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Interaction of Microbial β-Glucuronidase with Vegetable Pectins

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Supporting Information

ABSTRACT: The purpose of this study was to investigate the in vitro effects of vegetable carbohydrates on the activity of microbial β-glucuronidase (βG) and the adsorption of the enzyme on carbohydrates. This study used pectin−protein complexes (PPCs) with molecular weights of 300 kDa isolated under conditions simulating a gastric environment from cabbage (HCl − PPC_C and HCl + pepsin − PPC_{CP}) and sweet pepper (PPC_P and PPC_{PP}). As a sample for comparison, microcrystalline cellulose was used. The activity of βG from *Escherichia coli* was determined spectrophotometrically by the formation of the colored product from the breakdown of phenolphthalein-β-D-glucuronide. Adsorption of βG on biopolymers was studied by the retention of the enzyme on the membrane of a concentrator with a pore diameter of 300 kDa and by native PAGE. PPC_{CP} and PPC_C were established to increase the activity of βG by 50 and 100%, respectively. Cellulose had a weak effect, whereas pepper PPC had no effect. All studied carbohydrates adsorb on βG. The maximum βG adsorption (15%) was observed with PPC_C, whereas PPC_{CP} absorbed 5% of the enzyme. Pepper PPCs and cellulose adsorbed up to 10% of the enzyme. There was a positive correlation between the increase of βG activity in the presence of carbohydrates and enzyme adsorption on the polymers ($r = 0.80$; $P < 0.01$). The activity of the enzyme in the gel after electrophoresis of the PPC_C + βG mixture was inversely proportional to the concentration of PPC_C in the mixture. A model explaining the effects of cabbage PPCs on the excretion of estrogens is proposed.

KEYWORDS: white cabbage (*Brassica oleracea*), sweet pepper (*Capsicum annuum*), pectin−protein complex, microbial β-glucuronidase, adsorption, activity

INTRODUCTION

An elevated level of estrogens in postmenopausal women is a risk factor for hormone-dependent tumors, especially in cases of breast and endometrial cancer.[1−3] The digestive system plays an important role in the metabolism and excretion of estrogens. In humans, the metabolic conversion of estrogens and its subsequent excretion mainly in urine (70%) are observed during the process of enterohepatic circulation (EHC, Figure 1).[4] Foods rich in proteins and fats slow the excretion of estrogens[5] and increase the risk of breast cancer.[4,6] However, foods rich in plant fiber, such as pectin, interrupt the EHC of estrogens and accelerate its excretion from the organism. In this case, the main route of the excretion of estrogens (70%) is through feces.[4,7,8] Pectin may interrupt the EHC of estrogens in two possible ways: (1) the adsorption of estrogens on the pectins may prevent its reabsorption from the chyme into the bloodstream, or (2) adsorption of β-glucuronidase (βG) with pectin may reduce activity and/or accelerate its excretion.

We have shown previously[9] that pectin−protein complexes (PPCs) isolated from cabbage, sweet pepper, and cellulose adsorb estrogens under in vitro conditions. Cabbage PPCs absorb estrogens to a greater extent than pepper PPCs and cellulose. These results suggest that the interruption of the EHC of estrogens may be carried out through the first mechanism proposed above.

The purpose of this study was to investigate the in vitro effects of pectic carbohydrate from cabbage and pepper on the activity of β-glucuronidase and the adsorption of the carbohydrates to the enzyme to understand better the mechanism of estrogens excretion from the body.

MATERIALS AND METHODS

Reagents. *Enzyme* (βG), β-glucuronidase type IX-A from *Escherichia coli* (Sigma); *substrate*, 10 mM aqueous solution of phenolphthalein-β-D-glucuronide (Sigma); *standard*, 0.03 M solution of phenolphthalein in 70% (v/v) ethanol; *solution for incubation*, 0.1 M phosphate buffer, pH 7.0, and 0.1 mM EDTA; *alkaline mixture*, 200 mL of glycine buffer, 0.2 M, pH 11, added to 50 mL of 0.5 N NaOH; *sorbents*, microcrystalline cellulose (C) (Lachema Chemapol, Czech Republic) and the macromolecular fraction (>300 kDa) of vegetable PPCs isolated from white cabbage (PPC_C and PPC_{CP}) and sweet pepper (PPC_P and PPC_{PP}).

