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Letter

Genotoxicity evaluation of oil prepared from the internal organs of the Japanese giant scallop (*Patinopecten yessoensis*)

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ABSTRACT — Discarded scallop internal organs, especially the hepatopancreas, are rich in n-3 polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid. However, they have not been utilized because of their contamination with toxic substances, such as cadmium (Cd), and the occurrence of diarrheic shellfish toxins (DST). We have successfully prepared a high-quality scallop oil (SCO) from the internal organs of the Japanese giant scallop (*Patinopecten yessoensis*), including the hepatopancreas without Cd and DST. These pollutants were removed by liquid-liquid partitioning followed by adsorption to active carbon of fine particle size with high pore surface volume. In this study, we prepared SCO from scallop internal organs obtained from two different processing areas (Mutsu and Uchiura bays, Japan), and referred to them as SCO-M and SCO-U, respectively. Genotoxicity of SCO-M and SCO-U was evaluated by the *in vitro* bacterial reverse mutation test (Ames test) and *in vivo* micronucleus test in accordance with the Organisation for Economic Co-operation and Development guidelines. SCO-M and SCO-U showed negative results in the Ames test in the presence or absence of metabolic activation with S9 mix. In addition, no genotoxic effects of SCO-M and SCO-U were observed at all tested doses in the micronucleus test. Based on the results of the present study, it can be concluded that SCO-M and SCO-U are safe products in terms of genotoxicity under these experimental conditions.

Key words: n-3 Polyunsaturated fatty acids, Eicosapentaenoic acid, Docosahexaenoic acid, Scallop internal organs, Genotoxicity

INTRODUCTION

The n-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are used as supplements and pharmaceutical products. EPA and DHA have beneficial effects against hyperlipidemia (Eslick *et al*., 2009), cardiovascular diseases (Bowen *et al*., 2016), hypertension (Campbell *et al*., 2013), and Alzheimer's disease (Song *et al*., 2016). The European Food Safety Authority recommends the intake of 250 mg/day EPA and DHA for the maintenance of normal heart function, either 2 g/day DHA or 2 g/day EPA and DHA for the maintenance of normal blood triglyceride (TG) levels, and 3 g/day EPA and DHA for the maintenance of normal blood pressure. In addition, the American Heart Association also recommends the intake

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of 2-4 g/day EPA and DHA for patients with hypertriglyceridemia (Kruzynski, 2004). Due to the growing demand of n-3 PUFA as supplements and pharmaceutical products worldwide, there are concerns about the exhaustion of n-3 PUFA supply sources.

Fishery by-products are rich in n-3 PUFA and they are viable sources of n-3 PUFA. Japanese giant scallops (*Patinopecten yessoensis*) are produced in quantities of around 500,000 tons a year in Japan, but the edible part (adductor muscle) is only about 15% (wt/wt). The outer shells and internal organs are wastes. Previous studies reported that the scallop's hepatopancreas has an extremely high n-3 PUFA content, especially EPA (Hayashi, 1986, 1988). However, the scallop's internal organs, the hepatopancreas in particular, contains large amounts of cadmium (Cd) and sometimes diarrheic shellfish poison (DST), including dinophysis toxin and okadaic acid (Kruzynski, 2004; Matsushima *et al*., 2018). For this reason, the scallop's internal organs have not been used as n-3 PUFA source. Recently, we successfully prepared a high-quality scallop oil (SCO) from its internal organs that satisfies the specifications for its use as a food, by removing the toxic components, such as Cd and DST (Okuyama *et al*., 2019). The SCO has a higher EPA content than other fish oils and contains TG as well as phospholipids (PL) formed n-3 PUFA.

To the best of our knowledge, there is no publication describing the safety of SCO from the view point of genotoxicity studies. In this study, we prepared SCO from the internal organs of *P. yessoensis*, obtained from two different processing areas and referred to them as SCO-M (SCO from Mutsu bay, Aomori, Japan) and SCO-U (SCO from Uchiura bay, Hokkaido, Japan). In order to use SCO as a food ingredient and supplement for n-3 PUFA, the present study focused on the genotoxic potential of SCO-M and SCO-U, using the *in vitro* (the bacterial reverse mutation test) and *in vivo* genotoxicity tests (micronucleus test), which are accepted by the Organisation for Economic Co-operation and Development (OECD) guideline (OECD, 1997, 2014).

