Effect of high-fat diets supplemented with okara soybean by-product on lipid profiles of plasma, liver and faeces in Syrian hamsters

M.J. Villanueva a,⁎, W.H. Yokoyama b, Y.J. Hong b, G.E. Barttley b, P. Rupérez c

a Department of Nutrition and Food Science, Facultad de Farmacia, Universidad Complutense, Ciudad Universitaria, 28040 Madrid, Spain
b Processed Foods Research, Western Regional Research Center, Agricultural Research Service (ARS), United States Department of Agriculture (USDA), Albany, CA 94710, USA
c Department of Metabolism and Nutrition, Instituto del Frío (IF-ICTAN), CSIC, José Antonio Novais 10, Ciudad Universitaria, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 11 December 2009
Received in revised form 6 April 2010
Accepted 25 May 2010

Keywords:
Dietary fibre
Okara
Lipid profile
Syrian golden hamsters

ABSTRACT

The main components of okara, a by-product from soybean, are dietary fibre and protein. In this study we fed okara based diets with different amounts of fibre, protein and isolflavones to determine their effect on the lipid profile in the plasma, liver and faeces of an animal model. Male golden Syrian hamsters were fed high-fat diets supplemented with okara for 3 weeks. The supplemented diets contained 13% or 20% of okara fibre (OK-13 and OK-20), low-protein okara with 13% of fibre (OK1-13), and isoflavone-free okara with 13% of fibre (OK2-13). Okara supplemented diets did not produce significant differences in the feed intake or body weight gain (p > 0.05). The plasma levels of triglycerides, VLDL- plus LDL cholesterol and total cholesterol in hamsters fed OK-20 decreased significantly (p < 0.05) with respect to the 20% control group (C-20). However, no significant differences (p > 0.05) were found in LDL- and HDL-cholesterol plasma levels in all experiments. Total lipids, triglycerides, total and esterified cholesterol concentrations in liver were reduced by OK-20 diet. Regarding the hamsters fed OK-13 diets, the mean values of the total lipid, triglyceride and cholesterol in the plasma and liver decreased as compared to the control (C-13), but the differences were not statistically significant. All of the okara diets assayed increased the faecal excretion of total lipids, triglycerides, free cholesterol and total nitrogen (p < 0.05) compared to their respective controls. Our results suggest that the main components of okara, dietary fibre and protein, could be related with the total lipids and cholesterol decrease in the plasma and liver, as well as with the faecal output increase in high-fat fed hamsters. Okara might play an interesting role in the prevention of hyperlipidaemia and could be used as a natural ingredient or supplement for functional food preparation.

⁎ Corresponding author. Tel.: +34 91 394 1808; fax: +34 91 394 1799.
E-mail address: mjvilla@farm.ucm.es (M.J. Villanueva).

1. Introduction

Soy milk and tofu consumption are steadily increasing in Western countries. Soy foods are associated with health claims for improved cardiovascular health. However, the processing of soybeans into soy milk and tofu requires large volumes of water that produces a high moisture by-product that is commonly used as animal feed. For each kilogram of soybean processed into soy milk an output increase in high-fat fed hamsters. Okara might play an interesting role in the prevention of hyperlipidaemia and could be used as a natural ingredient or supplement for functional food preparation.

