

Effect of Extruded Products Made with Cassava Starch Blended with Oat Fiber and Resistant Starch on the Hypocholesterolemic Properties as Evaluated in Hamsters

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Abstract

To examine the cholesterol-lowering effects of extruded products made with cassava starch (CS) and blends of cassava starch with either resistant starch (CS-RS) or oat fiber (CS-OF) hamsters were fed with diets containing a high-cholesterol (2%) and high-fat (17%) diet for 20 days. Hamsters fed with a diet containing no cholesterol were used as a control. Total cholesterol (TC) levels in the CS-RS and CS-OF groups were significantly ($p > 0.05$) lower compared to the CS group by 11.5% and 8.5%, respectively. Also, the diets containing fibers decreased the value of low-density lipoproteins plus very low-density lipoproteins fraction by 32.4% (CS-RS diet) and 51.7% (CS-OF), respectively, as compared to the CS diet. Total lipid values were significantly ($p < 0.05$) lower in hamsters fed the CS-RS diet (916 mg/dL) and CS-OF diet (964 mg/dL) as compared to those fed the CS diet (1661 mg/dL). The results obtained in this study suggest that extruded products containing cassava starch blended with either resistant starch or oat fiber, could prevent health problems associated with high levels of cholesterol and hypertriglyceridemia induced by a high cholesterol and fat diet.

Key words: total cholesterol, low-density lipoproteins, very low-density lipoproteins, hypertriglyceridemia

INTRODUCTION

Dietary fiber (DF) is composed of the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. DF includes polysaccharides, oligosaccharides, lignin and associated plant substances (1). DF has been classified into two fractions, the insoluble fraction (IF) containing mainly cellulose, lignin and some hemicelluloses, and the soluble fraction (SF) comprised of pectin, some hemicelluloses and gums (2). IF normalizes the intestinal motility, stimulating peristaltic movements and accelerating intestinal food transit, preventing constipation and reducing diverticulosis (3-5). SF reduces the postprandial rise in blood glucose and insulin (6,7). The effect has been attributed to a slowing of gastric emptying due to the viscosity developed by SF. Moreover, SF is partially fermented in the large intestine, producing short chain fatty acids which are related to

cholesterol lowering (8,9), a risk factor in coronary heart disease (10).

Cardiovascular disease (CVD) is the main cause of human death in the world. CVD is the term used to describe a group of ailments that are involved in pathological changes in the blood vessels that feed the cardiac muscle, the brain and other parts of the body. A high level of blood cholesterol is considered as a major indicator of CVD risk.

Dietary fiber has hypocholesterolemic properties (11-14). Foods containing high levels of fiber reduce the absorption of cholesterol, as previously noted by (15). The mechanisms by which fibers lower blood cholesterol and lipid levels in the serum are not well understood. One possibility is that SF is hydrated in the gastrointestinal tract forming a gel matrix that has the capacity to bind lipids and cholesterol, interfering with their absorption (16,17). Another explanation is that increased sterol excretion is responsible for the cholesterol-lowering effects. Some di-

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etary fibers bind bile salts and neutral sterols (18), causing their removal from the body. Then, smaller amounts of bile salts are removed from the enterohepatic cycle, and are available for lipid absorption. Moreover, serum and liver cholesterol are used for bile acid synthesis to compensate depleted pools. Also, it has been assumed that dietary fibers could exercise their influence on the lipids of the blood by the reduction of the postprandial levels of glucose and insulin. Insulin increases both cholesterol and very low-density lipoproteins, which are highly harmful to the human. A reduction in the insulin concentration could reduce the new synthesis of lipids in the blood serum.

In recent years, great interest has been concentrated on the study and use of traditional fibers as food ingredients, such as wheat, rice, corn and oat fibers. However, new fiber sources, such as resistant starch, are becoming potential food ingredients. According to the definition of Englyst (19), starch that is undigested after 2 hours, passes through the body like fiber, and is classified as resistant starch. Since, resistant starch is neither digested nor absorbed in the small intestine, it is subsequently passed to the large intestine and broken down by colonic microflora to short-chain fatty acids, carbon dioxide, hydrogen and methane (20,21). The presence of these short-chain fatty acids in the large intestine inhibits the diffusion of cholesterol and bile acids at the microvillous boundary layer, interfering with their absorption into the body. This results in an enhanced bile acid excretion (21-24).

