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Influence of Feeding Malt, Bread Crust, and a Pronylated Protein on the Activity of Chemopreventive Enzymes and Antioxidative Defense Parameters in Vivo

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The aim of the present study was to investigate whether feeding of malt, bread crust, and a pronylated albumin modulates chemoprevention enzymes, such as glutathione-S-transferase (GST) and UDP-glucuronyl-transferase (UDP-GT), and antioxidative defense parameters in vivo and whether the intake of these foods rich in Maillard reaction compounds results in an accumulation of compounds formed in in vivo glycation reactions. After quantitation of pronylated lysine in malt and bread crust, male Wistar rats were fed a standard chow supplemented with 28% of protein containing different amounts of casein, bread crust, caraffa malt, or pronyl bovine serum albumin (BSA) for 15 days. GST activity in the kidneys was increased by 18% ($p > 0.05$) in animals of the bread crust group, while UDP-GT activity was elevated by 27% in the liver of animals administered pronyl-BSA. Contents of tocopherol in plasma were increased by 33, 14, and 14% in the bread crust, malt, and pronyl-BSA group compared to the control group, while the levels of thiobarbituric acid reactive substances were decreased and the total antioxidant capacity was increased. Parameters of endogenous glycation indicated a 32 and 46% higher load of advanced glycation end products in the kidneys after administration of the malt and the pronyl-BSA containing diet. However, the main systemic effects of dietary malt, bread crust, and pronyl-BSA were, for the first time, demonstrated to be the enhanced antioxidant capacity and the particulate increase in chemopreventive enzymes.

KEYWORDS: bread crust; malt; pronyl-BSA; glutathione-S-transferase; NADPH cytochrome *c* reductase; antioxidative capacity; tocopherol; advanced glycation end products (AGEs)

INTRODUCTION

The delicious aroma and the dark color of bread crust are chiefly responsible for the consumer's choice of freshly baked bread. Whereas the key aroma compounds of bread crust have been chemically identified (*1*, *2*), not very much is known about the chemical structure of the compounds responsible for the color formation. Although it is generally accepted that the colored compounds formed during heat treatment of the bread dough belong to the group of Maillard reaction products (MRPs) and melanoidins which result from interactions between reducing sugars and free amino groups of proteins or amino acids, the knowledge about their exact chemical structures as well as their health effects is relatively scarce. One health effect of MRPs has been demonstrated in an animal feeding trial on rats. Oral administration of the N-epsilon carboxymethyllysine, which is present in bread crust in a concentration of about 550 ppm, resulted in an induction of a chemopreventive Phase II enzyme, the glutathione S-transferase (GST) (*3*).

Current data suggest that the balance between the Phase I carcinogen-activating enzymes and the Phase II detoxifying enzymes is critical in determining an individual's risk for cancer. Human deficiencies in Phase II enzyme activity, specifically GST, have been identified and associated with increased risk for colon cancer (*4*). On the other hand, induction of GST or other Phase II enzymes, e.g., the UDP-glucuronyl-tansferase (UDP-GT), by, i.e., antioxidants, represents a promising strategy for cancer prevention. Although the molecular mechanisms by which antioxidants bind to an antioxidant responsive element resulting in the specific, monofunctional induction of Phase II enzymes has been intensively studied (*5*), it is still an open question which of the MRP or melanoidin structures may function as a Phase II inducer in a biological system.

