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Composition of Chicory Root, Peel, Seed and Leaf Ethanol Extracts and Biological Properties of Their Non-Inulin Fractions

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Summary

The chemical composition of the ethanol extracts of chicory root, peel, seed and leaf has been determined, in particular their inulin and phenolic fractions. The root and peel extracts were characterized by large mass fractions of inulin (60.1 and 46.8 g per 100 g of fresh mass, respectively), predominantly with degree of polymerization in the range from 3 to 10, while phenolics, determined as caffeoylquinic acids, made up 0.5 and 1.7 g per 100 g of fresh mass, respectively. The leaf and seed extracts had decidedly lower mass fractions of inulin (1.7 and 3.2 g per 100 g of fresh mass, respectively) and higher mass fractions of phenolics (9.6 and 4.22 g per 100 g of fresh mass, respectively) recognized as caffeoylquinic acids, chicoric acid and quercetin glucuronide. The biological properties of a non-inulin fraction from each extract were determined on Wistar rats fed with diets rich in fructose and saturated fat, as a model of metabolic changes related to westernization of human eating habits. The diets contained the same amount of inulin (6 %) with various phenolic fractions. Some changes were noted in the microbial enzymatic activity of the caecum after feeding for 4 weeks with the diet containing the highest mass fraction of phenolics (0.208 %), derived from the mixture of peel and seed extracts (decreased activity of β-galactosidase and β-glucuronidase), as well as with the diet containing leaf extract (decreased β-glucuronidase activity). All the diets showed no essential influence on the caecal concentration and profile of short-chain fatty acids, except acetate, whose concentration decreased significantly in rats fed with the diet enriched with root extract. The addition of peel and leaf extracts to the fructose diets significantly increased the serum antioxidant capacity of lipophilic substances. The study indicates that parts of chicory and its byproducts might be a source of valuable compounds to improve the physiological activity of inulin.

Key words: inulin, phenolic compounds, *Cichorium intybus*, chlorogenic acid, antioxidants, rat

Introduction

Chicory (*Cichorium intybus* L.) belongs to the Asteraceae family and is a biennial plant with many applications in the food industry. The dried and roasted roots are used for blending with coffee to add flavour; young leaves can be added to salads and vegetable dishes, while chicory extracts are used for the production of invigo-

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rating beverages (*1*). The roots are also a main source of linear β-(2→1)-linked fructose chains, which are indigestible and selectively fermented in the body. As such they are classified as part of dietary fibre with proven prebiotic properties, beneficially affecting large bowel function with health promoting impact on the rest of the organism (*2,3*). Chicory inulin (average chain length of 10 fructose units) and its partial hydrolysate fructooligosaccharides (average chain length of 4 fructose units) are nowadays used as an ingredient in a wide range of food applications such as dairy, bakery, cereals, desserts, dressings, *etc*. These prebiotics are used in over 5000 food products worldwide. However, apart from inulin, there are also many other phytochemicals present in the different parts of chicory, such as sesquiterpenes and phenolic compounds (*1*). Moreover, several biological attributes of chicory extracts have been reported in *in vivo* studies, such as antihepatotoxic activity (*4,5*) and reduction of cholesterol uptake (*6*).

On the other hand, large amounts of chicory wastes and residues (leaves and peels) are currently hardly used in industrial processing. The management of vegetable and fruit wastes seems to be advisable and is of growing scientific interest (*7*). Additionally, a recent study has demonstrated that the *Cichorium* genus possesses both pro- and antioxidants (*8*), and other authors have pointed to a phenolic fraction with potential antiradical activity present in chicory (*7,9*). However, not only phenolic compounds can be responsible for the *in vitro* antioxidant activity of chicory preparations. For instance, it is proposed that sugars themselves, especially sucrose and fructans, can also act as radical scavengers in plant cells (*10,11*). The foregoing data, in connection with a new scientific approach to antioxidant capacity as the major health-promoting property associated with dietary fibre (*12*), encouraged us to examine non-inulin fraction of chicory by-products as potential health promoting agents improving physiological activity of inulin.

