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Clinical safety and efficacy of a novel marine source of the long-chain omega-3 fatty acids

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Abstract : Squid is a sustainable source of long-chain omega 3 fatty acids. This study aims to assess the safety and triglyceride-lowering efficacy of refined oil derived from the squid(Todarodes pacificus) viscera. Male and female participants with elevated fasting serum lipids (i.e., total cholesterol of ≥5.2 mmol/L or fasting serum triglyceride of ≥1.65 mmol/L) were randomly allocated to the control (n = 52) or squid oil group (n = 52), and participants in the latter group were instructed to consume 3 g of squid oil daily for 60 days. None of the subjects reported adverse events associated with the consumption of squid oil. Baseline clinical chemistry and hematological parameter values and those toward the end of the treatment period were similar, and all values were within the normal range. Fasting cholesterol and triglyceride levels in the control and squid oil groups were similar; however, toward the end of the 60 day study period, these levels significantly reduced in the squid oil group relative to those in the control group (P< 0.01). However, high-density lipoprotein-cholesterol remained unchanged in both groups. Thus, it can be inferred that squid oil is a safe source of long-chain omega-3 fatty acids and has beneficial effects on the blood lipid levels. This is the first clinical study on squid oil usage, and suggests that it could be a sustainable source of omega 3 fatty acids.

Keywords : squid oil; eicosapentaenoic acid; docosahexaenoic acid; triglycerides

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviations.

Introduction

The cardio-protective effects of the long-chain omega-3 fatty acids EPA and DHA are well-documented; in fact, there is strong consensus in the scientific literature that the long-chain omega-3 fatty acids reduce fasting serum triglycerides [1-6], blood pressure [7-9], and the risk of coronary heart disease mortality [2,10-15]. The recommended daily intakes of the long-chain omega-3 fatty acids are a minimum of 1g for patients with heart disease and 250 to 500 mg for healthy individuals [13,15-18]. Blanket recommend- ations to increase the consumption of EPA and DHA long-chain omega-3 fatty acid

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s have raised concerns about fish oil sustainability [16,19,20].

Many fish species are in decline and the marine eco-system is being negatively impacted by human activity. Fish stocks, such as the Atlantic cod stocks of south eastern Canada and the eastern United States and the Pacific and Atlantic species of the bluefin tuna, have dropped worldwide due to overfishing [21-23]. According to the Food and Agriculture Organization of the United Nations, of the 600 marine fish stocks that they monitor, 52% are fully exploited; 17% are overexploited; and 7% are depleted (FAO).

Squid oil is emerging as a novel and sustainable alternative source of the long-chain omega-3 fatty acids. Much of the squid harvested for human consumption does not make it to market, but is discarded as "cut offs" during food preparation; these remaining or “left-over” trimmings are predominantly the squid viscera, which are rich in the long-chain omega-3 fatty acids EPA and DHA [24]. Thus, squid oil is manufactured solely from by-products obtained during the food production of squid. Squid are a uniquely sustainable source of the long-chain omega-3 fatty acids, given that they have a short lifespan of less than 450 d; mainly breed a single time in their lives and will normally perish after spawning; reach reproductive age early; have a low progeny mortality rate; and have a very efficient food to growth ratio of up to 2:1. The fisheries are managed to ensure safe levels of escapement and good recruitment. The main fishing method employed for catching squid is jigging. The fishing boats use bright lights at night to attract large schools to the surface and then deploy a vast number of un-baited hooks just below the surface. This makes for a highly eco-friendly fishery (*i.e.,* there is virtually zero by-catch, as the fishing method is highly selective, specifically targeting same size specimens, with no impact on the ocean floor or coral reefs).

Squid oil is not genotoxic [25], and in a 90-day safety study conducted in Sprague-Dawley rats, the no-observed-adverse-effect level was found to be 1,000 mL/kg body weight/day, the highest dose tested [26]. Human feeding trials of squid oil have not been conducted; thus, the objective of the current study was to assess, in humans, the safety of oil derived from squid, as well as the effects of squid oil on blood lipid levels.