MATERIALS AND METHODS

Chemicals and bacterial strains

The chemicals used as positive controls for the bacterial reverse mutation test were 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2), 9-aminoacridinehydrochloride hydrate (9-AA), sodium azide (SA), and 2-aminoanthracene (2-AA) and were purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan) and Sigma-Aldrich Co., LLC. (Missouri, USA). The cyclophosphamide monohydrate (CPA), used as positive control, and refined olive oil, used as negative control, in the micronucleus test were purchased from Sigma-Aldrich Co. LLC. and FUJIFILM Wako Pure Chemical Co., respectively. The bacterial strains *Salmonella typhimurium* (TA100, TA98, TA1535, and TA1537) and *Escherichia coli* (WP2*uvr*A) were obtained from the National Institute of Technology and Evaluation (Chiba, Japan). All other chemicals were of reagent grade and were obtained from Sigma-Aldrich Co. LLC. and NACALAI TESQUE, INC. (Kyoto, Japan).

Preparation of oil from the Japanese giant scallop's internal organs

The internal organs of the Japanese giant scallop (*P. yessoensis*) from Mutsu and Uchiura bays were provided by SATO CHIKURO Co. (Aomori, Japan) and Yakumo fishery cooperative (Hokkaido, Japan), respectively. By-produced giant scallop's internal organs obtained from Mutsu bay only consisted of the hepatopancreas and were obtained from October to November 2017. In contrast, the giant scallop's internal organs obtained from Uchiura bay contained hepatopancreas, gonads, gills, and mantles and were obtained from August to September 2017. In order to extract the crude oil from the giant scallop's internal organs, they were boiled first, then minced, and treated with *n*-hexane/95% (w/w) ethanol (5:2, v/v) containing 1% (w/v) d-*δ*-tocopherol and 1% (w/v) ascorbyl palmitate (MITSUBISHI-CHEMICAL FOODS CO., Tokyo, Japan) for 30 min at room temperature. Then, *n*-hexane layer (upper layer) was collected using solid-liquid separator (BM-20, Matsumoto Machine Manufacturing Co., Ltd., Osaka, Japan). Then, 5% active carbon by weight was added to the *n*-hexane layer and stirred for 30 min at room temperature. Filtration using a diatomaceous earth pre-coated filter press, was carried out to remove the active carbon. Cd, DST, and color were adsorbed on the active carbon. Then the *n*-hexane layer was evaporated. The resulting products were referred to as SCO-M (prepared from giant scallop internal organs by-produced in Mutsu bay area) and SCO-U (prepared from giant scallop internal organs by-produced in Uchiura bay area). The prepared SCO-M and SCO-U were stored in nitrogen headspace gas at -35°C until used. The detailed method of SCO preparation was described previously (Okuyama *et al*., 2019).

Lipid compositions of the test substances

After methylation of fatty acids (FA) with boron trifluoride methanol, the FA compositions of the SCO-M and SCO-U were determined using a gas chromatograph-

ic (GC) system (GC-2014; Shimadzu Co., Kyoto, Japan) equipped with Omegawax® capillary column (Merck KGaA, Darmstadt, Germany) employing tridecanoic acid (C13:0) as an internal standard as described previously (Fukunaga *et al*., 2016). The PL content of SCO-M and SCO-U was determined using phosphorus analyses as described previously (Rouser *et al*., 1970). Peroxide values (PV) of SCO-M and SCO-U were determined using the standard iodometric procedure (Japan Oil Chemists' Society, 2013). The FA and PL contents of SCO-M and SCO-U are shown in Table 1. We confirmed the contents of Cd (< 0.4 mg/kg), arsenic (< 2 ppm), mercury (< 0.4 ppm), dioxin (< 4 pg-TEQ/g), pesticide residues (< 0.01 ppm, respectively), polychlorobiphenyl (< 3 ppm), and DST (< 0.16 mg okadaic acid eq/kg) in SCO-M and SCO-U, using the official analytical methods performed by a commercial service (Japan Food Research Laboratories, Tokyo, Japan), and demonstrated that the specifications for their use as food are satisfied.

Table 1. Fatty acid content, phospholipid content, and peroxide value of the oil prepared from Japanese giant scallop's (*P. yessoensis*) internal organs.

