ELSEVIER

Food Chemistry 76 (2002) 1–6

Food Chemistry

www.elsevier.com/locate/foodchem

Cholesterol-lowering effect of extruded amaranth (*Amaranthus caudatus* L.) in hypercholesterolemic rabbits

Andrea Y.A. Plate, José A.G. Arêas*

Laboratório de Propriedades Funcionais, Departamento de Nutrição, Faculdade de Saúde Pública da Universidade de São Paulo—SP, Brazil

Received 14 June 2000; received in revised form 21 May 2001; accepted 21 May 2001

Abstract

Hypercholesterolemic rabbits, obtained by a special diet, were divided in three groups, and each group was fed a different diet for 21 days. The diets were: extruded amaranth diet (EAD), diet with amaranth oil (AOD) and a control diet. Growth rates of rabbits were similar in all groups. After being fed the experimental diets, total cholesterol and LDL-C concentrations were lower in rabbits fed the EAD than in those fed the AOD or the control diet. Triglycerides and VLDL-C concentrations were approximately 50% lower in rabbits fed the EAD and the AOD than in rabbits fed the control diet. No significant differences were found among HDL-C concentrations of the three groups. These results demonstrate that the consumption of extruded amaranth reduces LDL and total cholesterol levels and may be another option to prevent coronary heart diseases. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Extruded amaranth; LDL; Cholesterol; Hypercholesterolemic rabbits; Cardiovascular diseases

1. Introduction

Interest in amaranth has recently been aroused due to its high nutritive values. Its protein presents a high concentration of essential amino acids, especially lysine which is a limiting amino acid in other crops (Teutonico & Knorr, 1985). Amaranth has also a higher concentration of soluble fibre than many cereals, such as wheat, corn or oats.

Extrusion cooking has been recently optimized for the production of an amaranth snack (Chávez-Jáuregui, Silva, & Arêas, 2000) and this product was of a high acceptability compared with commercial brands. Nutritional evaluation of the snacks made in this laboratory showed that they exhibit a high nutritional value, measured by its protein biological value, bioavailability of minerals, especially calcium, and high content of fibre (Ferreira, 1999).

Some studies conducted with amaranth suggest that it offers cholesterol-lowering effects. In an experiment with rats, Grajeta (1997) observed a reduction of 37% and 33% of total cholesterol when the animals were fed diets with whole amaranth and defatted amaranth, respectively.

In the present study amaranth was used in its extruded form due to the fact that extrusion resulted in a highly nutritious product (Table 1) and is one of the best methods for obtaining the maximum nutritive value of several grains, including amaranth (Mendoza & Bressani, 1987). Our aim was to evaluate the cholesterol-lowering effect of extruded amaranth and amaranth oil in hypercholesterolemic rabbits.

2. Materials and methods

2.1. Amaranth

The amaranth species employed was *Amaranthus caudatus* L., variety Oscar Blanco, provided by CICA—Centro de Investigación en Cultivos Andinos (Perú). The seeds were milled in a knife mill (Mod. Termomatic, Marconi, Brazil), and defatted with n-hexane in Soxhlet apparatus to a final lipid concentration lower than 1% for further extrusion. The defatted flour had its proximate composition determined by conventional methods for moisture, protein, ash, and lipids, as described by AOAC (1990).

* Corresponding author. Tel./fax: +55-11-3066-7701.
E-mail address: jagareas@usp.br (J.A.G. Arêas).

PII: S0308-8146(01)00238-2

2.2. Extrusion

The extrusion was performed in a laboratory single-screw extruder L/D = 20:1, 20 mm diameter, at 70 g min^{-1} feed rate, screw (compression ratio 3.55:1) speed 200 r.p.m., 150 °C central zone temperature, die diameter of 4 mm. These operational conditions yielded an expanded product with a pleasant texture similar to the optimum described for snack production of amaranth (Chávez-Jáuregui et al., 2000). These expanded products were then milled in a blender (Mod. 35BL59, Jencons Scientific, USA) and incorporated into the experimental diets.

