

## Oat (*Avena sativa* L.) and amaranth (*Amaranthus hypochondriacus*) meals positively affect plasma lipid profile in rats fed cholesterol-containing diets

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### Abstract

Cereals are an important part of diets for hypercholesterolemic patients. However, some of these patients are allergic to these natural products. The purpose of the current study was to compare oatmeal with equal in nutritional values two allergy-free amaranth meals to determine whether this pseudocereal can be a substitute for allergic to cereals individuals. The total phenols of the samples were determined with the Folin–Chocalteu reagent, anthocyanins, and flavonoids spectrophotometrically. The antioxidant activities were estimated with nitric oxide scavenging radical (NO) and by  $\beta$ -carotene bleaching ( $\beta$ -carotene). It was found that the contents of different protein fractions, antioxidant compounds, and the antioxidant activities of oatmeal were significantly higher than those of the two amaranth samples. The results of kinetic reactions showed that samples differed in their capacities to quench these radicals, and oats have shown more antioxidant activity than amaranth. High correlation was observed between antioxidant activities and phenols ( $R^2 = 0.99$ ). In the in vivo part of the investigation, 60 male Wistar rats were divided into five diet groups of 12 animals each; these groups were designated as Control, Chol, Chol/Oat, Chol/AmarI, and Chol/AmarII. The rats of the Control group were fed basal diet (BD) only. To the BD of the four other groups were added the following: 1% of cholesterol (Chol), 10% of oat meal and 1% of cholesterol (Chol/Oat), 10% of amaranth I meal, and 1% of cholesterol (Chol/AmarI) and 10% of amaranth II meal and 1% of cholesterol (Chol/AmarII). After 32 days of different feeding, diets supplemented with oat meal and, to lesser degree, with amaranth I and amaranth II hindered the rise in the plasma lipids: a) TC: 3.14 vs. 4.57 mmol/L, – 31.3%; 3.31 vs. 4.57 mmol/L – 27.6%; and 3.40 vs. 4.57, – 25.6%, respectively b) LDL-C: 1.69 vs. 3.31 mmol/L, – 49.9%; 2.05 vs. 3.31 mmol/L, – 38.1%; and 2.16 vs. 3.31 mmol/L, – 34.8%, respectively; c) TG: 0.73 vs. 0.88 mmol/L, – 17.1%; 0.75 vs. 0.88 mmol/L, – 14.8%; and 0.79 vs. 0.88 mmol/L, – 10.2%, respectively. The HDL-PH was increased as follows: 0.79 vs. 0.63 mmol/L, – 25.3%; 0.75 vs. 0.63 mmol/L, – 23.0%; and 0.71 vs. 0.63 mmol/L, – 12.7% for the Chol/Oat, Chol/AmarI and Chol/AmarII, respectively. No significant changes in the concentrations of HDL-C and TPH were found; however the HDL-C in the Chol/Oat group was slightly higher than in other groups. No changes in the Control group were registered.

In conclusion, oat and amaranth meals positively affect plasma lipid profile in rats fed cholesterol-containing diets. The degree of this positive influence is directly connected to the contents of the bioactive components and the antioxidant activities of the studied samples. It is suggested that amaranth could be a valuable substitute for hypercholesterolemic patients allergic to cereals. © 2004 Elsevier Inc. All rights reserved.

**Keywords:** Oat and amaranth meals; Composition; Antioxidant potential; Rats; Plasma lipids

### 1. Introduction

Cereals and, to a lesser degree, pseudocereals meet the major dietary requirements in proteins, antioxidants, and

minerals and are an important part of diets for hypercholesterolemic patients [1–5]. However, more than 2% of the adult population of the developed countries experiences IgE-mediated hypersensitivity reactions after ingestion of foods, including cereals products [6–8]. Therefore, the investigation of allergy-free pseudocereals has been intensified and the use of amaranth recommended [9–11]. It has

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been shown that consumption of cereals leads to hypocholesterolemic effects [12,13]. However, a significant number of hypercholesterolemic patients cannot use this important natural product to which they are allergic. Therefore, we decided to investigate a popular cereal oat and, equal in its nutritional value, allergy-free amaranth to determine whether this pseudocereal can be a substitute for cereals. Bioactive compounds of oat meal and two amaranth meals were investigated in vitro and their antioxidant activities were determined by two different complementary tests. Their possible hypocholesterolemic effect was then investigated in experiment on rats fed cholesterol-containing and cholesterol-free diets. As far as we know, no such comparative investigations, including experiments in vivo, has been conducted to date.