	SCO-M[1]	SCO-U[2]
Fatty acid content (mg/g)		
C14:0	37	29
C16:0	104	124
C16:1	92	88
C18:0	4	20
C18:1n-9	16	45
C18:1n-7	33	45
C18:2n-6	40	19
C20:4n-6 (ARA)	73	10
C20:5n-3 (EPA)	297	216
C22:5n-3	3	7
C22:6n-3 (DHA)	58	103
Others	91	112
Phospholipid content (mg/g)	53.8	91.1
Peroxide value (meq/kg)	0.1	0

The contents of cadmium (< 0.4 mg/kg), arsenic (< 2 ppm), mercury (< 0.4 ppm), dioxin (< 4 pg-TEQ/g), pesticide residues (< 0.01 ppm, respectively), polychlorobiphenyl (< 3 ppm), and diarrheic shellfish poison (< 0.16 mg okadaic acid eq/kg) in SCO-M and SCO-U were determined using official analytical methods. The results confirmed that the specifications for their use as food, have been satisfied.

ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SCO, scallop oil.

[1] Oil prepared from internal organs of *P. yessoensis*, by-produced in Mutsu bay area (Aomori, Japan).

[2] Oil prepared from internal organs of *P. yessoensis*, by-produced in Uchiura bay area (Hokkaido, Japan).

Bacterial reverse mutation test (Ames test)

Two independent tests were performed, the dose-finding toxicity test and the main Ames test. The dose-finding toxicity test was performed to establish suitable exposure levels for the main Ames test. Ten-fold dilutions of SCO-M and SCO-U were prepared using acetone.

The *Salmonella typhimurium* strain TA100 was used for the dose-finding toxicity test with different doses of SCO-M and SCO-U (up to 5,000 μg/plate) in the presence and absence of metabolic activation with S9 mix (S9 mix for Ames test, IEDA TRADING CO., Tokyo, Japan) in accordance with the OECD test guideline 471 (OECD TG 471, 1997), and the lower doses were set with a common ratio of approximately 3. In the dose-finding toxicity test, we did not observe more than a 2-fold or a dose-dependent increase in the number of revertant colonies for SCO-M and SCO-U compared to those treated with acetone, as a negative control. Furthermore, there was no growth inhibition of the test strains (TA100) at concentrations up to 5,000 μg/plate in the presence and absence of metabolic activation. Therefore, the highest doses were set at 5,000 μg/plate of SCO-M and SCO-U for the main Ames test, while the lower doses were set with a common ratio of 2.

The main Ames test was performed using a pre-incubation method with and without metabolic activation (Yahagi *et al*., 1977). Each test strain was pre-incubated in 2.5% (w/w) nutrient broth No.2 (Sigma-Aldrich Co., LLC.) for 8 hr at 37°C, and then the resulting suspensions were used as bacterial suspension. The top agar for the culture of *S. typhimurium* was mixed with 0.5 mM L-histidine and agar solutions. In addition, the top agar for the culture of *E. coli* was mixed with 0.5 mM L-tryptophan and agar solutions. A test tube, containing 0.1 mL of the bacterial suspension, 0.5 mL of either 0.1 M sodium phosphate buffer (pH 7.4) or the S9 mix (for presence and absence of metabolic activation, respectively), and the test substances (SCO solutions or a positive and negative control solutions), was incubated at 37°C for 20 min. Then, 2 mL of the top agar was added to the test tube, and the mixture was overlaid onto a minimal glucose agar plate. The plates were subsequently placed in an incubator at 37 °C for 48 hr, and then the revertant colonies were counted. A response was determined as positive when the test substances caused a more than 2-fold dose-dependent increase in the mean number of revertant colonies per plate compared to the negative control. Triplicate plates were used for each dose to calculate the means and standard error of the means (SEM).

Micronucleus test in mice

The intake of SCO-M and SCO-U at a dose of 2,000 mg/kg body weight (BW) /day, which is the upper limit dose specified in the OECD Guideline for the Testing of Chemicals No. 474 (OECD TG 474, 2014), did not induce clinical signs or body weight changes in mice and rats (Sugimoto K. *et al*., unpublished data). Therefore, 2,000 mg/kg BW/day was set as the highest dose of the SCO-M and SCO-U for the micronucleus test, while the lower doses were 1,000 and 500 mg/kg BW/day. The dilution of the SCO-M and SCO-U were done by adding refined olive oil, which has no mutagenicity.