2.3. Animals and diets

Eighteen male New Zealand rabbits, weighing 1.5–2.0 kg each, were fed a casein diet (25%) for 50 days in order to raise their cholesterol levels. When hypercholesterolemia was detected, the rabbits were divided into three groups of six rabbits. Thereafter, each group was fed a different diet for 21 days. A diet with 12% of casein was used as control and the other two diets were prepared to incorporate amaranth oil (AOD) and extruded amaranth (EAD). All of the diets were isonitrogenous and isocaloric. For the EAD, 500 g/kg of extruded amaranth were introduced into the original balanced diet, and all other nutrients were adjusted to retain the original balance. For the AOD, 20 g/kg of sunflower oil in the original balanced diet was replaced with amaranth oil. The compositions of all diets are shown in Table 2. Diets were randomly assigned to the three experimental groups with unlimited access to water. The rabbits were housed individually in metal cages with controlled temperature and a 12-h light period, obtained by artificial light. The procedures concerning the animals were followed according to the National Research Council (1985). Diets were weighed every day of the experiment and rabbits were weighed every 7 days. Blood samples were collected at the start of the experiment and after 50 days of the administration of the casein diet. When the three experimental diets were introduced, blood samples were collected every 4 days until the end of the experiment. The animals were deprived of food for 12 h before the blood samples were collected. Growth rates were calculated from body and feed weights taken during the experiment.

Table 1
Composition of extruded amaranth at 150 °C and 13% of moisture

Grain components	%
Moisture	8.00
Proteins (N×6.25)	16.0
Carbohydrates	63.4
Lipids	0.70
Total fibres[a]	8.20
Ash	3.70

[a] Sum of soluble and insoluble fibre as determined by the AOAC method (AOAC, 1990).

2.4. Assays

Total cholesterol and triglycerides were assayed using kits COD 11539 and 11529, respectively, from BioSystems S.A. (Costa Brava, Barcelona). LDL- and VLDL-cholesterol were precipitated from 200 μl of plasma with 500 μL of a mixture of 0.4 mmol/l phosphotungstate and 20 mmol/l magnesium chloride with mild shaking and incubation for 10 min at room temperature (Burstein, Scholnick, & Morfin, 1970). The supernatant containing HDL-cholesterol was analysed with BioSystems reagent COD 11539. VLDL-cholesterol was calculated by the formula: triglycerides/5. LDL-cholesterol was calculated using Friedewald's formula: LDL-cholesterol = (total cholesterol) − (HDL-cholesterol) − (triglycerides/5).

2.5. Statistical analysis

A computer program was used for statistical calculations (Epi Info 6, version 6.04b, CDC/WHO, Switzerland, Dean et al., 1995). All variables were analysed by one-way ANOVA.

Table 2
Composition of diets fed to rabbits

Ingredients	Diets			
	Casein (g/kg)	Control (g/kg)	Amaranth oil (g/kg)	Extruded amaranth (g/kg)
Starch	186.5	550	550	184.3
Extruded amaranth	–	–	–	500
Amaranth oil	–	–	20	–
Sunflower oil	20	20	–	16.5
Casein	250	120	120	40
Cellulose	140	140	140	97.5
Mineral mixture[a]	56.5	56.5	56.5	42.7
Vitamin mixture[a]	2	2	2	2
Sucrose	345	111.5	111.5	111.5

[a] Reeves, Nielsen, and Fahey (1993).

3. Results

Growth rates of rabbits were similar in all groups. The body weight gain of all groups is illustrated in Fig. 1.

After a 21 day feeding period, the total cholesterol levels were lower in the group that was fed EAD than in the other two groups. The same was observed for the LDL-cholesterol levels. These results are presented in Fig. 2. However, no differences between the HDL-cholesterol levels were observed among groups. As for the VLDL-cholesterol levels and triglycerides, both the EAD and the AOD groups presented lower levels when compared with the control group. All these results and the statistical analysis are presented in Table 3.

