Food Research International 45 (2012) 60–68

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effect of protein hydrolysates from sardinelle (*Sardinella aurita*) on the oxidative status and blood lipid profile of cholesterol-fed rats

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ARTICLE INFO

Article history:
Received 7 July 2011
Accepted 5 October 2011

Keywords:
Sardinelle
Protein hydrolysates
Cholesterol-enriched diet
Hypocholesterolemic

ABSTRACT

This study was designed to test the hypolipidemic properties and antioxidative activities of sardinelle (*Sardinella aurita*) protein hydrolysates (SPHs) obtained by treatment with crude enzyme preparations from *Bacillus pumilus* A1(SPHA1), *Bacillus mojavensis* A21(SPHA21) and crude enzyme extract from sardinelle viscera (SPHEE).

Wistar rats were fed during 7 weeks a standard laboratory diet, a cholesterol-enriched diet (1%) or a cholesterol SPH-enriched diet. The hypercholesterolemic diet induced the elevation of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C). Supplementing cholesterol-enriched diet with SPHs or whole sardinelle protein (WSP) at a concentration of 5% (*w*/*w*) increased the serum level of high-density lipoprotein cholesterol (HDL-C) and HDL-C/TC ratio and decreased the serum levels of TC, TG, LDL-C and LDL-C/HDL-C ratio significantly.

The thiobarbituric acid-reactive substances (TBARS) level, as an indicator of lipid peroxidation, and the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were examined. The hepatic antioxidant enzymes activities were significantly decreased and the malondialdehyde (MDA) level was increased in rats fed a cholesterol-enriched diet compared to those fed a standard diet. The treatment of hypercholesterolemic (HCD) diet rats with SPHs reduced the MDA concentration and increased the antioxidant enzyme activities.

These results suggested that the hypolipidemic effect of SPHs might be due to their abilities to lower serum TC, TG, and LDL-C levels as well as to their antioxidant activities preventing the lipid peroxidation process.

1. Introduction

Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions such as coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease. A causal relationship between the elevated plasma lipids and the development of atherosclerotic plaques has been well established. Hyperlipidemia is an elevation of lipids in the bloodstream and these lipids include fats, fatty acids, cholesterol, cholesterol esters, phospholipids, and triglycerides (Jain, Kathiravan, Somani, & Shishoo, 2007).

Oxidative stress is currently suggested as a mechanism underlying hypercholesterolemia (Jiangwei, Zengyong, & Xia, 2011). Free radicals are continually produced in the body as the result of normal metabolic processes and interaction with environmental stimuli. Oxidative stress results from imbalance between the production of free radicals and the effectiveness of the antioxidant defense system (Chenni et al., 2007). Several defense systems have been developed by the cell in order to protect itself against free radicals such as non enzymatic antioxidant (ascorbic acid, glutathione) and antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) (McEligot, Yang, & Meyskens, 2005).

It is crucial to maintain serum cholesterol to an adequate level. Serum cholesterol levels are influenced by diet and cholesterol biosynthesis, uptake and secretion (Zhang & Beynen, 1993).

Several works have reported the importance of dietary proteins and protein hydrolysates in the regulation of cholesterol metabolism. To date, hypocholesterolemic properties have been reported for soy (Sugano et al., 1990) and whey (Zhang & Beynen, 1993). Soy protein hydrolysates were found to exhibit a higher hypocholesterolemic activity than the undigested proteins (Zhong, Liu, Ma, & Shoemaker, 2007). Wergedahl et al. (2004), reported that fish protein hydrolysate (FPH) have a role as a cardioprotective nutrient. In fact, FPH treatment

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doi:10.1016/j.foodres.2011.10.003

affected the fatty acid composition in liver, serum and triacylglycerol-rich lipoprotein. Proteins from different fish species have been proved to reduce the serum cholesterol level in the rat model when compared with casein (Shukla et al., 2006), but the reported effects of fish protein on hepatic cholesterol level in rats are not consistent. Wergedahl, Gudbrandsen, Røst and Berge (2009), reported a decrease in hepatic cholesterol concentration in obese rats by fish protein hydrolysate (FPH), although elevation and lowering of hepatic cholesterol have been reported in lean rats on fish protein diets (Zhang & Beynen, 1993). A number of studies have focused on other beneficial effects of fish protein, FPH, and bioactive peptides derived from FPH. Antihypertensive and antioxidant effects (Qian, Je, & Kim, 2007), immunomodulating properties (Duarte, Vinderola, Ritz, Perdigon, & Matar, 2006), and reparative properties in the intestine (Fitzgerald et al., 2005) have been reported.

In Tunisia, sardinelle catches were about 13,300 t in 2002 (FAO, 2004). During processing, solid wastes including heads and viscera are generated and constitute as much as 30% of the original material. These wastes, which represent an environmental problem to the fishing industry, constitute an important source of proteins. Traditionally, this material is transformed into powdered fish flour for animal feed (Ström & Eggum, 1981).

The antioxidative activity of sardinelle heads and viscera protein hydrolysates obtained by various enzymatic treatments was previously investigated by Bougatef et al. (2010). In the present study we investigated the potential of SPHs obtained by treatment with crude enzyme from *Bacillus pumilus* A1(SPHA1), *Bacillus mojavensis* A21(SPHA21) and crude enzyme extract from sardinelle viscera (SPHEE) and whole sardinelle protein (WSP), to lower serum cholesterol level in hypercholesterolemic rat and we investigated their beneficial role on antioxidant enzymes (SOD, CAT and GPx) and cytotoxic parameters (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and phosphatase alkaline).