Equipment. A Vivaspin 500 concentrator (Sartorius AG, Germany) with a pore diameter of 300 kDa, an Ultrospec 3000 spectrophotometer (Pharmacia Biotech, England), and a Microplate PowerWave 200 spectrophotometer (Bio-Tek Instruments) were used.

General Analytical Methods. The glucuronic acid content of the PPCs was determined using a reaction of 3,5-dimethylphenol in the presence of concentrated sulfuric acid and the calibration graph that was plotted for D-galacturonic acid (Sigma-Aldrich).[10] The protein content of the PPCs was determined using the Bradford assay[11] and the calibration graph plotted for BSA (MP Biomedicals).

Isolation of PPCs from Vegetables. Four fractions of PPCs were extracted under conditions simulating a gastric environment: two fractions were from cabbage (*Brassica oleracea*), denoted PPC_C and PPC_{CP}, and two fractions were from sweet pepper (*Capsicum annuum*),

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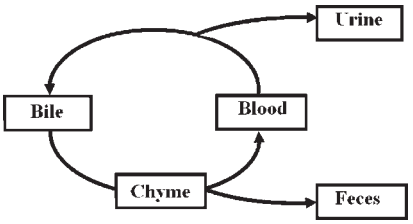

Figure 1. Scheme of estrogen's enterohepatic circulation (explanation in the text).

Table 1. General Characteristics of PPCs

		content,[a] %		
sample	yield,[b] %	protein	Gal A	NM[c]
PPC_C	0.2	26.2	34.1	19.3
PPC_{CP}	0.3	9.3	38.6	30.6
PPC_P	0.3	23.4	66.5	5.9
PPC_{PP}	0.3	10.0	74.1	7.7

[a] Mass percentages. [b] Yield was calculated per weight of fresh vegetables. [c] Total content of neutral monosaccharides.

denoted PPC_P and PPC_{PP}. Extractions of PPC_C and PPC_P were performed according to a previously described method.[12] Pepsin (Fluka, activity = 600 U/g) was added to separate the fractions of PPC_{CP} and PPC_{PP} in the extraction mixtures. The output and chemical characteristics of the obtained fractions are shown in Table 1.

Determination of βG Activity. The determination of the enzyme activity was conducted according to a previously described method[13,14] with modifications. βG (1 mg) was dissolved in 0.5 mL of phosphate buffer. The enzyme was incubated in a glass container with a flat bottom with constant shaking for 1 h at room temperature. In each well of the microplate, 75 μL of the incubation mixture and substrate was added. The reaction was performed at room temperature for 1 h and was stopped by adding 150 μL of the alkaline mixture. The optical density of samples was measured with a microplate spectrophotometer at 540 nm. Under these conditions, 1 mg of βG showed an activity equal to 80.1 ± 2.0 Sigma units (mean ± SD; $n = 6$).

Study of the Effect of PPCs on βG Activity. Each sample was analyzed in 5−12 independent experiments. PPCs were added at a final concentration of 0.05 or 0.1%. Enzyme activity in the presence of PPCs was measured as described above. βG activity in the presence of PPCs was expressed as a percentage of the activity of pure enzyme. Statistical analysis of the results is presented in Table 1S of theSupporting Information.

Study of the Adsorption of βG on PPCs. Incubation of the enzyme with PPCs was conducted in 5−12 independent experiments as described above. The incubation mixture was subsequently transferred to a concentrator and centrifuged for 30 min at 12000 rpm at room temperature. The βG activity of the solution that passed through the membrane of the concentrator was determined as described above. Statistical analysis of the results is presented in Table 2S of the Supporting Information.

