4:Standard Agar plating method
5:BGLB broth inoculating method

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Noriko Imaizumi
Principal Investigator

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No. 13064337001-02 1/1
July 22, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda, Saitama, 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. (b) (4)

Received date: July 10, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Cadmium	Not detected	0.01 ppm		1
Viable molds count	Negative/0.1g	-----		2
Viable yeasts count	Negative/0.1g	-----		2
Coagulase positive staphylococci	Negative/0.01g	-----		3
Salmonella	Negative/25g	-----		4

QL: Quantitation limit N: Notes M: Method

Method

1:Atomic absorption spectrometry
2:Potato Dextrose (10 %) Agar plating method
3:Surface spread plating method
4:Enrichment culture method

(b) (6)

JAPAN FOOD RESEARCH LABORATORIES

Noriko Imaizumi
Principal Investigator

Jul. 22, 2013
Date

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

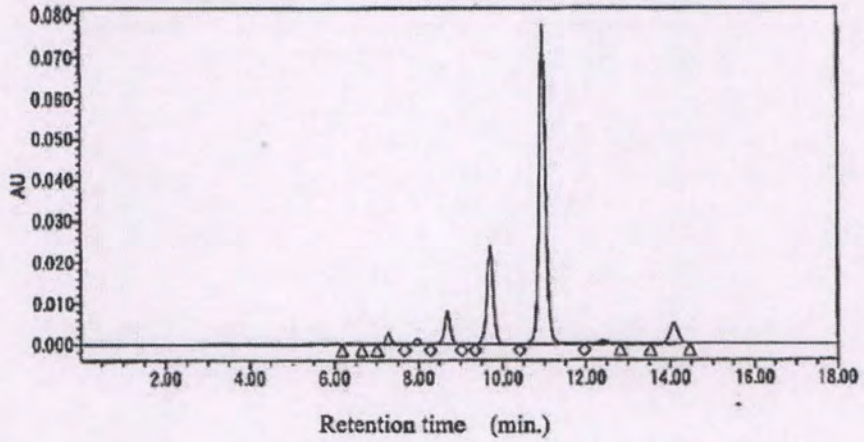
Lot No. (b) (4)

Date of Manufacturing : November 29, 2012

Date of Analysis : May 23, 2013

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	3.2
Echinenone	15.0
3-Hydroxyechinenone	8.7
Canthaxanthin	47.8
Adonirubin	177.1
Astaxanthin	602.0
Asteroidenone	8.5
Adonixanthin	45.8
Others	1.3
Total carotenoids	909.4



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(b) (6)

Sep. 9, 2013

Kazuaki Hirasawa

Senior Chemist

Advanced Materials R&D Group

Central Technical Research Laboratory

Date: September 9, 2013

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

Lot No. : (b) (2)

Date of Manufacturing : December 2, 2012

Test item	Results	Method
Appearance	Black-purple powder crystal	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	0.08 %	JP *2.41 (0.5g, 25°C, in vacuum, silica gel, 4hours)
Ethanol	0.088 %	JP* 2.46 (Gas chromatography)
Loose Bulk Density	0.28 g/ml	JP *3.01 (20ml cylinder)

* JP: The Japanese Pharmacopoeia 16th Edition

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 13, 2013

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Senior Chemist
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Central Technical Research Laboratory

Date: September 13, 2013

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No. 13049097003-02 1/1
June 11, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda Saitama 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. (b) (4)

Received date: May 31, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Arsenic (as As_2O_3)	Not detected	0.1 ppm		1
Heavy metals (as Pb)	Not detected	5 ppm		2
Lead	Not detected	0.05 ppm		1
Mercury	Not detected	0.01 ppm		3
Aerobic plate count	Not more than 300/g	—		4
Coliform bacteria	Negative/2.22g	—		5
Residue on ignition	0.5 %	—	1	

QL: Quantitation limit N: Notes M: Method

Notes

1:"Residue on Ignition Test (Sample 1 g, 600 ℃, Constant Weight)", General Tests, The Japanese Pharmacopoeia, Sixteenth Edition.

Method

1:Atomic absorption spectrometry
2:Sodium sulfide colorimetric method
3:Cold vapor atomic absorption spectrometry
4:Standard Agar plating method
5:BGLB broth inoculating method

(b) (6)

JAPAN FOOD RESEARCH LABORATORIES

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No. 13064337002-02 1/1
July 22, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda, Saitama, 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot (b) (4)

Received date: July 10, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Cadmium	Not detected	0.01 ppm		1
Viable molds count	Negative/0.1g	—		2
Viable yeasts count	Negative/0.1g	—		2
Coagulase positive staphylococci	Negative/0.01g	—		3
Salmonella	Negative/25g	—		4

QL: Quantitation limit N: Notes M: Method

Method

1:Atomic absorption spectrometry
2:Potato Dextrose (10 %) Agar plating method
3:Surface spread plating method
4:Enrichment culture method

(b) (6)

JAPAN FOOD RESEARCH LABORATORIES

Noriko Imaizumi
Principal Investigator

Jul. 22, 2013
Date

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

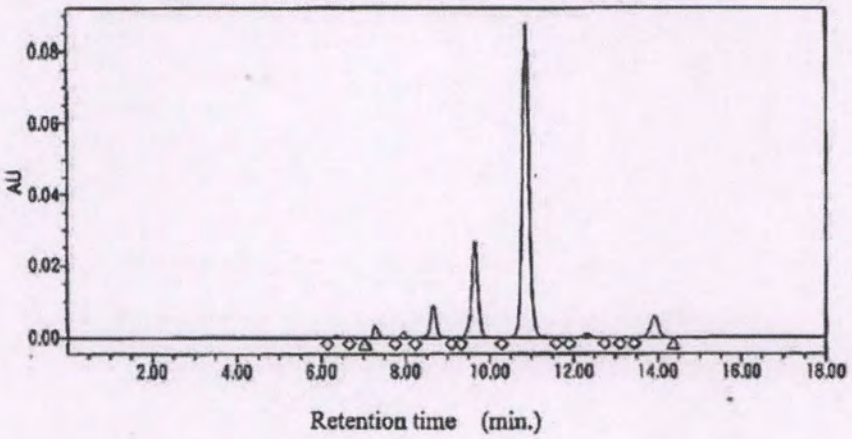
Lot No. : (b) (4)

Date of Manufacturing : December 2, 2012

Date of Analysis : May 28, 2013

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	4.0
Echinenone	16.3
3-Hydroxyechinenone	9.0
Canthaxanthin	48.3
Adonirubin	173.7
Astaxanthin	**591.8**
Asteroidenone	9.5
Adonixanthin	46.6
Others	3.1
Total carotenoids	902.3



JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 9, 2013

Kazuaki Hirasawa
Senior Chemist
Advanced Materials R&D Group
Central Technical Research Laboratory

Date: September 9, 2013

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

Lot No. : (b) (4)

Date of Manufacturing : December 5, 2012

Test item	Results	Method
Appearance	Black-purple powder crystal	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	0.08 %	JP *2.41 (0.5g, 25°C, in vacuum, silica gel, 4hours)
Ethanol	0.126 %	JP* 2.46 (Gas chromatography)
Loose Bulk Density	0.27 g/ml	JP *3.01 (20ml cylinder)

* JP: The Japanese Pharmacopoeia 16th Edition

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 13, 2013

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Date: September 13, 2013

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No. 13049097004-02 1/1
June 11, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda Saitama 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot(b) (4)

Received date: May 31, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Arsenic (as As_2O_3)	Not detected	0.1 ppm		1
Heavy metals (as Pb)	Not detected	5 ppm		2
Lead	Not detected	0.05 ppm		1
Mercury	Not detected	0.01 ppm		3
Aerobic plate count	Not more than 300/g	----		4
Coliform bacteria	Negative/2.22g	----		5
Residue on ignition	0.6 %	----	1	

QL: Quantitation limit N: Notes M: Method

Notes

1:"Residue on Ignition Test (Sample 1 g, 600 ℃, Constant Weight)", General Tests, The Japanese Pharmacopoeia, Sixteenth Edition.

Method

1:Atomic absorption spectrometry
2:Sodium sulfide colorimetric method
3:Cold vapor atomic absorption spectrometry
4:Standard Agar plating method
5:BGLB broth inoculating method

(b) (6)

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No. 13064337003-02 1/1
July 22, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda, Saitama, 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. (b) (4)

Received date: July 10, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Cadmium	Not detected	0.01 ppm		1
Viable molds count	Negative/0.1g	----		2
Viable yeasts count	Negative/0.1g	----		2
Coagulase positive staphylococci	Negative/0.01g	----		3
Salmonella	Negative/25g	----		4

QL: Quantitation limit N: Notes M: Method

Method
1:Atomic absorption spectrometry
2:Potato Dextrose (10 %) Agar plating method
3:Surface spread plating method
4:Enrichment culture method

(b) (6)

JAPAN FOOD RESEARCH LABORATORIES

Noriko Imaizumi
Principal Investigator

Jul. 22, 2013
Date

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

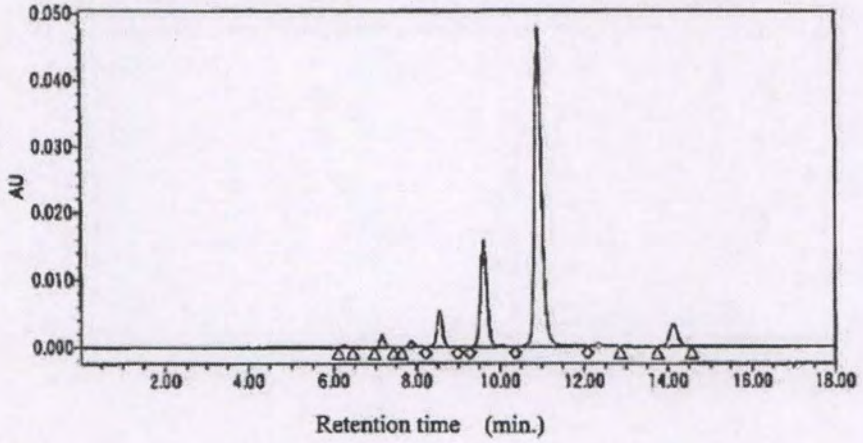
Lot No. : (b) (4)

Date of Manufacturing : December 5, 2012

Date of Analysis : February 18, 2013

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	3.5
Echinenone	16.1
3-Hydroxyechinenone	9.1
Canthaxanthin	48.1
Adonirubin	174.5
Astaxanthin	**593.8**
Asteroidenone	9.7
Adonixanthin	46.5
Others	0.8
Total carotenoids	902.2



JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 9, 2013

Kazuaki Hirasawa
Senior Chemist
Advanced Materials R&D Group
Central Technical Research Laboratory

Date: September 9, 2013

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

Lot No. : (b) (4)

Date of Manufacturing : December 8, 2012

Test item	Results	Method
Appearance	Black-purple powder crystal	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	0.04 %	JP *2.41 (0.5g, 25°C, in vacuum, silica gel, 4hours)
Ethanol	0.111 %	JP* 2.46 (Gas chromatography)
Loose Bulk Density	0.25 g/ml	JP *3.01 (20ml cylinder)

* JP: The Japanese Pharmacopoeia 16th Edition

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 13, 2013

Kazuaki Hirasawa
Senior Chemist
Advanced Materials R&D Group
Central Technical Research Laboratory

Date: September 13, 2013

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 13049097005-02 1/1
June 11, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda Saitama 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. CK-004

Received date: May 31, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Arsenic (as As_2O_3)	Not detected	0.1 ppm		1
Heavy metals (as Pb)	Not detected	5 ppm		2
Lead	Not detected	0.05 ppm		1
Mercury	Not detected	0.01 ppm		3
Aerobic plate count	Not more than 300/g	—		4
Coliform bacteria	Negative/2.22g	—		5
Residue on ignition	0.5 %	—	1	

QL: Quantitation limit N: Notes M: Method

Notes

1:"Residue on Ignition Test (Sample 1 g, 600 ℃, Constant Weight)", General Tests, The Japanese Pharmacopoeia, Sixteenth Edition.

Method

1:Atomic absorption spectrometry
2:Sodium sulfide colorimetric method
3:Cold vapor atomic absorption spectrometry
4:Standard Agar plating method
5:BGLB broth inoculating method

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Noriko Imaizumi
Principal Investigator

Jun. 11, 2013
Date

Japan Food Research Laboratories
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52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 13064337004-02 1/1
July 22, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda, Saitama, 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. CK-004

Received date: July 10, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Cadmium	Not detected	0.01 ppm		1
Viable molds count	Negative/0.1g	----		2
Viable yeasts count	Negative/0.1g	----		2
Coagulase positive staphylococci	Negative/0.01g	----		3
Salmonella	Negative/25g	----		4

QL: Quantitation limit N: Notes M: Method

Method

1:Atomic absorption spectrometry
2:Potato Dextrose (10 %) Agar plating method
3:Surface spread plating method
4:Enrichment culture method

(b) (6)

Noriko Imaizumi
Principal Investigator

Jul. 22, 2013
Date

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

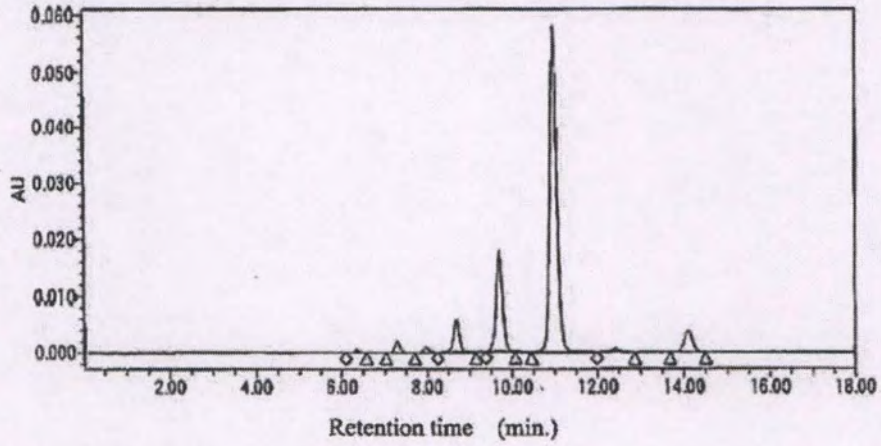
Lot No. : CK-004

Date of Manufacturing : December 8, 2012

Date of Analysis : May 23, 2013

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	3.2
Echinenone	15.5
3-Hydroxyechinenone	8.6
Canthaxanthin	46.9
Adonirubin	171.8
Astaxanthin	**593.2**
Asteroidenone	9.5
Adonixanthin	46.6
Others	1.0
Total carotenoids	896.3



JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 9, 2013

Kazuaki Hirasawa

Senior Chemist

Advanced Materials R&D Group

Central Technical Research Laboratory

Date: September 9, 2013

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

Lot No. : CK-005

Date of Manufacturing : December 11, 2012

Test item	Results	Method
Appearance	Black-purple powder crystal	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	0.10 %	JP *2.41 (0.5g, 25°C, in vacuum, silica gel, 4hours)
Ethanol	0.172 %	JP* 2.46 (Gas chromatography)
Loose Bulk Density	0.25 g/ml	JP *3.01 (20ml cylinder)

* JP: The Japanese Pharmacopoeia 16th Edition

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 5, 2013

Kazuaki Hirasawa

Senior Chemist

Advanced Materials R&D Group

Central Technical Research Laboratory

Date: September 13, 2013

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 13049097006-02 1/1
June 11, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda Saitama 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. CK-005

Received date: May 31, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Arsenic (as As_2O_3)	Not detected	0.1 ppm		1
Heavy metals (as Pb)	Not detected	5 ppm		2
Lead	Not detected	0.05 ppm		1
Mercury	Not detected	0.01 ppm		3
Aerobic plate count	Not more than 300/g	—		4
Coliform bacteria	Negative/2.22g	—		5
Residue on ignition	0.5 %	—	1	

QL: Quantitation limit N: Notes M: Method

Notes

1:"Residue on Ignition Test (Sample 1 g, 600 ℃, Constant Weight)", General Tests, The Japanese Pharmacopoeia, Sixteenth Edition.

Method

1:Atomic absorption spectrometry
2:Sodium sulfide colorimetric method
3:Cold vapor atomic absorption spectrometry
4:Standard Agar plating method
5:BGLB broth inoculating method

(b) (6)

JAPAN FOOD RESEARCH LABORATORIES

Noriko Imaizumi
Principal Investigator

Jun. 11, 2013
Date

Japan Food Research Laboratories
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52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 13064337005-02 1/1
July 22, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda, Saitama, 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. CK-005

Received date: July 10, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Cadmium	Not detected	0.01 ppm		1
Viable molds count	Negative/0.1g	---		2
Viable yeasts count	Negative/0.1g	---		2
Coagulase positive staphylococci	Negative/0.01g	---		3
Salmonella	Negative/25g	---		4

QL: Quantitation limit N: Notes M: Method

Method

1:Atomic absorption spectrometry
2:Potato Dextrose (10 %) Agar plating method
3:Surface spread plating method
4:Enrichment culture method

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Noriko Imaizumi
Principal Investigator

Jul. 22, 2013
Date

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

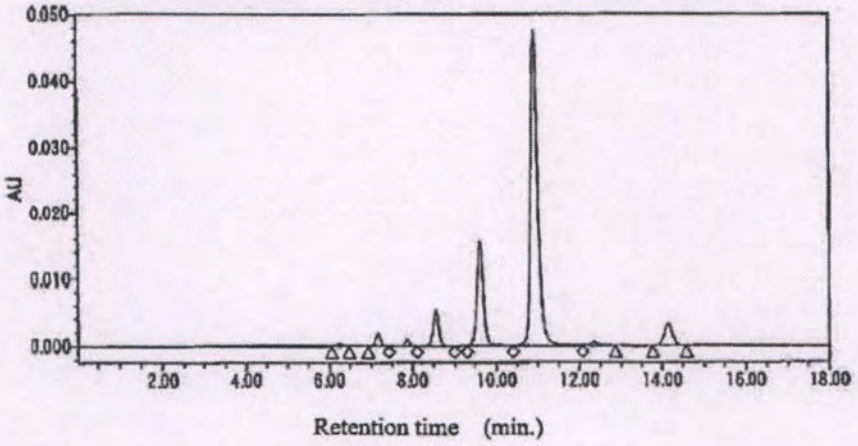
Lot No. : CK-005

Date of Manufacturing : December 11, 2012

Date of Analysis : February 18, 2013

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	3.3
Echinenone	15.7
3-Hydroxyechinenone	8.9
Canthaxanthin	47.2
Adonirubin	174.1
Astaxanthin	**596.2**
Asteroidenone	8.8
Adonixanthin	46.7
Others	1.6
Total carotenoids	902.3



JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 9, 2013

Kazuaki Hirasawa

Senior Chemist

Advanced Materials R&D Group

Central Technical Research Laboratory

Date: September 9, 2013

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

Lot No. : CK-006

Date of Manufacturing : December 14, 2012

Test item	Results	Method
Appearance	Black-purple powder crystal	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	0.08 %	JP *2.41 (0.5g, 25°C, in vacuum, silica gel, 4hours)
Ethanol	0.076 %	JP* 2.46 (Gas chromatography)
Loose Bulk Density	0.24 g/ml	JP *3.01 (20ml cylinder)

* JP: The Japanese Pharmacopoeia 16th Edition

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 13. 2013

Kazuaki Hirasawa
Senior Chemist
Advanced Materials R&D Group
Central Technical Research Laboratory

Date: September 13, 2013

Japan Food Research Laboratories
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52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 13049097007-02 1/1
June 11, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda Saitama 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. CK-006

Received date: May 31, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Arsenic (as As_2O_3)	Not detected	0.1 ppm		1
Heavy metals (as Pb)	Not detected	5 ppm		2
Lead	Not detected	0.05 ppm		1
Mercury	Not detected	0.01 ppm		3
Aerobic plate count	Not more than 300/g	---		4
Coliform bacteria	Negative/2.22g	---		5
Residue on ignition	0.6 %	---	1	

QL: Quantitation limit N: Notes M: Method

Notes

1: "Residue on Ignition Test (Sample 1 g, 600 °C, Constant Weight)", General Tests, The Japanese Pharmacopoeia, Sixteenth Edition.