2. Materials and methods

2.1. Materials

Sardinelle was purchased from the fish market of Sfax City, Tunisia. It was washed twice with water; muscle were separated, rinsed with cold distilled water, and then stored in sealed plastic bags at −20 °C until used.

2.2. Enzymes

The enzyme preparations used were: crude enzyme preparation from *B. pumilus* A1 (Fakhfakh-Zouari, Haddar, Hmidet, Frikha, & Nasri, 2010), alkaline proteases from *B. mojavensis* A21 (Haddar et al., 2009), and crude enzyme extract from viscera of sardinelle (Ben Khaled et al., 2008). The protease activity in the crude enzyme extract was determined by the method of Kembhavi, Kulkarni and Pant (1993) using casein as a substrate. One unit of protease activity was defined as the amount of enzyme required to liberate 1 μg of tyrosine per minute under the experimental conditions used.

2.3. Production of sardinelle muscle protein hydrolysates

Sardinelle muscle (500 g), in 1000 ml distilled water, was first minced, using a grinder (Moulinex Charlotte HV3, France) and then cooked at 90 °C for 20 min to inactivate endogenous enzymes. The cooked muscle sample was subsequently homogenized in a Moulinex® blender for about 2 min. The pH of the mixture was adjusted to the optimum activity value for the crude enzyme, then, the muscle proteins were digested (Ratio Enzyme/Substrate = 3). The optimum pH and temperature of sardinelle muscle proteins hydrolysis (SPHA1, SPHA21 and SPHEE) were 8.5, 50 °C; 10.0, 50 °C and 8.0, 45 °C, respectively. During the reaction, the pH of the mixture was maintained constant by continuous addition of 4 M NaOH solution. After the required digestion time the reaction was stopped by heating the solution at 80 °C over a 20 min period to inactivate the enzyme. The SPHs was then centrifuged at 5000 *g* for 20 min to separate insoluble and soluble fractions. Finally, the soluble phase was freeze-dried using a freeze-dryer (Bioblock Scientific Christ ALPHA 1–2, IllKrich-Cedex, France) and stored for further use for a few months.

2.4. Determination of the degree of hydrolysis

The degree of hydrolysis (DH), defined as the percent ratio of the number of peptide bonds cleaved (h) to the total number of peptide bonds in the substrate studied (h_{tot}), in each case, was calculated from the amount of base (NaOH) added to keep the pH constant during the hydrolysis (Adler-Nissen, 1986) as given below.

$$DH(\%) = \frac{h}{h_{tot}} \times 100 = \frac{B \times Nb}{MP} \times \frac{1}{\alpha} \times \frac{1}{h_{tot}} \times 100$$

where B is the amount of base consumed (ml) to keep the pH constant during the reaction. Nb is the normality of the base, MP is the mass (g) of protein (N × 6.25), and α is the average degree of dissociation of the α-NH2 groups released during hydrolysis expressed as:

$$\alpha = \frac{10^{pH-pK}}{1 + 10^{pH-pK}}$$

where pH and pk are the values at which the proteolysis was conducted. The total number of peptide bonds (h_{tot}) in a fish protein hydrolysate was assumed to be 8.6 meq/g (Adler-Nissen, 1986).

2.5. Animals treatment

Male Wistar rats weighing 200–250 g were purchased from the breeding center of the Central Pharmacy of Tunis (Tunisia). All animal procedures were conducted in strict conformation with the local Institute Ethical Committee Guidelines for the Care and Use of laboratory animals of our Institution, they were kept in an environmentally controlled breeding room (temperature: 22 ± 2 °C, humidity: 60 ± 5%, 12 h dark/light cycle). All rats had free access to tap water and alimentation.

The rats were divided into six groups (n = 6 rats each). Group 1 was fed a regular diet (Control group (CD)). Standard diet supplied by Society of Animals Nutrition, Sfax, Tunisia and composed of corn, soya and VMC (vitamins minerals compound). The characteristic of the standard diet was illustrated in the Table 1. Group 2 is hypercholesterolemia diet (HCD) which rats were fed a cholesterol-enriched diet during seven weeks (Wergedahl et al., 2009), which was prepared by adding 10 g cholesterol/kg diet + 1 g cholic acid/kg diet to standard diet; Groups 3, 4 and 5 were fed a cholesterol-enriched diet supplemented with SPHs (5%), while group 6 was fed a based diet supplemented with WSP. At the end of the feeding period, after overnight fasting (12 h), the rats were anesthetized by an intraperitoneal injection of ketamine, and sacrificed by decapitation. The trunk blood was collected and 2 ml of blood were distributed into dry tubes, followed by centrifugation at 4000 rpm and the serum was aliquotted into 1.5 ml vials, and frozen at −80 °C for determination of cholesterol.

Liver (500 mg) were homogenized in 5 ml of lysis buffer (50 mM Tris, 150 mM NaCl adjusted to pH 7.4) and centrifuged at 8000 *g* for 10 min at 4 °C. The supernatant was collected and used for the experiments. For histological examination, pieces of liver were immediately fixed in 10% formalin solution.