Hypolipidemic and antioxidant activities of oil from Antarctic krill (*Euphausia superba*)

Shuo Zhang
Jiangsu Ocean University
Li Chen (chenlhhit@sina.com)
Jiangsu Ocean University

Research

Keywords: Antarctic krill, hypolipidemic activity, antioxidant activity, lipids

Posted Date: March 23rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-18681/v1

Abstract

Background: This study aims to determine the hypolipidemic and antioxidant activities of oil from Antarctic krill (AK) (*Euphausia superba*) in rats.

Methods: Thirty-six rats were randomly assigned to three groups fed with diets containing high-fat (HF), AK oil (AKO) or normal fat for 6 weeks.

Results: Compared with the HF group, the AKO-diet group had decreased body weight gain, food efficiency ratio, plasma triacylglycerol and total cholesterol but increased faecal fat and cholesterol, plasma low-density lipoprotein cholesterol level and plasma phenoloxidase activity, superoxide dismutase (SOD) activity and glutathione peroxidase activity of rats.

Conclusions: These results indicated that AKO is a suitable alternative for hypolipidemic and antioxidant source for humans.

Introduction

Antarctic krill (AK) (*Euphausia superba*) lives in the Antarctic waters of the Southern Ocean. The biomass of AK is estimated to be 125–725 million tonnes and thus, it may be the most successful animal species on the planet [1]. AK plays an ecological role as the most important trophic link between primary production and vertebrate predators.

The oil extracted from AK consists of 49.30% triglycerides (TG), and 44.16% phospholipids [2]. Polyunsaturated fatty acids, such as long-chain omega-3 fatty acids, EPA and DHA account for 20.89% of the total fatty acids and have attracted considerable attention because of their health benefits [2-5]. Mao et al. [6] found that AK (*E. superba*) oil (AKO) facilitated bone formation in dexamethasone-treated mice. However, its effect on hypolipidemic and antioxidant activities was not frequently reported [7].

In this work, AKO was prepared by supercritical CO_2 extraction, and its hypolipidemic and antioxidant activities were investigated.

Materials And Methods

Materials

Frozen AK bricks were purchased from Xiangshan Han Wei Aquatic Products Co., Ltd (Ningbo, Zhejiang, China). Triacylglycerol (TG), total cholesterol (TC), low- and high-density lipoprotein cholesterol (LDL-C and HDL-C), phenoloxidase (PO), superoxide dismutase (SOD) and glutathione peroxidase (GPx) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Preparation of AKO

Frozen AKs were thawed, dried at 80 °C to a constant weight and crushed into a fine paste. AKO was prepared through supercritical CO_2 extraction (Model: HA 420-40-96, Jiangsu Huaan Scientific Instrument Co., Ltd., Jiangsu, China) under the following conditions: dried AKs, 20 g; approximate size of dried AKs, 0.49 mm; supercritical CO_2 flow rate, 20 L/min; pressure, 25 MPa; temperature, 35 °C; and time, 70 min.

The FFA content of AKO was assayed according to the method of Natseba et al. [8]. Fatty acid composition was determined according to a previous technique [9].

Test animals

Thirty-six male Sprague–Dawley rats with a mean body weight of 108 ± 7 g were used. The rats were fed individually in a metabolic cage equipped with a glass separator for faeces and urine collection and kept in an air-conditioned room maintained at 21 ± 1 °C in a 12-h light and dark cycle. All animal protocols were approved by the Institutional Animal Care and Use Committee of Jiangsu Ocean University (Jiangsu, China). The rats were allowed free access to water and food.

After being fed with commercial diet and water for 1 week, the 36 rats were randomly assigned to three groups (12 rats per group): normal-fat (NF) group fed with commercial diet conforming to GB14924.3; high-fat (HF) control group fed with HF diet containing 10% (w/w) lard, 15 % (w/w) egg yolk powder, 1% (w/w) cholesterol and 100% (w/w) commercial diet; and AKO group fed with 95% (w/w) HF diet plus 5% (w/w) AKO. The diets were prepared by blending the powdered commercial basal diet with other ingredients and test materials and subsequently pelleting the food.

Experimental design

The rats were starved overnight, and blood was collected to determine the initial plasma lipid levels and PO, SOD and GPx activities before the experiments. Food intake (FI) was recorded daily. Body weights were recorded initially and every other day. The body weight gains (WGs) of the rats after 6 weeks of experiments were calculated and recorded. The faeces of rats were collected for 3 continuous days of the 6th week and dried at 100 °C for 2 h to determine the fat and cholesterol contents. All rats were given diethyl ether after fasting for 18 h. The blood was collected, and the plasma was obtained through centrifugation at 3,000 × g for 15 min. The plasma samples were stored in a freezer at -18 °C until further analysis.