On the other hand, its efficient transport and utilisation throughout the body depends on the solubilising action of lipid bilayers, micelles and vesicles in bile and lipoprotein complexes in blood. When an unbalanced state between these two effects occurs, the conditions for its in vivo precipitation become favoured. Thus, gallstones and atherosclerotic deposits are formed and eventually, the health of the individual is endangered (Chen, Jiao, & Ma, 2008; Uskokovic, 2008). Therefore, food intake rich in dietary fibre in the daily diet is important to reduce or regulate plasma cholesterol and triacylglycerol levels and to promote health (Jenkins et al., 2002). The hypocholesterolaemic effect of dietary fibre has been reported in different animal models (rat, mouse, hamster) (Cho, Lee, & Ha, 2007; Martínez-Flores, Chang, Martínez-Bustos, & Sgarbieri, 2004; Van Bennekum, Nguyen, Schulthess, Hauser, & Phillips, 2005) and in humans (Keenan et al., 2007). Moreover, some investigations have been done with only one macromolecular type of isolated fibre, such as guar gum, pectin, β-glucan, carrageenan, soluble cellulose (Cho & Samuel, 2009; Hong, Turowski, Lin, & Yokoyama, 2007) in order to understand the relationship between hypocholesterolaemic and soluble polysaccharide properties.
Several positive effects have been attributed to soybeans, including a hypocholesterolaemic effect (Kerckhoffs, Brouns, Hornstra, & Mensink, 2002; Zhuo, Melby, & Watanabe, 2004). Some compounds found in soybean, such as protein, isoflavone, or dietary fibre have been reported to affect cholesterol metabolism (Clarkson, 2002; Lichtenstein et al., 2002; Matsumoto, Watanabe, & Yokoyama, 2007), but the hypocholesterolaemic effects observed in previous studies may depend on the conditions used for the assays. Therefore, some of them suggested that soy protein effectively lowered the plasma lipids in humans and animals (Adams et al., 2004; Fukui, Tachibana, Fukuda, Takamatsu, & Sugano, 2004), but some other studies did not report cholesterol-lowering effects (Ni et al., 1999; Thorp et al., 2008). Moreover, isoflavones of soybeans have antioxidant properties and they can contribute to the effects of dietary fibre on the lipid profile in blood plasma, liver and faecal lipid excretion in Syrian golden hamsters fed a high-fat diet supplemented with an okara by-product from soybean. In addition, since the hypocholesterolaemic effect of okara may come from different components (dietary fibre, protein or isoflavone), okara was prepared as low-protein okara and isoflavone-free okara in order to evaluate their effects on the lipid profile in hamsters.

2. Materials and methods

2.1. Materials

Okara was kindly provided by a soy foods processor (Toofu-Ya S.L., Arganda del Rey, Madrid, Spain). Okara has a high proportion of water, so it is a highly perishable material. It was collected and immediately frozen and freeze-dried (Labconco, Kansas City, MO). The dry okara was ground to a fine powder (particle size less than 1.0 mm) and vacuum packed to decrease lipid oxidation. Since several components of okara (protein, isoflavones or dietary fibre) might contribute to the hypocholesterolaemic effect, to evaluate their effects, okara ingredients were prepared by deproteinisation (low-protein okara), or solvent extraction (isoflavone-free okara) as described below.

Low-protein okara preparation: Commercial proteases (Novozyme, Sigma, St. Louis, MO) were selected for protein hydrolysis of okara: alkaline alcalase and neutral neuramidase endopeptidase from Bacillus licheniformis, and Bacillus amyloliquefaciens, respectively, and flavorzyme exopeptidase and endopeptidase complex from Aspergillus oryzae. These commercial enzymes were used because they are permitted in food applications. Hydrolysis conditions recommended by the manufacturer were taken into account as a starting point. Afterwards, different conditions were used to increase the soy protein isolation from okara. Hexane defatted okara was sequentially treated with the following enzymes and conditions: alcalase (pH 8/50 °C), neuramidase (pH 6/50 °C), flavorzyme (pH 7/50 °C), each enzyme for 3 h, with pH control and continuous stirring, to achieve maximum hydrolysis. The hydrolysis was terminated by addition of acid until the reaction mixture reached pH 5. Finally, the residue after proteolysis was removed by centrifugation (Sorvall RCS8 Plus, Dupont Company, Wilmington, MA) at 9000g for 20 min and 4 °C. The residues were washed with distilled water and freeze-dried. The percentage of hydrolysis and yield of protein from the original okara was calculated by the amount of protein (N × 6.25) remaining in the residue. This low-protein sample was prepared to determine the effect of okara fibre.

Isoflavone-free okara: The extraction of isoflavones from okara was done with 70% ethanol at 50 °C for 30 min. The presence of isoflavone in the extracts was assessed with the Folin–Ciocalteau reagent.

2.2. Nutritional data

Male Syrian golden hamsters (Mesocricetus auratus) were purchased from Charles River Laboratories (Wilmington, MA). The animals were individually housed in cages in a temperature-controlled room (20–22 °C) and 60% relative humidity, and a 12 h light–dark cycle. The experimental protocol for the animals was approved by the Animal Use Committee of USDA (WRRC, Albany, CA). Hamsters were fed a commercial chow (Purina Rodent, St. Louis, MO) for 1 week in order to acclimatise them to the new facility and environment. The hamsters were randomised (n = 7–9) and fed one of six experimental high-fat diets for 3 weeks. High-fat diets (controls and supplemented diets) contained 20% total fat (8% butterfat, 10% corn oil and 2% fish oil), and 0.15% w/w cholesterol. Fish oil was used in this study to increase VLDL and LDL cholesterol (German et al., 1996). The diets were designed to provide similar amounts of proteins, lipids, carbohydrates, vitamins and minerals as the control diets. The composition of the controls and supplemented okara diets is given in Table 1. The control diets (C-13, C-20) contained 13% or 20% (wt/wt) of microcrystalline cellulose as fibre control (Dyets, Inc., Bethlehem, PA). The okara supplemented diets were formulated with okara to 13% and 20% fibre (OK-13 and OK-20, respectively); with low-protein okara to 13% fibre (OK-13); and with isoflavone-free okara to 13% fibre (OK2-13). The composition of the three okara ingredients used in the diets is summarised in Table 3. The diets were stored at 4 °C until used. Hamsters were given food and water ad libitum. The diet intake per hamster was measured once a week and the animals were weighed weekly. Faeces were collected during the last three days of the study and freeze-dried. After 2 weeks on the experimental diets animals were sedated with isoflurane and their blood pressure was measured in the fore limb (model MK-2000A, Muro-machi Kikai Co., Ltd., Tokyo, Japan).