Materials and methods

This study was conducted at Chinese Center for Disease Control and Prevention through the Beijing Public Federation of Biological Sciences Technology. As the purpose for conducting the study was to seek functional food approval for squid oil supplements, the clinical trial protocol established by the Chinese State Food and Drug Administration and published in the 2003 Technical Standards for the Testing and Assessment of Health Foods for Blood Lipids Reduction was followed. All subjects provided written informed consent prior to participation in the clinical study. The clinical study was conducted in compliance with the World Medical Association Declaration of Helsinki.

Subjects

To be considered eligible for study participation, subjects had to be between 18 and 65 years of age with elevated fasting serum lipids, defined as a fasting serum total cholesterol of ≥5.2 mmol/l or fasting serum triglyceride of ≥1.65 mmol/l, assessed in two blood samples, collected within 6 months of each other. Subjects also had to have normal chest X-rays, electrocardiogram, and abdominal ultrasonography at screening. Excluded subjects were patients with serious cardiovascular, liver, kidney and or blood diseases; psychiatric patients; pregnant or lactating women; individuals with allergies to fish or seafood; and individuals who had recently participated in another clinical study. Subjects were instructed to maintain their habitual diets and lifestyles throughout the experimental period.

Study design

Subjects who were found to be eligible for study partic

ipation were randomly allocated to either the squid oil group or to the control group; randomisation was stratified according to baseline fasting serum total cholesterol and triglyceride levels. Subjects in the squid oil group were required to consume 3 g of squid oil daily for 60 d; this was achieved by consuming three 500-mg squid oil capsules, twice a day. Subjects in the control group did not consume any product. Following screening, subjects returned to the hospital twice for clinical assessment: once at baseline and once at the end of the 60-d study.

Study products

Squid oil capsules (500 mg/capsule) were supplied by Dongwoo Industrial Company, Ltd (South Korea). The oil was derived from the viscera of squid (*Todarodes pacificus*) by the refining process of previous study[26, 27]. The EPA and DHA contents of the squid oil, determined by the method of previous study[26,28] ranged between 120 to 160 and 260 to 300 mg/g, respectively. The capsules were soft gel-caps, and the squid oil was yellow in colour. The fatty acid composition of squid oil is summarized in Table 1.

Table 1 Fatty acid composition of refined squid oil[a]

Fatty acid	Area %
Myristic (C14:0)	2.71
Pentadecanoic (C15:0)	0.36
Palmitic (C16:0)	9.92
Palmitoleic (C16:1)	4.68
Heptadecanoic (C17:0)	0.42
Stearic (C18:0)	1.36
Oleic (C18:1n9c)	11.46
Linoleic (C18:2n6c)	1.60
cis-11-Eicosenoic (C20:1)	3.29
Linolenic (C18:3n3)	1.28
cis-11,14-Eicosadienoic (C20:2)	3.02
Erucic (C22:1n9)	0.31
cis-11,14,17-Eicosatrienoic (C20:3n3)	0.30
Tricosanoic (C23:0)	2.20
cis-13,16-Docosadienoic (C22:2)	1.01
cis-5,8,11,14,17-Eicosapentaenoic (C20:5n3)	15.23
Nervonic (C24:1)	0.35
cis-43,7,10,13,16,19-Docosahexaenoic (C22:6n3)	30.50

[a] Adapted from Park et al. (2011a).

Clinical and laboratory assessments

At the baseline and 60-day clinic visits, the following assessments were conducted: blood pressure; clinical chemistry (fasting serum triglyceride, total cholesterol, high-density lipoprotein (HDL)-cholesterol, albumin, total protein, glucose, creatinine, urea, AST, and ALT); and haematology (fasting erythrocytes, haemoglobin, and leukocytes).