Serum biochemical assay

Plasma TG, TC, LDL-C and HDL-C levels and PO, SOD and GPx activities were measured in triplicate using commercial assay kits.

Faecal fat and cholesterol

The dried faeces were pulverised and weighed, and their fat content was determined gravimetrically by Soxhlet method with diethyl ester as the solvent [10]. For faecal cholesterol determination, faecal lipid

was first extracted from 100 mg of dried faeces with 2 mL of chloroform-methanol (2:1 v/v) according to the method of Folch et al. [11]. The lower phase was dried under nitrogen gas. Faecal cholesterol was extracted as previously described [12].

Statistical analysis

All data were presented as mean ± S.D. ANOVA was used to compare the means of the two groups. Statistical significance at 95% and 99% probability levels were set at $p < 0.05$ and $p < 0.01$, respectively.

Results And Discussion

AKO characterisation

The fatty acid composition of AKO is presented in Table 1. The total saturated, polyunsaturated and polyunsaturated fatty acid contents were 37.94%, 40.16% and 19.70%, respectively. Total polyunsaturated fatty acids accounted for 17.90% of the total fatty acids which was similar to the results obtained by Yin et al. [2].

Body WG, FI and food efficiency ratio (FER)

No significant differences were observed in the FI of all groups ($p > 0.05$). HF diet increased the WG and FER of the rats compared with those of the NF control group (Table 2, $p < 0.05$). The WG and FER of the rats in the AKO group were lower than those of the HF group and comparable with those of the NF group. This result could be due to the hypolipidemic activities of the unsaturated fatty acids of AKO.

Faecal fat and cholesterol

The fat and cholesterol contents in the dried faeces collected for 3 continuous days of the 6th week are presented in Table 3. The AKO-treated group had increased faecal fat and cholesterol contents compared with the HF and NF groups ($p < 0.05$). This phenomenon occurred because AKO itself is a lipid and therefore has high fat and cholesterol binding capacity due to hydrophobic interaction.

Plasma lipid

HF diet increased the plasma TC, TG and LDL-C levels and decreased HDL-C level compared with the NF control group (Table 4, $p < 0.05$). However, AKO diet decreased the plasma TC, TG and LDL-C levels and decreased the HDL-C level compared with the HF control (Table 4, $p < 0.05$). Nevertheless, the plasma TC, TG and LDL-C levels of the AKO group were higher than those of the NF group (Table 4, $p < 0.05$), indicating that AKO cannot completely counteract the blood lipid increasing effects of HF diet. Similarly, Sun et al. [7] revealed that AKO decreases the LDL-C levels of rats fed with HF diet.

PO, SOD and GPx activities

The effects of different diets on the serum PO, SOD and GPx activities of rats are shown in Table 5. The PO, SOD and GPx activities of rats with HF diet were decreased compared with those of the NF control ($p < 0.05$). However, the AKO-diet group had increased PO, SOD and GPx activities compared with the HF and NF groups ($p < 0.05$). This finding could be due to the antioxidant activities of the unsaturated fatty acids of AKO, indicating that AKO diet could inhibit organ damage from lipid peroxidation.

Acknowledgements

This research was supported by A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

References

[1] Lee HY, Haque ASMT, Kim SB, Lee YB, Chun BS. Effect of reaction parameters on conversion of krill (*Euphausia superba*) oil by immobilized lipase ethanolysis. J Ind Eng Chem 2014;20:1097–1102

[2] Yin FW, Liu XY, Fan XR, Zhou DY, Xu WS, Zhu BW, Mura YY. Extrusion of Antarctic krill (*Euphausia superba*) meal and its effect on oil extraction. Int J Food Sci Technol 2015;50:633–639

[3] Haraldsson GG, Thorarensen A. Preparation of phospholipids highly enriched with n-3 polyunsaturated fatty acids by lipase. J Am Oil Chem Soc 1999;76:1143-1149

[4] Kidd PM. Omega-3 DHA and EPA for cognition, behavior, and mood: clinical findings and structural-functional synergies with cell membrane phospholipids. Altern Med Rev 2007;12:207-227

[5] Suzuki T, Shibata N. The utilization of Antarctic krill for human food. Food Rev Int 1990;6:119-147

[6] Mao L, Wang F, Li YY, Dai YF, Liu YJ, Wang JF, Xue CH. Oil from Antarctic krill (*Euphausia superba*) facilitates bone formation in dexamethasone-treated mice. Food sci biotechnol 2019;28:539-545