## 2. Methods and materials

### 2.1. Chemicals

Folin-Ciocalteu reagent,  $\alpha$ -amylase A-3306, protease P-3910 and amyloglucosidase A-991, sodium dodecyl sulfate (SDS),  $\beta$ -mercaptoethanol ( $\beta$ -ME), 6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxylic acid (Trolox), Greiss reagent, sodium nitroprusside,  $\beta$ -carotene, butylated hydroxyanisole (BHA),  $\beta$ -carotene, and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO). Deionized water was used throughout.

### 2.2. Samples

Oat (*Avena sativa* L.) from main region of cultivation, the Karpatian Mountains in Poland, and allergy-free amaranth (*Amaranthus hypochondriacus*) from two regions, the northeast (Lomza) and southeast (Zamosc) regions of Poland, were investigated. The meal samples of oat and amaranth were defatted in a Soxhlet extractor with *n*-hexane (extraction glassware—Soxhlet for ROT-X-TRACT-S, Organomation Associates Inc, MA 01503-1699, USA) for 10 hours and then were stored at 5°C after removal of hexane.

### 2.3. Dietary fiber content

Determination of total, soluble and insoluble dietary fibers was done according to Prosky et al. [14]. Samples were treated with heat-stable  $\alpha$ -amylase, protease, and amyloglucosidase, followed by centrifugation ( $3000 \times g$  for 15 minutes) to separate the soluble and insoluble fractions and dialysis against water.

### 2.4. Protein extraction

Total proteins from 40 mg of each defatted meals were extracted with 1 mL of a 1-mol/L quantity of Tris buffer pH 6.8, containing 1% sodium dodecyl sulphate (SDS) (w/v)

and 4% (v/v) 2-mercaptoethanol (2-ME). The extracts were allowed to stand overnight at room temperature and then were boiled for 5 minutes and centrifuged at  $18,000 \times g$  for 15 minutes at 15°C. The sequence of the used solvents for protein fraction extraction was as follows [15,16]: Saline-soluble proteins (SSP) (albumins [Alb] and globulins [Glo]) were extracted from defatted meals with 0.5 mol/L NaCl (1:10) at 4°C. The supernatants were dialyzed ( $M_w$  cutoff 6000) for 3 days against deionized water at 4°C. Total alcohol-soluble proteins (prolamin-like) were then extracted from the precipitates of the same meals with 55% (v/v) isopropanol (IP), containing 4% (v/v) 2-ME. The extracts were separated by centrifuging at  $10,000 \times g$  for 10 minutes. Each step was repeated twice. Extracts were combined and lyophilized.

Nitrogen content was determined by the micro-Kjeldahl method combined with a colorimetric procedure [17].

### 2.5. Determination of trace elements

Samples of oat and two amaranth meals, each 0.8 g, were mineralized in a microwave oven with concentrated  $HNO_3$ . The contents of trace elements were estimated by a Perkin-Elmer 5100 ZL atomic absorption spectrometer (Perkin-Elmer, Beaconsfield, Buckinghamshire, UK) as previously described [18], using the flame and flameless methods for Co, Se, Cu, I, Zn, and Mn.

### 2.6. Extraction of total phenols and their determination

Defatted samples were extracted from a 50-mg aliquot with 5 mL of 1.2 mol/L HCl in 50% methanol/water. Each sample was vortexed separately for 1 minute and heated at 90°C for 3 hours with vortexing every 30 minutes. After the samples were cooled, they were diluted to 10 mL with methanol and centrifuged for 5 minutes at  $5000 g$  with a bench-top centrifuge to remove solids [19]. The clear supernatants obtained from these extractions were used for determination of total polyphenols and antioxidant activity. Total phenols were measured at 765 nm using Folin-Ciocalteu reagent with gallic acid as a standard and were expressed as  $\mu g/g$  of dry weight (DW) of gallic acid equivalent [20].