Method

1: Atomic absorption spectrometry
2: Sodium sulfide colorimetric method
3: Cold vapor atomic absorption spectrometry
4: Standard Agar plating method
5: BGLB broth inoculating method

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Noriko Imaizumi
Principal Investigator

Jun. 11, 2013
Date

Japan Food Research Laboratories
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52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 13064337006-02 1/1
July 22, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda, Saitama, 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. CK-006

Received date: July 10, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Cadmium	Not detected	0.01 ppm		1
Viable molds count	Negative/0.1g	—		2
Viable yeasts count	Negative/0.1g	—		2
Coagulase positive staphylococci	Negative/0.01g	—		3
Salmonella	Negative/25g	—		4

QL: Quantitation limit N: Notes M: Method

Method

1:Atomic absorption spectrometry
2:Potato Dextrose (10 %) Agar plating method
3:Surface spread plating method
4:Enrichment culture method

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Noriko Imaizumi
Principal Investigator

Jul. 22, 2013
Date

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

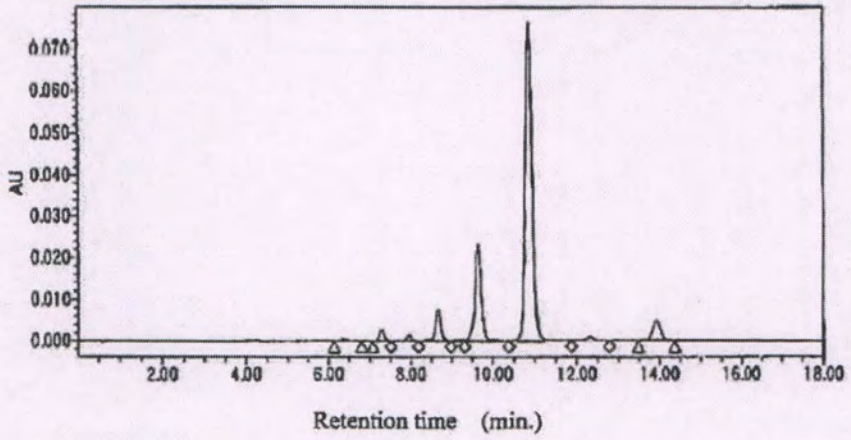
Lot No. : CK-006

Date of Manufacturing : December 14, 2012

Date of Analysis : May 28, 2013

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	3.8
Echinenone	16.3
3-Hydroxyechinenone	10.1
Canthaxanthin	49.0
Adonirubin	177.2
Astaxanthin	**600.2**
Asteroidenone	11.5
Adonixanthin	47.8
Others	2.5
Total carotenoids	918.3



JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 9, 2013

Kazuaki Hirasawa
Senior Chemist
Advanced Materials R&D Group
Central Technical Research Laboratory

Date: September 9, 2013

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

Lot No. : CK-007

Date of Manufacturing : December 25, 2012

Test item	Results	Method
Appearance	Black-purple powder crystal	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	0.23 %	JP *2.41 (0.5g, 25°C, in vacuum, silica gel, 4hours)
Ethanol	0.090 %	JP* 2.46 (Gas chromatography)
Loose Bulk Density	0.26 g/ml	JP *3.01 (20ml cylinder)

* JP: The Japanese Pharmacopoeia 16th Edition

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Sep. 13. 2013

Kazuaki Hirasawa
Senior Chemist
Advanced Materials R&D Group
Central Technical Research Laboratory

Date: September 13, 2013

Japan Food Research Laboratories
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No. 13049097001-02 1/1
June 11, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda Saitama 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot.CK-007

Received date: May 31, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Arsenic (as As_2O_3)	Not detected	0.1 ppm		1
Heavy metals (as Pb)	Not detected	5 ppm		2
Lead	Not detected	0.05 ppm		1
Mercury	Not detected	0.01 ppm		3
Aerobic plate count	Not more than 300/g	----		4
Coliform bacteria	Negative/2.22g	----		5
Residue on ignition	0.5 %	----	1	

QL: Quantitation limit N: Notes M: Method

Notes

1:"Residue on Ignition Test (Sample 1 g, 600 ℃, Constant Weight)", General Tests, The Japanese Pharmacopoeia, Sixteenth Edition.

Method

1:Atomic absorption spectrometry
2:Sodium sulfide colorimetric method
3:Cold vapor atomic absorption spectrometry
4:Standard Agar plating method
5:BGLB broth inoculating method

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Noriko Imaizumi
Principal Investigator

Jun. 11, 2013
Date

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No. 13064337007-02 1/1
July 23, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda, Saitama, 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. CK-007

Received date: July 10, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Cadmium	Not detected	0.01 ppm		1
Viable molds count	Negative/0.1g	----		2
Viable yeasts count	Negative/0.1g	----		2
Coagulase positive staphylococci	Negative/0.01g	----		3
Salmonella	Negative/25g	----		4

QL: Quantitation limit N: Notes M: Method

Method

1:Atomic absorption spectrometry
2:Potato Dextrose (10 %) Agar plating method
3:Surface spread plating method
4:Enrichment culture method

(b) (6)

Noriko Imaizumi
Principal Investigator

Jul. 23, 2013
Date

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

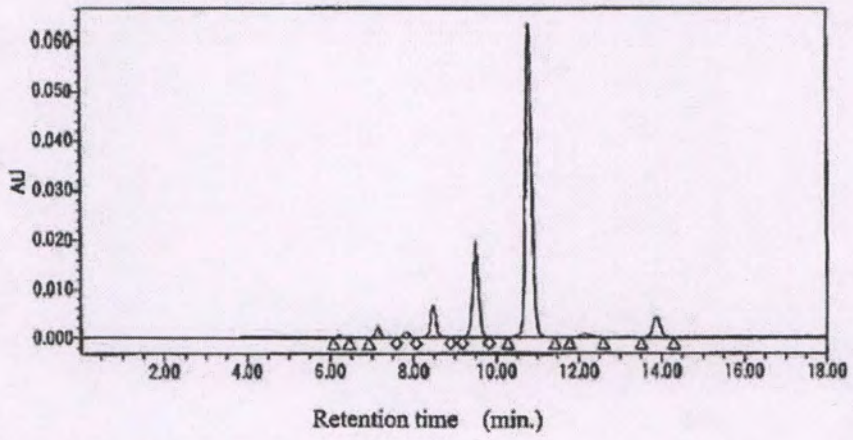
Lot No. : CK-007

Date of Manufacturing : December 25, 2012

Date of Analysis : December 27, 2012

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	3.3
Echinenone	15.8
3-Hydroxyechinenone	8.9
Canthaxanthin	48.1
Adonirubin	175.4
Astaxanthin	**596.0**
Asteroidenone	8.6
Adonixanthin	47.2
Others	1.6
Total carotenoids	904.8



JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Kazuaki Hirasawa

Senior Chemist

Advanced Materials R&D Group

Central Technical Research Laboratory

Sep. 9, 2013

Date: September 9, 2013

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

Lot No. : DK-001

Date of Manufacturing : November 18, 2013

Test item	Results	Method
Appearance	Black-purple powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	0.17 %	JP *2.41 (0.5g, 25°C, in vacuum, silica gel, 4hours)
Ethanol	0.076 %	JP* 2.46 (Gas chromatography)
Loose Bulk Density	0.23 g/ml	JP *3.01 (20ml cylinder)

* JP: The Japanese Pharmacopoeia 16th Edition

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Dec. 18, 2013

Kazuaki Hirasawa
Senior Chemist
Advanced Materials R&D Group
Central Technical Research Laboratory

Date: December 18, 2013

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

December 12, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda, Saitama, 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. DK-001

Received date: November 28, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Amino acids	----	----		
Arginine	23 mg/100g	----		1
Lysine	13 mg/100g	----		1
Histidine	6 mg/100g	----		1
Phenylalanine	27 mg/100g	----		1
Tyrosine	26 mg/100g	----		1
Leucine	40 mg/100g	----		1
Isoleucine	20 mg/100g	----		1
Methionine	22 mg/100g	----	1	1
Valine	60 mg/100g	----		1
Alanine	99 mg/100g	----		1
Glycine	44 mg/100g	----		1
Proline	66 mg/100g	----		1
Glutamic acid	92 mg/100g	----		1
Serine	22 mg/100g	----		1
Threonine	41 mg/100g	----		1
Aspartic acid	61 mg/100g	----		1
Tryptophan	3 mg/100g	----		2
Cystine	13 mg/100g	----	1	1
Arsenic (as As_2O_3)	Not detected	0.1 ppm		3
Heavy metals (as Pb)	Not detected	5 ppm		4
Lead	Not detected	0.05 ppm		3
Cadmium	Not detected	0.01 ppm		3
Mercury	Not detected	0.01 ppm		5
Sulfur	0.07 g/100g	----		6
Aerobic plate count	Not more than 300/g	----		7
Coliform bacteria	Negative/2.22g	----		8
Viable molds count	Negative/0.1g	----		9
Viable yeasts count	Negative/0.1g	----		9
Coagulase positive staphylococci	Negative/0.01g	----		10
Salmonella	Negative/25g	----		11

QL: Quantitation limit N: Notes M: Method

Notes

1:Before measurement, performic acid oxidation and hydrochloric acid hydrolysis were performed.

Japan Food Research Laboratories
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December 12, 2013

CERTIFICATE OF ANALYSIS

Method

1:Amino acid analyzer method
2:HPLC
3:Atomic absorption spectrometry
4:Sodium sulfide colorimetric method
5:Cold vapor atomic absorption spectrometry
6:Barium sulfate gravimetric method
7:Standard Agar plating method
8:BGLB broth inoculating method
9:Potato Dextrose (10 %) Agar plating method
10:Surface spread plating method
11:Enrichment culture method

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Takeko Arai
Principal Investigator

Dec. 12, 2013
Date

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

No. 13114242001-08 1/1
December 12, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda, Saitama, 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. DK-001

Received date: November 28, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Residue on ignition	0.3 %	----	1	

QL: Quantitation limit N: Notes M: Method

Notes

1:"Residue on Ignition Test (Sample 1 g, 600 °C, Constant Weight)", General Tests, The Japanese Pharmacopoeia, Sixteenth Edition.