[7] Sun DW, Zhang L, Chen HJ, Feng R, Cao PR, Liu YF. Effects of Antarctic krill oil on lipid and glucose metabolism in C57BL/6J mice fed with high fat diet. Lipids Health Dis 2017;16:218

[8] Natseba A, Lwalinda I, Kakura E, Muyanja C, Muyonga J. Effect of pre-freezing icing duration on quality changes in frozen Nile perch (*Lates niloticus*). Food Res Int 2005;38:469–474

[9] Ramírez MR, Morcuende D, Estévez M, López RC. Fatty acid profiles of intramuscular fat from pork loin chops fried in different culinary fats following refrigerated storage. Food Chem 2005;92:159–167

[10] Deuchi K, Kanauchi O, Imasato Y, Kobayashi E. Decreasing effect of chitosan on the apparent fat digestibility by rats fed on a high fat diet. Biosci Biotech Bioch 1994;58:1613-1616

[11] Folch J, Lee M, Stanley HS. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;22:497-509

[12] Zhao HL, Cho KH, Ha YW, Jeong TS, Lee WS, Kim YS. Cholesterol-lowering effect of platycodin D in hypercholesterolemic ICR mice. Eur J Pharmacol 2006;537:166-173

Tables

Table 1 Free fatty acid composition of Antarctic krill oil. SFA, saturated fatty acid; MUSFA, polyunsaturated fatty acid; PUSFA, polyunsaturated fatty acid.

Free fatty acid	Content (%)
C14:0	15.92 ± 0.35
C16:0	20.28 ± 0.31
C18:0	1.74 ± 0.02
C16:1	12.59 ± 0.13
C18:1	27.57 ± 0.32
C18:2	4.64 ± 0.03
C18:3	2.47 ± 0.02
C20:5	8.43 ± 0.16
C22:6	4.16 ± 0.09
Total SFA	37.94 ± 1.39
Total MUSFA	40.16 ± 1.16
Total PUSFA	19.70 ± 0.96

Values are the mean ± SD (n = 3).

Table 2. Body weight gain of rats (WG), Food intake (FI), food efficiency ratio (FER) of rats fed on high fat (HF), AK oil, and normal fat diets (NF).

Parameters	Dietary group		
	HF	AK oil	NF
WG (g)	14.2 ± 0.6^{a}	9.4± 0.4^{b}	9.1 ± 0.4^{b}
FI	128.4 ± 5.8^{a}	125.8 ± 4.9^{a}	126.3 ± 5.3^{a}
FER	11.1 ± 0.6^{a}	7.4 ± 0.4^{b}	7.2 ± 0.4^{b}

Values are expressed as mean ± SE (n=6). Means with different superscripts within a row indicate significant differences ($p<0.05$).

Table 3. Fecal fat and cholesterol excretion of rats fed on high fat (HF), Antarctic krill (AK) oil, and normal fat (NF) diets.

Parameters (g/100g dry weight)	Dietary group		
	HF	AK oil	NF
Fecal fat	10.1 ± 0.42^{b}	13.2± 0.53^{a}	7.9 ± 0.41^{c}
Fecal cholesterol	18.6 ± 0.64^{b}	21.5 ± 0.79^{a}	10.4 ± 0.61^{c}

Values are expressed as mean ± SD (n = 12). Means with different superscripts within a row indicate significant differences ($p < 0.05$).

Table 4. Plasma lipid concentrations of rats

Parameters	Dietary group		
	HF	AK oil	NF
TC (mmol/L)	3.08±0.23[a]	2.49±0.15[b]	1.93±0.11[c]
TG (mmol/L)	3.11±0.28[a]	2.51±0.16[b]	2.03±0.13[c]
LDL-C (mmol/L)	0.81±0.06[a]	0.71±0.05[b]	0.59±0.04[c]
HDL-C (mmol/L)	0.59±0.14[b]	0.78±0.21[a]	0.76±0.18[a]

Values are expressed as mean ± SE (n=6). Means with different superscripts within a row indicate significant differences ($p<0.05$).

Table 5. Phenoloxidase (PO) activity, superoxide dismutase (SOD) activity and glutathione peroxidase (GPx) activity of rats

Parameters	Dietary group		
	HF	AK oil	NF
PO activity (O.D. 490 nm)	0.52±0.06[c]	1.03±0.11[a]	0.74±0.09[b]
SOD (U mg protein^{-1})	36.17±2.28[c]	64.58±4.62[a]	51.43±3.16[b]
GPx (U g protein^{-1})	95.21±7.24[c]	156.72±14.18[a]	121.73±11.71[b]

Values are expressed as mean ± SE (n=6). Means with different superscripts within a row indicate significant differences ($p<0.05$).