### 2.7. Flavonoids

Total flavonoids content was determined by a colorimetric method. First, 0.25 mL of the sample extracts were diluted with 1.25 mL of distilled water. Then 75  $\mu L$  of a 5%  $NaNO_2$  solution was added to the mixture. After 6 minutes, 150  $\mu L$  of a 10%  $AlCl_3 \cdot 6H_2O$  solution was added, and the mixture was allowed to stand for another 5 minutes. A 0.5-mL quantity of 1 mol/L NaOH was added, and the total was made up to 2.5 mL with distilled water. The solution was well mixed, and the absorbance was measured immediately against the prepared blank at 510 nm using a spec-

trophotometer in comparison with the standards prepared similarly with known (+)-catechin concentrations. The results are expressed as mg/100 g DW [21,22].

## 2.8. Anthocyanins

Anthocyanins were estimated by a pH differential method [23]. Absorbance was measured in a Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using  $A = [(A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5}]$  with a molar extinction coefficient of cyanidin-3-glucoside of 29 600. Results were expressed as mg/100 g DW.

## 2.9. Total antioxidant potential determination

The total antioxidant potential was determined by two assays, as described below.

### 2.9.1. Scavenging activity against nitric oxide (NO test)

Nitric oxide interacts with oxygen to produce stable products, nitrite, and nitrate. Scavengers of nitric oxide compete with oxygen, leading to a reduced production of nitrite. The concentration of nitrite in aqueous solution was assayed spectrophotometrically by using the Greiss reagent, with which nitrite reacts to give a stable product absorbing at 542 nm [24,25].

Sodium nitroprusside solution was prepared immediately before the experiment, dissolving 10 mmol/L sodium nitroprusside in 20 mmol/L phosphate buffer, pH 7.4, previously bubbled with argon. The samples diluted in 20 mmol/L phosphate buffer, pH 7.4, to obtain optimal concentrations. At the beginning of the experiment, a 0.5-mL quantity of the samples (at various concentrations) was diluted with 0.5 mL of sodium nitroprusside solution and incubated at 25°C for 150 minutes. At the end of the incubation, 1 mL of Greiss reagent was added to each sample, and the absorbance was read at 542 nm. The nitrite concentration was calculated by referring to the absorbance of standard solutions of potassium nitrite. Results were expressed as percentage nitrite production with respect to control values (sample, 0  $\mu\text{L}$ ).

### 2.9.2. Antioxidant assay using $\beta$ -carotene linoleate model system ( $\beta$ -carotene)

$\beta$ -Carotene (0.2 mg) in 0.2 mL of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate; 200 mg) were mixed. Chloroform was removed at 40°C under vacuum, and the resulting mixture was diluted with 10 mL of water and mixed well. To this emulsion was added 40 mL of oxygenated water. Next, 4-mL aliquots of the emulsion were pipetted into different test tubes containing 0.2 mL of the sample extracts (50 and 100 ppm) and synthetic antioxidant butylated hydroxyanisole (BHA) in ethanol. BHA was used for comparative purposes. A control solution containing 0.2 mL of ethanol and

4 mL of the above emulsion was prepared. The tubes were placed at 50°C in a water bath, and the absorbance at 470 nm was taken at zero time ( $t = 0$ ). Measurement of absorbance was continued until the color of  $\beta$ -carotene disappeared in the control tubes ( $t = 180$  min) at an interval of 15 minutes. A mixture prepared as above without  $\beta$ -carotene served as blank. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of the  $\beta$ -carotene using the following formula:  $AA = 100 [1 - (A_t - A_c)/(A_0 - A_c)]$ , where  $A_0$  and  $A_c$  are the absorbency values measured at zero time of the incubation for test sample and control, respectively, and  $A_t$  and  $A_c$  are the absorbance measured in the test sample and control, respectively, after incubation for 180 minutes [26].