Nongradient Native PAGE. Highly porous acrylamide gels were prepared according to a previously described method[15] with modifications. Essentially, a stock solution containing 50% (w/v) acrylamide and 0.5% (w/v) bis-acrylamide (acrylamide/bis-acrylamide = 100:1, w/w) was prepared in distilled water and used for both stacking and resolving gels. The final acrylamide/bis-acrylamide concentrations of the stacking and resolving gels were 4 and 8% (w/w), respectively. Nongradient native PAGE was performed at room temperature using a Bio-Rad Mini-PROTEAN II electrophoresis cell. Running and gel buffers were prepared according to the protocol of Gallagher.[16] Briefly, a buffer containing 375 mM Tris-HCl, pH 8.8, was used for the stacking gel. The resolving gel was made with 125 mM Tris-HCl, pH 6.8, and the running buffer was 25 mM Tris and 192 mM glycine, pH 8.3. βG was dissolved in 0.4% (w/v) phosphate buffer and blended with PPC_C to obtain solutions with 0 (βG without pectin), 25, and 50% (w/w) PPC_C concentrations (as compared with the βG concentration). The solutions were incubated overnight at 4 °C to ensure complete hydration of the macromolecules. Before electrophoresis, 66 μL of the solutions was mixed with 33 μL of native PAGE loading buffer (three times) containing 375 mM Tris-HCl, pH 7.0, 20% (w/w) glycerol, and 1.2% (w/v) Coomassie brilliant blue G-250 to enhance the positive charge of nondenaturated enzymes.[17] Following sample loading (20 μL of sample mixtures with 80 μg of protein per well), the gel was run at 100 V until completion (80 min). Next, the stacking gel was carefully removed using a Teflon blade prior to in-gel activity staining.

In-Gel βG Activity Staining. Initially the vertical gel strip with the βG sample track was cut with a Teflon blade and was incubated with substrate to determine the position of enzyme in the gel. Next, the horizontal gel strip with βG was separated. The gel strips were incubated in substrate solution for 1 h and were subsequently developed in the alkaline mixture as described above. All steps were conducted at room temperature in a wet case.

The native PAGE gel was documented using a Canon Power Shot A620 camera (Canon Inc., Japan). Image correction was performed using free image processing software (Image J, version 1.44p, U.S. National Institutes of Health) with level adjustment, green channels split and inverting.

Statistical Analysis. The Statistica 6.0 software package was used for statistical analysis. Data are presented as the mean ± standard deviation (SD). Correlation, regression analysis, and factorial ANOVA were also performed.

RESULTS

Pectin−protein complexes were isolated from commonly used vegetables, specifically, cabbage and sweet pepper, using conditions simulating a gastric environment. Processing of the plant material was performed at 37 °C for 4 h in two parallel samples with and without pepsin, which approximated the composition of a gastric juice solution. As a result, two samples, PPC_C and PPC_P, were isolated from the cabbage and pepper solutions without pepsin, and two additional samples, PPC_{CP} and PPC_{PP}, were isolated from the solutions with pepsin. Chemical analysis of the samples showed that they contained both carbohydrate and protein components (Table 1). The carbohydrate portion identified as pectic polysaccharides contained the residues of D-galacturonic acid (Gal A) as main constituents. The PPC_{CP} and PPC_{PP} samples had significantly lower protein contents than did PPC_C and PPC_P samples (Table 1).

Effect of PPCs on βG Activity. βG activity increased significantly in the presence of polysaccharide ($F_{2,128} = 26.78$; $P = 0.000000$; partial $\eta^2 = 0.29$) (Figure 2). βG activity in the presence of sweet pepper PPCs did not change ($F_{1,54} = 2.55$; $P = 0.12$). However, cellulose increased the activity of βG ($F_{1,30} = 12.43$; $P = 0.0014$; partial $\eta^2 = 0.29$), and the maximum increase in βG activity was observed in the presence of cabbage PPCs ($F_{1,74} = 10.57$; $P = 0.0017$; partial $\eta^2 = 0.13$) (Figure 2). This significant increase was observed at 0.1% concentration of cabbage PPCs (post hoc comparisons, Tukey test, $P < 0.03$−0.0008).