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Takeko Arai
Principal Investigator

Dec. 12, 2013
Date

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

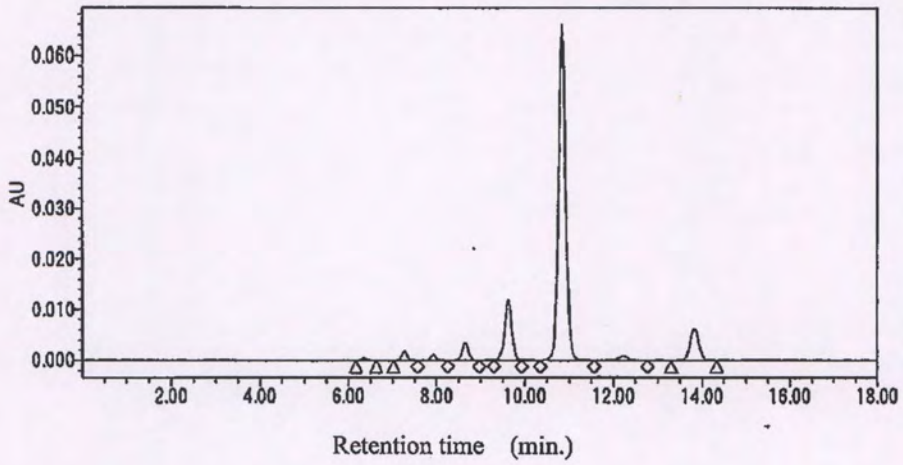
Lot No. : DK-001

Date of Manufacturing : November 18, 2013

Date of Analysis : November 27, 2013

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	3.6
Echinenone	13.0
3-Hydroxyechinenone	8.5
Canthaxanthin	28.6
Adonirubin	116.5
Astaxanthin	**664.9**
Asteroidenone	11.8
Adonixanthin	76.1
Others	3.9
Total carotenoids	926.8



JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Dec. 18, 2013

Kazuaki Hirasawa
Senior Chemist
Advanced Materials R&D Group
Central Technical Research Laboratory

Date: December 18, 2013

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

Lot No. : EK-001

Date of Manufacturing : November 23, 2013

Test item	Results	Method
Appearance	Black-purple powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	0.08 %	JP *2.41 (0.5g, 25°C, in vacuum, silica gel, 4hours)
Ethanol	0.031 %	JP* 2.46 (Gas chromatography)
Loose Bulk Density	0.26 g/ml	JP *3.01 (20ml cylinder)

* JP: The Japanese Pharmacopoeia 16th Edition

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Dec. 18, 2013

Kazuaki Hirasawa
Senior Chemist
Advanced Materials R&D Group
Central Technical Research Laboratory

Date: December 18, 2013

Japan Food Research Laboratories
Authorized by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan http://www.jfrl.or.jp/

December 12, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda, Saitama, 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. EK-001

Received date: November 28, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Amino acids	----	----		
Arginine	76 mg/100g	----		1
Lysine	43 mg/100g	----		1
Histidine	14 mg/100g	----		1
Phenylalanine	60 mg/100g	----		1
Tyrosine	115 mg/100g	----		1
Leucine	39 mg/100g	----		1
Isoleucine	21 mg/100g	----		1
Methionine	55 mg/100g	----	1	1
Valine	216 mg/100g	----		1
Alanine	185 mg/100g	----		1
Glycine	77 mg/100g	----		1
Proline	261 mg/100g	----		1
Glutamic acid	259 mg/100g	----		1
Serine	50 mg/100g	----		1
Threonine	141 mg/100g	----		1
Aspartic acid	181 mg/100g	----		1
Tryptophan	3 mg/100g	----		2
Cystine	15 mg/100g	----	1	1
Arsenic (as As_2O_3)	Not detected	0.1 ppm		3
Heavy metals (as Pb)	Not detected	5 ppm		4
Lead	Not detected	0.05 ppm		3
Cadmium	Not detected	0.01 ppm		3
Mercury	Not detected	0.01 ppm		5
Sulfur	0.09 g/100g	----		6
Aerobic plate count	Not more than 300/g	----		7
Coliform bacteria	Negative/2.22g	----		8
Viable molds count	Negative/0.1g	----		9
Viable yeasts count	Negative/0.1g	----		9
Coagulase positive staphylococci	Negative/0.01g	----		10
Salmonella	Negative/25g	----		11

QL: Quantitation limit N: Notes M: Method

Notes

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1:Before measurement, performic acid oxidation and hydrochloric acid hydrolysis were performed.

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December 12, 2013

CERTIFICATE OF ANALYSIS

Method
1:Amino acid analyzer method
2:HPLC
3:Atomic absorption spectrometry
4:Sodium sulfide colorimetric method
5:Cold vapor atomic absorption spectrometry
6:Barium sulfate gravimetric method
7:Standard Agar plating method
8:BGLB broth inoculating method
9:Potato Dextrose (10 %) Agar plating method
10:Surface spread plating method
11:Enrichment culture method

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Takeko Arai
Principal Investigator

Dec. 12, 2013
Date

Japan Food Research Laboratories
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No. 13114242002-08 1/1
December 12, 2013

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
17-35, Niizo Minami 3-chome, Toda, Saitama, 335-8502 JAPAN

Sample name: Astaxanthin-based Carotenoid Cocktail Lot. EK-001

Received date: November 28, 2013

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Residue on ignition	0.3 %	----	1	

QL: Quantitation limit N: Notes M: Method

Notes

1: "Residue on Ignition Test (Sample 1 g, 600 ℃, Constant Weight)", General Tests, The Japanese Pharmacopoeia, Sixteenth Edition.

JAPAN FOOD RESEARCH LABORATORIES

(b) (6)

Takeko Arai
Principal Investigator

Dec. 12, 2013
Date

CERTIFICATE OF ANALYSIS

Product : Astaxanthin-based Carotenoid Cocktail

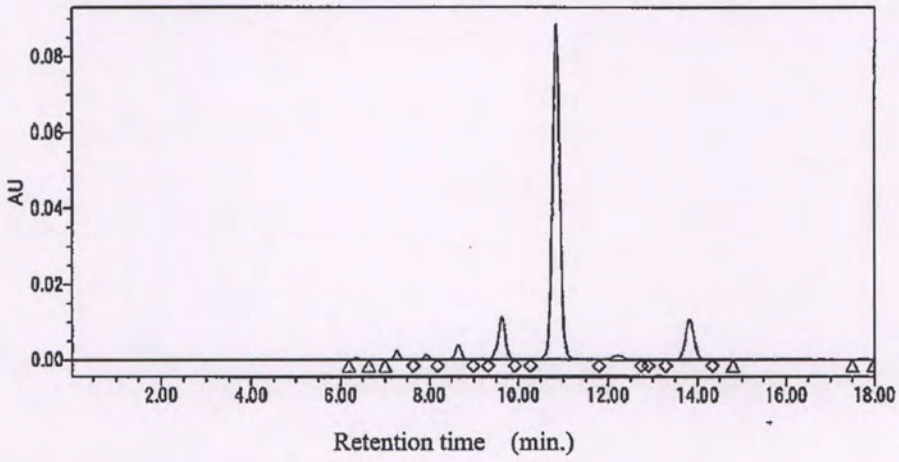
Lot No. : EK-001

Date of Manufacturing : November 23, 2013

Date of Analysis : November 27, 2013

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	3.5
Echinenone	12.9
3-Hydroxyechinenone	8.7
Canthaxanthin	23.6
Adonirubin	85.7
Astaxanthin	**661.6**
Asteroidenone	13.5
Adonixanthin	97.7
Others	6.5
Total carotenoids	**913.7**



JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

Dec. 18, 2013

Kazuaki Hirasawa

Senior Chemist

Advanced Materials R&D Group

Central Technical Research Laboratory

Date: December 18, 2013

Appendix 2

Certificates of Analysis for ARE-1P

CERTIFICATE OF ANALYSIS

Product : ARE-1P

Lot No. : SA001

Date of Manufacturing : July 28, 2015

Date of Analysis : December 15, 2015

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	0.1
Echinenone	0.2
3-Hydroxyechinenone	0.2
Canthaxanthin	0.4
Adonirubin	1.6
Asteroidenone	< 0.1
Astaxanthin	**12.3**
Adonixanthin	1.4
Others	< 0.1
Total carotenoids	16.2

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 8, 2016

Kazuaki Hirasawa
Senior Chemist
Biotechnology Development Group
Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 8, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-1P

Lot No. : SA009

Date of Manufacturing : October 15, 2015

Date of Analysis : December 17, 2015

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	0.1
Echinenone	0.2
3-Hydroxyechinenone	0.1
Canthaxanthin	0.4
Adonirubin	1.7
Asteroidenone	< 0.1
Astaxanthin	**11.9**
Adonixanthin	0.8
Others	< 0.1
Total carotenoids	15.2

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 8, 2016

Kazuaki Hirasawa
Senior Chemist
Biotechnology Development Group
Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 8, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-1P

Lot No. : SA007

Date of Manufacturing : October 14, 2015

Date of Analysis : December 17, 2015

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	0.1
Echinenone	0.2
3-Hydroxyechinenone	0.1
Canthaxanthin	0.4
Adonirubin	1.8
Asteroidenone	< 0.1
Astaxanthin	**12.1**
Adonixanthin	0.8
Others	< 0.1
Total carotenoids	15.5

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 8, 2016

Kazuaki Hirasawa
Senior Chemist
Biotechnology Development Group
Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 8, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-1P

Lot No. : SA005

Date of Manufacturing : October 13, 2015

Date of Analysis : December 15, 2015

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	0.1
Echinenone	0.2
3-Hydroxyechinenone	0.1
Canthaxanthin	0.4
Adonirubin	1.8
Asteroidenone	< 0.1
Astaxanthin	**11.8**
Adonixanthin	0.9
Others	< 0.1
Total carotenoids	15.3

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 8, 2016

Kazuaki Hirasawa
Senior Chemist
Biotechnology Development Group
Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 8, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-1P

Lot No. : SA003

Date of Manufacturing : October 9, 2015

Date of Analysis : December 15, 2015

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	0.1
Echinenone	0.2
3-Hydroxyechinenone	0.1
Canthaxanthin	0.4
Adonirubin	1.6
Asteroidenone	< 0.1
Astaxanthin	**11.9**
Adonixanthin	0.9
Others	< 0.1
Total carotenoids	15.2

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 8, 2016

Kazuaki Hirasawa
Senior Chemist
Biotechnology Development Group
Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 8, 2016

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http://www.jfrl.or.jp/

Date issued: October 30, 2015

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
8, Chidori-cho, Naka-ku, Yokohama-shi, Kanagawa 231-0815 JAPAN

Sample name: ARE-1P-SA001

Received date: October 16, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Moisture	2.15 g/100g	------		1
Protein	0.9 g/100g	------	1	2
Fat	31.7 g/100g	------		3
Ash	1.4 g/100g	------		4
Carbohydrate	63.9 g/100g	------	2	
Energy	545 kcal/100g	------	3	
Sodium	29.9 mg/100g	------		5
Salt (sodium as sodium chloride)	0.0759 g/100g	------	4	
Arsenic (as As_2O_3)	Not detected	0.1 ppm		5
Heavy metals (as Pb)	Not detected	5 ppm		6
Lead	0.55 ppm	------		5
Cadmium	Not detected	0.01 ppm		5
Mercury	Not detected	0.01 ppm		7
Aerobic plate count	Not more than 300/g	------		8
Coliform bacteria	Negative/2.22g	------		9
Viable molds count	40/g	------		10
Viable yeasts count	Negative/0.1g	------		10
Coagulase positive staphylococci	Negative/0.01g	------		11
Salmonella	Negative/25g	------		12

QL: Quantitation limit N: Notes M: Method

Notes

1:Nitrogen-to-protein conversion factor: 6.25.