The purpose of this study is to assess the biological properties of diets supplemented with extracts from chicory root, peel, seed and leaf, extracted with 75 % ethanol, with well-characterized carbohydrate and phenolic fractions. The diets, identical in terms of carbohydrates and the degree of polymerization (DP) of inulin, contained different amounts and structures of phenolic fraction, thus enabling us to check the effect of the non-inulin fraction of the tested extracts. The main focus was on the caecal fermentative processes, which are known to be favoured by dietary inulin, as well as on the antioxidant status and lipid profile of blood. In order to obtain an animal model of metabolic changes related to the westernization of human eating habits, such as the increase in consumption of refined sugars and saturated fats, we used a fructose- and cholesterol-rich diet supplemented with pork lard. A high-fructose diet is claimed to possess the ability to induce dyslipidemia, hyperinsulinemia, and oxidative stress in rats (*13*).

Materials and Methods

Chicory preparation

Raw chicory (*Cichorium intybus* L.) was provided by Cykoria Co. (Wierzchosławice, Poland), a company specializing in its processing. Preparation was carried out as previously reported (*14*). Briefly, after drying at <70 °C, the root cubes (2 kg), peels (2.5 kg), leaves (1.3 kg) and seeds (1 kg) were extracted with 75 % ethanol. Dried roots and peels were extracted once for 3 h in a continuous manner, while leaves and seeds were extracted consecutively (4 times) with 1:3 sample to solvent ratio. The total volume of ethanol used for obtaining root and peel extracts was 18 and 20 L, respectively. The extraction procedure was performed in hermetic containers and without light access from the outside. The ethanol was removed by vacuum distillation and the extracts were further freeze-dried for 24 h, beginning at –30 °C, followed by an additional drying at 40 °C for 2 h. The final extraction yields were 33.0 % from roots, 17.6 % from peels, 10.0 % from leaves and 3.5 % from seeds. The extracts contained different amounts of inulin and phenolics (Table 1).

Determination of dry matter, ash, protein, fat and antioxidant activity of the chicory preparations

Dry matter, ash, protein and fat contents in the extracts from roots, peels, seeds and leaves were determined according to the official methods of AOAC (*15–18*).

In vitro antioxidant activity was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical according to the modified method of Brand-Wiliams *et al.* (*19*). Briefly, the extracts were dissolved in 10 mL of 80 % ethanol, then 0.05 mL of the solution were added to 1.95-mL solution of DPPH radical (60.8 μmol/L), mixed and incubated for 30 min in the dark. The absorbance was measured at λ=515 nm and the results were expressed as nmol of Trolox equivalent per g of sample.

Determination of carbohydrates and phenolics in the chicory preparations

Carbohydrate determinations were performed using HPLC (Knauer Smartline with RI K-2301 Knauer detector, Berlin, Germany), Animex HPX 87C (300×7.8 mm) column and water as a mobile phase (flow rate 0.5 mL/min and temperature 85 °C). A sample of the tested preparation (0.5–1 g) was dissolved in water (10 mL) and passed through a column filled with a mixture of cation and anion exchangers (1:2 mass ratio). The first 5 mL of the filtrate were rejected and the next 4 mL were collected, then 2 of the 4 mL were used directly for the analysis of mono- and disaccharides. The remaining 2 mL were diluted twice and used for the determination of fructooligosaccharides, after previously desalting the filtrate and mixing it with acetonitrile (1:1 volume ratio) according to the procedure of Król and Grzelak (*20*).

Phenolic determinations were performed according to the procedure of Milala *et al.* (*14*), using HPLC (Dionex system with DAD detector, Germering, Germany) coupled with Synergi™ Fusion-RP 80A column (150×2 mm, 4 μm; Phenomenex, Torrance, CA, USA). Phase A was 0.05 % phosphoric acid in water, phase B was 0.05 % phosphoric acid in acetonitrile, flow rate was 0.25 mL/min, and temperature was 25 °C. Gradient: stabilization for 10 min with 4 % of phase B, 4–50 % B for 0–33 min, 50 % B for 33–34 min and 4 % B for 34–35 min. The absorbance was measured at 325 and 360 nm. Hyperoside, quercetin

