

EFFECT OF ADDITION OF ROMANESCO TYPE GREEN CAULIFLOWER TO DIET ON SERUM LIPID PROFILE IN RATS

Agnieszka Filipiak-Florkiewicz¹, Ewa Cieřlik¹, Renata B. Kostogrys²

¹The Malopolska Centre of Food Monitoring and Certification, ²Department of Human Nutrition, The Agricultural University of Cracow

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The aim of the present study was to establish the influence of raw and cooked (fresh and frozen) green cauliflower on some biochemical parameters (levels of total cholesterol, HDL, LDL and VLDL cholesterol and triacylglycerols) in rats. The experiment did not show any effect of cauliflower addition to diet on body weight of rats. The data indicated that AIN-93G diet containing 1% cholesterol increased the level of triacylglycerols and LDL + VLDL fractions in comparison with AIN-93 diet alone. Levels of total cholesterol were lower in animals fed the diet with addition of fresh cooked cauliflower as compared to rats given hypercholesterolemic diet with addition of raw vegetable.

INTRODUCTION

Cauliflower *Brassica L. var. Botrytis* belongs to cruciferous family *Cruciferae* (Brassicaceae), which comprises also: cabbage, broccoli, Brussels sprouts, turnip, Swedish turnip. Cauliflowers with white curds are the most important from the economic standpoint, but recently cauliflowers with differently colored curds can be encountered: green, yellow, and even orange and violet. Green varieties are still a novelty on our market, but they are very interesting to consumers [Gajewski, 1999].

Vegetables and fruits are the main source of antioxidants (vitamins: C, E, β -carotene and lycopene, polyphenols) in our diet. Potential health benefits of fruits and vegetables have been attributed also to the effect of other specific components: dietary fibre, minerals and glucosinolates [Broekmans *et al.*, 2000]. Due to the detection of many bioactive compounds in food with potential antioxidant activity, there has been increased interest in the relationship between these compounds and prevention of several diseases. The total pool of antioxidants supplied with diet depends on two factors: the stability of antioxidants during processing and their bioavailability in the human digestive tract. Prolonged cooking in large volume of water causes particularly high losses of antioxidants. Due to oxidation, their significant losses occur also during drying and deep-frying processes [Grajek, 2003].

Cauliflower belongs to the most valuable vegetables because it has high nutritive value, is tasty and easy to prepare. Low calorie value is its another advantage. It is considered to be the easily digestible brassicaceous vegetable and is recommended in diets for the sick and elderly [Rumpel, 2002;

Rutkowska, 1994]. In addition, cauliflower is characterised by one of the highest antioxidative activities among plants [Grajek, 2003]. Cieřlik *et al.* [2005] showed that fresh green cauliflower contains vitamin C (52.4 mg/100 g fresh mass), carotenoids (0.38 mg/100 g fresh mass), total polyphenols (calculated as equivalent of catechin – 144.0 mg/100 g fresh mass) and that it displays antioxidant potential (2.35 μ mol Trolox/g).

A number of experiments indicate that vegetables added to laboratory animals' diet had positive effects on lipid profile. Antioxidants from vegetables were shown to lower atherogenic cholesterol fraction (LDL +VLDL) concomitantly increasing HDL fraction, which is believed to be beneficial for the prevention of cardiovascular diseases [Gorinstein *et al.*, 2006; Leontowicz *et al.*, 2002; Nicolle *et al.*, 2004]. Rasmusen *et al.* [2005] suggested that polyphenols are slowing down metabolism of cholesterol in the liver, reducing excretion of VLDL to blood, which leads to lower cholesterol accumulation in the aorta.

The aim of the present study was to determine the influence of the addition of raw and cooked (fresh and frozen) green cauliflower to rats' diet on their biochemical parameters. In particular, we examined the effect of dietary cauliflower subjected to different technological processes on body weight gain, liver weight, and serum lipid profile, *i.e.* levels of total, HDL, LDL and VLDL cholesterol and triacylglycerols.

MATERIALS AND METHODS

The experiment was conducted on albino Wistar rats. Eight weeks old males were assigned to 5 groups (n = 6).

Control animals (group I) were fed a semisynthetic diet AIN-93G [Reeves *et al.*, 1993], group II was given AIN-93G diet containing 1% cholesterol, while the remaining groups received AIN-93G diet + 1% cholesterol with 10% addition of freeze-dried green cauliflower: raw (group III), fresh cooked (group IV) and frozen and cooked (group V) (Table 1). The raw Romanesco type cauliflower (cv. Amfora, cultivated in Krakowska Hodowla i Nasiennictwo Ogrodnicze "Polan" in 2004) was obtained by washing in tap water, drying on filter paper, crushing and then freezing at -22°C . After freezing, the cauliflower was immediately freeze-dried using Christ Alpha 1-4 freeze-dryer, and ground using Tecator Kniftec 1095 Sample Mill, until uniform powder was obtained. The boiling of fresh cauliflower was carried out for 10-15 min. Before freezing the vegetables were blanched at 80°C for approx. 3 min and then stored for 4 months at -22°C . Next, the frozen cauliflower was boiled for 5-8 min. The boiled (fresh and frozen) vegetables were freeze-dried and ground as described above.