Among all of the biological systems suitable for in vitro studies, the intestinal Caco-2 cell line is widely used to

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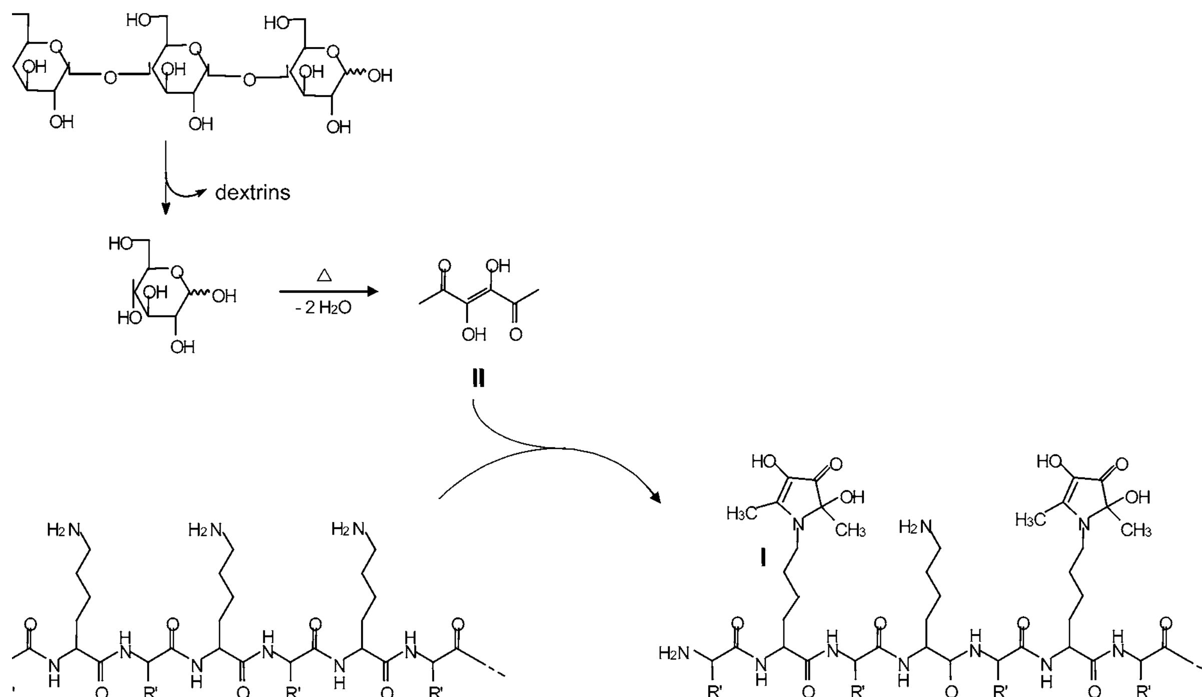

Figure 1. Reaction scheme on the formation of protein-bound pronyl-L-lysine (**I**) from proteins and starch via the key intermediate acetylformoin (**II**).

investigate the effects of dietary compounds on Phase I/II enzymes as the colon is clearly one of the most likely sites for the development of different types of dietary induced cancers (*6*, *7*). Thus, the chemopreventive action of bread crust was first investigated in intestinal Caco-2 cells which showed an induction of the Phase II enzyme GST after exposure to soluble bread crust extracts prepared by solvent extraction using water, ethanol, or 2-propanol (*8*). In that study, activity-guided fractionation of bread crust as well as suitable Maillard-type model systems led to the identification of the protein-bound 2,4-dihydroxy-2,5-dimethyl-1-(5-acetamino-5-methoxycarbonylpentyl)-3-oxo-2*H*-pyrrol (pronyl-L-lysine; **I** in **Figure 1**) as part of bread crust melanoidins (*8*). This compound was also demonstrated to be the key antioxidant of bread crust. Interestingly, the chemopreventive and the antioxidative effects were the same for pronyl-L-lysine and pronylated proteins as the active structure was identified as the pronyl rest.

During baking, glucose is thermally liberated from starch and was found to produce the pronyl-L-lysine by Maillard reactions via the transient intermediate and penultimate precursor acetylformoin (**II** in **Figure 1**) and lysine side chains or the N-terminus of the flour proteins. Moreover, it has been demonstrated that the content of such "pronylated" proteins is associated with the intensity of thermal treatment and the resulting dark colorization of the bread crust (*9*).