2.4. Blood and tissue preparation

After 3 weeks the animals were sacrificed under isoflurane anaesthesia. Blood samples were collected into EDTA tubes by cardiac puncture. The blood glucose-metre used was One Touch Ultra Life Scan, Inc. (Milpitas, CA). Livers and other tissues were extracted, weighed and immediately frozen in liquid nitrogen-chilled clamps and then transferred to freezer at −80 °C until analysis. Fresh plasma samples were separated after centrifugation at
1900 g for 40 min at 4 °C (Beckman GPR Centrifuge, Palo Alto, CA), and stored at -20 °C. Faecal samples were collected during the last 3 days of the experiment, weighed, and freeze-dried. Faeces were ground to produce a homogeneous powder and stored at -20 °C until analysis.

### 2.5. Triglycerides and lipoprotein analysis in plasma

Triglyceride concentrations were determined by an enzymatic method with a commercial kit (Thermo Electron, Pittsburgh, PA) at 37 °C for 5 min, and the absorbance measured at 525 nm (Beckman DU-64 Spectrophotometer, Fullerton, CA).

Very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) were performed by using size-exclusion HPLC (German et al., 1996). An aliquot was injected by an autosampler (WISP 7108, Waters Associates, Milford, MA) into an HPLC (Agilent 1100 chromatograph, Palo Alto, CA). The column was a 3 \( \times \) 2150 mm Superose 6HR HPLC (Pharmacia LKB Biotechnology, Piscataway, NJ). The mobile phase consisted of a buffer containing 0.15 M NaCl, pH 7.0, and 0.02% sodium azide at a flow rate of 0.5 mL/min (Waters model 510 solvent delivery pump). Bovine cholesterol lipoprotein standard (Sigma Aldrich, St. Louis, MO) was used for constructing the calibration curve using the peak areas. The concentrations of triglycerides and lipoproteins are expressed as mg/dL.

### 2.6. Triglycerides, total cholesterol and free cholesterol in liver

Livers were freeze-dried (Labconco, Kansas City, MO) and ground to a fine powder with a mortar and pestle. Total lipids from the liver was extracted with hexane/2-isopropanol (75:25; v:v) using an automated high pressure extractor (Dionex Accelerated Solvent Extraction System, Sunnyvale, CA) at 100 °C and 2000 psi, 25 min. The sample extracts were evaporated under nitrogen and

1900g for 40 min at 4 °C (Beckman GPR Centrifuge, Palo Alto, CA), and stored at -20 °C. Faecal samples were collected during the last 3 days of the experiment, weighed, and freeze-dried. Faeces were ground to produce a homogeneous powder and stored at -20 °C until analysis.

### 2.5. Triglycerides and lipoprotein analysis in plasma

Triglyceride concentrations were determined by an enzymatic method with a commercial kit (Thermo Electron, Pittsburgh, PA) at 37 °C for 5 min, and the absorbance measured at 525 nm (Beckman DU-64 Spectrophotometer, Fullerton, CA). Very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) were performed by using size-exclusion HPLC (German et al., 1996). An aliquot was injected by an autosampler (WISP 7108, Waters Associates, Milford, MA) into an HPLC (Agilent 1100 chromatograph, Palo Alto, CA). The column was a 3 \( \times \) 150 mm Superose 6HR HPLC (Pharmacia LKB Biotechnology, Piscataway, NJ). The mobile phase consisted of a buffer containing 0.15 M NaCl, pH 7.0, and 0.02% sodium azide at a flow rate of 0.5 mL/min (Waters model 510 solvent delivery pump). Bovine cholesterol lipoprotein standard (Sigma Aldrich, St. Louis, MO) was used for constructing the calibration curve using the peak areas. The concentrations of triglycerides and lipoproteins are expressed as mg/dL.