Statistical analyses and data interpretation

Data are expressed as means ± standard deviations. For normally distributed variables, a paired *t-test* was used to determine whether changes at the end of the 60-d experimental period differed significantly from baseline within each group; an unpaired *t-test* was used to determine whether results collected at the end of the 60-d experimental period differed significantly between the squid oil and control groups. For parameters that were not normally distributed, data are presented as median (minimum, maximum), and statistical evaluations were conducted using logarithmically-transformed data. For categorical variables (such as gender), a chi-squared test was used. For all statistical analyses, a p value less than 0.05 was considered significant.

A responder was defined as a subject who achieved >10% reduction from baseline in fasting serum total cholesterol, >15% reduction from baseline in fasting serum triglycerides, and >0.014 mmol/l increase in HDL cholesterol. The proportion of “responders” in the control group *versus* the squid oil group was compared using a chi-squared test.

According to the clinical trial protocol established by the Chinese State Food and Drug Administration and published in the 2003 Technical Standards for the Testing and Assessment of Health Foods for the function of Blood Lipids Reduction, 50 subjects per group are required. Thus, to account for potential subject attrition, we aimed to recruit and enrol 52 subjects per group.

Results

Subjects and demographics

One hundred and four subjects were randomised to either the control group or the squid oil group (52 control / 52 squid oil). There were no significant differences between the groups in gender distribution or age. The control group consisted of 23 males and 29 females, with a mean age of 54 ± 7 years. The squid oil group consisted of 26 males and 26 females, with a mean age of 54 ± 8 years. Study participants were similar with regards to baseline haematological and clinical chemistry parameters (Table 2) which were within the normal range. Subjects in the control and squid oil groups also were similar with regards to baseline lipid levels (Table 3)

Table 2. Fasting serum haematological and clinical chemistry parameters in control and squid oil groups (Mean values and standard deviations)

Parameter	Normal range	Control group (n=52)				Test group (n=52)			
		Baseline		End of trial		Baseline		End of trial	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Haematological									
Erythrocytes(X 1)	3.50 – 5.50	4.41	0.46	4.44	0.46	4.37	0.36	4.39	0.32
Leukocytes(X 1)	4.0 – 10.0	5.8	1.3	6.1	1.3	5.8	1.8	5.9	1.5
Haemoglobin(g/l)	110 – 160	135	12	136	11	138	10	139	11
Clinical chemistry									
AST (IU/l)	<40	29	13	28	13	28	10	26	8
ALT (IU/l)	<40	26	18	27	1 4	27	19	27	14
Total protein (g/l)	60.0 – 80.0	77.4	3.5	74.9	3.6	76.4	4.3	74.8	3.9
Albumin (g/l)	35.0 – 50.0	44.2	2.7	43.3	2.2	44.3	2.9	43.6	2.6
Blood sugar(μmol/l)	3.8 – 6.20	5.39	0.70	5.35	0.75	5.28	0.74	5.20	0.73
Creatinine(μmol/l)	20 - 200	82	26	78	17	84	16	76	15
Urea nitrogen(mmol/l)	2.50 – 6.85	4.99	1.46	4.84	1.40	4.44	1.15	4.20	1.22

Abbreviations: SD, standard deviation; AST, aspartate transaminase; ALT, alanine transaminase.

Table 3. Effects of squid oil on fasting serum lipids(Mean values and standard deviations)

	Number of subjects	Baseline		End of trial		Significance within groups	Proportion of subjects achieving target change[a]
		Mean	SD	Mean	SD		
Total cholesterol							
Control group	52	6.37	1.11	6.40	1.05	P=0.459	0/52 (0%)
Squid oil	52	6.38	0.85	5.65	0.70	P<0.001	35/52 (67.3%)
Significance between groups	-	P=0.977		P<0.001		-	P<0.01
Triglycerides							
Control group	52	2.66	1.30	2.70	1.20	P=0.211	2/52 (3.8%)
Squid oil	52	2.64	1.09	2.10	0.98	P<0.001	38/52 (73.1%)
Significance between groups	-	P=0.947		P=0.006		-	P<0.01
HDL cholesterol							
Control group	52	1.28	0.28	1.26	0.29	P=0.497	13/52 (25%)
Squid oil	52	1.29	0.33	1.30	0.35	P=0.726	15/52 (28.8%)
Significance between groups	-	P=0.949		P=0.573		-	P=0.658
LDL cholesterol[b]							
Control group	52	3.881		3.913		-	-