In earlier studies, exposure of Caco-2 cells to water-soluble malt extracts of different molecular weight ranges also resulted a modulation of the enzyme activity of Phase II GST but also of Phase I NADPH cytochrome *c* reductase (CCR) (*10*). In these studies, no key compound responsible for the enzyme-modulating activity was identified but from the chemical composition it may be hypothesized that ponylated proteins might also have been present in those malt samples (**Table 1**).

However, these results were obtained in in vitro studies, and it is still an open question whether the dietary intake of bread crust or pronylated proteins exert chemopreventive and antioxidant effects in vivo. In the present study, bread crust and malts as well as synthetically pronylated albumin were administered to rats at moderate, human diet equivalent doses in order to investigate their effects on chemopreventive Phase II enzymes and on parameters of the antioxidant defense system.

Table 1. Concentrations of Pronyl-L-lysine in Thermally Browned Foods and in Pronyl-BSA

sample	pronyl-L-lysine (mg/kg)
wheat bread crust	6.58
rye/wheat mixed bread (crust)[a]	62.2 (8)
full grain rye bread (crust)	40.8
pilsen malt	1.13
caraffa malt[a]	6.14
caramalt	16.6
caramunich malt	20. 8
pale beer (Pilsener type)	0.43
dark beer	0.92
dark wheat beer	1.07
roasted coffee (Arabica)	n.d.
roasted coffee (Robusta)	n.d.
pronylated-BSA	271

[a] Administered in the present feeding trial.

Another aspect which had to be considered was the putative effect of dietary bread crust and pronylated albumin on the formation of advanced glycation end products (AGEs) in vivo.

AGEs are formed in living organisms by Maillard-type reactions, called nonenzymatic glycation. The ability of AGE-modified proteins to form protein−protein cross links in collagen is a key determinant in the pathogenesis of the reduced vascular and myocardial compliance observed with aging and diabetes but also plays a role in the progression of associated complications such as nephropathy or retinopathy (*11*−*13*) as well as in Alzheimer's disease (*14*).

The pathophysiological significance of AGEs stems not only from their ability to modify the functional properties of proteins but also from their interaction with cells via AGE binding proteins or AGE receptors. The cellular interactions of AGEs are mediated through a specific receptor for AGEs (RAGE) on cell surfaces (*15*). AGE interaction with cellular RAGE results in receptor activation and receptor-mediated release of superoxide anions and pro-inflammatory cytokines (*15*, *16*). In our

Table 2. Composition of the Experimental Diets

components (%)	control	bread crust	malt	pronyl-BSA
altromin C1004-protein free	70.0	67.5	67.1	68.9
cellulose	2.00	2.00	2.00	2.00
casein	28.0	25.8	26.4	28.0
test compound		4.70	4.50	1.10

own experiments, exposure of RAGE-expressing Caco-2 cells to bread crust and to pronylated albumin resulted in a RAGE-mediated activation of mitogen activated kinases (*17*). However, the question whether dietary MRPs or melanoidins can pose risks to the vascular system and kidneys resulting in the progression of diabetes or aging is still not answered. Recently, Vlassara et al. (*18*) demonstrated that the intake of dietary MRPs in diabetics promote the formation of pro-inflammatory mediators, leading to tissue injury. Although restriction of dietary AGEs suppressed these effects, it is still not clear (i) which of the dietary MRP or melanoidin structures activate pro-oxidative and pro-inflammatory pathways after their absorption and (ii) whether the healthy organism can compensate for pathophysiological effects.

Therefore, the contents of AGEs in the kidneys, being the main site of AGEs' accumulation, were monitored and related on the overall systemic, chemopreventive, and antioxidative effects in rats after the oral administration of bread crust, malt, and pronylated albumin.

MATERIALS AND METHODS

Chemicals. Unless stated otherwise, reagents and chemicals were purchased from Sigma (Germany) and Merck (Germany). Bread and beer samples were purchased from local bakeries; malt and coffee samples were obtained from the German food industry.