The experimental protocol was reviewed and approved by the Animal Ethics Committee of Kansai University, and followed the "Guide for the Care and Use of Experimental Animals" issued by the Prime Minister's Office of Japan. Seven-weeks-old male ICR mice, obtained from Japan SLC, Inc. (Shizuoka, Japan), were kept in an air-conditioned room (temperature: 21-23°C; humidity: 50-70%; illuminated from 08:00-20:00). Forty-eight mice were divided into eight groups of six mice each. Mice were administered once daily for 2 days doses of 500, 1,000 and 2,000 mg/kg BW of SCO-M and SCO-U by gavage. The mice of the negative control group were administrated 2,000 mg/kg BW/day refined olive oil in the same manner as SCO-M and SCO-U administration. The mice of the positive control group were administered a single dose of 50 mg/kg BW/day CPA. Twenty-four hr after the last administration, mice were euthanized under isoflurane (Intervet K.K., Osaka, Japan) anesthesia, and then peripheral blood was collected from the inferior vena cava.

Peripheral blood samples were processed for flow cytometric evaluation of micronucleated polychromatic erythrocytes (MNPCE) as described previously (Balmus *et al*., 2015). Briefly, the obtained peripheral blood was immediately mixed with heparin solution, and then the heparin-blood mixture was transferred into cold methanol and stored at -80°C until later analysis. Peripheral blood cells were incubated with FITC labeled anti-CD71 antibody (cat NO. 11-0711-82, Thermo Fisher Scientific, Massachusetts, USA) and propidium iodide (PI) for 60 min at 4°C with gentle agitation. The numbers of positive cells were counted using flow cytometry (FACS Canto II, BD Biosciences, Mississauga, Canada) and analyzed by FlowJo software (TOMY DIGITAL BIOLOGY CO. LTD., Tokyo, Japan). Normochromatic erythrocytes (NCE) and polychromatic erythocytes (PCE) were enumerated concurrently during MNPCE analysis, and PCE/(PCE+NCE) ratio was calculated as a measure of bone marrow toxicity. For each peripheral blood sample, 2,000 PCE were analyzed to determine the frequency of MNPCE (MNPCE/PCE ratio).

Significance between the negative control group and each of the SCO-M and SCO-U treated groups or the positive control group in the micronucleus test was evaluated using one-way analysis of variance (ANOVA) and Dunnet's multiple comparison test. Significance was set at $p < 0.05$. Analyses were performed using GraphPad Prism7 software (GraphPad Software, California, USA).

RESULTS AND DISCUSSION

In the bacterial reverse mutation test, the dose range for the dose-finding toxicity test was determined in the presence or absence of metabolic activation with S9 mix. With SCO-M and SCO-U doses ranging from 62 to 5,000 μg per plate (with a common ratio of approximately 3), no increased numbers of bacterial revertant colonies over those occurring spontaneously could be observed in *S. typhimurium* strain TA100 (data not shown). Based on the results of the dose-finding toxicity test, the main Ames test was conducted using doses from 313 to 5,000 μg/plate with a common ratio of 2.

The main Ames test was performed on five different bacterial strains with either vehicle or five different doses of SCO-M and SCO-U (up to 5,000 μg per plate). The results of SCO-M and SCO-U are summarized in Table 2 (in absence of metabolic activation by S9 mix) and Table 3 (in presence of metabolic activation by S9 mix). No bacterial toxicity was observed at any of SCO-M and SCO-U doses. The positive controls were simultaneously evaluated under the Ames test conditions during all the experiments and showed marked positive responses, which validated the test results. SCO-M and SCO-U precipitated at doses over 2,500 μg/plate with and without metabolic activation. SCO-M and SCO-U did not cause a higher than two-fold increase in the number of colonies per plate, compared to the negative control in both the dose-finding toxicity test and main Ames tests. The results suggested that SCO-M and SCO-U have no genotoxic effect under the conditions of the present study.

The results of flow cytometric analysis of MNPCE and PCE frequencies in the micronucleus test to determine the genotoxic effects of SCO-M and SCO-U in mice are presented in Table 4. There were no significant differences in the PCE/(PCE+NCE) ratio and MNPCE/PCE ratio of the SCO-M and SCO-U groups compared with those of the negative control group. The positive control group (50 mg/kg BW CPA) significantly increased the MNPCE/PCE ratio and decreased the PCE/(PCE+NCE) ratio compared to the other groups. Additionally, there was no sta-

Table 2. Bacterial reverse mutation test (Ames test) without S9 mix of oil prepared from Japanese giant scallop's (*P. yessoensis*) internal organs.

