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Hepatoprotective and Anti-Obesity Properties of Sardine By-Product Oil in Rats Fed a High-Fat Diet

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ABSTRACT: Excess lipid intake can trigger liver lipid accumulation and oxidative responses, which can lead to metabolic disturbances and contribute to hepatic steatosis and obesity and increase the risk of cardiovascular disease. Production of fish oil rich in omega-3 is a good opportunity for valorizing fish by-products in the therapeutic field. In this study, we explored the effects of oil from *Sardina pilchardus* by-products on cardiometabolic and oxidative disorders caused by toxic effects of excess lipids in obese rats. Three groups of obese rats received either 20% sardine by-product oil (SBy-Ob-HS; experimental group), 20% fillet oil (SF-Ob-HS; positive control group), or a high-fat diet (Ob-HS). Normal weight rats received a standard diet (normal). There was a significant decrease in serum total cholesterol (TC), triacylglycerols (TG), and insulin concentrations in the SBy-Ob-HS group compared with the SF-Ob-HS group. Compared with the Ob-HS group, TC and TG, glycemia, glycosylated hemoglobin, and insulinemia were decreased in the SBy-Ob-HS (more notably) and SF-Ob-HS groups. Furthermore, hepatic lipids, low density lipoprotein-cholesterol (C), the non-esterified cholesterol/phospholipids ratio, serum transaminases activities and lipid peroxidation were lower and serum high density lipoproteins-C were higher in the SBy-Ob-HS and SF-Ob-HS groups compared with the Ob-HS group. Serum isoprostane concentrations were reduced in the SBy-Ob-HS (more notably) and SF-Ob-HS groups compared with the Ob-HS and normal groups. The activities of antioxidant enzymes in tissues were enhanced, particularly in the by-product oil group. The oil extracted from by-products demonstrate anti-obesity properties (hypolipemiant, hepatoprotective, antiatherogenic, antidiabetic, and antioxidant) that may be beneficial for the management of obesity and its complications, such as hepatic steatosis.

Keywords: by-products, hepatic lipid accumulation, obesity, oil, *Sardina pilchardus*

INTRODUCTION

High-fat diets induce obesity, hyperinsulinemia, dyslipidemia, hyperglycemia, hypertension, and liver damage in animals comparable to the phenotype observed in humans with non-alcoholic liver steatosis (NAFLD) (López et al., 2003; White et al., 2013; Recena Aydos et al., 2019). High-fat diets also affect the function of many organs in the body such as the liver, adipose tissue, heart, and kidney (Vincent and Taylor, 2006). Excess lipid intake can trigger liver lipid accumulation and an oxidative response, leading to metabolic disturbances that contribute to obesity and its complications, such as hepatic steatosis, which increases cardiovascular disease risk. In response to a hyperlipidic diet (HLD), rats develop obesity, characterized by weight gain, reduced satiety, increased blood and hepatic lipids disorders (Affane et al., 2018a; Louala and Lamri-Senhadji, 2019), and oxidative stress, particularly in adipose tissue (Hamza-Reguig et al., 2017).

The fisheries and aquaculture sector is one of the most important sectors of food production worldwide. Indeed, the fish industry includes several production processes, such as filleting, curing, salting, smoking, and canning. (Shahidi, 2006). World fish production was estimated at 179 million tons in 2018. However, only one part of the industry is used for direct human consumption, which generates a significant amount of waste, estimated at more than 50% of the total volume (FAO, 2020).

Production of high quality fish oil has great importance since marine oils rich in essential fatty acid omega-3 polyunsaturated fatty acids (PUFA; n-3), especially eicosapentaenoic acid (EPA; 20:5 n-3), and docosahexaenoic acid (DHA; 22:6 n-3), are increasing in popularity in many areas, such as pharmaceuticals and cosmetics industries

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(Rubio-Rodríguez et al., 2012; Huang et al., 2018). In addition, nutraceutical foods from natural sources (e.g., marine products) may help protect against the harmful effects of many chronic diseases. In the context, EPA and DHA have been considered to be functional foods, and are used as food supplements (Jenkins et al., 2008; Kaur et al., 2014; Huang et al., 2018).

Fisheries and aquacultures provide essential nutrition, and various compounds can be recovered from waste from the canning industries and used in the food and pharmaceutical industries, such as fish heads, skins, visceras, tails, and flesh. In order to be valorized, by-products must be considered noble materials in the same way as finished products. These by-products are very rich in lipids, minerals, enzymes, protein hydrolysates, and bioactive peptides (Ferraro et al., 2013), and large quantities of the by-products have great potential for extraction of biologically desirable valuable compounds. Most by-products are intended for use in flours and oils, the rest are distributed between protein hydrolysates, minced meats, and frozen foods. According to the International Fishmeal and Fish Oil Organization, in 2010 93% of these oils were intended for aquaculture, 15% for human nutrition and 2% for pharmaceutical use (IFFO, 2013).

Small pelagic species of fish, such as pilchard *Sardina pilchardus*, Walbaum 1792 (Clupeidae), are important Mediterranean commercial species and are essential food in the Mediterranean (Palomera et al., 2007). In Maghreb, Algeria, the sardine is the most important fish product in terms of sales volume. Indeed, sardines are widely consumed in the main urban centers of the country. According to the Ministry of Fisheries and Fishery Resources, 102,220 tons in 2013 of fish species (constituting 80% small pelagic fish such as sardines and anchovies and 20% of other species such as molluscs and crustaceans) were captured by the Algerian fish fleet in 2013 (MFMR, 2014).