Fiber content in the diet was balanced with cellulose (Table 1). Mineral and vitamin mixture composition was according to AIN-93G diet [Reeves *et al.*, 1993]. All the diets and water were available for rats *ad libitum*.

Rats were weighed once a week throughout the experiment. Water and diets were replenished daily. After 21 days, the rats were euthanized in compliance with requirements of the Local Ethics Commission. The time between last feeding and the section was 12 h. Blood was collected by cardiac puncture after chest incision. Levels of total cholesterol, and HDL fraction were determined in serum using an enzymatic method and reagents from BioVendor Cat. No. 108510 and 10855 (Czech Republic). The level of LDL + VLDL cholesterol was calculated from the difference between total and HDL cholesterol. Triacylglycerols level was assayed by the enzymatic method using BioVendor kit, Cat. No. 12805 (Czech Republic).

All data are presented as the mean \pm standard deviation. Significance of differences between the means was assessed with the Tukey's test. The differences were considered significant when $p \leq 0.05$.

RESULTS

It was shown that the addition of raw or cooked (fresh and frozen) green cauliflower to AIN-93G diet did not cause any significant differences in body weight gain of experimental animals. Mean rats' body weight gain of groups II, III, IV and V was 69.0 g during the experiment (Table 2).

Based on the results obtained, we observed that the addition of frozen and then cooked green cauliflower to AIN-93G + 1% cholesterol diet statistically significantly lowered liver weight in rats in comparison with the II, III and IV groups. Animals given the diet containing 10% of frozen and cooked cauliflower had lower liver weight (by 0.7 g/100 g body weight) than those given hypercholesterolemic diet (AIN-93G + 1% cholesterol) and diet containing fresh cooked cauliflower (Table 2).

Serum total cholesterol level was significantly lower in rats fed a diet with the addition of fresh cooked green cauliflower (IV) in comparison to animals given diet with raw vegetable (III). In the remaining groups, differences in total cholesterol contents were not statistically significant.

Serum HDL cholesterol level was the highest in rats fed AIN-93G diet. The largest decrease in serum HDL cholesterol level (by 24% vs. rats fed on AIN-93G diet) was observed in group IV, receiving the diet supplemented with fresh cooked cauliflower. This difference was statistically significant ($p=0.012$) (Table 3).

The results indicated that AIN-93G diet + 1% cholesterol and diet supplemented with raw cauliflower significantly elevated LDL-VLDL cholesterol level by 33.0% and 47.3%, respectively, vs. AIN-93G diet. The addition of fresh cooked

TABLE 1. Composition of experimental diets (g/kg).

Component	Group I AIN-93G	Group II AIN-93G+1% cho- lesterol	Group III AIN-93G+1% cho- lesterol + 10% raw cauliflower	Group IV AIN-93G+1% cho- lesterol + 10% fresh cooked cauliflower	Group V AIN-93G+1% cho- lesterol + 10% frozen cooked cauliflower
Corn starch	532.5	522.5	440.5	446.7	439.1
Casein (95% N x 6.25)	200	200	200	200	200
Sucrose	100	100	100	100	100
Soybean oil	70	70	70	70	70
Cellulose powder	50	50	31.9 [^]	25.8 [^]	33.4 [^]
Mineral mixture ^a	35	35	35	35	35
Vitamin mixture ^b	10	10	10	10	10
Choline	2.5	2.5	2.5	2.5	2.5
<i>Tert</i> -butylhydrochinon	0.014	0.014	0.014	0.014	0.014
Cholesterol	-	10	10	10	10
Romanesco type cauliflower	-	-	100 (18.07*) (9.6**)	100 (24.18*) (8.3**)	100 (16.62*) (6.3**)

* – cellulose content in 100 g of freeze-dried Romanesco cauliflower, ** dry matter content in raw cauliflower (g/100 g) [^] – fiber content in the diet was balanced with cellulose up to 50 g

TABLE 2. Body weight gain (g) and liver weight (g/100 g body weight) in experimental animals fed on cauliflower-supplemented diet.