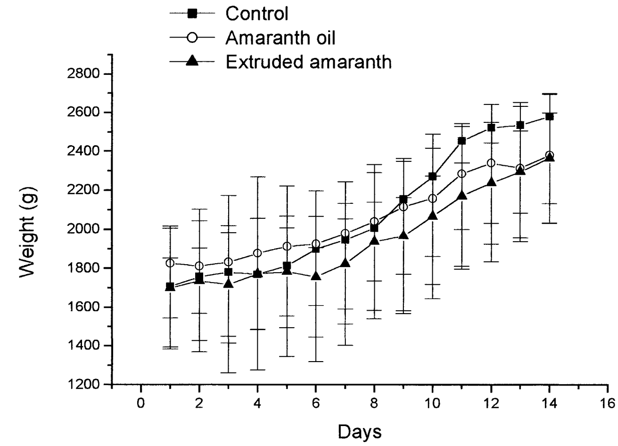

Fig. 1. Weight gain of the hypercholesterolemic rabbits of the three diet groups during the experiment.

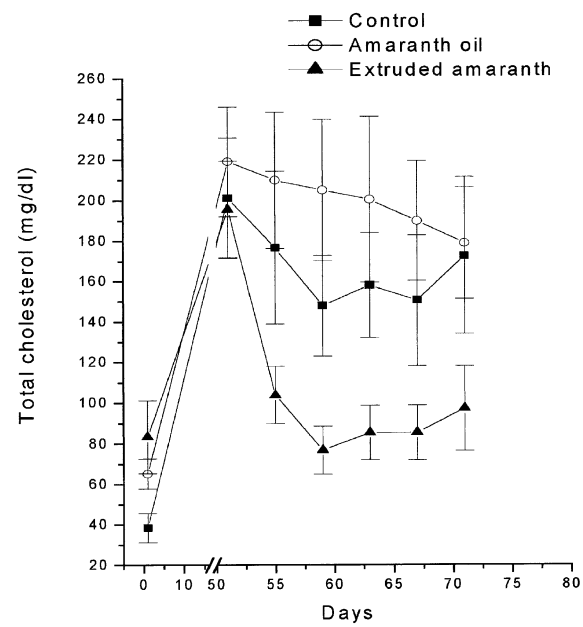

Fig. 2. Total cholesterol levels of the three different diets during the experiment.

4. Discussion

After 21 days, the diet with extruded amaranth reduced total cholesterol levels in rabbits by 50% whereas the control diet and the diet with amaranth oil reduced total cholesterol by 14% and 18%, respectively. Qureshi, Lehmann, and Peterson (1996) verified a reduction of 10–30% in total cholesterol of chickens when these were fed amaranth. Grajeta (1999) observed a reduction of 14% in total cholesterol of rats fed a diet with 30% of amaranth. These results indicate that extruded amaranth is more effective than the grain.

There are many different components in amaranth that could be related to its cholesterol-lowering effect. Qureshi et al. (1996) attributed this effect to amaranth's content of fibres. Danz and Lupton (1992) observed the effect of amaranth on the lipid levels and colon physiology of rats and suggested that it combined the positive benefits of insoluble fibres in the colon with the hypocholesterolemic properties of soluble fibres.

Lehmann, Putnam, and Qureshi (1994) conducted an experiment to determine amaranth's composition of tocotrienols and tocopherols—substances that have recently been shown to regulate cholesterol metabolism—and observed that it was similar to oat and barley. However, each species of amaranth has different amounts of tocotrienols and tocopherols and it was not possible to access the composition of these substances for *Amaranthus caudatus,* used in the present study. In addition, there are controversies about the effect of tocotrienols in reducing cholesterol. Qureshi et al. (1991) observed that total cholesterol and LDL-C were reduced by 15% and 8%, respectively, in subjects who took 200 mg of tocotrienols per day, for 8 weeks. On the other hand, Mensink, Van Houwelingen, Kromhout, & Hornstra (1999) verified no effect on lipid levels of mildly hypercholesterolemic men when they were treated with 140 mg of tocotrienols per day, for 6 weeks. These results may indicate that the action of tocotrienols is related to the amount consumed and period of time in which it is taken.