Sardines are major sources of fish oils and contain high contents of bioactive lipid omega-3 PUFAs, including EPA and DHA (Benyahia-Mostefaoui et al., 2014). Production of omega-3-rich fish oil is a good opportunity for valorizing sardine by-products. It is well demonstrated that fish oil extracted from the edible part of fish (muscle or fillet) has great potential in therapeutic and nutraceutical applications to combat important public health problems, especially in cardiovascular diseases. In contrast, the benefits of oil extracted from fish waste are not well demonstrated and still arouse reluctance for their consumption. In addition, very little has been reported on the content and composition of fatty acids in oils from fish waste compared with oils from fillets. Furthermore, few studies have reported the anti-diabetic, anti-atherogenic, anti-obesity, and antioxidant properties of sardine by-product oil (Boukhari et al., 2013), and there are no studies examining the effects of sardine by-product oil on preventing NAFLD progression and obesity-associated cardiovascular disease (CVD) risk. The few studies on by-products include investigation of bioactive peptide from *Oreochromis niloticus* skin gelatin: gelatin from fish by-products (Lee et al., 2017), peptides derived from fish viscera protein hydrolysate (*Mustelus mustelus*), and proteins and fish skin gelatin hydrolysates (Halldorsdottir et al., 2014; Choonpicharn et al., 2015; Villamil et al., 2017) in metabolic syndrome. Other studies have investigated the therapeutic effects [against hypertension, insulin resistance (IR), and CVDs] of commercial fish oils, in particular oils from cod, salmon, herrings, and krill.

It is currently not clear whether dietary consumption of sardine oil derived from by-products improves metabolic factors associated with obesity. Furthermore, the effect of sardine by-product oil on overall liver function, including activities of hepatic lipids and aminotransferases, membrane fluidity, lipoprotein profiles, and the pro/antioxidant balance (in both the liver and adipose tissue) is unclear.

The present study was designed to valorize sardine by-products oil by exploring their possible beneficial effects on serum levels of aminotransferases, hepatic lipid accumulation, glycemic and lipidic disorders, and oxidative damage induced by toxic effects of excess dietary lipids in rats fed a high-fat diet. To our knowledge, this is the first study to examine these parameters in rats fed a high-fat diet.

MATERIALS AND METHODS

Sardine oil extraction

Fresh sardines (*Sardina pilchardus*) were purchased daily from a local public fish market. Sardine oil was extracted from Sardines muscle (fillet) and by-products (head, scales, and viscera) according to a lipidic process of extraction (Métailler and Guillaume, 1999). By-products and fillets were previously washed and pressed. The general principle of oil extraction consists of separating water and oil from the dry matter. By-products and fillets were removed from fresh sardines, heated in an oven at 80～85°C [Tau Steril (S.N.C.), Fino Mornasco, Italy] for 20 min for the first separation [between a solid phase (coagulated proteins) and a liquid phase (water and oil)], and pressed. The press water was elutriated and centrifuged at 3,000 *g* (Centrifuge 5702, Eppendorf Corporate, Hamburg, Germany) to separate the oil.

Lipid and fatty acids (FAs) composition of sardine (fillet and by-product) oil

FA methyl esters were prepared by saponification with a methalonic-NaOH mixture followed by transesterification

in methanol (Morrison and Smith, 1964). Acid methyl ester (C23:0) was added as an internal standard. FA determination was conducted using gas chromatography (PerkinElmer, Inc., Waltham, MA, USA) equipped with a flame ionization detector and Omegawax 320 fused silica capillary column [30 m×0.25 mm (length×internal diameter)]. The separation was carried out with helium as the carrier gas (1.2 mL/min) using 3 mL of injected material. FAs were identified by comparing retention times with those of the standard 37 component FA mixture. Peak areas were determined using Varian software (Varian Medical Systems, Inc., Palo Alto, CA, USA).

Animal experiments

Male Wistar rats (Pasteur Institute of Algeria, Dély Ibrahim, Algérie) weighing 120±10 g were housed in stainless steel cages at 23±1°C and 55±5% humidity with a 12-h light/dark cycle. Obesity was induced with 20% mutton fat for 90 days. After this period, 24 obese rats (body weight of 400±20 g) were divided into three homogeneous groups: (1) consumption of 20% sardine by-products oil (inedible part) for 4 weeks; (2) consumption of sardine fillet oil (edible part) for 4 weeks (positive control); and (3) consumption of the HLD for 4 weeks (obese control). The normoponderal rats (normal group; n=8) consumed a standard diet throughout the study and served as a reference.

Body weight was measured once a week and food intake was estimated daily. Water and food were given *ad libitum* throughout the experiment. The diets were prepared in our laboratory and details of their compositions are shown in Table 1. The experimental procedure was approved by the Ethics Committee of our Institution (University Oran 1 Ahmed Ben Bella, Oran, Algérie) and the MESRS (Algerian Ministry of Higher Education and Scientific Research CNEPRU Code F01820120018; Approval number (LNCMW0911000/UO-CEEA-2016). The general councils concerning the animal experimentation were the European Directive 2010/63/EU on the protection of animals used for scientific purposes and laws on the welfare of animals, and General guidelines for the care and use of laboratory animals recommended by the Council of the European Union (1986). The Council Directive 86/609/EEC was strictly followed.

Food efficiency ratios (FERs) were calculated according to the following equation: FER=[body weight gain (g)/food intake (g)]×100 (De Sibio et al., 2013). Liver relative weight (RW) was determined according to the formula following: organs RW=[organ weight (g)/body weight (BW, g)]×100. Adiposity indexes were calculated as (total body fat/final BW)×100.

Blood and tissue collection

On day 28 of the study, after 12 h of fasting, 8 rats from each group were anesthetized with sodium pentobarbital (60 mg/kg BW) (Coopération Pharmaceutique Française, Melun, France). Blood was extracted from the abdominal aorta into dried tubes and serum was prepared by low-speed centrifugation at 1,000 *g* for 20 min at 4°C (Sigma 4K10, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). Serum aliquots were preserved in tubes containing 0.1% (W/V) Na_2ethylenediaminetetraacetic acid and 0.02% (W/V) sodium azide for assessment of biological parameters.