### 2.6. Triglycerides, total cholesterol and free cholesterol in liver

Livers were freeze-dried (Labconco, Kansas City, MO) and ground to a fine powder with a mortar and pestle. Total lipids from the liver was extracted with hexane/2-isopropanol (75:25; v:v) using an automated high pressure extractor (Dionex Accelerated Solvent Extraction System, Sunnyvale, CA) at 100 °C and 2000 psi, 25 min. The sample extracts were evaporated under nitrogen and
residues were weighed to determine the percentages of total lipids. The lipid residues were dissolved with chloroform/methanol (5:2; v:v), and an aliquot was mixed with 1% Triton X-100 in chloroform and evaporated to dryness under nitrogen. Total cholesterol in liver was determined using a highly specific enzymatic colorimetric method (Wako Diagnostics, Richmond, VA). Cholesterol esters in liver are hydrolysed by cholesterol esterase. The cholesterol produced and the free cholesterol already present in the liver are oxidised by cholesterol oxidase generating hydrogen peroxide. The compound formed participates in a quantitative oxidative condensation in the presence of peroxidase. The product of the reaction is a blue chromophore; the absorbance was measured at 600 nm (Beckman DU-64 Spectrophotometer, Fullerton, CA). Free cholesterol was determined with the same method, but without the previous hydrolysis step of the cholesterol esters (Wako Diagnostics, Richmond, VA). Triglycerides were analysed in the same way as described previously for plasma samples.

2.7. Total lipids, cholesterol, triglycerides and nitrogen in faeces

Total lipids from dried faecal samples were extracted with hexane/2-isopropanol (75:25; v:v) using an extractor (Dionex Accelerated Solvent Extraction System, Sunnyvale, CA) at 100 °C and 2000 psi. Total lipids in faeces were quantified gravimetrically after evaporating the organic solvent in the extracts. The nitrogen content was measured using a nitrogen autoanalyser (Elementary CHNOS Vario Macro, Hanau, Germany) based on the Dumas method. The content of triglycerides, free cholesterol and total cholesterol was determined with assay kits, as described above for liver samples.

2.8. Measurement of CYP51 and zPPAR mRNA

RNA extraction and purification: Total RNA from the liver tissue of hamsters was extracted and purified using an Invitrogen RNA-Plus kit according to the manufacturer’s instructions (Invitrogen Corporation, Carlsbad, CA). Firstly, an extraction was performed with trizol reagent for the liberation of RNA; secondly, the RNA was bound to a silica surface using a column purification to remove impurities, such as contaminating RNAses and residual phenol. The isolated RNA was reversely transcribed using M-MuLV reverse transcriptase as outlined in the manufacturer’s protocol (Applied Biosystems, Foster City, CA). The reaction took place in an Eppendorf Mastercycler gradient (Eppendorf Scientific, Inc., Westbury, NY) at the following conditions: reverse transcribe (42 °C/60 min), protein denaturing (99 °C/5 min) and cooling (4 °C/5 min).

Quantitative RT-PCR: The real-time PCR was performed using a SYBR Green supermix solution (Bio-Rad Laboratories). Real-time PCR reactions were carried out with 12.5 μL of the master mix, 0.75 μL of actin, 0.75 μL for each gene (CYP51 and zPPAR) and 10 μL of sterile water. The PCR programme was initiated by preheating at 95 °C for 5 min and then denaturing at 94 °C for 30 s, annealing for 1 min at 58 °C and extending at 72 °C for 30 s. This segment was repeated for 40 cycles (Stratagene La Jolla, CA, USA). The data obtained were analysed using a comparative cycle threshold (Ct) method, and were normalised by the actin expression value.

2.9. Statistical analysis

Results are expressed as mean values ± standard error of means (SEM). The statistical significance was determined using one-way analysis of variance (ANOVA). The differences between control and experimental diets were determined by the Dunnett’s test. Values of p < 0.05 were considered significant. JMP 7.02 (SAS Institute Inc., Cary, NC) was used for the statistical analysis.