The objective of this work was to study the effect of the hypocholesterolemic properties of diets based on extruded products made with a combination of oat fiber or resistant starch with cassava starch, as evaluated in hamsters.

MATERIALS AND METHODS

Materials

The cassava starch was obtained from B.R. Carvalho and Co., in Brazil. Oat fiber (300-58) was provided by Canadian Harvest, L.P., Canada. The resistant starch (Novelose 330) was supplied by the National Starch and Chemical Company, in Brazil.

Extrusion conditions

The cassava starch was blended either with the resistant starch or with the oat fiber for 10 minutes using a mixer (Kitchen Aid model K45SS, St. Joseph, Michigan, USA). Water was then added to the blends to reach moisture content of 18%. Samples were placed in sealed bags and maintained at 4°C for 16 hours. Samples were extruded using a single Brabender extruder 20 D/N-GNF 1014/2

(Brabender OHG, Duisburg, Germany). The extruder conditions were as follows: feed speed (70 g/min), screw speed (150 rpm), die exit diameter (4 mm), screw ratio (1 : 4). The temperatures of the three zones were 75°C, 100°C and 150°C. The extruded products were dried overnight at 45°C in a convection oven (FANEM, model 320-SE) and milled in a Brabender mill (Brabender OHG Duisburg, model TE 020) to obtain flour with a particle size lower than 250 µm.

Biological assay

Diets: Diets based on extruded cassava starch (CS), cassava starch extruded with oat fiber (CS-OF) and cassava starch extruded with resistant starch (CS-RS) were formulated to contain 20% casein, 3.5% minerals, 1% vitamins, 0.3% cystine, 0.3% choline bitartrate, 0.0014% *tert*-butylhydroquinone and 7% soybean oil, according to the American Institute of Nutrition-93 (25). The composition of the diets is given in Table 1. All the diets also contained 2% cholesterol and 10% coconut oil. The control diet, based on extruded cassava starch, was formulated as described above, but with 5% cellulose and without the cholesterol and coconut oil.

Animals: Eight weeks old male golden Syrian hamsters (Pathology Department, Veterinary Faculty of Sao Paulo University) were kept individually in wire-bottom cages in a controlled environment (21 ~ 22°C, 12-hr light and dark cycles). After three days of being fed on a fiber-cholesterol-free diet, the animals were weighed and assigned by selective randomization to four groups of eight animals each, and fed test diets. Treatment diets and water were provided *ad libitum* for a 21-day feeding period. The animals were weighed once a week and feed consumption measured twice weekly.

Blood serum sampling: At the end of the assay, the animals were fasted for 16 hr and anaesthetized using ether. Blood was drawn by cutting the portal vein and collected the blood in glass tubes. The blood was allowed to clot and then centrifuged at 1,500 × g for 30 min at 4°C to obtain the serum. Serum samples were analyzed

Table 1. Composition of the extruded materials (% dry basis)

Materials	Dietary fiber	Fat	Protein	Ash
Raw				
Cassava starch	0.4	0.11	0.09	0.12
Novelose 330	29.7	0.3	0.61	0.32
Oat fiber	93.8	0.12	0.12	0.43
Extruded				
CS ¹⁾	2.5	0.11	0.09	0.08
CS-RS ²⁾	17.4	0.25	0.31	0.15
CS-OF ³⁾	17.7	0.11	0.12	0.53

¹⁾CS. Extruded cassava starch.

²⁾CS-RS. Cassava starch extruded with resistant starch.

