

EFFECTS OF UNEXTRUDED AND EXTRUDED CRANBERRY POMACE ON SELECTED METABOLIC PARAMETERS IN HIGH-FAT DIET FED RATS*

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ABSTRACT

Aim. The effects of un-extruded (UCP) and extruded cranberry pomace (ECP) on fecal fat excretion, liver index, lipid and carbohydrate metabolism, and inhibition of oxidative stress due to a high-fat diet (HFD) in rats were studied.

Material and methods. The Wistar rats for 8 weeks received one of the four diets: (1) control (modified the American Institute of Nutrition: AIN based diet containing 7% fat), (2) HFD (AIN based diet containing 30% fat), (3) HFD with 3% un-extruded (UCP) and (4) HFD with 3% (ECP).

Result. Both UCP and ECP significantly improved the plasma antioxidant capacity and decreased lipid peroxidation in rats fed a HFD. However, only the addition of 3% UCP into the HFD significantly increased the fecal lipid excretion and considerably decreased serum triglycerides level in rats.

Conclusion. Further investigation is needed to determine the role of an individual components present in UCP and ECP in the improvement of metabolic conditions observed in the current study.

Keywords: cranberry pomace, extrusion, high-fat diet, metabolic parameters, rats

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INTRODUCTION

A number of studies have found an inverse relation between a sufficient intake of fruits high in phytochemical compounds and the risk of chronic diseases (Blumberg et al., 2013; Hung et al., 2004; Kim et al., 2013). However, there is not enough information to make specific recommendations for phytochemical intake (Slavin, 2012). Cranberry contains a relatively high level of phytochemicals, which have been studied extensively for their ability to reduce the onset and symptoms of urinary tract infections, inflammation, and cancer (Khanal, 2010; Pappas and Schaich, 2009). They are now fast becoming a subject of intense research in regards to their role in promoting cardiovascular health (Khanal, 2010; Pappas and Schaich, 2009). Cranberry pomace (CP) is a by-product of the cranberry-processing industry and is composed of the skin, seeds, and stems (White et al., 2010b). Due to low protein content, the applications of CP for animal feeds are limited (Blumberg et al., 2013). Since the CP still contains a high level of phenolic compounds, tocochromanols and dietary fiber, this by-product can be however use to fortify commonly consumed food-stuffs that have little nutritional value – for instance, muffins (Mildner-Szkudlarz et al., 2016) and extruded snacks (Khanal, 2010; Roopchand et al., 2013). This manipulation can significantly improve the nutritional value of these food items and ultimately the nutritional status of the population. However, the influence of food processing (including of the extrusion process) on the quantity and activity of CP phytochemicals and their antioxidant activity in the final food products and associated with this health benefits should be also taken into consideration. Although the primary function of food is to provide nutrients, its secondary function involves sensory attributes such as taste and flavour. In general, cranberries have a tart and astringent taste, which has a strong influence on the taste and flavour of food items it enriches, even at low substitution levels (Blumberg et al., 2013). The use of cranberry bioactives as functional food components is thus limited. According to White et al. (2010a) 30:70 ratio of CP to corn starch is desirable in producing well-accepted cranberry extrudates. Therefore, in the present study, extruded (ECP) and un-extruded cranberry pomace (UCP) were included into a high-fat diet (HDF)

at dose of 3% to determine their effectiveness on fecal excretion of total fat, liver index, lipid and carbohydrate metabolism, and inhibition of oxidative damage due to a high-fat diet in Wistar rats.

MATERIAL AND METHODS

Sample preparation

Cranberry pomace (CP) deriving from the 2013 crop of cranberries (*Vaccinium macrocarpon*) was provided by a juice producer in Poland (Polska Róża Ernest Michalski, Raszyn, Poland). The material was lyophilised under vacuum (Martin Christ-Alpha 1–4 freeze drier, Osterode am Harz, Germany). The composition of dried CP is presented in Table 1.

Table 1. The composition of dried CP

Components	Content in 100 g of CP sample, g
Ash	1.0 ±0.04
Protein	2.0 ±0.06
Total fat	10.5 ±0.14
Insoluble fibre	67.3 ±2.6
Soluble fibre	5.7 ±0.1
Other carbohydrates	
– glucose	6.7 ±0.11
– fructose	1.9 ±0.05

Values are means ±SDs of three replicates.