Urine were collected daily during the last week (days 21 to 28) using an antiseptic (10% thymol-isopropyl), filtered, and stored at 4°C. Liver and adipose tissue samples (total body fat was estimated by the sum of epididymis, retroperitoneal, and visceral fats) were homogenized for 5 min in Tris-buffered saline buffer (50 mM Tris-HCl and 150 mM NaCl, pH 7.4). Then, the homogenates were centrifuged at 1,000 *g* for 15 min and the supernatants were collected and used for biochemical estimations. All processes were carried out at 4°C.

Table 1. Composition of diets (g/kg diet)[1)]

Ingredients	Sardine fillet oil diet	Sardine by-product oil diet	Hyper-lipidic diet	Stand-ard diet
Casein[2)]	200	200	200	200
Corn starch[3)]	450	450	450	600
Sucrose[4)]	40	40	40	40
Sardine fillet oil[5)]	200	–	–	–
Sardine by-products oil[6)]	–	200	–	–
Mutton fat[7)]	–	–	200	–
Sunflower oil[8)]	–	–	–	50
Cellulose[9)]	50	50	50	50
Mineral mix[10)]	40	40	40	40
Vitamin mix[11)]	20	20	20	20

[1)]Semi-synthetic diets were prepared in our laboratory.
[2)]Prolabo, Fontenay/bois, France.
[3)]ONAB, Sidi Bel Abbes, Algeria.
[4)]Cevital SPA, Bejaia, Algeria.
[5,6)]Oil were purified from sardines in our laboratory.
[7)]Mutton fats were obtained from a local market.
[8)]Sunflower oil was obtained from Cevital SPA, Bejaia, Algeria.
[9)]Prolabo-Fontenay/S/bois, France.
[10)]UAR 205 B, Villemoisson. 91360. Epinay/S/Orge, France.
[11)]UAR 200, Epinay/S/Orge, France.

Biochemical analyses

***Glucose, insulin, and haemoglobin measurements*:** Glucose was measured by strip tests (Accu-Chek® Active, Roche Diabetes Care, Inc., Indianapolis, IN, USA). The percentage of glycosylated haemoglobin (HbA1c) was estimated by ion exchange chromatography kit (Biosystems, Barcelona, Spain). Insulin concentrations were measured by immuno-enzymatic kits (Bertin Bioreagent, Montigny-le-Bretonneux, France).

***Transaminase activity evaluation*:** Serum aspartate amino-

transferase (ASAT) and alanine aminotransferase (ALAT) activities were evaluated using a colorimetric method (Chronolab Systems, Barcelona, Spain).

***Serum and liver lipids measurements*:** Serum total cholesterol (TC) and triacylglycerols (TG) concentrations were evaluated using enzymatic colorimetric methods [glicerol-3-oxidasa (GPO)-peroxidasa (POD) kit, Spinreact, Girona, Spain]. In the liver, the amounts of total lipids (TL) were estimated using the Folch technique (Folch et al., 1957). TG contents were assessed by enzymatic colorimetric methods (GPO-POD kit, Spinreact) and non-esterified cholesterol (NEC) concentrations were evaluated using enzymatic methods [cholesterol oxidase (CHOD)-phenol 4-aminoantipyrine peroxidase (PAP); Biolabo S.A.S., Maizy, France]. Phospholipid (PL) concentrations were determined by enzymatic determination [cholesterol (CHO)-POD kit, Cypress Diagnostics, Hulshout, Belgium], and non-esterified fatty acid (NEFA) contents were estimated using the enzymatic colorimetric method (Sigma-Aldrich Co., St. Louis, MO, USA). The NEC/PL ratio was calculated to estimate membrane fluidity.

Lipoprotein fractions [high-density lipoprotein (HDL) and low-density lipoprotein (LDL)] were separated by precipitation methods (Burstein et al., 1970; Burstein et al., 1989). The cholesterol content of each fraction was evaluated by the enzymatic colorimetric method (CHOD-POD kit, Spinreact).

***Measurements for lipid peroxidation in tissues*:** Lipid peroxidation in livers and adipose tissues was estimated by measuring levels of thiobarbituric acid reactive substances (TBARS) (Quintanilha et al., 1982) using tetraethoxypropane (Prolabo SA, Fontenay-Sous-Bois, France) as a precursor of malondialdehyde (MDA), the principal marker of free radicals determination. Tetraethoxypropane reacts in acid and heat with two molecules of thiobarbituric acid (TBA) to give a pink colored complex that has an absorbance wavelength of 532 nm. The amount of TBARS was calculated using an extinction coefficient of 1.56×10^5 $M^{-1}\cdot cm^{-1}$ and expressed as nmol of MDA/mg of protein.

Lipid hydroperoxides (LPO) concentrations (another marker of lipid peroxidation) in livers and adipose tissues were estimated by an enzymatic method (Cayman Chemical, Ann Arbor, MI, USA). This method is based on a redox reaction with the ferrous ion.

Isoprostanes contents in serum and urine were determined by a competitive immunoenzymatic method (enzyme-linked immunosorbent assay kits, Oxford Biomedical Research, Metamora, MI, USA).

***Measurements for antioxidant enzyme activities in tissues*:** Glutathione peroxidase (GSH-Px; EC. 1.11.1.9), glutathione reductase (GSSH-Red; EC. 1.6.4.2) and superoxide dismutase (SOD; EC. 1.15.1.1) activities were determined using an enzymatic colorimetric method (Cayman Chemical). Catalase (CAT; EC. 1.11.1.6) activity was estimated by Aebi's method (Aebi, 1974). The enzyme activities were calculated via microplate titration with the microplate titrator iEMS Reader MF (Kirial International SA, Couternon, France).