## 2.10. Animals and diets

The protocol of this experiment was previously described [27]. The Animal Care Committee of the Warsaw Agricultural University had approved this study. Wistar rats ( $n = 60$ ), with a mean weight of 120 g at the beginning of the experiment, were provided by the Institute of Animal Physiology and Nutrition of Polish Academy of Sciences (Jablonna, Poland). They were housed in individual metal cages in an air-conditioned room (temperature 21–22°C, relative humidity 55–65%).

The rats were divided into five groups of 12 animals each. These groups were designated as Control, Chol, Chol/Oat, Chol/AmarI, and Chol/AmarII. During 32 days of the experiment the rats in all groups were fed a basal diet (BD), which included wheat starch, casein, soybean oil, vitamin and mineral mixtures [27]. In the Control group 12 rats were fed BD only. To the BD of the four other groups were added the following: 1% cholesterol (Chol), 10% oat meal and 1% cholesterol (Chol/Oat), 10% amaranth meal I and 1% cholesterol (Chol/AmarI), and 10% amaranth meal II and 1% cholesterol (Chol/AmarII). Cholesterol of USP analytical grade was obtained from Sigma Chemical (St. Louis, MO) and checked with high-performance liquid chromatography [28]. No cholesterol oxides were found. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats.

The diets contained energy percentages of 67% carbohydrates, 24% protein, and 9% fat. The calculated energy of the used diets was from 394.9 to 400.1 kcal/100 g, a difference that was statistically not significant.

All rats were fed once a day at 10 AM ad libitum, and had unrestricted access to drinking water. Food intake and body gains were monitored daily.

It is generally accepted that the most reliable data on blood lipid metabolism can be obtained from fasting animals, 14–16 hours after their last feeding. Therefore, food was removed from the cages at 6 PM 1 day before blood samples were drawn, and the samples were collected at 9 AM the next day. Before the experiment the blood samples were taken from the tail vein. After completion of the

Table 1  
Dietary fiber in oat and amaranth (% on dry-matter basis)

Meal	Total	Soluble	Insoluble
Oat	16.6 ± 1.1 <sup>a</sup>	6.9 ± 0.4 <sup>a</sup>	9.7 ± 0.7 <sup>a</sup>
Amaranth I	14.5 ± 0.9 <sup>a</sup>	6.3 ± 0.4 <sup>a</sup>	8.2 ± 0.6 <sup>a</sup>
Amaranth II	14.2 ± 0.9 <sup>a</sup>	6.1 ± 0.4 <sup>a</sup>	8.1 ± 0.6 <sup>a</sup>

Values are means ± SD of five measurements; means in columns without superscript letters in common differ significantly ( $P < 0.05$ ).

experiment the rats were fully anesthetized by an intraperitoneal injection of verbutal and the blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests.

Two time points were used in this experiment: before and after 32 days of feeding. At these time points a wide range of laboratory tests were performed, as previously described [27].

### 2.11. Statistical analysis

The results of this investigation *in vitro* are means ± SD of five measurements. When appropriate, differences between groups were tested by two-way analysis of variance. In the assessment of the antioxidant potential, the Spearman correlation coefficient ( $R$ ) was used. Linear regressions were also calculated.  $P$  values  $< 0.05$  were considered to be significant.

## 3. Results

### 3.1. *In vitro*

#### 3.1.1. Dietary fiber

The results of the determination of the content of the dietary fiber in oat and two samples of amaranth are summarized in Table 1. As can be seen, the contents of total, soluble, and insoluble dietary fibers are higher in oat than in the two samples of amaranth, but the differences are not significant.

#### 3.1.2. Essential trace elements

Results of the determination of the contents of the essential trace elements in the studied samples are summarized in Table 2. As can be seen, only the content of Mn is significantly higher in oat than in amaranth meals.

Table 2  
Essential trace elements in the studied samples (mg/kg dry matter)

Meal	Mn	Zn	Cu	Se	I	Co
Oat	42.3 ± 3.7 <sup>a</sup>	41.1 ± 3.6 <sup>a</sup>	7.6 ± 0.6 <sup>a</sup>	0.26 ± 0.02 <sup>a</sup>	0.11 ± 0.01 <sup>a</sup>	0.06 ± 0.001 <sup>a</sup>
Amaranth I	31.7 ± 2.9 <sup>b</sup>	38.7 ± 3.3 <sup>a</sup>	8.3 ± 0.7 <sup>a</sup>	0.23 ± 0.02 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.06 ± 0.001 <sup>a</sup>
Amaranth II	31.5 ± 2.8 <sup>b</sup>	38.3 ± 3.3 <sup>a</sup>	8.1 ± 0.7 <sup>a</sup>	0.22 ± 0.02 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.05 ± 0.001 <sup>a</sup>

Values are means ± SD of five measurements; means in columns without superscript letters in common differ significantly ( $P < 0.05$ ).