3. Results and discussion

3.1. Preparation and composition of okara samples

To prepare okara that was low-in-protein, several assays with commercial proteases (alcalase, neurase or flavorzyme) were carried out. Alcalase was the most effective individual enzymatic treatment; protein removal from defatted okara was 58%. The best results were obtained when the three enzymes were used in sequence (3 h incubation/each enzyme); then protein removal from okara was close to 80%. The protein extraction from okara was incomplete due to the interactions between protein and other components, such as hemicellulose or cellulose. The literature (Zhong, Zhang, Ma, & Shoemaker, 2007) described the use of enzymes to hydrolyse soy protein; however, the removal of protein from okara seemed to be more complicated than soy protein hydrolysis. The use of different conditions to remove isoflavones: solvent (ethanol, acetone), temperature (room temperature and 50 °C) and extraction time (15–40 min) were evaluated to prepare isoflavone-free okara. The results suggested that extraction with 70% ethanol at 50 °C for 30 min was the most effective. Rostagno, Palma, and Barroso (2003) studied the ultrasound-assisted extraction of soy isoflavones with various solvents and different water percentages (30–70%), and concluded that the best conditions are 50% aqueous ethanol at 60 °C for 20 min.

The proximate composition of untreated, low-protein and isoflavone-free okara that was included in the diets is shown in Table 2. These okara ingredients had a high protein content, an interesting profile of fat, and a high fibre content with a much higher proportion of insoluble than soluble dietary fibre (Redondo-Cuenca, Villanueva-Suárez, & Mateos-Aparicio, 2008). Several studies have assessed okara soybean by-products as potential hypolipidemic ingredients and for other health-promoting properties in animals (Jiménez-Escrig, Tenorio, Espinosa-Martos, & Rupérez, 2008; Matsumoto et al., 2007; Préstamo, Rupérez, Espinosa-Martos, Villanueva, & Lasunción, 2007).

3.2. Animals health, feed intake and body weight

Male Syrian golden hamsters were chosen because the fat, sterol and bile acid metabolism of this animal model is more similar to that of humans than either mice or rats (Martínez-Flores et al., 2004). Daily observation of all animals revealed that diets were well accepted and that the animals remained healthy during the experiment. The total feed intake was similar in all the hamster groups throughout the experimental period. There were no statistically significant differences (p > 0.05) between experimental diets and their respective cellulose controls. Then, the addition of the okara by-product did not affect the approximate feed intake of 10 g/day in all groups (Table 3).

3.3. Body weight and other parameters

Body weight increased in each group of animals, control and treated, after 3 weeks of feeding the experimental diets (Table 3). The addition of okara by-product to the diets did not change the body weight compared with the control diet. Hamsters fed OK1-13 and OK2-13 also had a similar body weight as compared to control. The weight gain per day was slightly higher in the OK-13 diet group (1.25 g) and lower in the OK1-13 group (1.09 g), but not significant differences (p > 0.05) were found among the groups.
Matsumoto et al. (2007) noticed significantly lower body weight in mice fed with 20% and 40% okara supplemented to their basal diet for 10 weeks, indicating a preventive effect in the obesity. Préstamo et al. (2007) and Jiménez-Escrig et al. (2008) observed a decrease in body weight gain from the second week in rats (Wistar Hannover) fed okara, compared to the control animals. The weight of adipose tissue was not significantly different among groups, but the liver weight of the OK-20 group was significantly different ($p < 0.05$) when compared to the control. The lower liver weight in the okara-fed groups suggested a higher accumulation of lipids in the control groups than in animals fed the okara supplemented diets. There were no significant changes in the capillary blood glucose in either hamster groups (Table 3). The systolic blood pressure of each animal was also measured before sacrificing. The mean systolic blood pressure among the groups was similar, without significant changes, except for the animals fed the OK-20 diet, which had a small increase (data not shown).

3.4. Triglycerides and lipoprotein cholesterol profile in plasma

The major lipid constituents of plasma are cholesterol and triglycerides. In hamsters and humans, cholesterol is synthesised in the liver and transported into the blood mainly in the form of lipoprotein particles, mainly LDL and HDL. Cholesterol is present for the most part as the esterified form in the blood. Plasma triglycerides and lipoprotein cholesterol levels in hamsters fed the 13% and 20% control and okara supplemented diets over 3 weeks are shown in Table 4. Feeding hamsters with the okara supplemented diets at 13% fibre (OK-13, OK1-13, and OK2-13) displayed no significantly lower plasma triglycerides compared to their control. The group fed the diet supplemented with 20% okara fibre (OK-20) had significantly lower ($p < 0.05$) plasma triglyceride levels than the 20% control group. Matsumoto et al. (2007) also reported that in mice, plasma triglycerides were significantly lowered by 40% okara supplemented diets, but not by 10% or 20% supplementation. Plasma triglycerides of rats fed 10% okara were not different from the controls (Jiménez-Escrig et al., 2008).