Extrusion

For extrusion, the CP was mixed with cornstarch (“Stanisław Grygier Mill”, Śmigiel, Poland) in an industrial kitchen mixture (Mesko-AGD, Skarżysko) at a 30:70 ratio of CP to cornstarch on a dry-weight basis (a total of 2 kg/bath). This ratio was taken into account when calculating the level of CP in the diet during the diet formulation for both ECP and UCP. The raw materials were brought to a moisture content of 14% by adding the appropriate amount of water, mixing, and resting for 1 h. The extrusion of the CP mixture was carried out using an S-54 single screw

extruder (Metalchem Gliwice, Poland). The following process parameters were applied: the temperature at individual zones of 135°C/175°C/135°C, screw rotations at 120 rpm and a nozzle diameter of 3.0 mm. Extrudates were allowed to cool, placed in sealed bags, and stored at –20°C for chemical analysis.

Tocochromanol (lipophilic antioxidants) determination

Tocochromanols were extracted in accordance with a previously developed method described by Górnaś et al. (2014a) and determined by RP-HPLC, according to an earlier validated method (Górnaś et al., 2014b).

Phenolic compounds (hydrophilic antioxidants) determination

The phenolic compounds were extracted from the defatted pomace and extrudates using a method described by Makarova et al. (2015) and determined using a Shimadzu HPLC system (Kyoto, Japan).

Evaluation of antioxidant capacity

Both fractions of antioxidants – the lipophilic (tocopherols and tocotrienols) and hydrophilic (polyphenols) – were combined, according to the sample weight for the extraction, and diluted by ethanol to obtain concentrations in the range of the developed calibration curves for the antioxidant assay.

DPPH[•] assay

The DPPH[•] assay was performed by a previously developed method (Górnaś et al., 2015).

Animal experiment

The animal study design was approved by the local bioethics committee on animal research at Poznań University of Life Sciences (approval 68/2010, 3 September 2010). 40 male Wistar rats (56 days old) were housed on a 12-h dark/12-h light cycle, at a temperature of 22° ±2°C. After one week of acclimatization the animals were randomly divided into four groups (*n* = 10 each) with equal average body weight and fed: (1) control (modified AIN based diet containing 7% fat), (2) high fat diet (AIN based diet containing 30% fat, HFD), (3) HFD with 3% un-extruded cranberry pomace (UCP-HFD) and (4) HFD with 3% extruded cranberry pomace (ECP-HFD) for 8 weeks

with *ad libitum* access to tap water and diet. The diets were freshly prepared at weekly intervals, stored in hermetic containers at –20°C. The composition of the animal diets is presented in Table 2. The food intake of the individual rats was checked every day by measuring the difference between the amount of diet supplied each day and the amount of diet remaining. Body mass was measured every seven days using an electronic balance. Feces were collected quantitatively from individual rats during the last 7 days of the eighth-period and dried for later analysis. At the end of the experiment, and after overnight fasting, the animals were euthanized by intraperitoneal injection of ketamine–xylazine. Blood was collected by cardiac puncture for future biochemical studies. The liver was dissected and weighted.

Macronutrient analysis of CP, diets and feces

Gross energy of the diet was measured by bomb calorimetry (Calorimeter KL-11 Mikado Microprocessor System, Precyzja, Bydgoszcz, Poland). Moisture and ash in CP were assessed according to standards procedures. Nitrogen in the total CP and in the animal diets was determined with the Kjeldahl method using a Tecator-Kjeltec system 1026 (Tecator AB, Höganäs, Sweden). To calculate the amount of protein in the evaluated samples, the results were multiplied by 6.25. Fat contents in the CP, animal diets and faeces were measured by extracting 5 g of each sample with petroleum ether in a Soxtec Avanti 2055 apparatus (Foss Tecator AB). The amounts of total dietary fibre (TDF), soluble dietary fibre (SDF), and insoluble dietary fibre (IDF) in the CP samples were established by an enzyme-gravimetric method described by Asp et al. (1983).