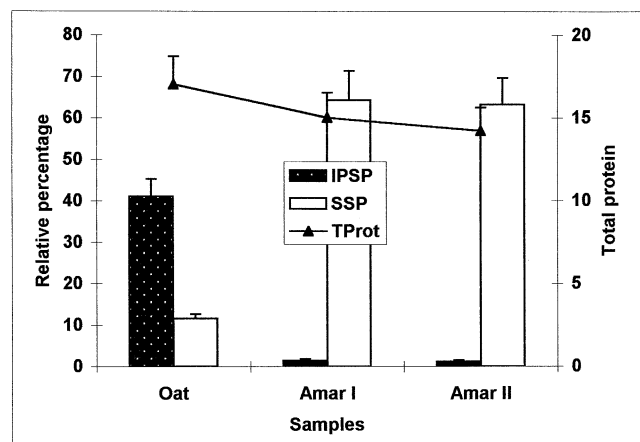


Fig. 1. Total protein (TProt) content (% nitrogen) and protein fractions distribution (relative percentage) of isopropanol-soluble proteins (IPSP) and saline-soluble proteins (SSP) in amaranth and oat samples. IPSP were extracted with 55% (v/v) isopropanol (IP), containing 4% (v/v) 2-ME; SSP with 0.5 mol/L NaCl.

#### 3.1.3. Proteins

Total proteins (% nitrogen) in amaranth I and II, and oat meals were 15%, 14.2%, and 17%, respectively. The relative proportions of nitrogen content between prolamin fraction and saline water-soluble protein fraction in amaranth I and II, and oat meals were about 64.2:1.4; 63.3:1.2, and 11.5:41.1, respectively (Fig. 1). As shown, the relative proportions of pseudocereals are different from those of cereals in the main prolamin fraction, which is characteristic only for cereals. The globulin and albumin fractions are the main proteins only for pseudocereals.

#### 3.1.4. Antioxidant compounds and antioxidant activities

The content of total phenols, flavonoids, anthocyanins, and antioxidative activities in amaranth and oat meals are summarized in Table 3. As can be seen, the content of all indices are significantly higher in oat than in amaranth I and II meals ( $P < 0.05$  in both cases).

The antioxidant activities of the three plant extracts and standard antioxidants at 0.2 mg/mL concentration, as measured by the bleaching of  $\beta$ -carotene, are presented in Fig. 2. The oat extract showed the highest antioxidant activity (34.6%), followed by amaranth I (26%) and amaranth II (23.2%). As can be seen, BHA (94.4%), BHT (84%) and Trolox (80.0%) have the maximum antioxidant activities. Oat extract showed stronger inhibition than the extracts

Table 3  
Antioxidant compounds in dry weight of the studied samples and the sample antioxidant activities

Meal	Polyphenols ( $\mu\text{g/g}$ )	Anthocyanins ( $\text{mg}/100\text{ g}$ )	Flavonoids ( $\text{mg}/100\text{ g}$ )	$\beta$ -Carotene (%)	NO (%)
Oat	$196.1 \pm 14.8^a$	$83.5 \pm 7.1^a$	$17.7 \pm 1.4^a$	$34.6 \pm 1.8^a$	$32.7 \pm 2.1^a$
Amaranth I	$149.1 \pm 13.2^b$	$62.5 \pm 6.1^b$	$14.3 \pm 1.1^b$	$26.0 \pm 2.1^b$	$25.1 \pm 1.9^b$
Amaranth II	$147.2 \pm 13.1^b$	$59.6 \pm 6.0^b$	$13.4 \pm 1.0^b$	$23.2 \pm 2.1^b$	$23.0 \pm 1.9^b$