2:The formula for carbohydrate, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, is: 100 - (Moisture + Protein + Fat + Ash).

3:The energy conversion factors, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, are: Protein, 4; Fat, 9; Carbohydrate, 4.

4:Salt (sodium as sodium chloride) = Sodium × 2.54.

Method

1:Karl Fischer method
2:Kjeldahl method
3:Acid hydrolysis method
4:Ashing method
5:Atomic absorption spectrometry
6:Sodium sulfide colorimetric method
7:Cold vapor atomic absorption spectrometry
8:Standard Agar plating method
9:BGLB broth inoculating method
10:Potato Dextrose (10 %) Agar plating method
11:Surface spread plating method
12:Enrichment culture method

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Date issued: October 30, 2015

CERTIFICATE OF ANALYSIS

JAPAN FOOD RESEARCH LABORATORIES

Signed for and on behalf of JFRL

(b) (6)

Michiyo Horiuchi
Section of Analysis Documentation

Oct. 30, 2015
Date

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52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan
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Date issued: November 06, 2015

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
8, Chidori-cho, Naka-ku, Yokohama-shi, Kanagawa 231-0815 JAPAN

Sample name: ARE-1P-SA003

Received date: October 27, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Moisture	2.46 g/100g	.....		1
Protein	0.9 g/100g	.....	1	2
Fat	30.2 g/100g	.....		3
Ash	1.3 g/100g	.....		4
Carbohydrate	65.1 g/100g	.....	2	
Energy	536 kcal/100g	.....	3	
Sodium	5.8 mg/100g	.....		5
Salt (sodium as sodium chloride)	0.015 g/100g	.....	4	
Arsenic (as As_2O_3)	Not detected	0.1 ppm		5
Heavy metals (as Pb)	Not detected	5 ppm		6
Lead	Not detected	0.05 ppm		5
Cadmium	Not detected	0.01 ppm		5
Mercury	Not detected	0.01 ppm		7
Aerobic plate count	Not more than 300/g	.....		8
Coliform bacteria	Negative/2.22g	.....		9
Viable molds count	Negative/0.1g	.....		10
Viable yeasts count	Negative/0.1g	.....		10
Coagulase positive staphylococci	Negative/0.01g	.....		11
Salmonella	Negative/25g	.....		12

QL: Quantitation limit N: Notes M: Method

Notes

1:Nitrogen-to-protein conversion factor: 6.25.

2:The formula for carbohydrate, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, is: 100 - (Moisture + Protein + Fat + Ash).

3:The energy conversion factors, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, are: Protein, 4; Fat, 9; Carbohydrate, 4.

4:Salt (sodium as sodium chloride) = Sodium × 2.54.

Method

1:Karl Fischer method
2:Kjeldahl method
3:Acid hydrolysis method
4:Ashing method
5:Atomic absorption spectrometry
6:Sodium sulfide colorimetric method
7:Cold vapor atomic absorption spectrometry
8:Standard Agar plating method
9:BGLB broth inoculating method
10:Potato Dextrose (10 %) Agar plating method
11:Surface spread plating method
12:Enrichment culture method

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Date issued: November 06, 2015

CERTIFICATE OF ANALYSIS

JAPAN FOOD RESEARCH LABORATORIES

Signed for and on behalf of JFRL
(b) (6)

Takeko Arai
Section of Analysis Documentation

Nov. 06, 2015
Date

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Date issued: November 10, 2015

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
8, Chidori-cho, Naka-ku, Yokohama-shi, Kanagawa 231-0815 JAPAN

Sample name: ARE-1P-SA005

Received date: October 27, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Moisture	2.16 g/100g	-----		1
Protein	0.8 g/100g	-----	1	2
Fat	29.9 g/100g	-----		3
Ash	1.2 g/100g	-----		4
Carbohydrate	65.9 g/100g	-----	2	
Energy	536 kcal/100g	-----	3	
Sodium	5.5 mg/100g	-----		5
Salt (sodium as sodium chloride)	0.014 g/100g	-----	4	
Arsenic (as As_2O_3)	Not detected	0.1 ppm		5
Heavy metals (as Pb)	Not detected	5 ppm		6
Lead	0.08 ppm	-----		5
Cadmium	Not detected	0.01 ppm		5
Mercury	Not detected	0.01 ppm		7
Aerobic plate count	Not more than 300/g	-----		8
Coliform bacteria	Negative/2.22g	-----		9
Viable molds count	Negative/0.1g	-----		10
Viable yeasts count	Negative/0.1g	-----		10
Coagulase positive staphylococci	Negative/0.01g	-----		11
Salmonella	Negative/25g	-----		12

QL: Quantitation limit N: Notes M: Method

Notes

1:Nitrogen-to-protein conversion factor: 6.25.

2:The formula for carbohydrate, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, is: 100 - (Moisture + Protein + Fat + Ash).

3:The energy conversion factors, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, are: Protein, 4; Fat, 9; Carbohydrate, 4.

4:Salt (sodium as sodium chloride) = Sodium × 2.54.

Method

1:Karl Fischer method
2:Kjeldahl method
3:Acid hydrolysis method
4:Ashing method
5:Atomic absorption spectrometry
6:Sodium sulfide colorimetric method
7:Cold vapor atomic absorption spectrometry
8:Standard Agar plating method
9:BGLB broth inoculating method
10:Potato Dextrose (10 %) Agar plating method
11:Surface spread plating method
12:Enrichment culture method

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Date issued: November 10, 2015

CERTIFICATE OF ANALYSIS

Signed for and on behalf of JFRL

(b) (6)

Michiyo Horiuchi
Section of Analysis Documentation

Nov. 10, 2015
Date

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http://www.jfrl.or.jp/

Date issued: November 06, 2015

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
8, Chidori-cho, Naka-ku, Yokohama-shi, Kanagawa 231-0815 JAPAN

Sample name: ARE-1P-SA007

Received date: October 27, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Moisture	2.27 g/100g	-----		1
Protein	0.8 g/100g	-----	1	2
Fat	30.2 g/100g	-----		3
Ash	1.3 g/100g	-----		4
Carbohydrate	65.4 g/100g	-----	2	
Energy	537 kcal/100g	-----	3	
Sodium	5.4 mg/100g	-----		5
Salt (sodium as sodium chloride)	0.014 g/100g	-----	4	
Arsenic (as As_2O_3)	Not detected	0.1 ppm		5
Heavy metals (as Pb)	Not detected	5 ppm		6
Lead	0.07 ppm	-----		5
Cadmium	Not detected	0.01 ppm		5
Mercury	Not detected	0.01 ppm		7
Aerobic plate count	Not more than 300/g	-----		8
Coliform bacteria	Negative/2.22g	-----		9
Viable molds count	Negative/0.1g	-----		10
Viable yeasts count	Negative/0.1g	-----		10
Coagulase positive staphylococci	Negative/0.01g	-----		11
Salmonella	Negative/25g	-----		12

QL: Quantitation limit N: Notes M: Method

Notes

1:Nitrogen-to-protein conversion factor: 6.25.

2:The formula for carbohydrate, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, is: 100 - (Moisture + Protein + Fat + Ash).

3:The energy conversion factors, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, are: Protein, 4; Fat, 9; Carbohydrate, 4.

4:Salt (sodium as sodium chloride) = Sodium × 2.54.

Method

1:Karl Fischer method
2:Kjeldahl method
3:Acid hydrolysis method
4:Ashing method
5:Atomic absorption spectrometry
6:Sodium sulfide colorimetric method
7:Cold vapor atomic absorption spectrometry
8:Standard Agar plating method
9:BGLB broth inoculating method
10:Potato Dextrose (10 %) Agar plating method
11:Surface spread plating method
12:Enrichment culture method

JFRL

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Date issued: November 06, 2015

CERTIFICATE OF ANALYSIS

JAPAN FOOD RESEARCH LABORATORIES

Signed for and on behalf of JFRL
(b) (6)

Takeko Arai
Section of Analysis Documentation

Nov. 06, 2015
Date

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Date issued: November 10, 2015

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
8, Chidori-cho, Naka-ku, Yokohama-shi, Kanagawa 231-0815 JAPAN

Sample name: ARE-1P-SA009

Received date: October 27, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Moisture	1.98 g/100g	------		1
Protein	0.9 g/100g	------	1	2
Fat	30.6 g/100g	------		3
Ash	1.2 g/100g	------		4
Carbohydrate	65.3 g/100g	------	2	
Energy	540 kcal/100g	------	3	
Sodium	6.2 mg/100g	------		5
Salt (sodium as sodium chloride)	0.016 g/100g	------	4	
Arsenic (as As_2O_3)	Not detected	0.1 ppm		5
Heavy metals (as Pb)	Not detected	5 ppm		6
Lead	0.06 ppm	------		5
Cadmium	Not detected	0.01 ppm		5
Mercury	Not detected	0.01 ppm		7
Aerobic plate count	Not more than 300/g	------		8
Coliform bacteria	Negative/2.22g	------		9
Viable molds count	Negative/0.1g	------		10
Viable yeasts count	Negative/0.1g	------		10
Coagulase positive staphylococci	Negative/0.01g	------		11
Salmonella	Negative/25g	------		12

QL: Quantitation limit N: Notes M: Method

Notes

1:Nitrogen-to-protein conversion factor: 6.25.

2:The formula for carbohydrate, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, is: 100 - (Moisture + Protein + Fat + Ash).

3:The energy conversion factors, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, are: Protein, 4; Fat, 9; Carbohydrate, 4.

4:Salt (sodium as sodium chloride) = Sodium × 2.54.