Adsorption of βG on PPC. The adsorption of βG on biopolymers was tested using two different methods: the retention

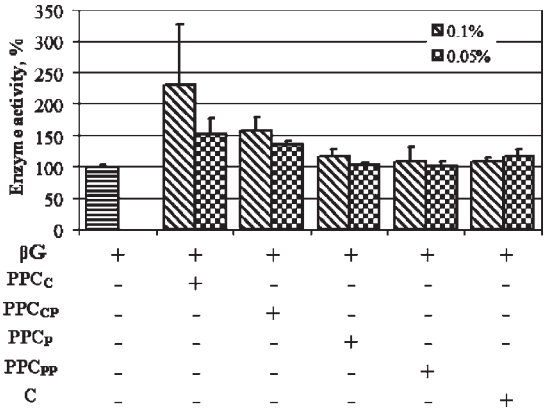

Figure 2. Effect of PPCs on βG activity. 0.05 and 0.1% are the concentrations of PPCs in the incubation mixture. Details are given under Materials and Methods. Error bars represent SD.

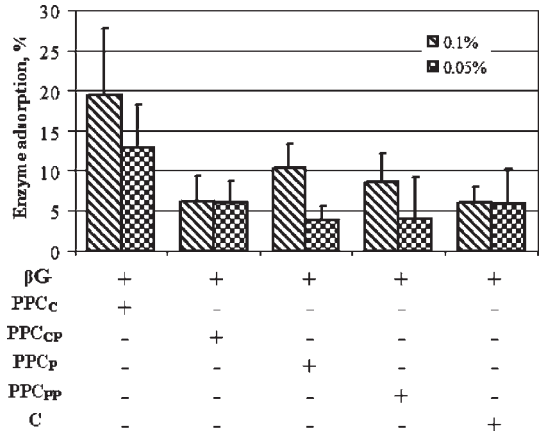

Figure 3. Adsorption of βG on PPCs. 0.05 and 0.1% are the concentrations of PPCs in the incubation mixture. Details are given under Materials and Methods. Error bars represent SD.

of PPC−βG complexes by the membrane of a concentrator and the identification of βG activity in a gel after PAGE. The first method showed that βG adsorbed on all three samples tested: cabbage PPC, pepper PPC, and cellulose ($F_{2,128}$ = 25.45; P = 0.000000; partial η^2 = 0.28). The maximum adsorption of βG was observed on cabbage PPCs (Figure 3). The adsorption of βG on PPC_C was significantly higher than on PPC_{CP} (post hoc comparison, Tukey test, $P < 0.01$), whereas the PPC_P and PPC_{PP} samples did not differ ($F_{1,40}$ = 4.07; P = 0.0504). Adsorption of βG on the cabbage PPCs ($F_{1,60}$ = 8.94; P = 0.004; partial η^2 = 0.13) and pepper PPCs ($F_{1,40}$ = 12.87; P = 0.0009; partial η^2 = 0.24) was dependent on the concentration of polysaccharide in solution. Adsorption of βG on cellulose was not dependent on the concentration of polysaccharide ($F_{1,20}$ = 1.44; P = 0.24).

In-gel staining of βG activity in the samples with PPC_C revealed less enzyme than in the control sample without PPC_C. Moreover, there was an inverse relationship between the intensity of the gel staining and the concentration of PPC_C (Figure 4).

Relationship between the Induction of βG Activity and Its Adsorption on the PPCs. There was a positive correlation between the ability of PPCs to enhance the activity of βG and the adsorption of the enzyme on PPCs (r = 0.80; $P < 0.01$; n = 10). Regression analysis indicated that linear regression between

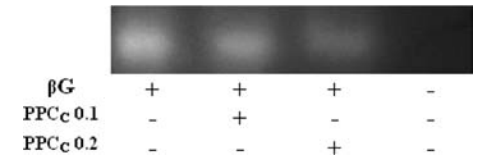

Figure 4. In-gel activity staining of βG. Green channel, inverted image; PPC_C 0.1, cabbage pectin 0.1%; PPC_C 0.2, cabbage pectin 0.2%.

adsorption and enzyme activity explained 65.5% of the variability (Supporting Information, Figure 1S). In addition, as shown in Figure 4, after PAGE of the βG + PPC_C samples, the in-gel βG activity decreased with increasing concentration of pectin. Consequently, there was an increase in the quantity associated with PPC and nongel βG.