³⁾CS-OF. Cassava starch extruded with oat fiber.

by enzymatic colorimetric procedures for total cholesterol (TC) (Cat. 60-2/100) and triglycerides (Cat. 59-4/50), using kits 60-2/10 and 59-4/50, respectively, from Lab Test Diagnostica (Lagoa Santa, MG, Brazil). High-density lipoproteins (HDL-cholesterol) were measured after phosphotungstic acid precipitation of very low-density lipoprotein (VLDL-cholesterol) and low-density lipoprotein (LDL-cholesterol) using kit 13 from Labtest Diagnostica (Lagoa Santa, MG, Brazil). The LDL + VLDL cholesterol fraction was obtained using the following equation:

$$\text{LDL} + \text{VLDL} = (\text{TC-cholesterol}) - (\text{HDL-cholesterol}).$$

Total lipids were analyzed using enzymatic kit 1691 from CELM (Barueri, SP, Brazil).

Analytical: Raw and extruded materials were analyzed for moisture, protein and ash, using the methods 44-9, 44-19 and 3020, respectively, of the AACC (26) methods. Insoluble and soluble dietary fibers were measured using the Prosky et al. (27) method. The chemical compositions of the raw and extruded materials are shown in Table 1.

Statistical analyses: The results were analyzed using the Statistical Analysis System (28) software.

RESULTS AND DISCUSSION

Experimental diets and animal weight

The control diet was formulated to contain 9.5% of dietary fiber (1.5% dietary fiber from extruded cassava starch plus 8% cellulose). The diet CS was formulated to contain 9.3% dietary fiber (1.3% from extruded cassava starch and 8% cellulose), plus 2% cholesterol. The CS-RS diet was formulated to contain 9.7% dietary fiber and 2% cholesterol. The CS-OF diet was formulated to contain 9.9% dietary fiber and 2% cholesterol (Table 2).

After three days of basal diet, the initial animal weights were similar for all treatments (100.9 ± 1.57). At the end of the trial, the animals fed on CS-RS and CS-OF showed greater weight gains than animals fed on the other diets, although the difference was not significant in all cases ($p > 0.05$) (Table 3).

TC serum

The values obtained for TC, HDL-cholesterol, LDL + VLDL-cholesterol, triglycerides and total lipids are shown in Table 4. Serum TC levels varied between 159 mg/dL (control group) and 270 mg/dL (CS group). Hamsters fed the control diet had lower total cholesterol serum than those fed on any other diet, since the control diet had no added cholesterol. Hamsters fed CS-RS and OF-RS diet had significantly (11.5% and 8.5%, respectively, $p > 0.05$) lower serum cholesterol as compared to the CS group. The serum cholesterol-lowering effect of the diet containing OF is in agreement with the studies done by Behall (29), Schinick et al. (30), Kahlon et al. (31), and Anderson et al. (32).

Table 2. Diet compositions (% dry basis)

Components	Diets			
	Control ¹⁾	CS ²⁾	CS-RS ³⁾	CS-OF ⁴⁾
Extruded product (EP) ⁵⁾	59.9	47.9	55.9	55.9
Starch from EP	58.2	46.6	46.2	46.0
Fiber from EP	1.5	1.3	9.7	9.9
Casein	20.0	20.0	20.0	20.0
cellulose	8	8	-	-
mineral salts	3.5	3.5	3.5	3.5
vitamins	1.0	1.0	1.0	1.0
cistine	0.3	0.3	0.3	0.3
choline bitartrate	0.3	0.3	0.3	0.3
tert-butylhydroquinone	0.0014	0.0014	0.0014	0.0014
Soybean oil	7.0	7.0	7.0	7.0
cholesterol	-	2.0	2.0	2.0
coconut oil	-	10.0	10.0	10.0

¹⁾Control. Diet containing extruded cassava starch extruded and 8% cellulose.

²⁾CS. Diet containing extruded cassava starch plus 8% cellulose, 10% coconut oil and 2% cholesterol.

³⁾CS-RS. Diet containing cassava starch extruded with resistant starch plus 10% coconut oil and 2% cholesterol.

⁴⁾CS-OF. Diet containing cassava starch extruded with oat fiber plus 10% coconut oil and 2% cholesterol.