Groups	Diets	Body weight (g)						Liver weight (g/100 g body weight)	
		at the beginning of experiment		at the end of experiment		gain (g)	SD	Mean	SD
		Mean	SD	Mean	SD				
I	AIN-93G	167.2	12.3	235.0	14.5	68.3 ^a	4.6	4.6 ^{ab}	0.3
II	AIN-93G + 1% cholesterol	137.0	17.9	205.8	18.5	68.8 ^a	4.8	5.1 ^b	0.3
III	AIN-93G + 1% cholesterol + 10% raw cauliflower	145.5	15.9	209.8	24.0	64.3 ^a	3.4	5.0 ^b	0.4
IV	AIN-93G + 1% cholesterol + 10% fresh cooked cauliflower	137.5	17.9	216.6	38.3	79.1 ^a	5.3	5.1 ^b	0.4
V	AIN-93G + 1% cholesterol + 10% frozen cooked cauliflower	126.0	8.5	190.7	13.1	64.7 ^a	4.9	4.4 ^a	0.3

The values in the same columns denoted with different letters: a, b, c, d, differ statistically significantly at $p \leq 0.05$.

TABLE 3. Serum lipid profile in experimental animals fed cauliflower-supplemented diet (mmol/L).

Groups	Diets	Total cholesterol		HDL cholesterol		LDL + VLDL cholesterol		Triacylglycerols	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
I	AIN-93G	2.1 ^{ab}	0.3	1.2 ^b	0.1	0.9 ^a	0.1	0.8 ^a	0.3
II	AIN-93G + 1% cholesterol	2.2 ^{ab}	0.2	1.0 ^{ab}	0.1	1.2 ^b	0.2	1.7 ^b	0.4
III	AIN-93G + 1% cholesterol + 10% raw cauliflower	2.4 ^b	0.2	1.0 ^{ab}	0.1	1.3 ^b	0.1	1.5 ^{ab}	0.35
IV	AIN-93G + 1% cholesterol + 10% fresh cooked cauliflower	1.9 ^a	0.11	0.9 ^a	0.0	1.1 ^{ab}	0.1	1.9 ^b	0.5
V	AIN-93G + 1% cholesterol + 10% frozen cooked cauliflower	2.1 ^{ab}	0.21	1.0 ^{ab}	0.1	1.1 ^{ab}	0.2	1.1 ^{ab}	0.1

The values in the same columns denoted with different letters: a, b, c, d, differ statistically significantly at $p \leq 0.05$.

cauliflower decreased LDL+VLDL fraction level by 10.7% in comparison with AIN-93G diet + 1% cholesterol. Frozen cooked cauliflower caused a similar drop of this parameter by 9.1%, however, these differences were not statistically significant (Table 3).

The experiment showed that rats fed on hypercholesterolemic diet had twice higher contents of triacylglycerols in comparison with AIN-93G diet. Fresh cooked cauliflower added to a hypercholesterolemic diet caused a similar statistically significant raise in this parameter. In contrast, raw or frozen and then cooked cauliflower lowered triacylglycerols contents by 8.8% and 35.3%, respectively, in relation to AIN-93G diet containing 1% cholesterol, but these values were not statistically significant (Table 3).

DISCUSSION

High intake of fruits and vegetables rich in antioxidants is recommended to prevent and treat degenerative diseases [Wartanowicz & Ziemiański, 1999]. Results of many studies have indicated that antioxidants exhibit anticarcinogenic and antiatherogenic properties [Broekmans *et al.*, 2000; Grajek, 2007].

The present experiment did not show a larger feed and water consumption over the control group. There were also no statistically significant changes in body weight gain between the study groups. Neither raw nor cooked (fresh or frozen) cauliflower added to the diet affected growth rate of animals.

Analysis showed that body weight gains in groups II, III, IV and V were similar.

Gorinstein *et al.* [2006] obtained similar results in their studies on the effect of raw and cooked garlic-supplemented diet in rats. They showed no statistically significant differences in body weight gain, as well. Leontowicz *et al.* [2002], who investigated the influence of antioxidants contained in apples, peaches and pears, also did not report any differences in body weight gain nor liver weight between study groups. On the other hand, Nicolle *et al.* [2004] demonstrated a statistically significant decrease in liver weight of animals fed a lettuce-containing diet in comparison with the control diet.

The present experiment showed that hypercholesterolemic diet (AIN-93G + 1% cholesterol) significantly elevated the level of LDL + VLDL cholesterol and triacylglycerols vs. AIN-93G diet. It was also revealed that cauliflower addition to the diet slightly lowered some parameters of lipid profile in comparison with hypercholesterolemic AIN-93G diet alone. Dietary cooked (fresh and frozen) cauliflower reduced serum total cholesterol level and its LDL + VLDL fraction. Results obtained by Kostogryś *et al.* [2006] showed that incorporation of curly kale (10% ww.) had no significant effect on serum total cholesterol and LDL-cholesterol in rats. At the same time, the HDL-cholesterol was significantly increased in serum from rats fed diets with cooked or blanched curly kale. Also the concentration of triacylglycerols decreased in rats fed these diets.