Quantitation of Pronyl-L-lysine. An aliquot (50 g dry weight) of ground bread crust, beer, malt, or ground roasted coffee was defatted with chloroform (3 × 100 mL), suspended in water (250 mL), and then derivatized with methyl hydrazine closely following the procedure detailed recently (*8*). By use of 1-methylpyrrolidone as the internal standard, the amount of pronyl-L-lysine was determined by means of HRGC/MS operating in the chemical ionization mode (*8*).

Animal Experiments. Male Wistar rats (Wistar Unilever HsdCpb: WU) were obtained from Harlan-Winkelmann (Germany). Adult animals weighing 326 ± 1.04 g were individually housed in metabolic cages. The temperature was maintained at 20 ± 0.8 °C and the humidity at 55 ± 2%. A 12-hour dark/light cycle was kept with artificial lighting. The animals were fed daily at 8:00 a.m. with an average of 30 ± 0.01 g of the diet per capita and were allowed free access to drinking water.

The experiment comprised four randomized groups of eight rats each, which were fed a standard chow (No. C1004, Altromin/Germany) supplemented with cellulose (Bilaney Consultants, Duesseldorf/Germany) and either casein purchased from New Zealand Milk Products, Rellingen/Germany (Control group), ground bread crust (Bread crust group) prepared from a standardized recipe (*8*), ground roasted caraffa malt (Malt group) (*10*), or pronylated bovine serum albumin (Pronyl-BSA group) (*8*) for 15 days. The composition of the individual diets is detailed in **Table 2**.

According to a previous study (*19*) in which 5% bread crust were fed to healthy and to nephrectomized rats analyzed for their AGE tissue contents, in the present study, the dosage of 5% bread crust was chosen again. The dosage of 5% malt was chosen accordingly and that of pronyl-BSA was adjusted to the pronyl-lysine content of the bread-crust, which was 62.2 mg/kg pronyl-lysine (*8*). The composition of the experimental diets is given in **Table 1**. The resulting content of pronyl-lysine in the bread crust, pronyl-BSA, and malt diet was 0.29% (w/w), 0.29% (w/w), and 0.028% (w/w), respectively.

The resulting daily intake of bread crust, malt, and pronyl-BSA in the individual diet groups was 3.14, 3.01, and 0.70 g per kilogram body weight, respectively.

During the feeding period, urine samples were collected every 24 h, pooled, and stored at −80 °C until analysis. After the feeding period, the animals were fasted for 24 h, narcotized with a CO_2/O_2 mixture, decapitated, and blood, liver, kidneys, and ceaca were collected for sample preparation. The experimental protocols and procedures were approved by the Animal Care and Use Committee at the University of Kiel.

Sample Preparation. Blood was collected into a heparinized tube and immediately centrifuged (3000 g, 10 min). The resulting plasma and erythrocyte samples were stored at −80 °C until further analyses. Liver and kidney samples were removed rapidly, weighed, and washed in ice-cold 0.9% NaCl and frozen at −80 °C until they were subjected to the following procedure. All tissue samples were homogenized with 3 volumes of ice-cold sodium−potassium−phosphate buffer (0.01M) containing 1.15% KCl (pH 7.4). The homogenate was centrifuged at 9000× g for 20 min at 4 °C. The pellet was discarded and the supernatant centrifuged at 105 000× g at 4 °C for 60 min. The supernatant, containing the cytosol, was decanted and stored at −80 °C until the GST activity was analyzed. The remaining pellets were suspended in KCl solution (0.15 M) and again centrifuged at 105 000× g for 60 min at 4 °C. This process was repeated only with kidney samples. Finally, the microsomal pellets were suspended in a sodium−potassium−phosphate buffer (0.05 M, pH 7.4) containing EDTA (0.001 M) and stored at −80 °C until analysis of the CCR activity.