Statistical analysis

Data were expressed as mean±standard error of mean (SEM) for 8 rats per group. Data were analyzed by the one-way analysis of variance (ANOVA), and differences between means were assessed using Duncan's new multiple range tests (Duncan, 1955) at $P<0.05$.

RESULTS

FA composition of sardine by-product and fillet oils

Lipid and FA (FAs as a percentage of the total FAs) compositions of sardine fillet and by-product oils are shown in Table 2. The distribution of SFA, MUFA, and PUFA in the of sardine by-product oil were 34.9%, 23.2%, and 41.9%, respectively (Table 2). Of the SFAs, palmitic acid (C16:0) accounted for 58% of the total SFA; palmitoleic acid (C16:17), oleic acid (C18:19) and MUFA were present in similar amounts. The PUFA percent in the sardine by-product oil was 1.2- and 1.8-fold higher than the percentage of SFA and MUFA, respectively. n-3 PUFA accounted for the largest share of PUFAs (76.4%), particularly EPA and DHA. EPA alone accounted for 34% of PUFAs and DHA for 32%. The FA distribution of the sardine fillet oil was 34.7%, 20.0%, and 35.6% for SFAs, MUFAs, and PUFAs, respectively (Table 2). In sardine fillet oil, the amount of PUFA was 1.8-fold higher than MUFA, and the n-3 PUFAs mainly included EPA and DHA (12.4% and 8.0%, respectively).

Sardine by-product oil contains the same proportion of SFAs as sardine fillet oil, whereas the MUFA and PUFA contents were 1.2-fold higher. In addition, the by-product oil was richer in EPA and DHA (14.3% and 13.6%, respectively) than the fillet oil.

Growth parameters and adiposity indexes

Diets including sardine fillet and by-product oils induced similar decreases in BW of obese rats (of 12%) compared to the HFD. This reduction was accompanied by a decrease in weight gain, food intake, and FER ($P<0.05$) (Table 3).

Liver RW was reduced in both the SF-Ob-HS and SBy-Ob-HS groups (−14% and −19%, respectively) compared with the Ob-HS group, but remained elevated compared with the normal group. Adiposity indexes were lower in the SBy-Ob-HS group compared with the positive control and obese control groups (−19% and −15%, respectively).

Glycemic and lipidic markers

At day 28, there was a decrease in glucose and HbA1c values in the SF-Ob-HS and SBy-Ob-HS groups compared with the Ob-HS group ($P<0.05$) (Table 4). However, compared to the normal group, glucose and HbA1c remain elevated in both the SF-Ob-HS and SBy-Ob-HS groups ($P<0.05$) (Table 4).

Insulin levels were lower in the SBy-Ob-HS group (−33%) compared with the SF-Ob-HS and Ob-HS groups, but increased by 50% compared with the normal group (Table 4).

Compared with the Ob-HS group, serum TC levels were reduced in the SF-Ob-HS and SBy-Ob-HS groups by 54% and 58%, respectively (Table 4). Moreover, the serum TC levels of the SBy-Ob-HS group was reduced compared with the SF-Ob-HS group.

TG levels in the SF-Ob-HS and SBy-Ob-HS groups were reduced by 52% and 69%, respectively, compared with the Ob-HS group. Furthermore, the TG levels of the SBy-Ob-HS group was 36% lower than that of the SF-Ob-HS group (Table 4).

Table 2. Fatty acid (FA) compositions of sardine by-product and fillet oils[1)] (unit: %)

Common name of fatty acid	Nomenclature	Sardine fillet oil	Sardine by-product oil
Saturated FA			
Myristic acid	C14:0	7.2	10.2
Pentadecanoic acid	C15:0	ND	0.9
Palmitic acid	C16:0	23.9	20.3
Stearic acid	C18:0	3.5	1.6
Arachidic acid	C20:0	ND	0.5
Heptadecanoic acid	C21:0	ND	0.2
Behenic acid	C22:0	ND	0.2
Others		–	0.9
Total		34.7	34.9
Monounsaturated FA			
Palmitoleic acid	C16:1n-7	5.0	11.2
Oleic acid	C18:1n-9	14.2	11.2
Gadoleic acid	C20:1n-9	0.8	0.8
Total		20.0	23.2
Polyunsaturated FA			
Linoleic acid	C18:2n-6	2.2	2.9
Methyl γ-linolenate	C18:3n-6	3.0	2.7
α-Linolenic acid	C18:3n-3	0.6	0.9
Eicosatetraenoic acid	C20:4n-3	0.8	0.1
Arachidonic acid	C20:4n-6	0.4	0.6
Eicosapentaenoic acid	C20:5n-3	12.4	14.3
Docosapentaenoic acid	C22:5n-3	2.8	1.8
Docosahexaenoic acid	C22:6n-3	8.0	13.6
Others		5.4	5.0
Total		35.6	41.9
Total FA unsaturated (%)		55.6	65.1

[1)]Fatty acids as percentages of total fatty acids.
ND, non-detected.

Transaminases activities, membrane fluidity, and hepatic and serum lipids profiles

Compared with the Ob-HS group, serum ALAT and ASAT activities were decreased by 44% and 8%, respectively, in the SF-Ob-HS group, and by 49% and 16%, respectively, in the SBy-Ob-HS group (Table 5). Moreover, the value noted for the SBy-Ob-HS group was slightly lower that of the SF-Ob-HS positive control group (Table 5).