Table 1. Composition and antioxidant activity of chicory extracts

	m/(g in 100 g of fresh mass)			
	RE	PE	SE	LE
Dry matter	(97.3±0.1)^b	(98.0±0.20)^a	(95.0±0.20)^c	(97.9±0.1)^a
ash	(3.8±0.03)^d	(8.2±0.04)^b	(8.4±0.05)^a	(6.6±0.06)^c
protein	(5.6±0.1)^c	(8.9±0.2)^b	(10.7±0.3)^a	(8.7±0.2)^b
fat	(1.73±0.06)^b	(0.66±0.07)^c	(14.4±0.2)^a	(1.42±0.06)^b
Mono- and disaccharides	(24.3±0.15)^d	(25.6±0.2)^c	(34.9±0.3)^b	(62.4±0.6)^a
glucose	(2.7±0.02)^d	(3.4±0.03)^c	(12.1±0.1)^b	(27.9±0.3)^a
fructose	(5.7±0.03)^d	(6.4±0.04)^c	(21.4±0.2)^b	(22.3±0.3)^a
sucrose	(15.9±0.1)^a	(15.8±0.1)^a	(1.4±0.02)^c	(12.2±0.1)^b
Inulin fraction	(60.1±0.3)^a	(46.8±0.2)^b	(1.7±0.04)^d	(3.2±0.02)^c
DP=3–10	(46.0±0.2)^a	(33.8±0.1)^b	(1.5±0.02)^d	(3.2±0.02)^c
DP>10	(14.1±0.1)^a	(13.0±0.1)^b	(0.3±0.02)^c	–
Phenolic fraction	(0.50±0.02)^d	(1.70±0.02)^c	(9.60±0.03)^a	(4.22±0.07)^b
CQAs	(0.50±0.02)^d	(1.70±0.02)^c	(9.60±0.03)^a	(1.35±0.03)^b
monoCQAs	(0.30±0.01)^d	(0.95±0.01)^c	(2.80±0.01)^a	(1.27±0.01)^b
diCQAs	(0.20±0.01)^c	(0.75±0.01)^b	(6.80±0.02)^a	(0.08±0.02)^d
chicoric acid	–	–	–	2.13±0.03
quercetin glucuronide	–	–	–	0.74±0.02
Antioxidant activity/(nmol/g of fresh mass)	(30.2±3.1)^d	(80.3±4.2)^c	(505.1±6.0)^a	(210.1±5.8)^b

RE=root extract, PE=peel extract, SE=seed extract, LE=leaf extract, DP=degree of polymerization, CQAs=caffeoylquinic acids; data are expressed as mean±standard deviation (*N*=3); values not sharing the same superscript within a row are different at $p \leq 0.05$

glucoside, apigenin and luteolin were purchased from Extrasynthese (ZI Lyon Nord, Genay, France). Chicoric acid (dicaffeoyltartaric acid) was isolated from leaves as described by Scarpati and Oriente (*21*) and its purity was confirmed by UV spectroscopy, optical rotation, and HPLC. Dicaffeoylquinic acid was a pure substance isolated by semipreparative HPLC (Phenomenex column, 250×10 mm, Torrance, CA, USA, injection volume 200 μL, flow rate 5 mL/min, eluent as above) from root extracts and was confirmed by MS spectrum (FAB MS $[M–H]^-$ 515.3 *m/z*). Quercetin glucuronide was isolated from leaf extract by semipreparative HPLC and was confirmed by UV and MS spectra (Phenomenex column, 250×10 mm, Torrance, CA, USA, injection volume 200 μL, flow rate 5 mL/min, eluent as above). All aforementioned substances were used as identification standards by comparison of retention times and UV spectra and additionally compared with the data obtained previously (*22–25*). All hydroxycinnamic acids were calculated as chlorogenic acid (5-caffeoylquinic acid) and all polyphenolic glycosides as quercetin (Sigma, St. Louis, MO, USA). Additionally, the total phenolic content was also determined using Folin-Ciocalteu reagent (*26*).