Groups	Dose (μg/plate)	Number of revertant colonies/plate				
		Base-pair substitution mutations			Frameshift mutations	
		TA100	TA1535	WP2*uvr*A	TA98	TA1537
Negative control[1]	-	54 ± 11	9 ± 2	19 ± 1	65 ± 5	27 ± 2
SCO-M[2]	312.5	73 ± 2	13 ± 2	22 ± 2	85 ± 11	16 ± 3
	625	69 ± 8	12 ± 4	28 ± 3	63 ± 14	25 ± 3
	1250	78 ± 6	15 ± 3	29 ± 3	95 ± 10	23 ± 3
	2500	81 ± 6	15 ± 2	21 ± 2	61 ± 26	16 ± 7
	5000	72 ± 10	8 ± 4	20 ± 1	44 ± 13	17 ± 4
SCO-U[3]	312.5	76 ± 11	12 ± 2	21 ± 4	90 ± 8	19 ± 2
	625	72 ± 6	10 ± 1	20 ± 1	91 ± 16	21 ± 4
	1250	72 ± 10	10 ± 1	23 ± 1	71 ± 20	26 ± 7
	2500	69 ± 10	12 ± 1	22 ± 2	46 ± 1	17 ± 4
	5000	88 ± 5	7 ± 2	23 ± 1	39 ± 4	14 ± 1
Positive controls						
AF-2	1	551 ± 25				
SA	2		126 ± 30			
AF-2	10			633 ± 24		
AF-2	0.5				617 ± 36	
9-AA	2					179 ± 11

Data show means ± SEM (n = 3).

[1] Acetone was used as a negative control.

[2] Oil prepared from internal organs of *P. yessoensis,* by-produced in Mutsu bay area (Aomori, Japan).

[3] Oil prepared from internal organs of *P. yessoensis,* by-produced in Uchiura bay area (Hokkaido, Japan).

AF-2, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide; 9AA, 9-aminoacridinehydrochloride hydrate; SA, sodium azide; SCO, scallop oil; 2AA, 2-aminoanthracene.

Table 3. Bacterial reverse mutation test (Ames test) with S9 mix of oil prepared from Japanese giant scallop's (*P. yessoensis*) internal organs.

Groups	Dose (μg/plate)	Number of revertant colonies/plate				
		Base-pair substitution mutations			Frameshift mutations	
		TA100	TA1535	WP2*uvr*A	TA98	TA1537
Negative control[1]	-	74 ± 3	12 ± 1	20 ± 2	43 ± 2	12 ± 1
SCO-M[2]	312.5	97 ± 7	16 ± 2	25 ± 1	45 ± 2	16 ± 2
	625	105 ± 2	15 ± 2	26 ± 4	41 ± 2	16 ± 2
	1250	89 ± 4	8 ± 1	19 ± 3	42 ± 4	9 ± 1
	2500	84 ± 12	11 ± 2	26 ± 2	41 ± 3	15 ± 1
	5000	95 ± 7	11 ± 2	24 ± 4	51 ± 2	15 ± 2
SCO-U[3]	312.5	80 ± 11	11 ± 1	24 ± 2	53 ± 2	17 ± 2
	625	93 ± 8	10 ± 2	29 ± 1	47 ± 2	17 ± 4
	1250	95 ± 5	12 ± 1	23 ± 1	46 ± 5	12 ± 3
	2500	101 ± 5	10 ± 1	27 ± 4	46 ± 2	15 ± 3
	5000	99 ± 5	12 ± 2	25 ± 1	45 ± 4	12 ± 3
Positive control						
2-AA	1	448 ± 29				
2-AA	2		144 ± 16			125 ± 10
2-AA	10			647 ± 101		
2-AA	0.5				494 ± 78	

Data show means ± SEM (n = 3).

[1] Acetone was used as a negative control.

[2] Oil prepared from internal organs of *P. yessoensis,* by-produced in Mutsu bay area (Aomori, Japan).

[3] Oil prepared from internal organs of *P. yessoensis,* by-produced in Uchiura bay area (Hokkaido, Japan).

SCO, scallop oil; 2AA, 2-aminoanthracene.

Table 4. Micronucleus test results in mice administered with oil prepared from Japanese giant scallop's (*P. yessoensis*) internal organs.