Becker et al. (1981) observed the presence of significant amounts of squalene in the amaranth species studied (6.7%) and Chatuverdi, Sarojini, & Devi (1993) attributed the hypocholesterolemic effect of amaranth to the squalene.

Another component that might have influenced the effect of extruded amaranth on the cholesterol levels of rabbits is its protein. West et al. (1984) suggested that the tertiary structure of dietary proteins plays an important role in maintaining proper serum cholesterol levels. Vahouny et al. (1985) and Ratnayake, Sarwar, & Farhey (1997) believe that soybean protein has a hypercholesterolemic effect, due to its lysine/arginine ratio, which is 0.9. When compared to soybean protein,

Table 3
Total cholesterol, LDL-cholesterol, HDL-cholesterol and VLDL-cholesterol levels (mg/dl) of animals consuming all diets at 21 days on diet after hypercholesterolemia had been induced[a]

Diet type	Lipid type	Days on diet					
		1	5	9	13	17	21
Control	Total cholesterol	201±29.6	177±37.6	148±25.1d	158±26.0d	151±32.5f	173±38.8f
	LDL-chol	159±26.2	129±37.8	117±22.7c	129±26.6d	123±30.5f	148±36.6f
	HDL-chol	34±4.01	40.11±5.33	23.6±2.81	23.2±3.66	19.1±2.62	15.4±1.75
	VLDL-chol	8.23±1.06	8.03±1.34	6.94±1.12	7.41±0.62	8.43±0.77b	9.36±1.02b
Amaranth oil diet (AOD)	Total cholesterol	219±27.1	210±33.5d	205±34.8c	200±40.9f	190±29.5c	179±27.6f
	LDL-chol	183±27.5	159±33.4e	166.7±35.1c	156±38.6f	149±27.6c	145±26.7e
	HDL-chol	28.3±1.45	41.9±4.72	20.5±2.66	29.0±5.19	25.9±5.21	20.8±4.10
	VLDL-chol	7.32±1.27	6.43±0.41	7.20±1.56	6.52±0.81	6.73±0.74	4.08±0.39c
Extruded amaranth diet (EAD)	Total cholesterol	196±23.7	104±14.1fd	76.8±11.8dc	85.3±13.3df	85.5±13.3fc	97.3±20ff
	LDL-chol	162±20.9	60.8±11.8e	51.7±9.91cc	62.4±11.1df	64.2±12.3fc	72.8±20.8fe
	HDL-chol	31.3±4.33	35.2±3.14	23.6±1.57	22.8±2.15	21±0.77	18.1±1.37
	VLDL-chol	8.28±1.94	9.41±1.57	5.57±0.83	6.93±0.84	4.93±0.68b	4.52±0.72b

[a] Means±S.E.M.; values in the same column are significantly different, indicated by the letters b for $P<0.01$, c for $P<0.02$, d for $P<0.05$, e for $P<0.06$ and f for $P<0.1$.

amaranth protein presents a similar lysine/arginine ratio (0.8) and according to Marcone (1999) both grains present similar molecular weight distributions of subunits of purified 11S globulins. In a clinical studies review about the cholesterol-lowering effects of soybean, Carroll (1991) asserted that substitution of soybean protein for casein increases fecal steroid excretion and the rate of turnover of plasma cholesterol in rabbits. Okita and Sugano (1981) attributed the hypocholesterolemic effect of soybean protein to the subunit structure of the globulin storage protein. Sugano, Ishwak, and Nakashima (1984) attributed this effect to its amino acid sequence. We believe that a similar situation might occur when amaranth protein is substituted for casein.