Measurement of the CIE Lab Color Space in Urine. Urine color was measured in CIE Lab space (*20*) by reflection spectrometry using a Spectro Color Pen (Dr. Lange, Germany). Data are reported as L*, uniform lightness, and the chromaticness coordinates a* (+red to −green) and b* (+yellow to −blue). To exclude the confounding factor of urine dilution, urine samples were standardized for their creatinine content prior to analysis. Urine creatinine content was determined by using Jaffé's method (*21*). Data are expressed relative to controls with chromaticness coordinates set at 100%.

Quantitation of α-Tocopherol Equivalents, Cholesterol, and Thiobarbituric Acid Reactive Substances (TBARS) in Plasma. Concentrations of tocopherol equivalents in plasma were determined by HPLC with UV−vis detection according to a method reported in the literature (*22*) with some modifications of the HPLC separation. Plasma samples (100 μL) were mixed with a mixture (40/10, v/v) of *n*-hexane and ethanol and centrifuged (3000× g, 5 min). The *n*-hexane phase was then removed in vacuo, and the residue obtained was dissolved in a mixture (85/15, v/v; 700 μL) of methanol and dichloromethane. α- and γ-Tocopherol were separated isocratically using methanol/dichloromethane (85/15, v/v) as mobile phase (flow rate 0.8 mL/min) and a SpheriGROM ODS 1.5-μm analytical column (250 mm length, 4.6 mm internal diameter, 5 μm particle size, GROM, Germany). Detection of α- and γ-tocopherols was performed by UV−vis (λ = 295 nm). Both tocopherol isomers were quantified by external standard calibration. Tocopherol equivalents were calculated according to the method of Jacob and Elmadfa (*22*), taking into account that the bioactivity of γ-tocopherol in vivo is about 25% of that of α-tocopherol (mg α-tocopherol + mg γ-tocopherol × 0.25 = mg tocopherol equivalents). As a reference parameter for tocopherol, the contents of cholesterol were measured using a colorimetric assay (Monotest cholesterol kit no. 237574; Roche Diagnostics, Basel, Switzerland).

The content of TBARS was measured as the malondialdehyde−thiobarbituric acid adduct, which was separated by HPLC and detected by fluorescence at excitation and emission wavelengths of 532 and 553 nm, respectively (*23*).

Antioxidant Activity of Plasma. The antioxidant activity (AOX) of plasma samples was determined in vitro by measuring their inhibitory effect on linoleic acid peroxidation closely following the procedure of Bright et al. (*24*) with some modifications as reported recently (*8*). An aliquot (120 μL) of the plasma sample was diluted with ethanol (30 μL) and then used for antioxidant analysis. The results were related to the absorption of a standard solution of trolox (1 mmol/L) in water/ethanol (50/50; v/v) and were expressed as trolox equivalents (TE values). Each of the experiments was performed in triplicate.

Analysis of Chemopreventive Enzymes in Liver and Kidneys. The enzymatic CCR activity of liver and kidney microsomes was analyzed according to the method of Masters et al. (*25*). The activity

of liver and kidney cytosolic GST with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate was determined as described by Habig et al. (*26*). Protein was analyzed by the method of Lowry et al. (*27*). Cytosolic UDP-GT activity was determined using the substrate 4-methylumbelliferon (*28*).

AGE Analysis by Western Blotting in Kidneys. Dot blot analysis of pentosidine (PENT), N-ϵ-carboxymethyllysine (CML) and total AGEs were performed as described recently (*29*). Briefly, 10 μg of kidney protein of each sample (duplicates) were transferred under vacuum to a nitrocellulose membrane (Schleicher & Schuell GmbH, Dassel, Germany). AGEs were detected with monoclonal primary AGE specific antibodies (anti-CML 011, anti-pentosidine 012, anti-AGE 013, Biologo, Kronshagen, Germany) at a concentration of 0.25 μg/μl. Conjugates were visualized with anti-mouse alkaline−phosphatase antibody (1:20 000 Dianova, Hamburg, Germany) and ECF detection (Amersham Pharmacia Biotech, Buckinghamshire, England) using a Fluor-Imager 595. For data analyzes, the Image-Quant software (Molecular Dynamics, Amersham) was used.