Groups	Dose (mg/kg/day)	PCE/(PCE+NCE) (%)	MNPCE/PCE (%)
Negative control[1]	2000	1.86 ± 0.11 [a]	1.54 ± 0.08 [b]
SCO-M[2]	500	1.58 ± 0.12 [a]	1.76 ± 0.06 [b]
	1000	1.78 ± 0.16 [a]	1.87 ± 0.12 [b]
	2000	1.77 ± 0.05 [a]	1.70 ± 0.11 [b]
SCO-U[3]	500	1.59 ± 0.09 [a]	1.96 ± 0.11 [b]
	1000	1.72 ± 0.09 [a]	1.88 ± 0.10 [b]
	2000	1.79 ± 0.11 [a]	1.83 ± 0.13 [b]
Positive control[4]	50	1.12 ± 0.18 [b]	4.11 ± 0.58 [a]

Data show means ± SEM (n = 6). Values in the same column not sharing a common superscript are significantly different at $p < 0.05$ using Dunnet's multiple comparison test.

[1] Refined olive oil was used as a negative control.

[2] Oil prepared from internal organs of *P. yessoensis,* by-produced in Mutsu bay area (Aomori, Japan).

[3] Oil prepared from internal organs of *P. yessoensis,* by-produced in Uchiura bay area (Hokkaido, Japan).

[4] Cyclophosphamide monohydrate was used as a positive control.

MNPCE, micronucleated polychromatic erythrocytes; NCE, normochromatic erythrocytes; PCE, polychromatic erythrocytes; SCO, scallop oil.

tistically significant difference in the body weights of animals among the SCO-treated groups and negative control group. No mortality and abnormal clinical signs were observed among the SCO-treated and negative control group (data not shown). Therefore, SCO-M and SCO-U did not show genotoxicity or bone marrow toxicity in mice under the experimental conditions of the present study.

Lipid components, such as TG, PL, or n-3 PUFA, have been consumed as food and supplements. It has been proven that the intake of TG and PL does not induce any genotoxicity based on the safety assessments (Johnson, 2001; Robertson *et al*., 2014). In addition, TG containing n-3 PUFA showed negative results in Ames assay at concentrations of 5-5,000 μg TG containing n-3 PUFA/plate (Belcher *et al*., 2011; Blum *et al*., 2007). On the other hand, FA hydroperoxide and its secondary autoxidation products showed a mutagenic response in TA97 and TA100 strains in the Ames test with metabolic activation (Hageman *et al*., 1989; Yamaguchi and Yamashita, 1979). In the present study, the PV, an initial oxidation index, of SCO-M and SCO-U were 0.1 and 0, respectively (Table 1). Therefore, SCO-M and SCO-U are considered not to contain any FA hydroperoxide.

The results of the present study indicated that SCO-M and SCO-U did not induce any genotoxic effects, as no genotoxicity were observed in the main Ames test and in the *in vivo* micronucleus test, respectively. In both tests, the highest doses used were the maximum levels recommended by their respective OECD guidelines. Furthermore, the negative and positive controls used in each test exhibited the expected responses, validating the methodologies. The results revealed that SCO-M and SCO-U are a safe source of n-3 PUFA in terms of genotoxicity under the present experimental conditions. SCO-M and SCO-U are rich in PL containing EPA and DHA recommended for long term consumption. The toxicological potential of SCO in a chronic long-term study must be the studied in the future.

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Conflict of interest---- All authors declare that there is no conflict of interest.

REFERENCES

Balmus, G., Karp, N.A., Ng, B.L., Jackson, S.P., Adams, D.J. and McIntyre, R.E. (2015): A high-throughput *in vivo* micronucleus

assay for genome instability screening in mice. Nat. Protoc., **10**, 205-215.

Belcher, L.A., MacKenzie, S.A., Donner, M., Sykes, G.P., Frame, S.R. and Gillies, P.J. (2011): Safety assessment of EPA-rich triglyceride oil produced from yeast: genotoxicity and 28-day oral toxicity in rats. Regul. Toxicol. Pharmacol., **59**, 53-63.

Blum, R., Kiy, T., Tanaka, S., Wong, A.W. and Roberts, A. (2007): Genotoxicity and subchronic toxicity studies of DHA-rich oil in rats. Regul. Toxicol. Pharmacol., **49**, 271-284.