Values are means  $\pm$  SD of five measurements; means in columns without superscript letters in common differ significantly ( $P < 0.05$ ).

from amaranth I and II. The best correlation ( $R^2 = 0.9946$  and  $0.9891$ ) was between total phenols and total antioxidant activity as determined by NO and  $\beta$ -carotene (Fig. 3A). Good correlation levels were also observed for anthocyanins:  $R^2 = 0.9908$  and  $R^2 = 0.9841$ , as determined by NO and  $\beta$ -carotene tests, respectively (Fig. 3B). Flavonoids showed similar high levels of correlation ( $R^2 = 0.9999$  and  $0.9985$ ) (Fig. 3C). However, the correlation of soluble fibers is relatively lower than in polyphenols, anthocyanins, and flavonoids:  $R^2 = 0.5773$  and  $R^2 = 0.5469$ , as determined by NO and  $\beta$ -carotene tests, respectively (Fig. 3D).

### 3.2. In vivo

The addition of oat and amaranth meals and/or cholesterol to the diets have no significantly different impact on the food intake and body weight gain (data not shown).

Diets supplemented with oat, amaranth I, and amaranth II and fed to rats given added cholesterol significantly hindered the rise of plasma lipids (Table 4), as follows: a) TC, 3.14 vs. 4.57 mmol/L,  $-31.3\%$ ; 3.31 vs. 4.57 mmol/L,  $-27.6\%$ ; and 3.40 vs. 4.57,  $-25.6\%$ , respectively; b) LDL-C, 1.69 vs. 3.31 mmol/L,  $-49.9\%$ ; 2.05 vs. 3.31 mmol/L,  $-38.1\%$ ; and 2.16 vs. 3.31 mmol/L,  $-34.8\%$ , respectively; c) TG, 0.73 vs. 0.88 mmol/L,  $-17.1\%$ ; 0.75

vs. 0.88 mmol/L,  $-14.8\%$ ; and 0.79 vs. 0.88 mmol/L,  $-10.2\%$ ; and increased HDL-PH: 0.79 vs. 0.63 mmol/L,  $+25.3\%$ ; 0.75 vs. 0.63 mmol/L,  $+23.0\%$ ; and 0.71 vs. 0.63 mmol/L,  $+12.7\%$  for the Chol/Oat, Chol/AmarI, and Chol/AmarII, respectively. No significant changes in the concentrations of HDL-C and TPH were found; however, the HDL-C in the Chol/Oat group was slightly higher than in other groups.

The above-mentioned results show that the bioactivities of the studied samples were as follow: oat > amaranth I  $\geq$  amaranth II meals.

### 4. Discussion

It is known that the bioactive components of cereals positively influence the plasma lipids both in hypercholesterolemic animals and in humans [29–33]. However, some

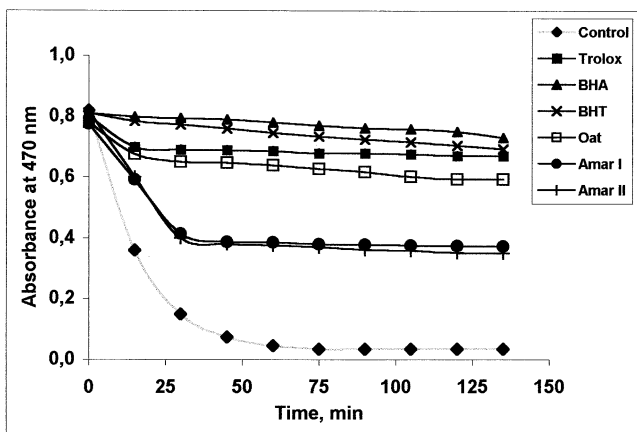


Fig. 2. Reaction kinetics: three plant extracts with 50% methanol/water/1.2 mol/L HCl from: oat meal (Oat), amaranth I (Amar I), amaranth II (Amar II) meals, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and trolox (Trolox) with  $\beta$ -carotene bleaching. The  $\beta$ -carotene concentration was 0.004 mg/mL and the samples were at 0.2 mg/mL in the reaction mixtures.