Morita, Oh-hashi, Takei, Ikai, Kasoka, and Kiriyama (1997) observed that the methionine content of soybean protein influences the reduction of total cholesterol levels in rats, especially by reducing HDL-C levels. Extruded amaranth has a methionine/lysine ratio similar to soybean, which could also reduce HDL-C secretion. However, unlike rats, which transport more than 50% of cholesterol as HDL-C, rabbits transport only 10–15% of cholesterol as HDL-C (Brattsand, 1976). The low methionine content of extruded amaranth might have influenced the reduction of the HDL-C levels in the rabbits, but it is not likely to have reduced total cholesterol levels.

As for the role of amaranth oil on lipid levels, the reductions of total cholesterol and LDL-cholesterol are significant after 21 days. This may be due to unsaturated fatty acid composition. However, Chávez-Jáuregui (1999) showed that fatty acid composition of amaranth oil is similar to the sunflower oil used in the control diet. Further studies should thus be conducted, using different concentrations of amaranth oil, because its influence on lipid levels of animals is not very clear.

References

Association Official Analytical Chemists. (1990). *Official methods of analysis*. Washington, D.C: AOAC.

Becker, R., Wheeler, E. L., Lorenz, K., Stafford, A. E., Grosjean, O. K., Betschart, A. A., & Saunders, R. M. (1981). A compositional study of amaranth grain. *Journal of Food Science*, *46*, 1175–1180.

Brattsand, R. (1976). Distribution of cholesterol and triglycerides among lipoprotein fractions in fat-fed rabbits at different levels of serum cholesterol. *Atheosclerosis*, *23*, 97–110.

Burstein, M., Scholnick, H. R., & Morfin, R. (1970). Rapid method for isolation of lipoproteins from human serum by precipitation with polyanions. *Journal of Lipid Research*, *11*(6), 583–595.

Carroll, K. K. (1991). Review of clinical studies on cholesterol-lowering response to soy protein. *Journal of the American Dietetic Association*, *91*(7), 820–827.

Chatuverdi, A., Sarojini, G., & Devi, N. L. (1993). Hypocholesterolemic effects of amaranth seed (*Amaranthus esculantus*). *Plant Foods for Human Nutrition*, *44*, 63–70.

Chávez-Jáuregui, R. N. (1999) Produção e avaliação de alimento expandido pela extrusão termoptastica do amaranto (*Amaranthus caudatus* L.). PhD thesis, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, 125pp.

Chávez-Jáuregui, R. N., Silva, M. E. M. P., & Arêas, J. A. G. (2000). Extrusion cooking process for amaranth (*Amaranthus caudatus* L.). *Journal of Food Science*, *65*(6), 1009–10015.

Danz, R. A., & Lupton, J. R. (1992). Physiological effects of dietary amaranth (*Amaranthus cruentus*) on rats. *Cereal Foods World*, *37*(7), 489–494.

Dean, A. G., Dean, J. A., Coulombier, D., Brendel, K. A., Smith, D. C., Burton, A. H., Dicker, R. C., Sullivan, K., Fagan, R. F., & Arner, T. G. (1995). *Epi Info, Version 6: A Word-Processing, Database, and Statistics Program for Public Health on IBM-compatible Microcomputers*. Atlanta, Georgia, USA: Centers for Disease Control and Prevention.

Ferreira, T.A.P.C. (1999) Avaliação nutricional do amaranto (*Amaranthus caudatus* L.) extrusado em diferentes condições de umidade e temperatura. PhD Thesis, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Brazil, pp.154.

Grajeta, H. (1997). Effects of amaranthus (*Amaranthus cruentus*) seeds on lipid metabolism in rats. *Bromatologia i Chemia Toksykologiczna*, *30*(1), 25–30.

Grajeta, H. (1999). Effect of amaranth and oat bran on blood serum and liver lipids in rats depending on the kind of dietary fats. *Nahrung*, *43*(2), S114–S117.