DISCUSSION

The aim of this study was to determine the influence of cabbage PPCs (PPC_C and PPC_{CP}) and sweet pepper PPCs (PPC_P and PPC_{PP}) on the function of *E. coli* βG at biopolymer concentrations of 0.05 and 0.1%, which previous work suggests are similar to the concentration of PPCs present in human chyme after the consumption of 1−2 g/day of pectin.[9] Epidemiological evidence suggests that the average intake of pectin is 1−7 g/day.[18]

We found that PPC_C and PPC_{CP} interactions with microbial βG increased the activity of the enzyme. Under the same conditions, cellulose had a weak effect, and PPC_P and PPC_{PP} had no effect on βG activity. The maximum stimulating effect was found with PPC_C. The PPC_{CP} sample, which was also isolated from cabbage and is distinguished by low protein content, had a significantly lower stimulating effect than did PPC_C. This result may reflect the influence of the proteins from the PPCs on specific interactions with βG.

The activity of βG was found to be 2 times higher in the presence of cabbage pectin than in the presence of pepper pectin. The content of neutral monosaccharides was shown to be significantly higher in the cabbage pectin compared with the pepper pectin. Therefore, we can presume that the neutral monosaccharides are involved in the interaction of cabbage pectin with βG. Conformational changes of βG induced by the interaction with pectin macromolecules are supposed to result in an increase of enzyme activity.

One of the universal mechanisms of inactivation of dangerous substances, such as carcinogens and toxins, is the glucuronidation of low molecular weight compounds, which is catalyzed by UDP-glucuronosyltransferases.[19] Hydrolysis of glucuronides in the colon, catalyzed by microbial βG, leads to the reactivation of these compounds and their reabsorption into the blood, which is potentially harmful to the body.

Previous experiments on laboratory animals and research with human volunteers have yielded conflicting data on the effect of pectin on the activity of microbial βG. In most cases,[14,20−22] the researchers observed an increase in βG activity in the colon, cecum, and feces when pectins were added to food regardless of the source (citrus, apple) or structure (high- and low-methoxylated forms) of the pectin. One previous study noted the suppression of βG activity when apple pectin was added to an animal's diet.[23]

However, it has been shown that the consumption of pectin lowers cholesterol levels,[24] and a positive correlation has also

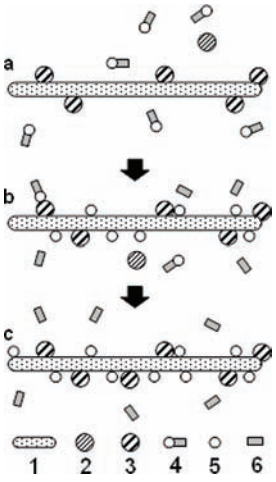

Figure 5. Mechanism of cabbage PPC action on the excretion of estrogen (for details, see text): 1, cabbage PPCs; 2, βG; 3, activated βG; 4, estrogen glucuronides; 5, free estrogens; 6, glucuronic acid.

been found between the level of pectin consumption and the level of fecal estrogens.[25]

In general, there appears to be a contradictory effect: the consumption of pectin increases the activity of βG and simultaneously interrupts the EHC of estrogens, increasing its excretion. The results of our present study may help to explain this contradiction. We have noted that an increase in βG activity occurs when the enzyme interacts with PPCs, which may result in a local increase of βG activity in chyme but not elsewhere. Given that the cabbage PPCs actively adsorb free estrogens,[9] we suggest the following mechanism of action of cabbage PPCs on the excretion of estrogens (Figure 5): (a) in the large intestine, cabbage PPCs adsorb microbial βG and causes a local increase in enzyme activity, (b) estrogens glucuronides located in the aqueous fraction of chyme come into contact with βG and are hydrolyzed, becoming insoluble in water, (c) due to a high affinity for cabbage PPCs, βG and free estrogens are adsorbed on the PPCs and, together with the undigested components of food, are excreted in feces. Thus, cabbage PPCs interrupt the EHC of estrogens, thereby preventing the reabsorption of estrogens from the large intestine into the bloodstream.