Blood biochemical analysis

The levels of total cholesterol (TC), and triglycerides (TG) were determined using an automatic biochemical Olympus AU 560 analyser (Olympus Japan, Tokyo, Japan). Fasting glucose (GLU) concentrations were measured using the hexokinase method (Olympus AU 2700). Insulin (INS) levels were measured with specific radioimmunoassay Rat Insulin RIA Kit RI-13K (Millipore Corporation, St. Charles, MO, USA) according to the manufacturer's protocol. β -cell function (HOMA-BCF) was calculated as 20·FPI/(FPG-3.5).

Table 2. Experimental diets composition per 1000 g of diet

Ingredients	Diets			
	control	HFD	3% UCP-HFD	3% ECP-HFD
Casein	120	120	120	120
Sucrose	100	100	97.1	97.1
Sunflower oil	70	100	100	100
Lard	0	200	200	200
Cellulose	50	50	25	25
Cornstarch	612	382	309.9	309.9
L-cysteine	3	3	3	3
AIN 93 vitamin mix	10	10	10	10
AIN 93 mineral mix	35	35	35	35
UCP	0	0	100	0
ECP	0	0	0	100
Total	1000	1000	1000	1000
Energy, kcal/100 g	417.1	530.9	532.3	531.8
% energy from protein	13.0	11.0	11.0	11.0
% energy from fat	15.0	51.0	51.0	51.0
% energy from carbohydrate	72.0	38.0	38.0	38.0

UCP – un-extruded cranberry pomace, ECP – extruded cranberry pomace, HFD – high-fat diet, UCP-HFD – high-fat diet enriched with 3% unextruded cranberry pomace, ECP-HFD – high-fat diet enriched with 3% extruded cranberry pomace.

The homocysteine (Hcy) and glutathione (GSH) concentrations were measured using HPLC, according to the method of Chmurzynska et al. (2013). Chemical antioxidants presented in rat plasma were examined using ferric reducing ability (FRAP) methodology elaborated by Benzie and Strain (1996). As a marker of lipid peroxidation products, ThioBarbituric Acid Reactive Substances (TBARS) concentration was measured using the method of Ohkawa et al. (1979).

Statistical analysis

Data are presented as means, standard deviation (SD), or standard errors (SEM) where appropriate. The normality of data and homogeneity of variances were tested using the Shapiro-Wilk test. The significance of differences among the groups was assessed using one-way analysis of variance and Tukey's posthoc test

or a non-parametric Kruskal-Wallis test. Statistical significance was assumed for $p < 0.05$ (Statistica 10.0 software, StatSoft, Kraków, Poland).

RESULTS AND DISCUSSION

Effect of extrusion process on the phenolic and lipophilic antioxidant compound contents of UCP and ECP

The total flavonol content of ECP was almost eight times lower than that of UCP, whereas anthocyanins were found only in UCP (Table 3). While by Khanal et al. (2010) and White et al. (2010a), higher levels of total flavonols after the extrusion process were reported. The difference between the present study and study mentioned above (White et al., 2010a) may be the result of applied different parameters during extrusion process

Table 3. Content of hydrophilic and lipophilic antioxidants of UCP and ECP with their antioxidant potentials (DPPH[•] assay), mg/100 g