Biological assessment of non-inulin fractions

The animal protocol used in this study was approved by the Institutional Animal Care and Use Committee, University of Warmia and Mazury, Poland. The assessment was conducted on 30 male Wistar rats divided into 5 groups of 6 animals each. All animals were housed individually over 4 weeks in standard conditions with free access to water and semi-purified casein diets (Table 2). All experimental diets were similar in terms of dietary ingredients except for the phenolic fraction, which was constituted mainly of caffeoylquinic acids (CQAs). The diets were adjusted to the level of carbohydrates and DP of inulin with calculated mass fractions of phenolics, and contained, among other items, the same quantity of fructose (60 %), lard (8 %), cholesterol (1 %), and inulin (6 %). Inulin was adjusted using commercial preparations Frutafit® TEX! with DP>10 (Sensus, Roosendaal, the Netherlands) and Raftilose® P95 with DP 3–7 (BENEO-Orafti, Tienen, Belgium). The control diet did not contain phenolics. A diet supplemented with 10 % of root extract (RE) contained 0.05 % of CQAs (monoCQAs/diCQAs 60:40), a diet supplemented with 6.5 % of peel extract (PE) contained 0.107 % of CQAs (monoCQAs/diCQAs 54:46), a diet supplemented with 8 % of PE and 0.8 % of seed extract (SE) contained 0.208 % of CQAs (PSE diet, monoCQAs/diCQAs 45:55), and a diet supplemented with 2.5 % of leaf extract (LE) contained 0.106 % of total phenolics. The mass ratio of phenolic compounds in the last diet was as follows: monoCQAs/diCQAs/chicoric acid/quercetin glucuronide 30:2:50:18. In all experimental diets the extracts were added at the expense of sucrose, inulin and corn starch.

Analysis of biological material

On termination of the experiment, rats were anaesthetized with sodium pentobarbital according to the recommendations for euthanasia of experimental animals. After laparotomy, blood samples were taken from the *inferior vena cava* into test tubes, and then the caecum was removed and weighed. The pH of caecal digesta was measured using a microelectrode and a pH/Ion meter

Table 2. Composition of the diets

	m/(g in 100 g of fresh mass)				
	Control	RE	PE	PSE	LE
Ingredients					
casein	14.80	14.80	14.80	14.80	14.80
DL-methionin	0.20	0.20	0.20	0.20	0.20
lard	8.00	8.00	8.00	8.00	8.00
cholesterol	1.00	1.00	1.00	1.00	1.00
cholic acid	0.20	0.20	0.20	0.20	0.20
mineral mix recommended for AIN-93G diet	3.50	3.50	3.50	3.50	3.50
vitamin mix recommended for AIN-93G diet	1.00	1.00	1.00	1.00	1.00
choline chloride	0.20	0.20	0.20	0.20	0.20
chicory root extract	–	10.00	–	–	–
chicory peel extract	–	–	6.50	8.00	–
chicory seed extract	–	–	–	0.80	–
chicory leaf extract	–	–	–	–	2.50
inulin with DP=3–7 (Raftilose® P95)	4.63	–	2.45	1.80	4.55
inulin with DP>10 (Frutafit® TEX!)	1.38	–	0.53	0.34	1.38
sucrose	2.40	–	0.80	–	0.90
fructose	60.00	60.00	60.00	60.00	60.00
corn starch	2.69	1.10	0.82	0.16	1.77
Calculated content of selected fractions					
mono- and disaccharides/(% of diet)	62.40	62.43	62.46	62.32	62.46
inulin fraction/(% of diet)	6.010	6.010	6.010	6.010	6.010
inulin with DP=3–7/(% of fraction)	77.00	77.00	77.00	77.00	77.00
inulin with DP=3–7/(% of fraction)	23.00	23.00	23.00	23.00	23.00
phenolic fraction/(% of diet)	–	0.050	0.107	0.208	0.106
monoCQAs/(% of fraction)	–	60.00	54.00	45.00	30.00
diCQAs/(% of fraction)	–	40.00	46.00	55.00	2.00
chicoric acid/(% of fraction)	–	–	–	–	50.00
quercetin glucuronide/(% of fraction)	–	–	–	–	18.00

RE=root extract, PE= peel extract, PSE=peel and seed extract, LE=leaf extract, DP=degree of polymerization, CQAs=caffeoylquinic acids; all diets had the same content of inulin with similar DP

(model 301, Hanna Instruments, Inc, Woonsocket, RI, USA), while dry matter was determined at 105 °C. Fresh caecal digesta were used for analyses of ammonia concentration, whereas microbial glycolytic activities, as well as the concentration of short-chain fatty acids (SCFAs) were determined after storage of samples at –70 °C. Ammonia was extracted and trapped in a solution of boric acid, then determined by direct titration with sulphuric acid (27). The microbial glycolytic activities (α- and β-glucosidase, α- and β-galactosidase, β-glucuronidase) were measured by the rate of *p*- or *o*-nitrophenol released from their nitrophenyl glucosides, and expressed as μmol of product formed per h per g of caecal digesta (2). The concentration of SCFAs was measured using gas chromatography under the conditions described previously (2).