There was a significant decrease in the liver TL contents of the SF-Ob-HS and SBy-Ob-HS groups compared with the Ob-HS (−23% and −30%, respectively) and normal (−9% and −17%, respectively) groups. Similarly, decreases in hepatic TG (−54%), NEC (−81%), and NEF (−27%) levels were observed in the SBy-Ob-HS group compared with the Ob-HS group. In contrast, the liver PL contents was higher in SF-Ob-HS and SBy-Ob-HS

Table 3. Effect of 4 weeks sardine by-product oil diet on growth parameters and adiposity indexes in diet-induced obese rats

Parameters	Normal	Ob-HS	SF-Ob-HS	SBy-Ob-HS
Initial body weight (g)	119.50±10.80	122.11±5.01	120.05±10.10	119.10±11.02
Final body weight (g)	326.01±13.05^{c}	431.11±17.08^{a}	380.21±11.10^{b}	378.17±9.01^{b}
Food intake (g/d/rat)	24.01±0.56^{c}	29.50±1.49^{a}	27.50±0.23^{b}	26.62±0.13^{b}
Weight gain (g/d/rat)	1.60±0.92^{a}	1.03±0.31^{b}	−0.78±0.80^{c}	−0.76±0.70^{c}
FER (%)	6.66±0.20^{a}	3.49±0.10^{b}	−2.84±0.61^{c}	−2.86±0.55^{c}
Organ weights				
Liver				
Absolute weight (g)	7.95±0.60^{c}	15.17±0.60^{a}	11.51±0.47^{b}	10.81±0.23^{b}
Relative weight (%)	2.79±0.16^{d}	3.52±0.30^{a}	3.03±0.20^{b}	2.86±0.19^{c}
Adipose tissue				
Absolute weight (g)	4.46±1.54^{d}	16.20±1.20^{a}	14.88±1.52^{b}	11.81±0.95^{c}
Adiposity index (%)	1.37±0.44^{c}	3.76±0.13^{a}	3.92±0.49^{a}	3.18±0.30^{b}

Data show mean±SEM of 8 rats per group.
Means with different letters (a-d) in the same row are considered significantly different ($P<0.05$).
Normal, normal control group; SBy-Ob-HS, obese group fed sardine byproducts oil; SF-Ob-HS, obese group fed sardine fillet oil; Ob-HS, obese control group.
Food efficiency ratio (FER)=[body weight gain (g)/food intake]×100.
Adiposity index=total body fat/final body weight×100.

Table 4. Effect of 4 weeks sardine by-product oil diet on glycemic and lipids markers in diet-induced obese rats by high fat diet for 90 days

Parameters	Normal	Ob-HS	SF-Ob-HS	SBy-Ob-HS
Glucose (mmol/L)	4.75±0.33^{c}	12.80±0.11^{a}	10.89±0.20^{b}	10.69±0.20^{b}
HbA1c (%)	2.48±0.41^{c}	8.90±0.81^{a}	7.39±0.11^{b}	7.78±0.04^{b}
Insulin (ng/mL)	0.20±0.03^{c}	0.61±0.18^{a}	0.60±0.02^{a}	0.40±0.04^{b}
Total cholesterol (mmol/L)	1.23±0.07^{d}	3.60±0.22^{a}	1.64±0.09^{b}	1.50±0.06^{c}
Triacylglycerols (mmol/L)	0.76±0.01^{b}	1.65±0.03^{a}	0.80±0.03^{b}	0.51±0.05^{c}

Data show mean±SEM of 8 rats per group.
Means with different letters (a-d) in the same row are considered significantly different (P<0.05).
Normal, normal control group; SBy-Ob-HS, obese group fed sardine byproducts oil; SF-Ob-HS, obese group fed sardine fillet oil; Ob-HS, obese control group.
HbA1c, glycosylated hemoglobin.

Table 5. Effect of 4 weeks sardine by-product oil diet on liver lipid profiles, lipoproteins-cholesterol contents, membrane fluidities, and transaminases activities in diet-induced obese rats by high fat diet for 90 days

Groups	Normal	Ob-HS	SF-Ob-HS	SBy-Ob-HS
Liver lipids contents				
TL (mg/g)	166.81±2.16^{b}	198.32±1.05^{a}	152.00±0.70^{c}	138.00±0.10^{d}
TG (μmol/g)	42.40±1.89^{b}	57.07±1.38^{a}	56.61±1.17^{a}	26.30±4.15^{c}
FC (μmol/g)	38.30±0.56^{b}	65.64±0.67^{a}	16.13±0.47^{c}	12.45±0.94^{d}
PL (μmol/g)	41.79±0.43^{c}	43.01±0.50^{b}	93.02±4.78^{a}	98.09±1.23^{a}
NEFA (μmol/g)	38.30±0.56^{c}	65.64±0.56^{a}	69.00±0.12^{a}	48.00±0.07^{b}
Lipoprotein-cholesterol concentrations				
LDL-C (mmol/L)	0.40±0.02^{b}	1.57±0.02^{a}	0.12±0.01^{c}	0.11±0.01^{c}
HDL-C (mmol/L)	0.93±0.03^{c}	0.31±0.02^{d}	1.36±0.01^{a}	1.23±0.03^{b}
Membrane fluidity				
NEC/PL	0.39±0.02^{d}	1.62±0.10^{b}	1.44±0.25^{c}	0.29±0.01^{a}
Transaminases activities				
ALAT (U/L)	14.91±0.70^{c}	39.07±0.70^{a}	21.85±0.20^{b}	19.87±0.70^{b}
ASAT (U/L)	20.81±0.43^{c}	37.63±0.83^{a}	34.54±1.23^{b}	31.50±1.20^{b}

Data show mean±SEM of 8 rats per group.
Means with different letters (a-d) in the same row are considered significantly different (P<0.05).
Normal, normal control group; SBy-Ob-HS, obese group fed sardine byproducts oil; SF-Ob-HS, obese group fed sardine fillet oil; Ob-HS, obese control group.
ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein cholesterol; FC, free cholesterol; NEFA, non-esterified fatty acids; PL, phospholipids; TL, total lipids; TG, triacylglycerols.

groups (+54% and +56%, respectively) compared with the Ob-HS group (Table 5).