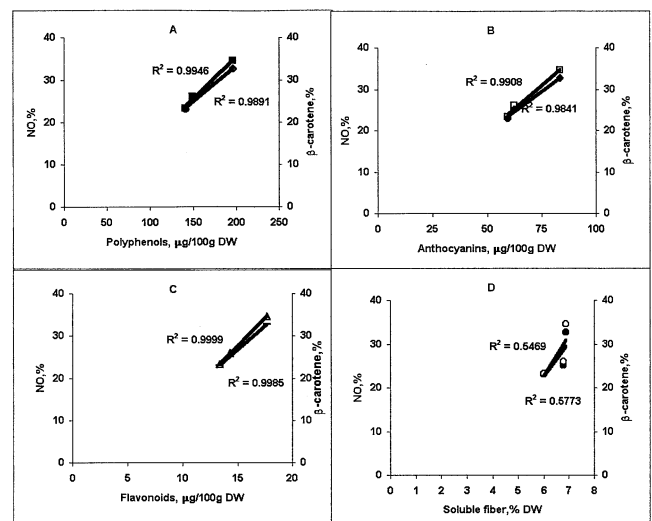


Fig. 3. Relationship, calculated by linear regression analysis for plant extracts between the following: (A)  $\blacksquare$  Polyphenols ( $\mu\text{g}$  gallic acid/100 g DW, X) to NO (%),  $Y_1$ ) and  $\blacklozenge$  Polyphenols ( $\mu\text{g}$  gallic acid/100 g DW, X) to  $\beta$ -carotene bleaching effect (%),  $Y_2$ ). (B)  $\blacklozenge$  Anthocyanins ( $\mu\text{g}/100\text{ g}$  DW, X) to NO (%),  $Y_1$ ) and  $\square$  anthocyanins ( $\mu\text{g}/100\text{ g}$  DW, X) to  $\beta$ -carotene bleaching effect (%),  $Y_2$ ); (C) Flavonoids ( $\mu\text{g}/100\text{ g}$  DW, X) to NO (%),  $Y_1$ ) and  $\triangle$  Flavonoids ( $\mu\text{g}/100\text{ g}$  DW, X) to  $\beta$ -carotene bleaching effect (%),  $Y_2$ ). (D)  $\bullet$  Soluble fiber ( $\mu\text{g}/100\text{ g}$  DW, X) to NO (%),  $Y_1$ ) and  $\circ$  soluble fiber (% DW, X) to  $\beta$ -carotene bleaching effect (%),  $Y_2$ ). Plant matter extracted with 1.2 mol/L HCl in 50% methanol/water. Polyphenols expressed as  $\mu\text{g}$  gallic acid/100 g dry weight (DW).

Table 4  
Plasma lipid concentrations in rats fed diets with and without 1% cholesterol and with and without added meals

Diet	Plasma lipids (mmol/L)					
	TC	LDL-C	HDL-C	TG	TPH	HDL-PH
Control	2.82 ± 0.14 <sup>c</sup>	0.97 ± 0.05 <sup>c</sup>	1.84 ± 0.11 <sup>b</sup>	0.69 ± 0.02 <sup>c</sup>	1.77 ± 0.10 <sup>a</sup>	1.05 ± 0.03 <sup>c</sup>
Chol	4.57 ± 0.21 <sup>a</sup>	3.31 ± 0.12 <sup>a</sup>	1.22 ± 0.08 <sup>a</sup>	0.88 ± 0.02 <sup>a</sup>	1.84 ± 0.10 <sup>a</sup>	0.63 ± 0.02 <sup>a</sup>
Chol/Oat	3.14 ± 0.17 <sup>b</sup>	1.69 ± 0.05 <sup>b</sup>	1.42 ± 0.09 <sup>a</sup>	0.73 ± 0.02 <sup>b</sup>	1.81 ± 0.09 <sup>a</sup>	0.79 ± 0.02 <sup>b</sup>
Chol/AmI	3.31 ± 0.18 <sup>b</sup>	2.05 ± 0.05 <sup>b</sup>	1.25 ± 0.07 <sup>a</sup>	0.75 ± 0.02 <sup>b</sup>	1.79 ± 0.09 <sup>a</sup>	0.75 ± 0.02 <sup>b</sup>
Chol/AmII	3.40 ± 0.19 <sup>b</sup>	2.16 ± 0.05 <sup>b</sup>	1.24 ± 0.07 <sup>a</sup>	0.79 ± 0.02 <sup>b</sup>	1.78 ± 0.09 <sup>a</sup>	0.71 ± 0.02 <sup>b</sup>
<i>P</i> -value (2-way ANOVA)						
Chol	<0.001	<0.001	NS	<0.001	NS	<0.001
Oat + Chol	<0.001	<0.001	NS	<0.001	<0.005	<0.025
AmI + Chol	<0.050	<0.050	NS	<0.050	<0.010	<0.050
AmII + Chol	<0.050	<0.050	NS	<0.050	<0.010	<0.050