Serum concentration of triglycerides, total cholesterol, and its high-density lipoprotein fraction were estimated with reagents from Alpha Diagnostics Ltd. (Warsaw, Poland). The activity of glutathione peroxidase (GPx) in the heparinized blood and superoxide dismutase (SOD) in erythrocyte lysate were determined using reagents from Randox Laboratories Ltd. (Crumlin, UK). Serum ACW (antioxidant capacity of water-soluble substances, Analytik Jena AG kit, Jena, Germany) and ACL (antioxidant capacity of lipid-soluble substances, Analytik Jena AG kit) were determined with a photochemiluminescence detection method using a Photochem (Analytik Jena AG). In the photochemiluminescence assay, the generation of free radicals was partially eliminated by the reaction with antioxidants present in serum samples, and the remaining radicals were quantified by luminescence generation. Ascorbate and Trolox calibration curves were used in order to evaluate ACW and ACL, respectively, and the results were expressed as μmol of ascorbate or Trolox equivalent per mL of serum.

Statistics

Results were refined statistically using a 1-way analysis of variance and the Duncan's multiple range test. Differences were considered significant at $p \leq 0.05$. Standard error of the mean (SEM) was pooled through division of a standard deviation from all rats by the square root of rat number.

Results and Discussion

A comparison of the chemicals present in the chicory preparations used in our study reveals the freeze-dried seed extract (SE) as the richest source of minerals, protein, fat, and phenolic compounds (Table 1). A phenolic fraction in the SE was identified as CQAs; its content equaled 9.6 % of the preparation with the predominant part being diCQAs (6.8 %). The LE was also rich in phenolics (4.2 %); however, in this preparation chicoric acid, monoCQAs, and quercetin glucuronide were present in 2.1, 1.3, and 0.74 %, respectively. The lowest level of phenolics identified as CQAs was in RE (0.5 %), whereas PE contained more than threefold CQAs (1.7 %) than the RE. In all tested extracts the main monoCQA was identified as chlorogenic acid. The total phenolic content in the extracts corresponds to their antioxidant activity, which was the highest in SE followed by LE, PE and RE (Table 1). The highest content of well-absorbable carbohydrates was present in LE (62.4 %), especially glucose and fructose were present in large quantities (27.9 and 22.3 %, respectively). A similar situation was evident in the seed preparation where glucose (12.1 %) and fructose (21.4 %) were also the main source of these carbohydrates (34.9 %); in RE and PE a predominant member of well-absorbable carbohydrates was sucrose (approx. 16 % in both cases). The inulin fraction was the highest in root and peel preparations (60.1 and 46.8 %, respectively) and consisted mainly of chains with DP<10; SE and LE were characterized by decidedly lower mass fractions of inulin (1.7 and 3.2 %, respectively).

The chemical composition of the root and peel extracts, with extraction yields of 33 and 17 g per 100 g of raw material, respectively, indicates that the method used in this study might be an alternative tool for obtaining functional preparations rich in inulin, especially with lower DP and CQAs. On industrial scale inulin with both low and high DP is efficiently isolated with hot water extraction, after which a purification and partial enzymatic hydrolysis are performed in order to obtain fructooligosaccharide preparations. Moreover, the extraction of chicory leaves and seeds with 75 % ethanol seems to be a useful way to isolate such bioactive compounds as CQAs and chicoric acid. According to Reinke *et al.* (*28*) chicoric acid is active against human immunodeficiency virus type 1, whereas CQAs are recognized as analgesic, antiviral and antihypertensive agents (*29–31*). Furthermore, it must be emphasized that Schmidt *et al.* (*32*) did not note any toxic effects in rats after oral administration of chicory root preparation rich in sesquiterpene lactones, obtained by 95 % ethanolic extraction, followed by *n*-heptane and ethyl acetate treatment.

In the biological assessment of non-inulin components from chicory extracts there were no statistically significant differences in diet intake and animal growth (Table 3). The tested diets did not have any significant effect on caecal tissue and digesta relative mass, as well as pH and ammonia concentration in digesta, while dry matter decreased significantly in the digesta of rats fed RE and PSE diets, when compared to those fed the control one (Table 3). Caecal activity of bacterial α- and β-glucosidase, as well as α-galactosidase did not differ among all rats, while β-galactosidase decreased significantly in the rats fed a diet enriched with the highest amount of phenolics (PSE diet), when compared to those fed control and LE diet. The highest β-glucuronidase activity was in rats fed control diet and it decreased considerably in animals fed PSE and LE diet. All the dietary treatments had no influence on the caecal concentration and profile of SCFAs, except acetate concentration, which was significantly decreased after the consumption of RE diet in comparison with control, yet with no statistical influence on its proportion in the SCFA profile.