Statistical Analysis. On the basis of the K−S test, a normal distribution of the data was excluded for all parameters. Therefore, the nonparametric Mann Whitney-U test was performed after elimination of outliers by the Nalimov test. For all parameters, the level of significance was set at $p < 0.05$ (*) and $p < 0.01$ (**). Data are presented as mean values ± SEM.

RESULTS

Pronyl-L-lysine in Thermally Browned Foods. To gain some insights into the amounts of pronyl-L-lysine present in various thermally treated food products, the antioxidant was quantified in the crusts of a selection of breads, malts, beers, as well as roasted coffee using the derivatization procedure reported earlier (*9*). The data, summarized in **Table 1**, show that the highest amount of 40.81 mg/kg was found in the crust of full grain rye bread, followed by a rye/wheat mixed bread, both of which are sourdough fermented breads. Also the cara munich malt as well as the caramalt malt contained rather high amounts of 20.78 and 16.63 mg/kg pronyl-L-lysine. Somewhat lower amounts were quantified in the extremely dark caraffa malt (6.14 mg/kg) and a pale pilsen malt (1.13 mg/kg). These data are well in line with previous findings showing that the pronyl-L-lysine formation is strongly depending on the intensity of thermal treatment (*9*). Compared to bread crust and the various malts, pale and dark beers contained just between 0.43 and 1.07 mg/kg pronyl-L-lysine, and roasted coffee was lacking in pronyl-L-lysine.

Aimed at the in vivo verification of the antioxidant and chemopreventive activity observed for bread crust, pronylated proteins, and malt in cell culture studies (*3*, *8*, *10*), these compounds were administered to rats and key markers of chemopreventive and antioxidative effects were analyzed in biological compartments.

Feeding Study. Body weight gain and food intake during the experimental period were not different among the groups (**Table 3**), and any effects by these parameters on the metabolic changes can be ruled out. Water consumption (individual group data not shown) averaging 31.4 ± 3.60 mL per day was also not different among the groups, which was reflected in an equal urine excretion (**Table 3**). But the urine color of animals fed the malt containing diet was significantly different from that of controls (**Figure 2**), which was analyzed as a shift to the red (chromaticness coordinate a*) and yellow (chromaticness coordinate b*). The increase in uniform lightness (chromaticness coordinates L) and the red shift of the urine color of animals on the bread crust diet did not reach the level of significance.

The amount of feces excreted per day again was not different, but the caeca volumes were increased by 9% ($p = 0.073$), 21% ($p < 0.05$), and 37% ($p < 0.01$) after administration of the experimental diet containing bread crust, malt, and pronyl-BSA, respectively (**Table 3**).

Kidney, liver, spleen, and heart weights related to body weight were also not different among the experimental groups (data not shown).

The enzyme activities Phase I CCR in liver and kidney microsomes was not effected by administration of either diet (**Table 4**), although feeding of the bread crust diet resulted in a 30% but statistically nonsignificant increase. In contrast, Phase II GST activity in the kidneys was increased by 18% in animals of the bread crust group in comparison with animals fed the control diet (**Table 4**). Phase II UDP-GT activity in the liver of animals administered pronyl-BSA was also increased by 27% compared to the data obtained for controls (**Table 4**).

The cholesterol-adjusted plasma content of tocopherol equivalents was elevated by 33, 14, and 14% in the bread crust, malt,

Table 3. Food Intake, Body Weight Gain, and Volumes of Urine, Feces, and Caecum of Rats after Oral Administration of Bread Crust, Malt, and Pronyl-BSA for 15 days ($n = 8$ per Group)

	food intake (g)	body weight gain (g)	urine volume (g/d)	feces volume (g/d)	caecum volume (g/bw)[a]
control	24.3 ± 1.60	29.3 ± 4.91	15.7 ± 0.94	7.49 ± 0.37	0.43 ± 0.02
bread crust	22.8 ± 0.94	20.2 ± 4.12	21.2 ± 2.14	7.90 ± 0.09	0.47 ± 0.03
malt	23.3 ± 1.11	19.8 ± 3.65	14.8 ± 1.84	8.00 ± 0.32	0.52 ± 0.03[b]
pronyl-BSA	23.2 ± 0.58	24.6 ± 4.51	16.7 ± 1.19	7.72 ± 0.16	0.59 ± 0.02[c]