The VLDL and LDL cholesterol values of hamsters fed okara supplemented diets (OK-13 and OK-20) decreased (Table 4), but the differences were not statistically significant ($p > 0.05$) in comparison with the corresponding controls after 3 weeks. However, the cholesterol levels of the combined plasma fractions (VLDL + LDL) in hamsters fed OK-20 were significantly ($p < 0.05$) lower (20%) than the control (C-20). Matsumoto et al. (2007) showed that a diet containing 40% okara reduced the total and LDL cholesterol significantly in mice after 10 weeks. The mean values of HDL cholesterol were no different ($p > 0.05$), among the different groups of hamsters. Higher HDL levels are considered beneficial since HDL picks up cholesterol from peripheral tissues and transports cholesterol in the blood back to the liver. The diets supplemented with 13% okara fibre (OK-13, OK1-13, OK2-13) did not have the capacity to significantly reduce the plasma cholesterol of the hamsters fed a high-fat diet. Total cholesterol in the plasma of hamsters fed OK-20 diet was significantly ($p < 0.05$) lower (21%) compared to the cellulose-control group.

The higher supplementation of okara fibre, 20% vs. 13%, reduced significantly total cholesterol (Table 4). However, it was not clear that fibre alone was responsible for the cholesterol-lowering property of okara, since the protein content was higher in OK-13 and OK2-13 diets (Table 2). These results indicated that not only fibre but other components of okara, such as protein, could be involved in the reduction of serum lipids. Other studies have shown that soy protein consumption has beneficial effects on serum lipid concentrations in animal models (Torres, Torre-Villalvazo, & Tovar, 2006; Zhong et al., 2007). Moreover, Zhan and Ho (2005) observed that soy protein containing isoflavones significantly reduced the serum total cholesterol, LDL-C, and triacylglycerol, and significantly increased HDL-C. Rossi et al. (2008) reported a lipid-lowering effect in hypercholesterolaemic rats fed isoflavone-supplemented soy yogurt, but the HDL-C and triglyceride concentrations did not differ significantly among the groups. However, Adams et al. (2004) concluded that the consumption of diets rich in soy protein ($\beta$-conglycinin) have an inhibitory effect on the development of atherosclerosis in mice. They also found that the cholesterol-lowering effect did not depend on the variation in isoflavone content, suggesting that isoflavones were not involved. In a recent analysis of 22 randomised clinical trials, the findings showed a small decrease in LDL cholesterol by a soy protein supplement, but no change in cholesterol levels when isolated soy isoflavone was consumed by humans (Sacks et al., 2006).

3.5. Total lipids, triglycerides and cholesterol in liver

Table 5 shows that the liver’s total lipids concentration was significantly lower ($p < 0.05$) in the hamsters fed the okara supplemented diets, OK-13 (20%), OK2-13 (16%) and OK-20 (30%), than in those fed cellulose-control diets. The liver triglycerides of hamsters were not significantly reduced ($p > 0.05$) in any of the groups supplemented with 13% okara fibre. However, the liver triglycerides were significantly ($p < 0.05$) decreased by 32% in the group fed 20% okara fibre (OK-20), compared to the control (C-20). The liver’s total cholesterol for the okara fibre supplemented diet