Method

1:Karl Fischer method
2:Kjeldahl method
3:Acid hydrolysis method
4:Ashing method
5:Atomic absorption spectrometry
6:Sodium sulfide colorimetric method
7:Cold vapor atomic absorption spectrometry
8:Standard Agar plating method
9:BGLB broth inoculating method
10:Potato Dextrose (10 %) Agar plating method
11:Surface spread plating method
12:Enrichment culture method

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Date issued: November 10, 2015

CERTIFICATE OF ANALYSIS

JAPAN FOOD RESEARCH LABORATORIES

Signed for and on behalf of JFRL

(b) (6)

Michiyo Horiuchi
Section of Analysis Documentation

Nov. 10, 2015
Date

CERTIFICATE OF ANALYSIS

Product : ARE-1P

Lot No. : SA001

Date of Manufacturing : July 28, 2015

Test item	Results	Method
Appearance	Deep to vivid yellowish red powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	2.9 %	JSSFA * (105°C, 5 hours)
Loose Bulk Density	0.31 g/ml	JP **3.01 (20ml cylinder)

* JSSFA: Japan's Specifications and Standards for Food Additives

* JP: The Japanese Pharmacopoeia

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 9, 2016

Kazuaki Hirasawa
Senior Chemist
Biotechnology Development Group
Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 9, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-1P

Lot No. : SA009

Date of Manufacturing : October 15, 2015

Test item	Results	Method
Appearance	Deep to vivid yellowish red powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	3.2 %	JSSFA * (105°C, 5 hours)
Loose Bulk Density	0.33 g/ml	JP **3.01 (20ml cylinder)

* JSSFA: Japan's Specifications and Standards for Food Additives

* JP: The Japanese Pharmacopoeia

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 9. 2016

Kazuaki Hirasawa
Senior Chemist
Biotechnology Development Group
Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 9, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-1P

Lot No. : SA007

Date of Manufacturing : October 14, 2015

Test item	Results	Method
Appearance	Deep to vivid yellowish red powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	3.1 %	JSSFA * (105°C, 5 hours)
Loose Bulk Density	0.32 g/ml	JP **3.01 (20ml cylinder)

* JSSFA: Japan's Specifications and Standards for Food Additives

* JP: The Japanese Pharmacopoeia

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 9, 2016

Kazuaki Hirasawa
Senior Chemist
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Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 9, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-1P

Lot No. : SA005

Date of Manufacturing : October 13, 2015

Test item	Results	Method
Appearance	Deep to vivid yellowish red powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	2.8 %	JSSFA * (105°C, 5 hours)
Loose Bulk Density	0.33 g/ml	JP **3.01 (20ml cylinder)

* JSSFA: Japan's Specifications and Standards for Food Additives

* JP: The Japanese Pharmacopoeia

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 9, 2016

Kazuaki Hirasawa
Senior Chemist
Biotechnology Development Group
Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 9, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-1P

Lot No. : SA003

Date of Manufacturing : October 9, 2015

Test item	Results	Method
Appearance	Deep to vivid yellowish red powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	3.3 %	JSSFA * (105°C, 5 hours)
Loose Bulk Density	0.32 g/ml	JP **3.01 (20ml cylinder)

* JSSFA: Japan's Specifications and Standards for Food Additives

* JP: The Japanese Pharmacopoeia

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 9, 2016

Kazuaki Hirasawa
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Specialty Chemicals & Materials Company

Date: March 9, 2016

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No. 16013214001-0201 1/1
Date issued: February 23, 2016

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
1-2 Otemachi 1-chome, Chiyodaku, Tokyo 100-8162, Japan

Sample name: ARE-1P-SA001

Received date: February 09, 2016

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethanol	Not detected	50 ppm	1	1

QL: Quantitation limit N: Notes M: Method

Notes
1:The quantitation limit was set at 50 ppm due to the small size of the sample.

Method
1:Gas chromatography

JAPAN FOOD RESEARCH LABORATORIES

Signed for and on behalf of JFRL
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Takeko Arai
Section of Analysis Documentation

Feb. 23, 2016
Date

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No. 16013214005-0201 1/1
Date issued: February 23, 2016

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
1-2 Otemachi 1-chome, Chiyodaku, Tokyo 100-8162, Japan

Sample name: ARE-1P-SA009

Received date: February 09, 2016

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethanol	Not detected	50 ppm	1	1

QL: Quantitation limit N: Notes M: Method

Notes

1:The quantitation limit was set at 50 ppm due to the small size of the sample.

Method

1:Gas chromatography

JAPAN FOOD RESEARCH LABORATORIES

Signed for and on behalf of JFRL

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No. 16013214004-0201 1/1
Date issued: February 23, 2016

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
1-2 Otemachi 1-chome, Chiyodaku, Tokyo 100-8162, Japan

Sample name: ARE-1P-SA007

Received date: February 09, 2016

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethanol	Not detected	50 ppm	1	1

QL: Quantitation limit N: Notes M: Method

Notes

1:The quantitation limit was set at 50 ppm due to the small size of the sample.

Method

1:Gas chromatography

JAPAN FOOD RESEARCH LABORATORIES

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No. 16013214003-0201 1/1
Date issued: February 23, 2016

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
1-2 Otemachi 1-chome, Chiyodaku, Tokyo 100-8162, Japan

Sample name: ARE-1P-SA005

Received date: February 09, 2016

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethanol	Not detected	50 ppm	1	1

QL: Quantitation limit N: Notes M: Method

Notes
1:The quantitation limit was set at 50 ppm due to the small size of the sample.

Method
1:Gas chromatography

JAPAN FOOD RESEARCH LABORATORIES

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Feb. 23, 2016
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No. 16013214002-0201 1/1
Date issued: February 23, 2016

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
1-2 Otemachi 1-chome, Chiyodaku, Tokyo 100-8162, Japan

Sample name: ARE-1P-SA003

Received date: February 09, 2016

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethanol	Not detected	50 ppm	1	1

QL: Quantitation limit N: Notes M: Method

Notes

1:The quantitation limit was set at 50 ppm due to the small size of the sample.

Method

1:Gas chromatography

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Appendix 3

Certificates of Analysis for ARE-10P

CERTIFICATE OF ANALYSIS

Product : ARE-10P

Lot No. : SA002

Date of Manufacturing : July 27, 2015

Date of Analysis : December 14, 2015

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	1
Echinenone	2
3-Hydroxyechinenone	1
Canthaxanthin	4
Adonirubin	17
Asteroidenone	1
Astaxanthin	**128**
Adonixanthin	23
Others	< 1
Total carotenoids	178

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 8, 2016

Kazuaki Hirasawa
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Specialty Chemicals & Materials Company

Date: March 8, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-10P

Lot No. : SA010

Date of Manufacturing : October 8, 2015

Date of Analysis : December 14, 2015

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	1
Echinenone	2
3-Hydroxyechinenone	1
Canthaxanthin	5
Adonirubin	22
Asteroidenone	1
Astaxanthin	**127**
Adonixanthin	18
Others	< 1
Total carotenoids	178

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(b) (6)

March 8, 2016

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Specialty Chemicals & Materials Company

Date: March 8, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-10P

Lot No. : SA008

Date of Manufacturing : October 7, 2015

Date of Analysis : December 14, 2015

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	1
Echinenone	2
3-Hydroxyechinenone	1
Canthaxanthin	5
Adonirubin	22
Asteroidenone	1
Astaxanthin	**122**
Adonixanthin	17
Others	< 1
Total carotenoids	171

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 8, 2016

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Date: March 8, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-10P

Lot No. : SA006

Date of Manufacturing : October 6, 2015

Date of Analysis : December 11, 2015

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	1
Echinenone	2
3-Hydroxyechinenone	1
Canthaxanthin	5
Adonirubin	22
Asteroidenone	1
Astaxanthin	**122**
Adonixanthin	17
Others	< 1
Total carotenoids	172

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 8, 2016

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Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 8, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-10P

Lot No. : SA004

Date of Manufacturing : October 5, 2015

Date of Analysis : December 11, 2015

Method : HPLC

Carotenoids	Content (mg/g)
β-Carotene	1
Echinenone	2
3-Hydroxyechinenone	1
Canthaxanthin	5
Adonirubin	21
Asteroidenone	1
Astaxanthin	**118**
Adonixanthin	17
Others	< 1
Total carotenoids	167

JX NIPPON OIL & ENERGY CORPORATION

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Date issued: October 30, 2015

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
8, Chidori-cho, Naka-ku, Yokohama-shi, Kanagawa 231-0815 JAPAN

Sample name: ARE-10P-SA002

Received date: October 16, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Moisture	2.83 g/100g	------		1
Protein	0.6 g/100g	------	1	2
Fat	5.4 g/100g	------		3
Ash	0.4 g/100g	------		4
Carbohydrate	90.8 g/100g	------	2	
Energy	414 kcal/100g	------	3	
Sodium	101 mg/100g	------		5
Salt (sodium as sodium chloride)	0.257 g/100g	------	4	
Arsenic (as As_2O_3)	Not detected	0.1 ppm		5
Heavy metals (as Pb)	Not detected	5 ppm		6
Lead	Not detected	0.05 ppm		5
Cadmium	Not detected	0.01 ppm		5
Mercury	Not detected	0.01 ppm		7
Aerobic plate count	Not more than 300/g	------		8
Coliform bacteria	Negative/2.22g	------		9
Viable molds count	Negative/0.1g	------		10
Viable yeasts count	Negative/0.1g	------		10
Coagulase positive staphylococci	Negative/0.01g	------		11
Salmonella	Negative/25g	------		12

QL: Quantitation limit N: Notes M: Method

Notes

1:Nitrogen-to-protein conversion factor: 6.25.

2:The formula for carbohydrate, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, is: 100 - (Moisture + Protein + Fat + Ash).

3:The energy conversion factors, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, are: Protein, 4; Fat, 9; Carbohydrate, 4.

4:Salt (sodium as sodium chloride) = Sodium × 2.54.