There was a significant reduction in serum LDL cholesterol (LDL-C) (−92%) in the SF-Ob-HS and SBy-Ob-HS groups compared with the Ob-HS group. Similarly, LDL-C was reduced by −70% and −73% in the SF-Ob-HS and SBy-Ob-HS groups, respectively, compared with the normal group. Furthermore, HDL-C was increased in the SF-Ob-HS and SBy-Ob-HS groups compared with the Ob-HS group (by 77% and 75%, respectively) and the normal group (by 32% and 24%, respectively).

The NEC/PL ratio was decreased in the SF-Ob-HS and SBy-Ob-HS groups (−11% and −82%, respectively) compared with the Ob-HS group. Moreover, the NEC/PL ratio was 80% lower in the SBy-Ob-HS group compared with the SF-Ob-HS group (Table 5).

TBARS and LPO levels in tissues

Compared with the Ob-HS group, in the SF-Ob-HS and SBy-Ob-HS groups, TBARS levels were decreased by 39% and 28%, respectively, in the liver and by 68% and 62%, respectively, in the adipose tissue (Table 6).

Furthermore, LPO levels were decreased in liver (−43% and −29%) and in adipose tissue (−30% and −31%) in SF-Ob-HS and SBy-Ob-HS groups, respectively, compared with the Ob-HS group (Table 6). In addition, compared with the SBy-Ob-HS group, lipid LPO contents in the SF-Ob-HS group were higher in the liver (+19%) and similar in adipose tissues (Table 6).

Serum and urine isoprostanes levels

In serum and urine, isoprostanes concentrations were reduced by 45% and 73%, respectively, in the SBy-Ob-HS group compared with the SF-Ob-HS group (Table 6). Furthermore, serum and urine isoprostanes concentrations

Table 6. Effect of 4 weeks sardine by-product oil diet on tissues TBARS, LPO, and Iso in serum and urine in diet-induced obese rats by high fat diet for 90 days

Parameters	Normal	Ob-HS	SF-Ob-HS	SBy-Ob-HS
Liver				
TBARS (mmol/g tissue)	1.04±0.70^{d}	13.53±1.77^{a}	8.22±0.50^{c}	9.75±0.34^{b}
LPO (μmol/g tissue)	5.98±3.30^{d}	75.97±4.79^{a}	43.53±8.18^{c}	54.00±0.88^{b}
Adipose tissue				
TBARS (mmol/g tissue)	4.11±2.70^{d}	87.4±1.4^{a}	27.61±3.03^{c}	32.88±1.88^{b}
LPO (μmol/g tissue)	3.44±0.87^{c}	48.99±2.71^{a}	34.16±0.22^{b}	34.00±0.44^{b}
Serum-Iso (ng/mL)	477.51±28.55^{b}	680.41±71.67^{a}	298.03±87.60^{c}	164.45±95.40^{d}
Urine-Iso (ng/mL)	128.09±4.48^{b}	274.58±7.14^{a}	115.30±43.01^{c}	31.50±7.02^{d}

Data show mean±SEM of 8 rats per group.
Means with different letters (a-d) in the same row are considered significantly different (P<0.05).
Normal, normal control group; SBy-Ob-HS, obese group fed sardine byproducts oil; SF-Ob-HS, obese group fed sardine fillet oil; Ob-HS, obese control group.
Iso, isoprostanes; LPO, lipid hydroperoxides; TBARS, thiobarbituric acid reactive substances.

Table 7. Effect of 4 weeks sardine by-product oil diet on tissue antioxidant enzyme activities in diet-induced obese rats by high fat diet for 90 days

Parameters	Normal	Ob-HS	SF-Ob-HS	SBy-Ob-HS
Liver				
GSH-Px (nmol/min/mL)	169.37±1.80^{c}	161.81±3.00^{c}	331.10±36.02^{b}	598.43±18.01^{a}
GSSH-Red (nmol/min/mL)	138.81±1.80^{c}	113.59±6.20^{d}	206.94±13.55^{b}	423.42±31.51^{a}
SOD (U/mL)	16.82±0.54^{a}	7.18±0.52^{c}	16.57±1.67^{a}	9.18±2.02^{b}
CAT (U/mg/min)	133.90±3.70^{NS}	125.22±42.80^{NS}	143.00±3.00^{NS}	172.00±45.00^{NS}
Adipose tissue				
GSH-Px (nmol/min/mL)	164.28±23.41^{c}	71.87±3.12^{d}	343.83±18.01^{a}	216.49±18.01^{b}
GSSH-Red (nmol/min/mL)	127.35±7.20^{b}	79.16±3.58^{d}	191.20±45.02^{a}	97.59±13.50^{c}
SOD (U/mL)	27.43±0.60^{c}	18.02±0.18^{d}	33.40±10.70^{b}	49.00±0.80^{a}
CAT (U/mg/min)	115.50±3.60^{c}	93.70±0.20^{d}	210.00±5.00^{b}	250.00±4.00^{a}

Data show mean±SEM of 8 rats per group.
Means with different letters (a-d) in the same row are considered significantly different (P<0.05).
Normal, normal control group; SBy-Ob-HS, obese group fed sardine byproducts oil; SF-Ob-HS, obese group fed sardine fillet oil; Ob-HS, obese control group.
GSH-Px, glutathione peroxidase; GSSH-Red, glutathione reductase; SOD, superoxide dismutase; CAT, catalase.
NS: not significant.

were reduced in the SF-Ob-HS (−56% and −58%, respectively) SBy-Ob-HS (−76% and −88%, respectively) groups compared with the normal group (−30% and −53%) (Table 6).