Epidemiological studies have suggested that the consumption of cruciferous vegetables reduces the risk of hormone-dependent cancer in humans.[26] One of the possible mechanisms of the anticancer action of cabbage is its ability to influence estrogens metabolism. Indole-3-carbinol, which is found in cabbage, shifts the metabolism of estrogens toward the formation of the less carcinogenic 2-hydroxylated forms.[27] Data presented in this paper suggest that cabbage pectin can also affect the rate of estrogens excretion from the body by adsorption with βG. The data presented in this paper suggest another anticancer mechanism through the ingestion of cabbage. Cabbage pectin may lead to the interruption of the EHC of estrogens and accelerate its removal from the body by adsorbing βG and free estrogens. These effects may explain the anticancer action of cabbage in relation to hormone-dependent tumors.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Tables 1S and 2S and Figure 1S. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

βG β-glucuronidase; PPC_C pectin−protein complex isolated from white cabbage using HCl; PPC_{CP} pectin−protein complex isolated from white cabbage using HCl + pepsin; PPC_P pectin−protein complex isolated from sweet pepper using HCl; PPC_{PP} pectin−protein complex isolated from sweet pepper using HCl + pepsin; C cellulose; EHC enterohepatic circulation.

■ REFERENCES

(1) Bernstein, L.; Ross, R. K. Endogenous hormones and breast cancer risk. *Epidemiol. Rev.* **1993**, *15*, 48–65.

(2) Ursin, G.; Wilson, M.; Henderson, B. E.; Kolonel, L. N.; Monroe, K.; Lee, H. P.; Seow, A.; Yu, M. C.; Stanczyk, F. Z.; Gentzschein, E. Do urinary estrogen metabolites reflect the differences in breast cancer risk between Singapore Chinese and United States African-American and white women? *Cancer Res.* **2001**, *61*, 3326–3329.

(3) Lepine, J.; Audet-Walsh, E.; Gregoire, J.; Tetu, B.; Plante, M.; Menard, V.; Ayotte, P.; Brisson, J.; Caron, P.; Villeneuve, L.; Belanger, A.; Guillemette, C. Circulating estrogens in endometrial cancer cases and their relationship with tissular expression of key estrogen biosynthesis and metabolic pathways. *J. Clin. Endocrinol. Metab.* **2010**, *95*, 2689–2698.

(4) Adlercreutz, H. Western diet and Western diseases. Some hormonal and biochemical mechanisms and associations. *Scand. J. Clin. Invest.* **1990**, *50*, 3–23.

(5) Adlercreutz, H. Diet and sex hormone metabolism. In *Nutrition, Toxicity and Cancer*; Rowland, I. R., Ed.; CRC Press: London, U.K., 2000; pp 170−175.

(6) Wu, A. H.; Yu, M. C.; Tseng, C.-C.; Stanczyk, F. Z.; Pike, M. C. Dietary patterns and breast cancer risk in Asian American women. *Am. J. Clin. Nutr.* **2009**, *89*, 1145–1154.

(7) Goldin, B. R.; Adlercreutz, H.; Gorbach, S. L.; Woods, M. N.; Dwyer, J. T.; Conlon, T.; Bohn, E.; Gershoff, S. N. The relationship between estrogen levels and diets of Caucasian American and Oriental immigrant women. *Am. J. Clin. Nutr.* **1986**, *44*, 945–953.

(8) Gorbach, S. L.; Goldin, B. R. Diet and the excretion and enterohepatic cycling of estrogens. *Prev. Med.* **1987**, *16*, 525–531.

(9) Borisenkov, M. F.; Golovchenko, V. V.; Vityazev, F. V. *In vitro* adsorption of estrogens on pectic fractions from sweet pepper and white cabbage. *Chem. Plant Raw Mater.* **2011** (in Russian, in press).

(10) Usov, A. I.; Bilan, M. I.; Klochkova, N. G. Polysaccharides of Algae. 48. Polysaccharide composition of several calcareous red algae: isolation of alginate from *Corallina pilulifera* P. et R. (Rhodophyta, Corallinaceae). *Bot. Mar.* **1995**, *38*, 43–51.

(11) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.