⁵⁾Extruded product as starch and fiber source.

Table 3. Initial and final weights, weight gain and feed intake of hamsters

Diet ¹⁾	Final weight (g)	Weight gain (g/20 days)	Feed intake (g/20 days)
Control ²⁾	114.4 ± 5.13^{ab}	11.5 ± 4.15^a	124.0 ± 10.58^a
CS ³⁾	114.2 ± 7.59^a	13.1 ± 7.64^a	121.0 ± 11.15^a
CS-RS ⁴⁾	120.3 ± 10.09^a	18.8 ± 9.78^a	130.6 ± 13.72^a
CS-OF ⁵⁾	117.9 ± 10.75^a	18.1 ± 10.22^a	131.2 ± 12.30^a

¹⁾All diets contained 20% casein, 3% minerals, 1% vitamins, 7% oil soybean, 0.3% cysthine, 0.3% of bitartrate of choline, 0.0014% tert-butylhydroquinone.

²⁾Control. Diet containing extruded cassava starch.

³⁾CS. Diet containing extruded cassava starch plus 2% cholesterol.

⁴⁾CS-RS. Diet containing cassava starch extruded with resistant starch plus 2% cholesterol.

⁵⁾CS-OF. Diet containing cassava starch extruded with oat fiber plus 2% cholesterol.

⁶⁾Means with different letters within a column indicate significant differences ($p < 0.05$).

On the other hand, Lopez et al. (33) and Cheng et al. (34) found that resistant starch from rice diets significantly lowers serum cholesterol in rats. Anderson and Chen (35) observed that the reduction of the TC value could be due to the greater excretion of both fecal bile salts and neutral sterols. Other mechanisms that could explain the cholesterol lowering effect of fermentable fibers, such as RS and OF, are summarized by Younes et al. (21): (1) inhibition of diffusion of cholesterol and bile acids at the microvillous boundary layer, (2) steroid binding capacities in the small intestine, (3) impairment of passive reabsorp-

Table 4. Effect of diets on total cholesterol (TC), high-density lipoprotein (HDL), low-density plus very low-density lipoproteins (LDL+VLDL), triglycerides (TG) and total lipids (TL) in the serum and liver of hamsters

Diets ¹⁾	Serum					Liver		
	TC (mg/dL)	HDL (mg/dL)	LDL+VLDL (mg/dL)	HDL/ (LDL+VLDL)	TG (mg/dL)	TL (mg/dL)	TC (mg/g)	TG (mg/g)
Control ²⁾	159 ± 6 ^{c6)}	119 ± 5 ^c	37 ± 5 ^c	3.22	130 ± 18 ^c	829 ± 29 ^c	3.4 ± 0.34 ^b	14.0 ± 1.55 ^b
CS ³⁾	291 ± 17 ^a	171 ± 22 ^b	114 ± 30 ^a	1.50	218 ± 35 ^a	1661 ± 86 ^a	8.9 ± 0.46 ^a	18.7 ± 2.17 ^a
CS-RS ⁴⁾	239 ± 27 ^b	158 ± 9 ^b	77 ± 11 ^b	2.05	198 ± 34 ^{ab}	916 ± 118 ^{bc}	3.3 ± 0.26 ^b	11.2 ± 1.03 ^c
CS-OF ⁵⁾	247 ± 11 ^b	195 ± 13 ^a	55 ± 14 ^{bc}	3.54	178 ± 26 ^b	964 ± 48 ^c	2.9 ± 0.19 ^{bc}	9.1 ± 0.74 ^c

¹⁾All diets contained 20% casein, 3% minerals, 1% vitamins, 7% oil soybean, 0.3% cysthine, 0.3% bitartrate of choline, 0.0014% tert-butylhydroquinone.

²⁾Control. Diet containing extruded cassava starch.

³⁾CS. Diet containing extruded cassava starch plus 2% cholesterol.

⁴⁾CS-RS. Diet containing cassava starch extruded with resistant starch plus 2% cholesterol.