Tissue antioxidant enzyme activities

Compared with the Ob-HS group, GSSH-Red activity in the SF-Ob-HS and SBy-Ob-HS groups was increased in liver (+45% and +73%, respectively) and adipose tissue (+59% and +19%, respectively) (Table 7). Similarly, SOD activity in SF-Ob-HS and SBy-Ob-HS groups was improved in the liver (+57% and +22%, respectively) and adipose tissue (+46% and +63%, respectively), and GSH-Px activity was increased in both the liver (+51% and +73%, respectively) and adipose tissue (+79% and +67, respectively) (Table 7). Furthermore, compared with the Ob-HS group, CAT activity was similar in the livers of the SF-Ob-HS and SBy-Ob-HS groups. However, in adipose tissue, this activity was increased (by +55% and +63%, respectively) (Table 7).

In the SF-Ob-HS group, liver SOD activity was lower (−45%) and liver GSH-Px and GSSH-Red activities were higher (45% and 51%, respectively) compared with those in the SBy-Ob-HS group. In adipose tissue, SOD and CAT activities were higher (+32% and +16%, respectively) in rats fed the fillet compared with by-products oil; however, GSH-Px and GSSH-Red activities were decreased (−37% and −49% respectively).

DISCUSSION

Fish and fish products are consumed all over the world due to their nutritional values and health benefits. However, valorization of fish by-products due to their richness in bioactive compounds (Affane et al., 2016; Affane et al., 2018b) also may represent an alternative to marine products, in the context of economic profitability and sustain-

able development. The use of fish by-products in the production of omega-3-rich oils has become an increasingly common practice in recent years. Indeed, the fish industry tends to value by-products of fish varieties such as tuna, salmon, or sardine (Dumay et al., 2006). Valorization of fish by-products by recovering their oil is of great interest to the fish industry, especially for oil with a high content of omega-3 PUFA.

In this context, the extraction process used to obtain oils rich in omega-3 is important for obtaining the best oil quality in terms of lipid oxidation, pollutant content, and sensory properties. In addition, the extraction method may not only affect the oil extraction yield and quality, but also the quality of the fish protein or meal obtained, which is of great interest as an added value ingredient (Affane et al., 2018a). The experimental model chosen in our study to induce obesity has been widely used in several studies (Boukhari et al., 2013; Hamza-Reguig et al., 2017; Louala and Lamri-Senhadji, 2019) since it is particularly suitable for elucidating certain mechanisms that contribute to the development of CVD risk complications, such as hepatic steatosis associated with obesity. In response to a high fat and calorie diet, rats develop obesity by increasing food intake, body weight and lipid storage in liver (Louala and Lamri-Senhadji, 2019).

Unlike those of fish by-products, the beneficial health effects of fish and fish oil consumption are well documented. Both lean and fatty fish may have a protective role through positive effects on satiety and by providing a variety of different nutrients (Uhe et al., 1992; Tørris et al., 2018).

Similarly, the role of fish oil rich in n-3 PUFAs on weight loss is well demonstrated (Buckley and Howe, 2010). Indeed, some studies have reported that fish oil is associated with reduced body weight in obese animal models fed a HLD (Janovská et al., 2013). Furthermore, in overweight and obese subjects, PUFAs help modulate postprandial satiety (Burdge and Calder, 2005).

One of the first results observed in our study was that the SBy-Ob-HS and SF-Ob-HS groups showed similar significant decreases in body weight, weight gain, and food intake as the obese control group. Moreover, obese rats consuming fillet and by-product sardine oils had negative FER values compared with control obese and normal rats; FER ratios indicate the animal's ability to transform energy consumed into body mass (De Sibio et al., 2013). Fatty fish such as sardines help the body burn fat in addition to generating a feeling of satiety. This satietogenic effect may explain the decrease in food intake in obese groups consuming sardine oil and explains the negative FER values. Our results suggest that the oils from both sardine by-products and fillets do not have toxic effects in rats, since hepatic function indicated by transaminase activity was not negatively altered (a reduction was observed). Saka et al. (2011) showed that elevation of transaminase activities, in particular aspartate aminotransferases (AST), is linked to liver damage. Moreover, experimental studies have shown beneficial effects of diets rich of PUFAs on transaminase (asparate aminotransferase and AST) activities (Ketsa and Marchenko, 2014).

In human, fish oil can ameliorate obesity and metabolic syndrome features, including anthropometric parameters, blood pressure, blood lipid concentrations (Shabrina et al., 2020), IR and inflammation biomarkers (Nettleton et al., 2005; Yashodhara et al., 2009). In addition, hypotriglyceridemic properties of fish oil in human and animals are well demonstrated (Harris, 1996; Boukhari et al., 2013; Benyahia-Mostefaoui et al., 2014). Furthermore, n-3 PUFAs have been reported to improve insulin sensitivity and glucose homeostasis in animal models of IR (Martins et al., 2018). The quality of lipids incorporated into diets plays an important role. Indeed, in rats, consumption of a HLD containing sheep fat [high saturated fat (58%), including 30% palmitic acid] induces obesity by increasing lipid storage, mainly in adipose tissue and liver (Hamza-Reguig et al., 2017), with an increase in adipocyte size (Bergouignan et al., 2010). In contrast, fish oil rich in n-3 PUFAs has been reported to improve insulin sensitivity and glucose homeostasis in animal models of IR. These authors suggest that dietary fish oil attenuates the deleterious effects of the HLD (obesity and IR) by improving skeletal muscle mitochondrial function (Martins et al., 2018). The anti-obesity effect of n-3 PUFA may result, at least in part, in increasing fat catabolism and decreasing lipogenesis in adipocytes (Todorčević and Hodson, 2015). Obesity is strongly associated with IR, which itself is a major risk factor for the development of type 2 diabetes in human (Hardy et al., 2012). High-fat and high carbohydrate diets induced obesity and severe hyperglycemia associated with hyperinsulinemia in Wistar rats (Cerf et al., 2012). Moreover, de las Heras et al. (2013) demonstrated that consumption of a HLD for 7 weeks induces hyperglycemia and hyperinsulinemia.