Squid oil	52	3.890	3.395	-	-

Abbreviations: SD, standard deviation; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a] The target changes for the fasting blood lipid levels were as follows: total cholesterol, >10% reduction from baseline; triglycerides, >than 15% reduction from baseline; and HDL cholesterol>0.104 mmol/l increase from baseline.

[b] Using average total cholesterol, HDL cholesterol, and triglyceride values obtained at baseline and at the end of the trial, the Friedwald equation [LDL cholesterol = total cholesterol − HDL cholesterol − Triglycerides/2.2] was used to calculate crude estimates of LDL cholesterol.

Serum lipids and lipoproteins

Fasting serum levels of total cholesterol, triglycerides, HDL-cholesterol, and low-density lipoprotein (LDL)-cholesterol before and after the 60-d trial period are summarized in Table 3. At baseline, subjects in the control and squid oil groups had similar lipid levels; by the end of the 60-day study period, total cholesterol and triglyceride levels were significantly lower in the squid oil group relative to the control group ($P<0.01$). As can be seen in Figure 1, total cholesterol and triglyceride levels decreased significantly from baseline in the squid oil group (by 11.4% and 20.5%, respectively; $P<0.001$), but were unchanged in the control group. HDL-cholesterol remained unchanged relative to baseline, both in the control and squid oil groups. Using Friedewald's equation, we calculated baseline and end-of-treatment LDL-cholesterol levels in the control and squid oil groups (Table 3). The reduction in total cholesterol levels in the squid oil group could be explained by a reduction in LDL-cholesterol levels (Figure 1)

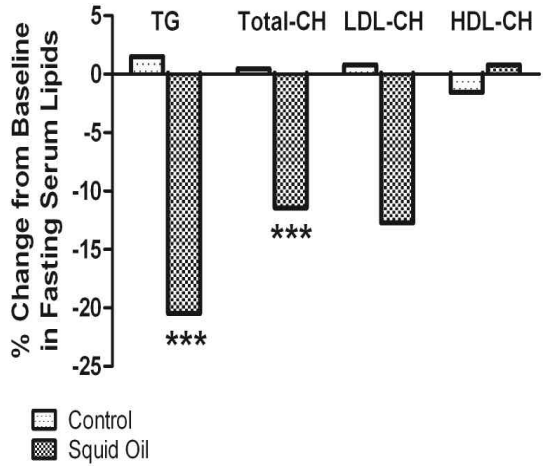

Figure legend

Figure 1 Changes from baseline in fasting serum lipid levels

The proportion of subjects achieving the target reduction in total cholesterol (>10% reduction from baseline) and triglycerides (>15% reduction from baseline) was significantly greater in the squid oil group relative to the placebo group; however, the proportion of subjects achieving the target reduction in HDL-cholesterol was similar between the squid oil and placebo groups (Table 3).

Safety

No statistically or clinically significant disparities from baseline were detected in the squid oil or control subjects in various haematological and clinical chemistry parameters (Table 2). At the end of the 60-d intervention period, all haematological and clinical chemistry parameters remained within the normal range. None of the subjects reported adverse events.

Discussion

Blanket recommendations to increase the consumption of the long-chain omega-3 fatty acids EPA and DHA have raised concerns about fish oil sustainability [27,19-20]. The oil derived from the viscera of squid is rich in DHA and EPA [24,28] thus, squid represents a possible alternate source of the long-chain omega-3 fatty acids. Moreover, squid are a sustainable source of the long-chain omega-3 fatty acids, given that they have a short lifespan of less than 450 d; mainly breed a single time in their lives and will normally perish after spawning; reach reproductive age early; have a low progeny mortality rate; and have a very efficient food to growth ratio of up to 2:1. The fisheries are managed to ensure safe levels of escapement to ensure good recruitment.