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>Control C-13</th>
<th>13% Okara fibre OK-13</th>
<th>OK1-13</th>
<th>OK2-13</th>
<th>Control C-20</th>
<th>20% Okara fibre OK-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>22.1 ± 1.6</td>
<td>18.2 ± 1.4</td>
<td>19.2 ± 1.4</td>
<td>18.1 ± 1.0</td>
<td>22.2 ± 1.3</td>
<td>15.3 ± 1.1*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>234 ± 9.3</td>
<td>207 ± 9.3</td>
<td>227 ± 14.5</td>
<td>202 ± 11.0</td>
<td>249 ± 5.9</td>
<td>197 ± 7.9*</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>30.8 ± 3.2</td>
<td>23.8 ± 2.7</td>
<td>28.7 ± 3.2</td>
<td>25.3 ± 7.4</td>
<td>27.2 ± 1.5</td>
<td>22.1 ± 2.9</td>
</tr>
<tr>
<td>LDL-C</td>
<td>121 ± 5.8</td>
<td>112 ± 8.4</td>
<td>118 ± 8.6</td>
<td>102 ± 13.6</td>
<td>120 ± 4.3</td>
<td>96 ± 7.0</td>
</tr>
<tr>
<td>VLDL-C + LDL-C</td>
<td>152 ± 6.4</td>
<td>136 ± 10.7</td>
<td>147 ± 29.9</td>
<td>127 ± 18.0</td>
<td>149 ± 4.5</td>
<td>118 ± 7.0*</td>
</tr>
<tr>
<td>HDL-C</td>
<td>82.7 ± 5.5</td>
<td>70.9 ± 3.2</td>
<td>80.3 ± 5.1</td>
<td>75.0 ± 7.8</td>
<td>100 ± 8.99</td>
<td>78.5 ± 4.0</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM.
C-13: control diet with 13% of cellulose.
C-20: control diet with 20% of cellulose.
OK-13: diet supplemented with 13% of dietary fibre from okara.
OK1-13: diet supplemented with 13% of dietary fibre from defatted and low-in-protein okara.
OK2-13: diet supplemented with 13% of dietary fibre from okara isoflavone-free okara.
OK-20: diet supplemented with 20% of dietary fibre from okara.
* Values are significantly different from control C-20 (Dunnett’s test).
(OK-20) and its corresponding control (C-20) was significantly \((p < 0.05)\) lower by about 30\%. The free cholesterol in the liver did not differ \((p > 0.05)\) within the fibre supplemented groups. The liver's esterified cholesterol levels for the OK-20 hamsters decreased significantly by 48\%, in comparison with the control diet (C-20), but a non significant effect was observed among the OK-13, OK1-13, OK2-13 groups, and their control. Some researchers have suggested that the cholesterol-lowering effect is related to the ability of the dietary fibre to increase the faecal neutral sterol and bile acid excretion, thus increasing the conversion of cholesterol to bile acids in the liver, and the cholesterol uptake from the circulation (Chen et al., 2008; Erkkiä & Lichtenstein, 2006; Hong et al., 2007). Other studies have shown that fibre undergoes fermentation in the colon to produce a series of short-chain fatty acids including acetic, propionic, and butyric acids, and inhibits hepatic cholesterol biosynthesis (Wong, de Souza, Kendall, Emam, & Jenkins, 2006). Fermentability may not be required for cholesterol lowering, as Carr, Wood, Hassel, Bahl, and Gallaher (2003) reported reduced liver- and plasma cholesterol in hamsters and rats fed HPMC (hydroxypropyl methylcellulose), a non-fermentable fibre.

### 3.6. Total lipids, triglycerides, cholesterol and nitrogen in faeces

All the hamster groups fed the okara supplemented diets had a significantly \((p < 0.05)\) total lipids content in faeces than those fed the control diets (Table 6). The total lipids excretion was markedly increased (90\%) in the faeces from animals fed 20\% of okara fibre (OK-20), compared to the control. Diets enriched in certain dietary fibres (oat bran, barley fibre, pectins, gums, wheat germ) increase faecal fat excretion in humans or experimental animals (Lairon, Play, & Jourdheuil-Rahmani, 2007). The faecal triglycerides were increased significantly, up to 2-fold in the OK-20 supplemented group, in all the hamster groups fed supplemented diets. The excretion of the faecal total cholesterol levels increased in all the animal groups, but these changes were only significantly \((p < 0.05)\) higher by 60\% for the hamsters fed 20\% okara fibre. The free cholesterol concentration in faeces was significantly enhanced in all the groups, and generally twice as much cholesterol was excreted compared to each control diet. Hamsters fed soluble dietary fibres such as HPMC increase significantly the faecal cholesterol excretion, as well as other neutral sterols (Hong et al., 2007). The excretion of faecal nitrogen enhanced significantly in all the groups fed the supplemented okara diets, compared with the controls. These remarkable amounts of nitrogen was excreted in the faeces could be attributed to the increase in faecal bacterial nitrogen (Cho & Samuel, 2009), and might be due to a higher proliferation of the colonic microbiota in the hamsters fed the okara supplemented diets. The prebiotic effect of okara has been reported in rats (Jiménez-Escrig et al., 2008; Préstamo et al., 2007).

### 3.7. CYP51 and PPAR\(\alpha\) mRNA by Real-Time PCR

These analyses were performed to measure the following genes: CYP51 and PPAR\(\alpha\). Cholesterol biosynthesis is a tightly regulated pathway consisting of several steps that are catalyzed by different enzymes, including CYP51, which is involved in the initial step of cholesterol synthesis.

### Table 5

Effect of the high-fat diets supplemented with okara on liver triglyceride and cholesterol concentrations in hamsters (mg/g dry weight).