Method

1:Karl Fischer method
2:Kjeldahl method
3:Acid hydrolysis method
4:Ashing method
5:Atomic absorption spectrometry
6:Sodium sulfide colorimetric method
7:Cold vapor atomic absorption spectrometry
8:Standard Agar plating method
9:BGLB broth inoculating method
10:Potato Dextrose (10 %) Agar plating method
11:Surface spread plating method
12:Enrichment culture method

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Date issued: October 30, 2015

CERTIFICATE OF ANALYSIS

JAPAN FOOD RESEARCH LABORATORIES

Signed for and on behalf of JFRL

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Michiyo Horiuchi
Section of Analysis Documentation

Oct. 30, 2015
Date

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Date issued: November 10, 2015

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
8, Chidori-cho, Naka-ku, Yokohama-shi, Kanagawa 231-0815 JAPAN

Sample name: ARE-10P-SA004

Received date: October 27, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Moisture	2.14 g/100g	------		1
Protein	0.4 g/100g	------	1	2
Fat	5.2 g/100g	------		3
Ash	0.3 g/100g	------		4
Carbohydrate	92.0 g/100g	------	2	
Energy	416 kcal/100g	------	3	
Sodium	68.4 mg/100g	------		5
Salt (sodium as sodium chloride)	0.174 g/100g	------	4	
Arsenic (as As_2O_3)	Not detected	0.1 ppm		5
Heavy metals (as Pb)	Not detected	5 ppm		6
Lead	Not detected	0.05 ppm		5
Cadmium	Not detected	0.01 ppm		5
Mercury	Not detected	0.01 ppm		7
Aerobic plate count	Not more than 300/g	------		8
Coliform bacteria	Negative/2.22g	------		9
Viable molds count	Negative/0.1g	------		10
Viable yeasts count	Negative/0.1g	------		10
Coagulase positive staphylococci	Negative/0.01g	------		11
Salmonella	Negative/25g	------		12

QL: Quantitation limit N: Notes M: Method

Notes

1:Nitrogen-to-protein conversion factor: 6.25.

2:The formula for carbohydrate, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, is: 100 - (Moisture + Protein + Fat + Ash).

3:The energy conversion factors, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, are: Protein, 4; Fat, 9; Carbohydrate, 4.

4:Salt (sodium as sodium chloride) = Sodium × 2.54.

Method

1:Karl Fischer method
2:Kjeldahl method
3:Acid hydrolysis method
4:Ashing method
5:Atomic absorption spectrometry
6:Sodium sulfide colorimetric method
7:Cold vapor atomic absorption spectrometry
8:Standard Agar plating method
9:BGLB broth inoculating method
10:Potato Dextrose (10 %) Agar plating method
11:Surface spread plating method
12:Enrichment culture method

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Date issued: November 10, 2015

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(b) (6)

Michiyo Horiuchi
Section of Analysis Documentation

Nov. 10, 2015
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CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
8, Chidori-cho, Naka-ku, Yokohama-shi, Kanagawa 231-0815 JAPAN

Sample name: ARE-10P-SA006

Received date: October 27, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Moisture	1.98 g/100g	-----		1
Protein	0.4 g/100g	-----	1	2
Fat	4.9 g/100g	-----		3
Ash	0.4 g/100g	-----		4
Carbohydrate	92.3 g/100g	-----	2	
Energy	415 kcal/100g	-----	3	
Sodium	67.7 mg/100g	-----		5
Salt (sodium as sodium chloride)	0.172 g/100g	-----	4	
Arsenic (as As_2O_3)	Not detected	0.1 ppm		5
Heavy metals (as Pb)	Not detected	5 ppm		6
Lead	Not detected	0.05 ppm		5
Cadmium	Not detected	0.01 ppm		5
Mercury	Not detected	0.01 ppm		7
Aerobic plate count	Not more than 300/g	-----		8
Coliform bacteria	Negative/2.22g	-----		9
Viable molds count	Negative/0.1g	-----		10
Viable yeasts count	Negative/0.1g	-----		10
Coagulase positive staphylococci	Negative/0.01g	-----		11
Salmonella	Negative/25g	-----		12

QL: Quantitation limit N: Notes M: Method

Notes

1:Nitrogen-to-protein conversion factor: 6.25.

2:The formula for carbohydrate, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, is: 100 - (Moisture + Protein + Fat + Ash).

3:The energy conversion factors, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, are: Protein, 4; Fat, 9; Carbohydrate, 4.

4:Salt (sodium as sodium chloride) = Sodium × 2.54.

Method

1:Karl Fischer method
2:Kjeldahl method
3:Acid hydrolysis method
4:Ashing method
5:Atomic absorption spectrometry
6:Sodium sulfide colorimetric method
7:Cold vapor atomic absorption spectrometry
8:Standard Agar plating method
9:BGLB broth inoculating method
10:Potato Dextrose (10 %) Agar plating method
11:Surface spread plating method
12:Enrichment culture method

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Nov. 10, 2015
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Date issued: November 06, 2015

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Client: JX Nippon Oil & Energy Corporation
8, Chidori-cho, Naka-ku, Yokohama-shi, Kanagawa 231-0815 JAPAN

Sample name: ARE-10P-SA008

Received date: October 27, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Moisture	1.65 g/100g	-----		1
Protein	0.4 g/100g	-----	1	2
Fat	4.4 g/100g	-----		3
Ash	0.3 g/100g	-----		4
Carbohydrate	93.3 g/100g	-----	2	
Energy	414 kcal/100g	-----	3	
Sodium	68.1 mg/100g	-----		5
Salt (sodium as sodium chloride)	0.173 g/100g	-----	4	
Arsenic (as As_2O_3)	Not detected	0.1 ppm		5
Heavy metals (as Pb)	Not detected	5 ppm		6
Lead	Not detected	0.05 ppm		5
Cadmium	Not detected	0.01 ppm		5
Mercury	Not detected	0.01 ppm		7
Aerobic plate count	Not more than 300/g	-----		8
Coliform bacteria	Negative/2.22g	-----		9
Viable molds count	Negative/0.1g	-----		10
Viable yeasts count	Negative/0.1g	-----		10
Coagulase positive staphylococci	Negative/0.01g	-----		11
Salmonella	Negative/25g	-----		12

QL: Quantitation limit N: Notes M: Method

Notes

1:Nitrogen-to-protein conversion factor: 6.25.

2:The formula for carbohydrate, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, is: 100 - (Moisture + Protein + Fat + Ash).

3:The energy conversion factors, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, are: Protein, 4; Fat, 9; Carbohydrate, 4.

4:Salt (sodium as sodium chloride) = Sodium × 2.54.

Method

1:Karl Fischer method
2:Kjeldahl method
3:Acid hydrolysis method
4:Ashing method
5:Atomic absorption spectrometry
6:Sodium sulfide colorimetric method
7:Cold vapor atomic absorption spectrometry
8:Standard Agar plating method
9:BGLB broth inoculating method
10:Potato Dextrose (10 %) Agar plating method
11:Surface spread plating method
12:Enrichment culture method

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Date issued: November 06, 2015

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Signed for and on behalf of JFRL

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Section of Analysis Documentation

Nov. 06, 2015
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Date issued: November 06, 2015

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
8, Chidori-cho, Naka-ku, Yokohama-shi, Kanagawa 231-0815 JAPAN

Sample name: ARE-10P-SA010

Received date: October 27, 2015

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Moisture	1.54 g/100g	------		1
Protein	0.4 g/100g	------	1	2
Fat	4.8 g/100g	------		3
Ash	0.2 g/100g	------		4
Carbohydrate	93.1 g/100g	------	2	
Energy	417 kcal/100g	------	3	
Sodium	68.3 mg/100g	------		5
Salt (sodium as sodium chloride)	0.173 g/100g	------	4	
Arsenic (as As_2O_3)	Not detected	0.1 ppm		5
Heavy metals (as Pb)	Not detected	5 ppm		6
Lead	Not detected	0.05 ppm		5
Cadmium	Not detected	0.01 ppm		5
Mercury	Not detected	0.01 ppm		7
Aerobic plate count	Not more than 300/g	------		8
Coliform bacteria	Negative/2.22g	------		9
Viable molds count	Negative/0.1g	------		10
Viable yeasts count	Negative/0.1g	------		10
Coagulase positive staphylococci	Negative/0.01g	------		11
Salmonella	Negative/25g	------		12

QL: Quantitation limit N: Notes M: Method

Notes

1:Nitrogen-to-protein conversion factor: 6.25.

2:The formula for carbohydrate, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, is: 100 - (Moisture + Protein + Fat + Ash).

3:The energy conversion factors, according to the Cabinet Office Ordinance No. 10 (2015) on Labelling Standards for Food, are: Protein, 4; Fat, 9; Carbohydrate, 4.

4:Salt (sodium as sodium chloride) = Sodium × 2.54.

Method

1:Karl Fischer method
2:Kjeldahl method
3:Acid hydrolysis method
4:Ashing method
5:Atomic absorption spectrometry
6:Sodium sulfide colorimetric method
7:Cold vapor atomic absorption spectrometry
8:Standard Agar plating method
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10:Potato Dextrose (10 %) Agar plating method
11:Surface spread plating method
12:Enrichment culture method

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Date issued: November 06, 2015

CERTIFICATE OF ANALYSIS

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Signed for and on behalf of JFRL

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Takeko Arai
Section of Analysis Documentation

Nov. 06, 2015
Date

CERTIFICATE OF ANALYSIS

Product : ARE-10P

Lot No. : SA002

Date of Manufacturing : July 27, 2015

Test item	Results	Method
Appearance	Very dark red powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	3.0 %	JSSFA * (105°C, 5 hours)
Loose Bulk Density	0.42 g/ml	JP **3.01 (20ml cylinder)

* JSSFA: Japan's Specifications and Standards for Food Additives

* JP: The Japanese Pharmacopoeia

JX NIPPON OIL & ENERGY CORPORATION

(b) (6)

March 9, 2016

Kazuaki Hirasawa

Senior Chemist

Biotechnology Development Group

Biotechnology Business Unit

Specialty Chemicals & Materials Company

Date: March 9, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-10P

Lot No. : SA010

Date of Manufacturing : October 8, 2015

Test item	Results	Method
Appearance	Very dark red powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	2.6 %	JSSFA * (105°C, 5 hours)
Loose Bulk Density	0.40 g/ml	JP **3.01 (20ml cylinder)

* JSSFA: Japan's Specifications and Standards for Food Additives

* JP: The Japanese Pharmacopoeia

JX NIPPON OIL & ENERGY CORPORATION

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March 9. 2016

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Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 9, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-10P

Lot No. : SA008

Date of Manufacturing : October 7, 2015

Test item	Results	Method
Appearance	Very dark red powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	2.4 %	JSSFA * (105°C, 5 hours)
Loose Bulk Density	0.41 g/ml	JP **3.01 (20ml cylinder)

* JSSFA: Japan's Specifications and Standards for Food Additives

* JP: The Japanese Pharmacopoeia

JX NIPPON OIL & ENERGY CORPORATION

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March 9. 2016

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Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 9, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-10P