Components	UCP	ECP
Cyanidin 3- <i>O</i> -galactoside	5.2 ±0.1	ND
Cyanidin 3- <i>O</i> -arabinoside	5.6 ±0.2	ND
Peonidin 3- <i>O</i> -galactoside	8.3 ±0.2	ND
Peonidin 3- <i>O</i> -glucoside	4.8 ±0.01	ND
Peonidin 3- <i>O</i> -arabinoside	8.1 ±0.04	ND
Total anthocyanins	32.1 ±0.5	–
Myricetin 3- <i>O</i> -galactoside	4.1 ±0.2	0.3 ±0.03
Myricetin 3- <i>O</i> -arabinoside	6.1 ±0.2	0.2 ±0.04
Myricetin	12.1 ±0.3	1.5 ±0.10
Quercetin	23.6 ±0.5	4.0 ±0.15
Quercetin 3- <i>O</i> -benzoyl galactoside	4.6 ±0.2	0.3 ±0.02
Quercetin 3- <i>O</i> -rhamnoside	8.3 ±0.1	1.2 ±0.02
Total flavonols	58.7 ±1.5	7.5 ±0.43
δ-T3	0.5 ±0.01	0.6 ±0.03
γ-T3	4.7 ±0.1	1.6 ±0.2
α-T3	0.9 ±0.1	0.6 ±0.03
δ-T	0.3 ±0.02	0.1 ±0.01
β-T	0.3 ±0.02	0.1 ±0.01
γ-T	1.8 ±0.1	0.6 ±0.03
α-T	8.7 ±0.1	2.4 ±0.2
Total tocochromanols	17.2 ±0.5	6.0 ±0.3
DPPH [•] assay, µg GAE	7.1 ±0.1	6.5 ±0.8

Values are means ±SDs of three replicates. ND – not detected, ECP – extruded cranberry pomace, UCP – unextruded cranberry pomace, DPPH[•] – assays measure antioxidant capacity using 1,1-diphenyl-2-picrylhydrazyl (DPPH[•]) free radicals.

between studies, as well as the bio composition of the raw material. The observed reduction of anthocyanin levels in the extruded product is related to the negative impact of high temperature. It was reported previously that content of anthocyanins is considerably reduced by heat treatment and depend on the time and temperature of the process (Górnaś et al., 2016; Mildner-Szkudlarz et al., 2016). Since the CP contains seeds,

which are a valuable source of lipophilic antioxidants such as tocopherols and tocotrienols, the levels of these bioactive compounds were determined in both UCP and ECP samples. The total tocopherol and tocotrienol contents of ECP were three times lower than those of UCP. A significant reduction in tocochromanols during heat treatment has also been observed in muffins enriched with CP (Mildner-Szkudlarz et al., 2016).

Although the content of hydrophilic and lipophilic antioxidants in ECP was lower than in UCP, the DPPH-scavenging activity of the tested samples was similar, at 7.1 ± 0.1 and 6.5 ± 0.8 $\mu\text{g GAE}/100$ mg in UCP and ECP, respectively (Table 3). These results can be explained by the impact of Maillard reaction products in the extrudates. It is widely accepted that those products influence the antioxidant activity of foods, but only the soluble fraction – high molecular weight melanoidins and low molecular weight heterocyclic compounds contribute to the reducing power (Billar and Ekielski, 2015; Sharma et al., 2012).

Effect of UCP and ECP on food intake, body weight, liver weight, and total fecal fat excretion in rats fed a HFD

A significantly higher ($P < 0.05$) food intake was noted in the control group than in the other groups (Table 4). This higher food intake was observed because this diet has a lower energy density than any of the HFDs (Table 2). There was no significant difference in body mass between the groups before and after the experiment, though we can observe a tendency to decreased body weight gain by 6.7% in 3% UCP HFD as compared with control HFD group. Incorporation of HFD led to significant increase of liver index, however only the introduction of the UCP into the basal HFD significantly affected this index. All the HFD fed groups of rats had higher percentages of faecal fat content than the rats from the control group ($P < 0.05$). However,

only the addition of UCP into the HFD significantly increased the faecal lipid excretion as compared with rats fed the HFD (Table 4). It seems that the differences observed can be explained by the presence of phytochemicals in UCP. For example, Martin-Carron et al. (1997) fed male Wistar rats a standard diet supplemented with 10% grape pomace over an eight-week period. The rats showed higher faecal weight as well as increased fat and protein excretion in the faeces than rats fed a control diet. Mildner-Szkudlarz and Bajerska (2013) also observed that a diet based on wheat bread enriched with dried powdered skins of grape by-products, or with freeze-dried extract of these, significantly increased faecal fat in rats, decreasing the apparent digestion of fat compared with a control HFD and a HFD enriched with control bread. Takahashi et al. (2014) indicated that anthocyanin-rich phytochemicals in chokeberry fruits significantly inhibited pancreatic lipase activity, hence reducing lipid absorption and enhancing fat excretion in faeces.