Lehmann, J. W., Putnam, D. H., & Qureshi, A. A. (1994). Vitamin E isomers in grain amaranth (*Amaranthus* spp.). *Lipids*, *29*(3), 177–181.

Marcone, M. F. (1999). Biochemical and biophysical properties of plant storage proteins: a current understanding with emphasis on 11S seed globulins. *Food Research International*, *32*, 79–92.

Mendoza, C., & Bressani, R. (1987). Nutritional and functional characteristics of extrusion-cooked amaranth flour. *Cereal Chemistry*, *64*(4), 218–222.

Mensink, R. P., Van Houwelingen, A. C., Kromhout, D., & Hornstra, G. (1999). A vitamin E concentrate rich in tocotrienols had no effect on serum lipids, lipoproteins, or platelet function in men with mildly elevated serum lipid concentrations. *American Journal of Clinical Nutrition*, *69*, 213–219.

Morita, T., Oh-hashi, A., Takei, K., Ikai, M., Kasaoka, S., & Kiriyama, S. (1997). Cholesterol-lowering effects of soybean, potato and rice proteins depend on their low methionine contents in rats fed a cholesterol-free purified diet. *Journal of Nutrition*, *127*, 470–477.

National Research Council. Institute of Laboratory Animal Resources Commission on Life Sciences, Committee on Care and Use of Laboratory Animals. (1985). *Guide for the care and use of laboratory animals*. Washington, DC: National Institutes of Health publication No. 8523 (rev.), Government Printing Office Government Printing Office.

Okita, T., & Sugano, M. (1981). Effects of dietary soybean globulins on plasma and liver lipids and on fecal excretion of neutral sterols in rats. *Journal of Nutritional Science Vitaminology*, *27*, 379–388.

Qureshi, A. A., Lehmann, J. W., & Peterson, D. M. (1996). Amaranth and its oil inhibit cholesterol biosynthesis in 6-week-old female chickens. *Journal of Nutrition*, *126*, 1972–1978.

Qureshi, A. A., Qureshi, N., Wright, J. J. K., Shen, Z., Kramer, G., Gapor, A., Chong, Y. H., DeWitt, G., Ong, A. S. H., Peterson, D. M., & Bradlow, B. A. (1991). Lowering of serum cholesterol in hypercholesterolemic humans by tocotrienols (palmvitee). *American Journal of Clinical Nutrition*, *53*(Suppl), 1021–1026.

Ratnayake, W. M. N., Sarwar, G., & Laffey, P. (1997). Influence of dietary protein and fat on serum lipids and metabolism of essential fatty acids in rats. *British Journal of Nutrition*, *78*, 459–467.

Reeves, P. G., Nielsen, F. H., & Fahey, G. C. (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition*, *123*, 1939–1951.

Sugano, M., Ishiwaki, N., & Nakashime, K. (1984). Dietary protein-dependent modification of serum cholesterol level in rats. Significance of the lysine/arginine ratio. *Annals of Nutrition and Metabolism*, *28*, 192–199.

Teutonico, R. A., & Knorr, D. (1985). Amaranth: composition, properties and applications of a rediscovered food crop. *Food Technology*, *39*, 49–60.

Vahouny, G. V., Adamson, I., Chalcarz, W., Satchithanandam, S., Muesing, R., Klurfeld, D. M., Tepper, A. S., Sanghvi, A., & Kritchevsky, D. (1985). Effects of casein and soy protein on hepatic and serum lipids and lipoprotein lipid distributions in rat. *Atherosclerosis*, *56*, 127–137.

West, C. E., Beynen, A. C., Scholz, K. E., Terpstra, A. H. M., Schuttle, J. B., Deuring, K., & Van Gils, L. G. M. (1984). Treatment of dietary casein with formaldehyde reduces its hypocholesterolemic effect in rabbits. *Journal of Nutrition*, *114*, 17–25.