Squid oil was recently demonstrated to be safe in a 90-day toxicity study conducted in Sprague-Dawley rats, with the no-observed-adverse-effect level was found to be 1,000 mL/kg body weight/day, the highest dose tested [26]. As there have been no human feeding trials of squid oil, the objective of the current study was to examine the clinical safety of refined oil obtained from the viscera of squid (*Todarodes pacificus*). The daily consumption of 3 g of this squid oil for 60 d by male and female subjects who were hyperlipidaemic but otherwise healthy was not associated with adverse effects on blood pressure or haematological and clinical chemistry measures.

Statistically significant reductions in fasting serum lipid levels were observed in study participants supplemented with squid oil relative to control subjects. Specifically, reductions in fasting serum levels of total cholesterol and triglycerides were 11.9% and 22.0%, respectively, after correcting for the corresponding changes in the control group. HDL cholesterol levels were unaffected by squid oil supplementation, and so it can be inferred that the reduction in total cholesterol was caused by a reduction in LDL-cholesterol levels. Using the Friedewald equation, we calculated crude estimates of LDL-cholesterol levels in the squid oil and control groups, and using these data, we estimated the reduction in LDL-cholesterol to be 13.5%, after correcting for the LDL-cholesterol change in the control group.

EPA and/or DHA have been shown to statistically significantly reduce fasting serum levels of triglycerides, regardless of their marine source (*e.g.,* fish oil, krill oil, or DHA algal oil) [29-31]. While there is consensus in the scientific literature that the long-chain omega-3 fatty acids reduce fasting serum triglyceride levels, their effects on cholesterol levels are less clear. In our study, relative to the control group, the daily consumption of 1.26 g EPA+DHA resulted in a statistically significant reduction in total cholesterol, with no effect on HDL-cholesterol, thereby implying a benefit in significantly reducing LDL-cholesterol. Bunea et al. [30] reported that the daily consumption of 1.0 to 3.0 g/d krill oil resulted in statistically significant reductions in total and LDL-cholesterol, and a statistically significant increase in HDL-cholesterol, relative to a placebo. In a meta-analysis of the effects of DHA from algal oil on cardiovascular risk factors, the daily consumption of a mean dose of 1.68 g DHA was associated with statistically significant increases in LDL- and HDL-cholesterol relative to the consumption of a placebo [31]. The reasons for these discrepancies are unclear.

Wei and Jacobson performed a meta-analysis of 21 randomised, placebo-controlled trials to determine whether EPA and DHA, when used as monotherapy, have differing effects on fasting lipid levels [32]. Although both fatty acids were found to significantly reduce fasting triglyceride levels, differences between the two long-chain omega-3 fatty acids were identified, with DHA found to significantly increase LDL- and HDL-cholesterol levels, and EPA found to non-significantly reduce LDL-cholesterol levels and to elicit small and non-significant increases in HDL-cholesterol. The authors suggested that EPA and DHA may have differential effects on the regulation and transcription of genes responsible for fatty acid uptake and metabolism.

The refined squid oil used in our study contained more DHA than EPA (260 to 300 mg/g *versus* 120 to 160 mg/g, respectively). Thus, the effects observed in our study (*i.e.,* a reduction in LDL-cholesterol and no effect on HDL-cholesterol) would not have been predicted by the results of Wei and Jacobson [32]. It is unclear if the efficacy of EPA and DHA is affected by their ratio, or if there are other unrelated factors that could affect lipid outcomes. Our study was not placebo- controlled, and although subjects were asked to maintain their dietary and lifestyle behaviors, neither physical activity nor dietary intakes were assessed in our study. Thus, there always exists the possibility that there were confounding variables (*e.g.,* changes in diet, physical activity levels) in the squid oil group that could account for the reductions in total- (and LDL-) cholesterol levels. Other placebo-controlled studies of squid oil are needed in order to confirm the resulted reported herein of a significant reduction in total (and LDL-) cholesterol levels.