Red cabbage, which is rich in anthocyanin, can stimulate increased faecal lipid excretion and prevent elevation of serum and tissue lipids which is induced by atherogenic diet (Mohamed, 2014). Yet it is not only that phytochemicals can affect intestinal lipid absorption. In this terms, the changing in the ratio of insoluble to soluble fibre due to extrusion process should be also taken into consideration. Extrusion process decreases the IDF content and increases the SDF content in final products (Qian and Ding, 1996). The IDF causes

Table 4. Food intake, body weight, and liver weight in rats fed the control diet, an HFD, and HFDs enriched with UCP or ECP

Parameters	Groups			
	control	HFD	3% UCP-HFD	3% ECP-HFD
Food intake, g/d	20.5 \pm 0.5 ^b	16.9 \pm 0.2 ^a	18.2 \pm 0.4 ^a	17.6 \pm 0.4 ^a
Initial body weight, g	217.1 \pm 9.4	217.3 \pm 9.4	217.8 \pm 8.8	217.3 \pm 9.2
Final body weight, g	456.5 \pm 12.3	473.2 \pm 19.9	456.6 \pm 11.6	453.8 \pm 12.0
Liver weight, g/100 g BW	2.5 \pm 0.06 ^a	2.8 \pm 0.08 ^b	2.5 \pm 0.07 ^a	2.6 \pm 0.05 ^{ab}
Faecal lipids, % dried feces	2.7 \pm 0.2 ^a	3.8 \pm 0.1 ^b	4.3 \pm 0.1 ^c	4.0 \pm 0.2 ^{bc}

Values are expressed as means \pm SEM ($n = 40$). Values in the same row with different letters in superscript indicate significant differences at $P < 0.05$. BW – body weight, CP – cranberry pomace, HFD – high-fat diet, 3% HFD-UCP – high-fat diet enriched with 3% un-extruded cranberry pomace, 3% ECP-HFD – high-fat diet enriched with 3% extruded cranberry pomace.

an increased rate of passage through the gastrointestinal tract and this results in reduced digestion and absorption of nutrients (Lattimer and Haub, 2010).

Effects of UCP and ECP on lipid oxidation and antioxidant capacity of rats fed a HFD

The incorporation of UCP or ECP into the HFDs significantly raised the plasma antioxidant capacities measured as FRAP and GSH (Table 5). The TBARS level, regarded as a marker of lipid peroxidation, was found to be elevated by the HF diet; however, the inclusion of either ECP or UCP to the HFD significantly decreased this parameter. This effect is in agreement with the study conducted by Kim et al. (2013) where CP was used. It was recognized that treatment with 5% of freeze-dried CP increased HDL cholesterol and reduced protein carbonyl, as well as TBARS levels, in rats fed an atherogenic diet (Kim et al., 2013). In the present study, the improvement in plasma antioxidant activity of the rats fed the HFD enriched with UCP was presumably due to the incorporation of compounds with strong antioxidant activity – mainly anthocyanins (cyanidin 3-*O*-galactoside) and flavonols (quercetin), but also lipophilic antioxidants such as tocopherols and tocotrienols. However, it should be mentioned that bioavailability of anthocyanins is usually low. The improvement of the antioxidant potential in rats fed the HFD enriched with ECP could be explained by the fact that the extrusion process mechanically disrupts cell walls of plants, improves the bioavailability of polyphenols (Singh et al., 2007). Gu et al. (2008) stated that procyanidins (especially the polymers)

usually bind onto other macromolecules, such as fibre and protein. It is unclear how extrusion affects this binding, but it may degrade these macromolecules into smaller fragments and release bound procyanidins.