Another result observed in our study was that the SBy-Ob-HS and SF-Ob-HS groups exhibited similar significant decreases in glucose, HbA1c (a marker of protein glycation) and insulin compared with the Ob-HS group. In addition, insulin level was significantly decreased in rats fed the SBy-Ob-HS diet. The high content of PUFAs (+10%), particularly in EPA and DHA (Table 2), may improve glucose homeostasis and prevent development of IR in response to a HLD (Chacińska et al., 2019).

In our experimental design, sardine fillet and by-product oils reduced hypercholesterolemia and hypertriglyceridemia in rats compared with the normal group. In addition, by-product oil appears to be more effective than the fillet oil for regulating serum TC and TG levels. The fatty acid composition of sardines justifies the functional

properties of sardine by-product oil compared to that of sardine fillet oil. Moreover, hepatic lipids, LDL-C, and NEC/PL ratios were lower in the SBy-Ob-HS (more notably) and SF-Ob-HS groups compared with the Ob-HS groups, whereas serum HDL-C concentrations were enhanced. This could be explained by the FA composition of by-product oil being rich in omega-3, particularly in EPA, compared with fillet oil (Table 2). Indeed, in by-product oil, n-3 PUFA accounted for the largest share of PUFAs, particularly EPA and DHA (34% and 32%, respectively). On the other hand, the FA composition of the sardine fillet oil showed that the arctic ground squirrels, MUFAs, and PUFAs comprised 34.7%, 20.0%, and 35.6%, respectively. Furthermore, EPA and DHA comprised 12.4% and 8.0%, respectively, of PUFAs, lower than the proportions in the sardine by-products the oil (Table 2).

Numerous studies have shown that sardine oil rich in n-3 FA has hypocholesterolemia and hypotriglyceridemia properties (Yashodhara et al., 2009; Njinkoue et al., 2017). Indeed, a diet rich in PUFAs induces a significant reduction in liver fat content (Tobin et al., 2018), and n-3 FAs help modulate inflammation and metabolic health (Zivkovic et al., 2011). Our results suggest that sardine oil diets (particularly by-product oil) improve lipid metabolism by modifying expression of lipid metabolism-related genes in the liver and increasing fecal lipid excretion. Indeed, serum transaminases activities were lower in the SBy-Ob-HS and SF-Ob-HS groups compared with the Ob-HS group. This reduction is probably due to a decrease in total liver lipids, which confirms the protective effect of PUFAs against cell damage, in particular hepatic damage, induced by a HLD. These data are consistent with *in vitro* and experimental data in Wistar rats consuming a high-fat diet, which showed that n-3 PUFAs inhibit lipogenesis by increasing β-oxidation (Ukropec et al., 2003).

A relationship was established between oxidative stress and the degree of obesity (Fernández-Sánchez et al., 2011). Indeed, in obesity, impaired carbohydrate and lipid metabolism was observed by large production of reactive oxygen species (ROS) and reduced antioxidant defense (Pou et al., 2007). Obesity induced by a HLD is correlated with mitochondrial dysfunction and increased oxidative stress (Yuzefovych et al., 2013). Markers of lipid peroxidation, such as TBARS, isoprostanes, and LPO, as well as markers of protein oxidation, such as carbonyl proteins, indicate ROS-induced oxidative damage (Uzun et al., 2007; Janicka et al., 2010). Oxidative damage is aggravated by a decrease in antioxidant enzyme activities, such as SOD, CAT, GSH-Px, and GSSH-Red, which act as free radical scavengers in conditions associated with oxidative stress (Blokhina et al., 2003).

In our study, evaluation of ROS showed that both sardine oils decreased TBARS content in the liver and adipose tissue, isoprostanes in serum and LPO in tissues in the SBy-Ob-HS and SF-Ob-HS groups compared with the normal group. The reductions in TBARS, LPO, and isoprostanes (one of the most compelling markers of oxidative stress) can be explained by increased activity of some antioxidant enzymes. Indeed, both SF-Ob-HS and SBy-Ob-HS diets increased almost all the antioxidant activities tested compared with the normal group. The sardine by-product oil decreased key biomarkers of lipid peroxidation, in particular isoprostanes, compared with the sardine fillet oil. This may be due to the richness of this oil in bioactive compounds (PUFA n-3, particularly EPA and DHA). Our results suggested that by-products oil releases bioactive fatty acids with antioxidant properties more efficiently in the digestive tract. Fish oil prepared from viscera, skin, and head of sardine by-products also appears to have antioxidant properties and, thus, inhibitory activity against lipid peroxidation. Our results support those of Godwin and Prabhu (2006) on the beneficial effects of fish oil on lipid peroxidation.

In conclusion, due to its richness in certain lipid bioactive compounds, especially EPA and DHA, sardine by-product oil improves cardio metabolic and oxidative biomarkers in HFD-fed rats by inducing weight loss, reducing levels of aminotransferases, lipids, and glycemic markers, reducing liver lipids accumulation, and improving redox status of liver and adipose tissue. Sardine by-product oil may be beneficial for the prevention or treatment of cardiometabolic diseases. Thus, by-products oil should be valorized due to its remarkable nutritional and functional properties.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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