Squid oil is a safe source of the long-chain omega-3

fatty acids, with beneficial effects on blood lipid levels. While the significant reductions in fasting triglyceride levels with squid oil consumption were expected, the reductions in total (and LDL-) cholesterol levels were unexpected and require confirmation in future studies.

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References

1. AHA (2011) Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association [Miller M, Stone NJ, Ballantyne C et al.]. *Circulation* **123**, 2292-2333.
2. Balakumar P & Taneja G (2012) Fish oil and vascular endothelial protection: bench to bedside. *Free Radic Biol Med* (Epublication ahead of print version).
3. Balk EM, Lichtenstein AH, Chung M *et al.* (2006) Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. *Atherosclerosis* **189**, 19–30.
4. Bernstein AM, Ding EL, Willett WC *et al.* (2012) A meta-analysis shows that docosahexaenoic acid from algal oil reduces serum triglycerides and increases HDL-cholesterol and LDL-cholesterol in persons without coronary heart disease [includes supplemental material]. *J Nutr* **142**, 99–104.
5. Brunner EJ, Jones PJ, Friel S *et al.* (2009) Fish, human health and marine ecosystem health: policies in collision. *Int J Epidemiol* **38**, 93–100.
6. Bunea R, El Farrah K, Deutsch L (2004) Evaluation of the effects of Neptune Krill Oil on the clinical course of hyperlipidemia. *Altern Med Rev* **9**, 420–428.
7. Campbell F, Dickinson HO, Critchley JA *et al.* (2012) A systematic review of fish-oil supplements for the prevention and treatment of hypertension. *Eur J Prev Cardiol* (Epublication ahead of print version).
8. Cicero AF, Ertek S, Borghi C (2009) Omega-3 polyunsaturated fatty acids: their potential role in blood pressure prevention and management. *Curr Vasc Pharmacol* **7**, 330–337.
9. Cudmore WW (2009) *The Decline of Atlantic Cod – A Case Study*. Salem, OR: Northwest Center for Sustainable Resources, Chemeketa Community College. available at: http://www.ncsr.org/ Downloads/fisheriesatlanticcod.pdf.
10. Di Minno MN, Tremoli E, Tufano A *et al.* (2010) Exploring newer cardioprotective strategies: ω-3 fatty acids in perspective. *Thromb Haemost* **104**, 664–680.
11. Druon JN (2010) Habitat mapping of the Atlantic bluefin tuna derived from satellite data: Its potential as a tool for the sustainable management of pelagic fisheries. *Marine Policy* **34**, 293–297.
12. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2009) Scientific Opinion on the substantiation of health claims related to EPA, DHA, DPA and maintenance of normal blood pressure (ID 502), maintenance of normal HDL-cholesterol concentrations (ID 515), maintenance of normal (fasting) blood concentrations of triglycerides (ID 517), maintenance of normal LDL-cholesterol concentrations (ID 528, 698) and maintenance of joints (ID 503, 505, 507, 511, 518, 524, 526, 535, 537) pursuant to Article 13 (1) of Regulation (EC) No 1924/2006 on request from the European Commission. (Question No EFSA-Q-2008-1289, EFSA-Q-2008-1290, EFSA-Q-2008-1292, EFSA-Q-2008-1294, EFSA-Q-2008-1298 …, [etc.]. *EFSA J* **7**, 1263.
13. Hamazaki K, Itomura M, Huan M *et al.* (2003) n-3 Long-chain FA decrease serum levels of TG and remnant-like particle-cholesterol in humans. *Lipids* **38**, 353–358.