Moreover, animals fed the hypercholesterolemic diet supplemented with fresh cooked cauliflower had lower total cholesterol level than the animals receiving the diet with raw cauliflower. Intensification of the beneficial effect of diet supplemented with cooked vegetables on lipid profile can be attributed to a positive effect of high temperature on antioxidant components by increasing their bioavailability. This phenomenon has been observed, *e.g.* for lycopene example [Grajek, 2003]. On the other hand, some methods of food processing like heating and freezing considerably decreased antioxidants concentration *e.g.* vitamin C [Grajek, 2007]. Cooking practice, such as blanching, may greatly influence the loss of antioxidant components in leafy vegetables [Yadav & Sehgal, 1995]. A loss of approximately 50% of antioxidant activity is caused by blanching [Hunter & Fletcher, 2002]. However, the lowered content of antioxidants in vegetables is not always connected with reducing their antioxidant potential. Results obtained by Amin *et al.* [2006] showed that the total phenolic content for all the spinach species tends to decrease after blanching. The statistical analysis revealed significant differences between raw and 10-min-blanching samples of three out of four spinach species. Simultaneously, significant differences between samples (two from four examined species of spinach) were shown in the case of total antioxidant activity.

Nicolle *et al.* [2004] reported that addition of 25% of lettuce and 0.25% of cholesterol to the diet significantly lowered total cholesterol level by 7%. LDL cholesterol level was also observed to decrease in relation to HDL fraction, whereas triacylglycerols level remained unchanged.

Similar results were obtained by Gorinstein *et al.* [2006]. They revealed that supplementation of control diet with 25 mg of lyophilized garlic/kg body weight obtained from raw or cooked garlic significantly diminished serum total cholesterol level (by 16%) and LDL fraction (by 30%) in rats. In addition, this diet decreased triacylglycerol level by 14% and caused a slight increase in HDL concentration (by 6%).

As demonstrated by Leontowicz *et al.* [2002], the addition of 10% of apples, peaches or pears to the hypercholesterolemic diet caused a statistically significant drop in total cholesterol (by 18%), LDL fraction (by 30%) and triacylglycerols (by 15%). Those authors did not observe any changes in HDL level.

All the above-cited experiments indicate that vegetables added to laboratory animals' diet had a positive effect on their lipid profile. This confirmed opinions about health-promoting properties of antioxidants present in vegetables.

CONCLUSIONS

1. Addition of raw or cooked green cauliflower to the atherogenic diet was not shown to affect body weight of rats.

2. The results indicate that AIN-93G diet containing 1% of cholesterol increased contents of triacylglycerols and LDL + VLDL cholesterol in comparison to the control diet AIN-93G.

3. Serum total cholesterol level was statistically significantly lower in animals fed the diet with the addition of fresh cooked cauliflower in comparison with rats receiving hypercholesterolemic diet with the addition of raw vegetable.

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WPLYW DIETY Z DODATKIEM KALAFIORA ZIEŁONEGO TYPU ROMANESCO NA PROFIL LIPIDOWY SZCZURÓW

Agnieszka Filipiak-Florkiewicz¹, Ewa Cieślak¹, Renata B. Kostogryś²

¹Małopolskie Centrum Monitoringu i Atestacji Żywności, ²Katedra Żywienia Człowieka, Akademia Rolnicza w Krakowie

Celem pracy było określenie wpływu diety z dodatkiem kalafiora zielonego świeżego oraz gotowanego (świeżego i mrożonego) na wybrane parametry biochemiczne (poziom cholesterolu całkowitego, lipoprotein HDL, LDL i VLDL oraz triacylogliceroli) szczurów. W przeprowadzonym doświadczeniu nie stwierdzono istotnych zmian w przyrostach masy ciała szczurów. W surowicy krwi zwierząt żywionych dietą hipercholesterolemiczną (AIN-93G + 1% cholesterolu) oznaczono wyższy poziom triacylogliceroli oraz lipoprotein LDL+VLDL w porównaniu ze szczurami karmionymi standardową dietą AIN93-G. Niższy poziom cholesterolu całkowitego wykazano we krwi zwierząt żywionych dietą z udziałem kalafiora gotowanego (świeżego) w porównaniu do szczurów otrzymujących dietę hipercholesterolemiczną z dodatkiem warzywa surowego.