⁵⁾CS-OF. Diet containing cassava starch extruded with oat fiber plus 2% cholesterol.

⁶⁾Means with different letters within a column indicate significant differences ($p < 0.05$).

tion in the large intestine by insolubilization of bile acids and, (4) metabolic effects on hepatic lipid metabolism.

Serum HDL-cholesterol and LDL + VLDL-cholesterol

HDL-cholesterol, known as "good cholesterol", was higher in the group fed CS-OF (195 mg/dL) and was significantly different from the groups fed on the CS-RS diet (158 mg/dL) and CS diet (171 mg/dL). A previous study of Rieckhoff et al. (36) showed that HDL-cholesterol increased in the hamster serum when oat fiber was used as an ingredient of the diet. In this study, the lower value corresponded to the control diet (119 mg/dL).

A higher content of HDL cholesterol is very important, because it is correlated to a reduced coronary heart disease risk. On the other hand, LDL + VLDL cholesterol serum values were reduced in hamsters fed on CS-RS and CS-OF diets, as compared to those obtained for hamsters fed on the CS diet. Ranhotra et al. (37) also found reduced values of serum LDL + VLDL-cholesterol in hamsters fed on diets based on resistant starch and cellulose. Diets containing fibers decreased the value by 32.4% (CS-RS diet) and 51.7% (CS-OF), respectively, as compared to those consuming the CS diet. Reductions in LDL + VLDL-cholesterol are beneficial in reducing coronary heart disease risk. As a result of the increases in HDL-cholesterol and decreases in LDL + VLDL-cholesterol, the ratio HDL/ LDL + VLDL increased in diets containing fibers (2.05 for CS-RS diet and 3.04 for CS-OF diet). The CS and control diets had ratios of 1.50 and 3.22, respectively.

Serum triglycerides and total lipids

Hamsters fed CS-RS and CS-OF diets had significantly lower serum triglycerides than the control hamsters. The CS-RS and CS-OF diets showed decreases of 9.2% and 18.3% respectively, in the serum triglyceride concentrations of the hamsters as compared to the CS group. Pos-

sibly, this occurred due to the higher starch content in the control diet. These results are in agreement with those obtained by Ranhotra et al. (37). The control diet showed a lower value (829 mg/dL), since this diet had no added coconut oil, and cellulose was included as a fiber source. The fiber in the CS-RS and CS-OF diets, was the main factor responsible for reducing the lipid and triglyceride contents in the serum of the hamsters. Another factor is the capacity of fibers to bind lipids, triglycerides, bile salts and cholesterol, thus inhibiting their absorption in the small intestine (38-41).

Liver TC and triglycerides

TC and TG as evaluated in the liver, showed the same trends as TC and triglycerides in the serum. Significant differences ($p < 0.05$) in liver TC occurred between hamsters fed the CS diet (8.9 mg/g) and those fed on CS-RS (3.3 mg/g) and CS-OF (2.9 mg/g). Liver TC with the control diet was 3.4 mg/g and there was no significant difference from the values obtained with the CS-RS and CS-OF diets. Reductions in liver TC in hamsters fed on the oat fiber diet were in agreement with Schinnick et al. (30) and Kahlon et al. (31). The CS-RS diet (11.2 mg/g) and CS-OF diet (9.1 mg/g) showed lower liver triglyceride values than those obtained with the CS diet (18.7 mg/g). The liver triglyceride concentration in hamsters fed the control diet (14.0 mg/g) was higher than for those fed with fiber diets. This was probably due to the higher starch content in the control diet. Usually, starch is converted into triglycerides in the liver.

CONCLUSIONS

Diets containing cassava starch blended with either resistant starch or oat fiber showed hypocholesterolemic properties, significantly lowering the levels of total cholesterol in the serum and liver, as compared to the diet

made with cassava starch without added fiber. Diets containing fiber positively improved the ratio of high-density lipoproteins to low-density lipoproteins plus very low-density lipoproteins. This is very important because these products could reduce coronary heart disease risk.

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