Effects of ECP and UCP on metabolic parameters in rats fed a HFD

It was pointed out that a rodent is generally accepted as a suitable lipid metabolic model for human (Buettner et al., 2006; Kobayashi et al., 2016). Lard is one of the most widely consumed foods rich in saturated fatty acids and it is often chosen in animal studies to test the deleterious effects of HFD. Levels of both TC and TG in serum from the HFD group of rats were significantly ($P < 0.05$) higher than those in control (Table 6). Administration 3% UCP and 3% ECP into the HFD had a tendency to decrease TC level. However, only 3% UCP-HFD group had significantly decreased levels of TG compared with control HFD. Neither the ECP nor the UCP significantly influenced either the Hcy level or the HOMA-BCF. In opposition to our findings, Khanal et al. (2010) indicated that ECP at a dose of 3% of the diet was more effective than UCP (given at the same level) in controlling the effects of high-fructose feeding. For instance, the inclusion of ECP, though not UCP, effectively lowered TC compared not only to the HFD but also to the starch-based diet. Similarly, Khanal et al. (2010) revealed that ECP was more effective in reducing the HOMA insulin resistance, TG, and insulin levels. Khanal et al. (2010) hypothesised that low molecular weight procyanidins found in ECP could be responsible for minimizing or

Table 5. Antioxidant capacity and lipid oxidation marker in rats fed the control diet, HFD, and HFDs enriched with UCP and ECP

Parameters	Groups			
	control	HFD	3% UCP-HFD	3% ECP-HFD
FRAP, $\mu\text{mol FeII/L}$	377.7 \pm 12.5 ^{ab}	353.3 \pm 19.3 ^a	438.1 \pm 24.7 ^b	447.7 \pm 17.1 ^b
GSH, $\mu\text{mol/L}$	41.1 \pm 3.2 ^{ab}	37.4 \pm 3.6 ^a	49.5 \pm 2.9 ^b	46.9 \pm 2.5 ^b
TBARS, $\mu\text{mol MDA/L}$	9.3 \pm 0.3 ^{ab}	10.1 \pm 0.2 ^a	8.8 \pm 0.2 ^b	8.4 \pm 0.4 ^b

Values are expressed as means \pm SEM ($n = 40$). Values in the same row with different letters in superscript indicate significant differences at $P < 0.05$. FRAP – ferric-reducing ability of plasma, GSH – glutathione, TBARS – thiobarbituric-acid-reactive species, MDA – malondialdehyde, HFD – high-fat diet, 3% UCP-HFD – high-fat diet enriched with 3% unextruded cranberry pomace, 3% ECP-HFD – high-fat diet enriched with 3% extruded cranberry pomace.

Table 6. The TC, TG, Hcy, and HOMA-BCF levels in rats fed the control diet, the HFD, and the HFDs enriched with UCP or ECP

Parameters	Groups			
	control	HFD	3% UCP-HFD	3% ECP-HFD
TC, mg/dL	84.8 ±4.4 ^a	117.7 ±5.0 ^b	103.4 ±6.0 ^{ab}	103.2 ±4.0 ^{ab}
TG, mg/dL	56.8 ±3.9 ^a	102.3 ±5.2 ^c	70.2 ±4.6 ^b	87.4 ±4.3 ^{bc}
Hcy, µmol/L	4.9 ±0.4	5.7 ±0.3	5.3 ±0.5	5.3 ±0.4
HOMA-BCF	106.2 ±21.9	170.5 ±31.3	141.8 ±24.4	136.5 ±16.1

Values are expressed as means ±SEM ($n = 40$). Values in the same row with different letters in superscript indicate significant differences at $P < 0.05$. TC – total cholesterol, TG – triglycerides, Hcy – homocysteine, HOMA-BCF – homeostasis model assessment for β -cell function, CP – cranberry pomace, HFD – high-fat diet, 3% UCP-HFD – high-fat diet enriched with 3% unextruded cranberry pomace, 3% ECP-HFD – high-fat diet enriched with 3% extruded cranberry pomace.

ameliorating some metabolic anomalies associated with a high fructose diet.

CONCLUSION

Cranberry pomace (CP; given in unextruded or extruded form, at dose of 3%) was effective in mitigating some of the negative effects of HFD feeding. Both UCP and ECP significantly improved the plasma antioxidant capacity and decreased lipid peroxidation in rats fed an HFD. However, only the addition of 3% UCP into the HFD significantly increased the faecal lipid excretion and considerably decreased liver index and TG level in rats. The further investigation is needed to determine the role of individual components present in UCP and ECP in the improvement of metabolic conditions observed in the current study.

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