<table>
<thead>
<tr>
<th></th>
<th>Control C-13</th>
<th>13% Okara fibre OK-13</th>
<th>OK1-13</th>
<th>OK2-13</th>
<th>Control C-20</th>
<th>20% Okara fibre OK-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipids</td>
<td>242 ± 8.5</td>
<td>192 ± 9.3*</td>
<td>218 ± 10.6</td>
<td>203 ± 8.9*</td>
<td>239 ± 12.1</td>
<td>168 ± 9.6*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>25.4 ± 1.6</td>
<td>20.4 ± 2.1</td>
<td>22.9 ± 2.1</td>
<td>21.5 ± 3.1</td>
<td>30.2 ± 2.0</td>
<td>20.6 ± 1.3*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>22.2 ± 1.7</td>
<td>18.4 ± 1.9</td>
<td>19.6 ± 1.0</td>
<td>18.5 ± 1.9</td>
<td>23.8 ± 1.4</td>
<td>17.5 ± 0.9*</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>6.1 ± 0.4</td>
<td>7.6 ± 0.5</td>
<td>8.2 ± 1.4</td>
<td>7.9 ± 0.6</td>
<td>6.5 ± 0.7</td>
<td>8.6 ± 1.1</td>
</tr>
<tr>
<td>Esterified cholesterol</td>
<td>16.1 ± 1.6</td>
<td>10.7 ± 1.8</td>
<td>11.4 ± 1.4</td>
<td>10.6 ± 1.4</td>
<td>17.3 ± 1.5</td>
<td>8.9 ± 0.7*</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM.
C-13: control diet with 13% of cellulose.
C-20: control diet with 20% of cellulose.
OK-13: diet supplemented with 13% of dietary fibre from okara.
OK1-13: diet supplemented with 13% of dietary fibre from defatted and low-in-protein okara.
OK2-13: diet supplemented with 13% of dietary fibre from isoflavone-free okara.
C-20: control diet with 20% of cellulose.

* Values are significantly different from control C-13.
* Values are significantly different from control C-20 (Dunnett’s test).
mechanism and involves over 20 enzymatic steps. The first sterol intermediate of the pathway is lanosterol catalysed by lanosterol 14α-demethylase (CYP51) (Rozman, Seliskar, Cotman, & Fink, 2005). PPARα (peroxisome proliferative activated receptor) is a nuclear receptor regulating the cell metabolism of carbohydrates, lipids and proteins. Fatty acids are the main natural ligands of PPARα, which then activates the genes for fatty acid catalysis (Francis, Fayard, Picard, & Auwerx, 2003). Since total cholesterol was lowered by 20% okara feeding, CYP51 and PPARα expression was determined. There was a small but not significant effect (p < 0.05) in the expression of CYP51 and PPARα genes in the liver tissues compared to their controls (7% and 24%, respectively). Matsumoto et al. (2007) reported that okara intake induced a down-regulation of the fatty acid synthetase gene and an up-regulation of the CYP7A1 gene in the liver of mice fed with okara for 10 weeks, suggesting a beneficial effect for the prevention of obesity. CYP7A1 regulates bile acid biosynthesis. The results suggested excretion of bile acids, thus resulting in the up-regulation of CYP7A1. The dietary fibre of okara may accelerate faecal bile acid excretion, and the increase in the conversion of cholesterol to bile acid, finally resulting in a decrease in the plasma cholesterol levels. Chan and Heng (2008) observed that high-fibre diets with Psyllium husks had an effect on the expression levels of hepatic genes related to cholesterol synthesis, such as CYP51, and suggested that a cholesterol-lowering effect was most likely mediated by increased bile acid synthesis.

4. Conclusion

The okara soybean by-product showed hypcholesterolaemic and hypolipidemic effects when hamsters were fed high-fat diets supplemented with 20% of okara fibre (OK-20) over 3 weeks. The consumption of 20% okara fibre decreased (p < 0.05) the triglyceride and total cholesterol concentrations in plasma and liver, and increased (p < 0.05) the faecal lipids, triglycerides, total cholesterol and nitrogen excretion. The levels of plasma and liver lipids in the diets supplemented with 13% of okara fibre showed a non statistical decrease (p > 0.05). Results indicated that both dietary fibre and protein could play a role for the plasma lipids and cholesterol decrease in high-fat fed hamsters. Okara could improve the nutritional quality of other foods and is a promising functional ingredient that can be used in the development of fibre-rich foods.

Acknowledgements


M. J. Villanueva et al. / Food Chemistry 124 (2011) 72–79