Lot No. : SA006

Date of Manufacturing : October 6, 2015

Test item	Results	Method
Appearance	Very dark red powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	2.7 %	JSSFA * (105°C, 5 hours)
Loose Bulk Density	0.39 g/ml	JP **3.01 (20ml cylinder)

* JSSFA: Japan's Specifications and Standards for Food Additives

* JP: The Japanese Pharmacopoeia

JX NIPPON OIL & ENERGY CORPORATION

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March 9, 2016

Kazuaki Hirasawa
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Biotechnology Business Unit
Specialty Chemicals & Materials Company

Date: March 9, 2016

CERTIFICATE OF ANALYSIS

Product : ARE-10P

Lot No. : SA004

Date of Manufacturing : October 5, 2015

Test item	Results	Method
Appearance	Very dark red powder	Visual test
Odor	Characteristic faint odor	Organoleptic examination
Loss on Drying	2.8 %	JSSFA * (105°C, 5 hours)
Loose Bulk Density	0.39 g/ml	JP **3.01 (20ml cylinder)

* JSSFA: Japan's Specifications and Standards for Food Additives

* JP: The Japanese Pharmacopoeia

JX NIPPON OIL & ENERGY CORPORATION

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Date: March 9, 2016

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No. 16013214006-0201 1/1
Date issued: February 23, 2016

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
1-2 Otemachi 1-chome, Chiyodaku, Tokyo 100-8162, Japan

Sample name: ARE-10P-SA002

Received date: February 09, 2016

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethanol	0.75 %	-----	1	1

QL: Quantitation limit N: Notes M: Method

Notes

1:The quantitation limit was set at 50 ppm due to the small size of the sample.

Method

1:Gas chromatography

JAPAN FOOD RESEARCH LABORATORIES

Signed for and on behalf of JFRL

(b) (6)

Takeko Arai
Section of Analysis Documentation

Feb. 23, 2016
Date

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No. 16013214010-0201 1/1
Date issued: February 23, 2016

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
1-2 Otemachi 1-chome, Chiyodaku, Tokyo 100-8162, Japan

Sample name: ARE-10P-SA010

Received date: February 09, 2016

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethanol	1.01 %	----	1	1

QL: Quantitation limit N: Notes M: Method

Notes

1:The quantitation limit was set at 50 ppm due to the small size of the sample.

Method

1:Gas chromatography

JAPAN FOOD RESEARCH LABORATORIES

Signed for and on behalf of JFRL

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No. 16013214009-0201 1/1
Date issued: February 23, 2016

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
1-2 Otemachi 1-chome, Chiyodaku, Tokyo 100-8162, Japan

Sample name: ARE-10P-SA008

Received date: February 09, 2016

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethanol	1.00 %	----	1	1

QL: Quantitation limit N: Notes M: Method

Notes

1:The quantitation limit was set at 50 ppm due to the small size of the sample.

Method

1:Gas chromatography

Signed for and on behalf of JFRL

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Feb. 23, 2016
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No. 16013214008-0201 1/1
Date issued: February 23, 2016

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
1-2 Otemachi 1-chome, Chiyodaku, Tokyo 100-8162, Japan

Sample name: ARE-10P-SA006

Received date: February 09, 2016

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethanol	0.93 %	.....	1	1

QL: Quantitation limit N: Notes M: Method

Notes

1:The quantitation limit was set at 50 ppm due to the small size of the sample.

Method

1:Gas chromatography

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Signed for and on behalf of JFRL

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No. 16013214007-0201 1/1
Date issued: February 23, 2016

CERTIFICATE OF ANALYSIS

Client: JX Nippon Oil & Energy Corporation
1-2 Otemachi 1-chome, Chiyodaku, Tokyo 100-8162, Japan

Sample name: ARE-10P-SA004

Received date: February 09, 2016

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Ethanol	0.94 %	.....	1	1

QL: Quantitation limit N: Notes M: Method

Notes

1:The quantitation limit was set at 50 ppm due to the small size of the sample.

Method

1:Gas chromatography

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Signed for and on behalf of JFRL

(b) (6)

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Feb. 23, 2016
Date

Appendix 4

Expert Panel Statement

Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of Astaxanthin Rich Extracts derived from *Paracoccus carotinifaciens*

December 19, 2016

INTRODUCTION

At the request of JX Nippon Oil & Energy (JX Nippon), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of human and animal food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information on its blends of carotenoids derived from the gram-negative proteobacteria *Paracoccus carotinifaciens* (*P. carotinifaciens*). The Panel was asked to determine whether the use of these extracts in food products for the general human population might be considered Generally Recognized as Safe (GRAS) through scientific procedures. JX Nippon manufactures three versions of the extract, astaxanthin-rich carotenoid extract derived from *P. carotinifaciens* (ARE-C), ARE-1P, and ARE-10P. The Panel consisted of the below-signed qualified scientific experts: Dr. David H. Bechtel (Bechtel Consulting, Inc.), Robert J. Nicolosi (RJ Nicolosi, LLC), and Dr. John A. Thomas (Tom-Tox, LLC).

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data compiled from the publicly available literature and other published sources based on searches of the published scientific literature conducted through August of 2016. Consideration was given to the status of astaxanthin as a naturally-occurring substance already present in the human diet, and to information from GRAS notices GRN 294 and GRN 580 (Fuji Chemical Industry Co., Ltd., 2009; U.S. FDA, 2010, 2015; INNOBIO Limited, 2015). In addition, the Panel considered other information deemed appropriate or necessary, including data and information provided by JX Nippon The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and comprehensive literature on the safety of the components of ARE-C, ARE-1P, and ARE-10P, including a number of other naturally occurring carotenoids (*i.e.,* astaxanthin, adonirubin, adonixanthin, canthaxanthin, β-carotene, echinenone, asteroidenone, and 3-hydroxyechinenone).

Following independent, critical evaluation of such data and information, the Panel unanimously concluded that the intended uses described herein of JX Nippon's ARE-C, ARE-1P, and ARE-10P, manufactured consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate feed-grade specifications, are GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

SUMMARY AND BASIS FOR GRAS

JX Nippon's ARE-C is manufactured under cGMP by extracting carotenoids from dehydrated *P. carotinifaciens* (DPC). Approximately 90% of ARE-C is carotenoids; the remaining 10% of ARE-C is mostly comprised of DPC matrix (*ca.* 8%), ethanol (*ca.* 0.1%) and moisture (*ca.* 0.2%). ARE-C is diluted and emulsified using gum arabic, maltodextrin, *dl*-α-tocopherol, medium chain triglycerides, and L-ascorbic acid to produce ARE-1P. ARE-10P is produced by diluting ARE-C with maltodextrin, L-ascorbic acid, and starch sodium octenyl succinate (modified starch). The Panel noted that the manufacturing process for each ingredient process results in high purity products that reproducibly meet product specifications.

ARE-C, ARE-1P, and ARE-10P are intended to be used as sources of dietary astaxanthin in baked goods, baking mixes, beverages, beverage bases, energy, sports, isotonic drinks, non-milk based meal replacements, cereals, cereal products, chewing gum, coffee and tea, dairy product analogs, frozen dairy desserts and mixes, hard candy, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and soft candy, at levels providing 0.15 mg astaxanthin/serving. The Panel noted these intended uses are identical to those described by INNOBIO in a prior GRAS notice filed by FDA as GRN No. 580 (INNOBIO Limited, 2015; U.S. FDA, 2015) and that consumer exposure to astaxanthin is likely to be comparable. INNOBIO calculated the mean and 90th percentile, users-only, dietary exposures to astaxanthin by the general population to be 0.96 mg/person/day (16 µg/kg body weight/day) and 1.62 mg/p/day (28 µg/kg body weight/day), respectively.

The Panel noted that the organism used in the production of ARE-C, ARE-1P, and ARE-10P, *P. carotinifaciens*, is a non-pathogenic and non-toxigenic organism. In addition, the Panel considered a series of toxicology studies on dehydrated *P. carotinifaciens,* from which the ARE ingredients are produced and concluded these studies revealed no evidence of mutagenicity or genotoxicity (Inveresk Research, 2004; SafePharm Laboratories, 2004a,b, 2005, 2006). Additionally, the Panel noted that administration of DPC to rats at concentrations of up to 5% in the diet for 90 days was associated with no toxicity or pathological effect (SafePharm Laboratories, 2006).

The Panel reviewed a series of safety studies on ARE-C. The Panel noted that ARE-C is of low acute toxicity, with an oral median lethal dose (LD_{50}) value in rats that is higher than 2,000 mg/kg body weight/day. The Panel also considered a 90-day, repeated-dose, oral toxicity

study of ARE-C in rats (Katsumata *et al.*, 2014) and noted that no test material-related changes on clinical observations, manipulative test, grip strength, motor activity, body weight, food consumption, ophthalmological examination, urinalysis (including water intake), hematological parameters, blood chemistry, organ weights, necropsy, or histopathological examination were observed following administration of up to 1,000 mg/kg body weight/day. The Expert Panel observed that ARE-C was non-mutagenic in the Ames assay (Bozo Research Center, 2013a), and did not induce chromosome aberrations in Chinese hamster lung fibroblast cells (Bozo Research Center, 2013b). In addition to the product-specific toxicology data, the Panel considered data related to the safety of the individual carotenoids present in ARE-C as supportive of the safety of GRAS substances.

CONCLUSION

Having considered all the relevant information, it is our opinion as qualified experts that there is reasonable certainty that no harm will result from the intended use of astaxanthin-rich carotenoid extract derived from *P. carotinifaciens* (ARE-C), ARE-1P, and ARE-10P, meeting food grade specifications and manufactured in accordance with current Good Manufacturing Practices (cGMP), for use in the U.S. as sources of dietary astaxanthin in baked goods, baking mixes, beverages, beverage bases, energy, sports, isotonic drinks, non-milk based meal replacements, cereals, cereal products, chewing gum, coffee and tea, dairy product analogs, frozen dairy desserts and mixes, hard candy, milk products, processed fruits and fruit juices, processed vegetables and vegetable juices, and soft candy, at levels providing 0.15 mg astaxanthin/serving. Such use would be considered Generally Recognized as Safe (GRAS) through scientific procedures, making ARE-C, ARE-1C, and ARE-10P exempt from the premarket approval requirements outlined in section 201(s) of the Federal Food, Drug, and Cosmetic Act.

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01/12/2017

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Date

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Jan 13, 2017

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Date

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01/16/17

David H. Bechtel, Ph.D., DABT
President,
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Monroe, NJ

Date

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