Bowen, K.J., Harris, W.S. and Kris-Etherton, P.M. (2016): Omega-3 Fatty Acids and Cardiovascular Disease: Are There Benefits? Curr. Treat. Options Cardiovasc. Med., **18**, 69.

Campbell, F., Dickinson, H.O., Critchley, J.A., Ford, G.A. and Bradburn, M. (2013): A systematic review of fish-oil supplements for the prevention and treatment of hypertension. Eur. J. Prev. Cardiol., **20**, 107-120.

Eslick, G.D., Howe, P.R., Smith, C., Priest, R. and Bensoussan, A. (2009): Benefits of fish oil supplementation in hyperlipidemia: a systematic review and meta-analysis. Int. J. Cardiol., **136**, 4-16.

Fukunaga, K., Hosomi, R., Fukao, M., Miyauchi, K., Kanda, S., Nishiyama, T. and Yoshida, M. (2016): Hypolipidemic Effects of Phospholipids (PL) Containing n-3 Polyunsaturated Fatty Acids (PUFA) Are Not Dependent on Esterification of n-3 PUFA to PL. Lipids, **51**, 279-289.

Hageman, G., Kikken, R., ten Hoor, F. and Kleinjans, J. (1989): Linoleic acid hydroperoxide concentration in relation to mutagenicity of repeatedly used deep-frying fats. Lipids, **24**, 899-902.

Hayashi, K. (1986): Seasonal Changes in Eicosapentaenoic Acid Content of Hepatopancreas of Scallop (*Patinopecten yessoensis*). Nippon Suisan Gakkaishi, **52**, 1559-1563.

Hayashi, K. (1988): Eicosapentaenoic Acid-Rich Triglycerides of Scallop Hepatopancreas. Nippon Suisan Gakkaishi, **54**, 1449-1449.

Japan Oil Chemists'Society (2013): The JOCS Standard Methods for the Analysis of Fats, Oils and Related Materials, Second English Edition, 2.5.2: Peroxide Value.

Johnson, W. Jr. (2001): Final report on the safety assessment of trilaurin, triarachidin, tribehenin, tricaprin, tricaprylin, trierucin, triheptanoin, triheptylundecanoin, triisononanoin, triisopalmitin, triisostearin, trilinolein, trimyristin, trioctanoin, triolein, tripalmitin, tripalmitolein, triricinolein, tristearin, triundecanoin, glyceryl triacetyl hydroxystearate, glyceryl triacetyl ricinoleate, and glyceryl stearate diacetate. Int. J. Toxicol., **20**, 61-94.

Kruzynski, G.M. (2004): Cadmium in oysters and scallops: the BC experience. Toxicol. Lett., **148**, 159-169.

Matsushima, R., Uchida, H., Watanabe, R., Oikawa, H., Kosaka, Y., Tanabe, T. and Suzuki, T. (2018): Distribution of Diarrhetic Shellfish Toxins in Mussels, Scallops, and Ascidian. Food Safety, **6**, 101-106.

OECD, (1997): OECD guideline for the testing of chemicals TG 471 bacterial reverse mutation test.

OECD, (2014): OECD Guideline for the Testing of Chemicals TG 474 mammalian erythrocyte micronucleus test.

Okuyama, J., Yajima, T., Takahashi, K. and Yoshioka, T. (2019): Lipid composition and method for producing same. Patent nr. US10246663B2.

Robertson, B., Burri, L. and Berge, K. (2014): Genotoxicity test and subchronic toxicity study with Superba krill oil in rats. Toxicol. Rep., **1**, 764-776.

Rouser, G., Fkeischer, S. and Yamamoto, A. (1970): Two dimensional then layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. Lipids, **5**, 494-496.

Song, C., Shieh, C.H., Wu, Y.S., Kalueff, A., Gaikwad, S. and Su, K.P. (2016): The role of omega-3 polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic acids in the treatment of major depression and Alzheimer's disease: acting separately or synergistically? Prog. Lipid Res., **62**, 41-54.

Yahagi, T., Nagao, M., Seino, Y., Matsushima, T. and Sugimura, T. (1977): Mutagenicities of N-nitrosamines on Salmonella. Mutat. Res., **48**, 121-129.

Yamaguchi, T. and Yamashita, Y. (1979): Mutagenic Activity of Autoxidized Linolenic and Linoleic Acid. Agric. Biol. Chem., **43**, 2225-2226.