It is well known that the intake of inulin can, among others, increase faecal biomass, stimulate acidification of digesta, lead to more saccharolytic and less proteolytic fermentation and increase microbial production of SCFAs in the large intestine (*3*). However, recent studies have shown that phenolics from plant extracts can act as antimicrobial agents *in vitro* (*33*). Such an activity could limit the aforementioned positive effects of inulin, provided that chicory phenolics can reach the hindgut. From all hydroxycinnamates identified in our study, the bioavailability of chlorogenic acid (monoCQA) is the most recognized. According to Gonthier *et al.* (*34*), the bioavailability of chlorogenic acid depends largely on its metabolism by the gut microflora. Moreover, the net absorption in the rat small intestine accounted for 8 % of the perfused chlorogenic acid, whereas its unchanged form was detected neither in plasma nor in bile (*35*). On the other hand, chlorogenic acid in its intact form was absorbed in the rat stomach, while 15–32 % passed to the caecum (*36*). It might be supposed that the other CQAs, especially diCQAs, as well as chicoric acid, due to their higher molecular mass, could reach the hindgut in comparable or even higher quantities than chlorogenic acid. In our study, besides the lowered activity of β-galactosidase after the ingestion of the PSE diet, we did not observe any inhibitory effects of the tested extracts on fermentative processes in the caecum. Additionally, the decreased β-glucuronidase activity after the intake of LE and PSE diets should be considered as a desired change, since this enzyme is recognized as responsible for the hydrolysis of glucuronide conjugates in the gut, and is thus an important factor in the generation of toxic and carcinogenic substances (*2*). Interestingly, tumour inhibitory activity of chicory RE in mice has been observed (*37*).

Lipid profile and antioxidant status of rat serum are shown in Table 4. In our study, all tested non-inulin fractions of the extracts possessing different content of phenolic compounds had no essential influence on the lipid profile of the animals; however, there is a tendency to reduce total cholesterol concentration in experimental groups, especially in those fed the PSE diet ($p=0.08$). The tendency is of importance in a context of the well-documented triglyceride-lowering effect of inulin (*3*), therefore the non-inulin fraction tested in the present study could be an additional factor in the modulation of lipid metabolism. Support for this supposition may be in the report by Meehye (*6*), who observed reduced cholesterol uptake through administration of chicory RE into perfused gut of rats. In our study, the experimental diets had no influence on SOD and GPx activity, compared to the control. This observation was also reflected in the lack of differences in ACW among all animals. The low-

Table 3. Effect of non-inulin components from chicory extracts on animal growth and caecal metabolism

	Diet					
	Control	RE	PE	PSE	LE	Pooled SEM
Animal growth						
initial body mass/g	103.1	103.7	103.2	103.2	103.1	0.738
diet intake/g	341.1	353.2	355.2	345.2	362.0	4.833
body mass gain/g	111.5	116.3	124.0	106.7	111.5	2.754
final body mass/g	214.6	209.9	221.6	209.9	221.6	2.883
Caecal parameters						
tissue mass/(g per 100 g of body mass)	0.502	0.537	0.504	0.504	0.503	0.013
digesta mass/(g per 100 g of body mass)	1.192	1.552	1.531	1.704	1.351	0.081
pH of digesta	5.720	5.790	5.460	5.550	5.540	0.054
dry matter/(% of digesta)	27.479^{a}	20.941^{b}	21.925^{ab}	19.111^{b}	22.732^{ab}	0.879
ammonia/(mg/g of digesta)	0.391	0.304	0.357	0.340	0.333	0.017
bacterial enzymes activity/(μmol/(h·g))						
α-glucosidase	23.01	19.79	17.21	16.16	22.70	1.236
β-glucosidase	5.05	4.97	4.75	3.71	4.32	0.367
α-galactosidase	9.23	6.98	7.40	5.80	8.62	0.785
β-galactosidase	37.88^{a}	26.6^{ab}	30.31^{ab}	14.82^{b}	33.04^{a}	2.662
β-glucuronidase	4.85^{a}	4.19^{ab}	3.23^{ab}	2.06^{c}	2.68^{bc}	0.342
concentration of SCFAs/(μmol/g)						
acetate	63.06^{a}	43.38^{b}	52.41^{ab}	55.28^{ab}	55.0^{ab}	2.161
propionate	39.1	39.19	50.61	41.60	35.42	1.990
isobutyrate	0.06	0.07	0.04	0.04	0.02	0.009
butyrate	1.98	1.29	2.05	1.02	1.29	0.199
isovalerate	0.31	0.22	0.26	0.20	0.20	0.021
valerate	0.17	0.10	0.16	0.12	0.13	0.009
total	104.6	84.26	105.52	98.25	92.04	3.098
profile of SCFAs/(% of total)						
acetate	59.93	51.66	49.76	55.89	61.38	1.615
propionate	37.68	46.33	47.91	42.76	36.83	1.692
butyrate	1.86	1.53	1.90	0.99	1.41	0.184