Values are means ± SD, *n* = 12.

Means in columns without superscript letters in common differ significantly (*P* < 0.05).

Am = amaranth; Chol = nonoxidized cholesterol; HDL-C = HDL cholesterol; HDL-PH = HDL phospholipids; LDL-C = LDL cholesterol; NS = not significant (*P* ≥ 0.05); TC = total cholesterol; TG = triglycerides; TPH = total phospholipids.

individuals with hypocholesterolemia are allergic to cereals. Therefore, in the current study, a popular cereal in the form of oat meal was compared with two different allergy-free amaranth meals to determine whether amaranth can be used as a substitute for cereals in allergic individuals. Therefore, the contents of dietary fibers, trace elements, proteins, antioxidant compounds, and the antioxidant potential of oat meal were determined and compared with these indices in two different amaranth meals.

It was found that the contents of dietary fibers in the studied samples are comparable. Our data are in accordance with others [34,35].

The results of the investigation of the essential trace elements [36] showed that only the content of Mn is significantly higher in oat meal. These results are also in accordance with the results of others [37,38].

The contents of the antioxidant compounds and the antioxidant activities of the studied samples were as follows: oat meal > amaranth I ≥ amaranth II. These results also agree with the data of others [39].

In our recent investigations we have studied total antioxidant potential, using the total radical-trapping antioxidative potential test [27,40]. There are many assays for total antioxidant determination and each has its limitations [27,40]. According to Ou et al. [41], some antioxidant assays give different antioxidant activity trends. Therefore, in this investigation, the free radical scavenging properties of the studied samples were determined by two different tests (NO and β-carotene).

The results of the determination of the antioxidant potential by these assays have shown the same trends: the antioxidant potential was higher in samples with higher contents of the antioxidant compounds. Therefore, we cannot support the claims of Ou et al. [41]. Like others [42,43] and like our previous investigation [44], we have found a linear correlation between content of the antioxidant compounds and the antioxidant potential of the studied samples.

The results of the present investigation have shown that an increase in dietary cholesterol intake led to hypercholesterolemia. These results are in accordance with the data of others, who have found in experiments on animals a nutritionally induced hypercholesterolemia [13,32].

After 32 days of the investigation we observed that diets supplemented with oat, amaranth I, and II and fed to rats given added cholesterol significantly hindered the rise of plasma lipids. These data correspond with the data of others, who have found that cereal- and pseudocereal-supplemented diets counteracted development of hypercholesterolemia in cholesterol-fed animals as well as hypercholesterolemic patients [32,39].

Like other investigators we have found that variety of cereal and pseudocereal products have different effects on serum lipid levels [39]. The cholesterol-lowering effect of amaranth I was slightly higher than of the amaranth II. However, contrary to the above-mentioned studies, we did not find unfavorable serum lipid responses to oat meal.

In conclusion, in this study we were able to show the following: 1) the contents of total, soluble, and insoluble dietary fibers and essential trace elements excluding Mn are comparable in the studied samples (i.e., oat, amaranth I, and amaranth II); 2) the contents of the protein fractions, antioxidant compounds, and the antioxidant activity in oat meal is higher than in the two studied amaranth meals; 3) diets supplemented with all three studied samples significantly hindered the rise of plasma lipids in rats given added cholesterol. Our findings suggest that amaranth and its products could be a valuable substitute for cereals in allergic hypercholesterolemic patients.

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