(12) Popov, S. V.; Ovodova, R. G.; Golovchenko, V. V.; Popova, G. Yu.; Viatyasev, F. V.; Shashkov, A. S.; Ovodov, Yu. S. Chemical composition and anti-inflammatory activity of a pectic polysaccharide isolated from sweet pepper using a simulated gastric medium. *Food Chem.* **2011**, *124*, 309–315.

(13) Goldin, B. R.; Gorbach, S. L. The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. *J. Natl. Cancer Inst.* **1976**, *57*, 371–375.

(14) Freeman, H. J. Effects of differing purified cellulose, pectin, and hemicellulose fiber diets on fecal enzymes in 1,2-dimethylhydrazine-induced rat colon carcinogenesis. *Cancer Res.* **1986**, *46*, 5529–5532.

(15) Doucet, J. P.; Trifaro, J. M. A discontinuous and highly porous sodium dodecyl sulfate−polyacrylamide slab gel system of high resolution. *Anal. Biochem.* **1988**, *168*, 265–271.

(16) Gallagher, S. R. One-dimensional SDS gel electrophoresis of proteins. *Curr. Protoc. Immunol.* **2006**, *75*, 8.4.1–8.4.37.

(17) Schagger, H.; von Jagow, G. Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. *Anal. Biochem.* **1991**, *199*, 223–231.

(18) Linseisen, J.; Schulze, M. B.; Saadatian-Elahi, M.; Kroke, A.; Miller, A. B.; Boeing, H. Quantity and quality of dietary fat, carbohydrate, and fiber intake in the German EPIC cohorts. *Ann. Nutr. Metab.* **2003**, *47*, 37–46.

(19) Lampe, J. W. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *Am. J. Clin. Nutr.* **1999**, *70*, 475S–490S.

(20) Bauer, H. G.; Asp, N. G.; Dahlqvist, A.; Fredlund, P. E.; Nyman, M.; Oste, R. Effect of two kinds of pectin and guar gum on 1,2-dimethylhydrazine initiation of colon tumors and on fecal β-glucuronidase activity in the rat. *Cancer Res.* **1981**, *41*, 2518–2523.

(21) Ross, J. K.; Leklem, J. E. The effect of dietary citrus pectin on the excretion of human fecal neutral and acid steroids and the activity of 7-α-dehydroxylase and β-glucuronidase. *Am. J. Clin. Nutr.* **1981**, *34*, 2068–2077.

(22) Licht, T. R.; Hansen, M.; Bergström, A.; Poulsen, M.; Krath, B. N.; Markowski, J.; Dragsted, L. O.; Wilcks, A. Effects of apples and specific apple components on the cecal environment of conventional rats: role of apple pectin. *BMC Microbiol.* **2010**, *10*, 13.

(23) Ohkami, H.; Tazawa, K.; Yamashita, I.; Shimizu, T.; Murai, K.; Kobashi, K.; Fujimaki, M. Effects of apple pectin on fecal bacterial enzymes in azoxymethane-induced rat colon carcinogenesis. *Jpn. J. Cancer Res.* **1995**, *86*, 523–529.

(24) Fernandez, M. L. Soluble fiber and indigestible carbohydrate effects on plasma lipids and cardiovascular risks. *Curr. Opin. Lipidol.* **2001**, *12*, 35–40.

(25) Pusateri, D. J.; Roth, W. T.; Ross, J. K.; Shultz, T. D. Dietary and hormonal evaluation of men at different risks for prostate cancer: plasma and fecal hormone-nutrient interrelationships. *Am. J. Clin. Nutr.* **1990**, *51*, 371–377.

(26) Higdon, J. V.; Delage, B.; Williams, D. E.; Dashwood, R. H. Cruciferous vegetables and human cancer risk: Epidemiologic evidence and mechanistic basis. *Pharmacol. Res.* **2007**, *55*, 224–236.

(27) Michnovicz, J. J.; Adlercreutz, H.; Bradlow, H. L. Changes in levels of urinary estrogen metabolites after oral indole-3-carbinol treatment in humans. *J. Natl. Cancer Inst.* **1997**, *89*, 718–723.