RE=root extract, PE=peel extract, PSE=peel and seed extract, LE=leaf extract, SEM=standard error of the mean, SCFAs=short-chain fatty acids; data are expressed as means (N=6 per group); values not sharing the same superscript within a row are different at $p \leq 0.05$

Table 4. Effect of non-inulin components from chicory extracts on serum lipid profile and antioxidant status

	Diet					
	Control	RE	PE	PSE	LE	Pooled SEM
Lipid profile						
total cholesterol/(mg/dL)	380.48	361.02	309.44	283.57	325.22	12.651
HDL-cholesterol/(mg/dL)	50.60	43.9	48.31	46.74	45.71	1.395
triglycerides/(mg/dL)	80.16	74.30	89.42	84.32	71.82	5.530
Antioxidant status						
GPx/(U/mL)	19.39	20.07	20.91	20.64	22.10	0.599
SOD/(U/mL)	300.32	330.43	322.79	340.32	301.22	9.208
ACW/(μmol/dL)	4.03	4.15	4.15	5.38	5.63	0.327
ACL/(μmol/dL)	7.33^{c}	7.88^{c}	9.79^{a}	8.16^{bc}	8.66^{ab}	0.202

RE=root extract, PE=peel extract, PSE=peel and seed extract, LE=leaf extract, SEM=standard error of the mean, HDL=high-density lipoprotein, GPx=glutathione peroxidase, SOD=superoxide dismutase, ACW=antioxidant capacity of water-soluble substances, ACL=antioxidant capacity of lipid-soluble substances; data are expressed as means (N=6 per group); values not sharing the same superscript within a row are different at $p \leq 0.05$

est ACL was in the rats fed control and RE diet; PE and LE diets significantly increased ACL, whereas the effect of PSE diet was insignificant.

Recently published *in vitro* studies indicate that the constituents of *Cichorium intybus* L., especially from the red varieties, possess potential anti-free radical activity (*8*,*9*). Our *in vitro* results showed relatively high antioxidant activity of the extracts from leaves, and especially seeds, when compared to root and peel ones; however, the potency was verified by animal study. It shows that non-inulin components from green leaves and root peels may, indeed, have an ability to increase protection against lipid peroxidation in rat organism with disturbed metabolism. This ability is a confirmation of an earlier *in vitro* study by Kim and Yang (*38*), who observed protective effects of chicory RE against low-density lipoprotein oxidation. In addition, some authors (*4*,*5*) have reported antihepatotoxic effects of chicory root callus and seed extracts against carbon tetrachloride-induced hepatic damage in rats, which is generated by free radicals, thus the evidence is strong that antioxidative compounds with strong biological activity are present in chicory. However, in our study the favourable elevation of ACL was not observed through an increase in the amount of phenolics in the PSE diet (*vs.* PE diet). Possibly other compounds present in the seeds played a role in establishing the blood antioxidant status.

Conclusion

The chemical composition of the 75 % ethanol extracts of root, peel, leaf and seed presented in this study indicates that different parts of chicory and its by-products might be good sources of functional compounds (inulin, chicoric acid, quercetin glucuronide, chlorogenic acid and other caffeoylquinic acids). The lack of inhibitory effects on fermentative processes in the caecum, in connection with their ability to increase the protection against lipid peroxidation, points that chicory non-inulin fractions, rich in phenolics, extracted from leaves and root peels might be an additional factor in improving the physiological activity of inulin. Finally, we hold that chicory should once again regain research and industrial attention on account of the possible nutritional and therapeutic use of its extracts.

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