14. Harris WS (1997) N-3 fatty acids and serum lipopro teins: Human studies. *Am J Clin Nutr* **65**, Suppl., 164 5S–1654S.
15. He K, Song Y, Daviglus ML *et al.* (2004) Accumul ated evidence on fish consumption and coronary hear t disease mortality: a meta-analysis of cohort studies. *Circulation* **109**, 2705–2711.
16. Jenkins DJ, Sievenpiper JL, Pauly D *et al.* (2009) Are dietary recommendations for the use of fish oils sustainable? *CMAJ* **180**, 633–637.
17. König A, Bouzan C, Cohen JT *et al.* (2005) A quant itative analysis of fish consumption and coronary hea rt disease mortality. *Am J Prev Med* **29**, 335–346.
18. Kris-Etherton PM, Harris WS, Appel LJ (2002) Fish consumption, fish oil, omega-3 fatty acids, and cardio vascular disease (for the Nutrition Committee) . *Circ ulation* **106**, 2747–2757 & [erratum, 2003, **107**, 512].
19. Lee JH, O'keefe JH, Lavie CJ *et al.* (2009) Omega-3 fatty acids: cardiovascular benefits, sources and susta inability. *Nat Rev Cardiol* **6**, 753–758.
20. Li D & Hu X (2009) Fish and its multiple human health effects in times of threat to sustainability and affordability: are there alternatives? *Asia Pac J Clin Nutr* **18**, 553–563.
21. Mason F (2002) The Newfoundland cod stock colla pse: a review and analysis of social factors. *Electron Green J*, **17**. available at: http://escholarship.org/uc/it em/19p7z78s#page-1.
22. Mori TA (2010) Omega-3 fatty acids and blood pres sure. *Cell Mol Biol (Noisy-le-grand)* **56**, 83–92.
Mozaffarian D & Wu JH (2011) Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol* **8**, 2047–2067.
23. Mozaffarian D, Prineas RJ, Stein PK *et al.* (2006) Dietary fish and n-3 fatty acid intake and cardiac electrocardiographic parameters in humans. *J Am Col l Cardiol* **48**, 478–484.
24. Musa-Veloso K, Binns MA, Kocenas AC *et al.* (201 0) Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid dose-dependently red uce fasting serum triglycerides. *Nutr Rev* **68**, 155–16 7.
25. Musa-Veloso K, Binns MA, Kocenas A, *et al.* (201 1) Impact of low *v*. moderate intakes of long-chain *n*-3 fatty acids on risk of coronary heart disease. *Br J Nutr* **106**, 1129–1141.
26. Park JH, Lee H, Lee GY *et al.* (2011a) Developmen t of Refined Cuttlefish (*Todarodes pacificus*) Oil and Its Use as Functional Ingredients. *Food Sci. Biotechn ol.* **20**, 389-394.
27. Park JH, Park YC, Lee H *et al*. (2011b) Safety Evaluation of Refined Cuttlefish (*Todarodes pacificu s*) Oil. *Food Sci. Biotechnol*. **20**, 905-912.
28. Park, JH. (2017) Quality Characteristics of Refined Squid (Todarodes pacificus) Oil as an Alternative Re source for Omega-3 Fatty Acids. Journal of Food Pro cessing and Preservation, 41(2), e12780.
29. Park JH, Musa-Veloso K, Lynch B, *et al.* (2012) 13-Week oral toxicity study of oil derived from squid (*Todarodes pacificus*) in Sprague-Dawley rats. *Regul Toxicol Pharmacol* **64**, 195-204.
30. Skulas-Ray AC, West SG, Davidson MH *et al.* (200 8) Omega-3 fatty acid concentrates in the treatment of moderate hypertriglyceridemia. *Expert Opin Phar macother* **9**, 1237–1248.
31. Wei MY & Jacobson TA (2011) Effects of eicosape ntaenoic acid versus docosahexaenoic acid on serum lipids: a systematic review and meta-analysis. *Curr Atheroscler Rep* **13**, 474–483 & [Erratum, 2012, **14**, 93].
32. Whelton SP, He J, Whelton PK *et al.* (2004) Meta-a nalysis of observational studies on fish intake and coronary heart disease. *Am J Cardiol* **93**, 1119–1123.