[a] Related to body weight. [b] $p < 0.05$ (Mann Whitney U Test) vs control. [c] $p < 0.01$ (Mann Whitney U Test) vs control.

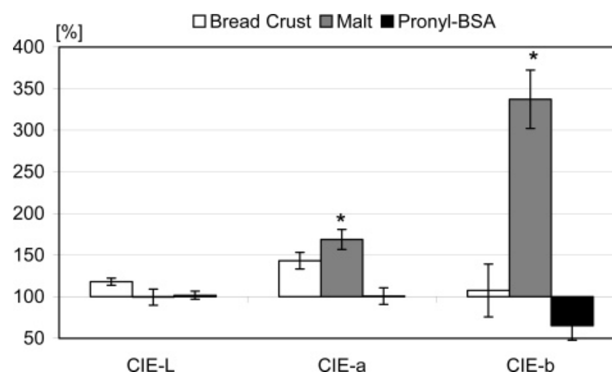

Figure 2. Relative CIE data on urine color formation in rats after oral administration of bread crust, malt, and pronyl-BSA for 15 days. Chromaticness coordinates +a* or +b*, and L represents red and yellow color as well as uniform lightness; absorption units per mg creatinine analyzed for urine samples of controls were L = 68.2 (7.3; a = –2.79 (0.66; b = 4.54 ± 1.53; *$p < 0.05$ (Mann Whitney U Test) vs control ($n = 7–8$ per group).

Table 4. Enzyme Activities of Phase I NADPH CCR as Well as Phase II GST and UDP-GT in the Liver and Kidneys of Rats after Oral Administration of Bread Crust, Malt, and Pronyl-BSA for 15 days ($n = 7–8$ per Group)

	liver			kidneys	
	CCR	GST	UDP-GT	CCR	GST
control	56.6 ± 6.4	300 ± 24.2	26.3 ± 3.2	13.8 ± 1.0	67.7 ± 5.9
bread crust	73.8 ± 7.2	307 ± 38.3	27.6 ± 3.3	13.7 ± 1.1	80.0 ± 4.0[a]
malt	59.2 ± 5.8	308 ± 40.6	26.0 ± 3.0	14.9 ± 2.6	69.0 ± 4.5
pronyl-BSA	54.8 ± 4.4	341 ± 38.0	33.4 ± 2.2[a]	12.9 ± 1.0	72.1 ± 3.6

[a] $p = 0.05$ (Mann Whitney U Test) vs control.

Table 5. Contents of α-Tocopherol Equivalents (TOC), Cholesterol (CHOL), Malondialdehyde (MDA), and Total Antioxidant Capacity in Plasma of Rats after Oral Administration of Bread Crust, Malt, and Pronyl-BSA for 15 days ($n = 6$–7 per Group)

		plasma		
	TOC (μg/mg cholesterol)	CHOL (mg/dL)	AOX TROLOX equiv (μM)	TBARS (μM)
control	10.6 ± 0.36	43.8 ± 2.16	0.30 ± 0.30	1.98 ± 0.21
bread crust	14.1 ± 0.89[a]	50.3 ± 1.70[a]	0.41 ± 0.36[a]	1.24 ± 0.08[b]
malt	12.1 ± 0.60[a]	51.2 ± 2.94[a]	0.36 ± 0.31	1.35 ± 0.15[a]
pronyl-BSA	12.1 ± 0.50[a]	55.2 ± 2.78[a]	0.44 ± 0.05[a]	1.29 ± 0.09[a]

[a] $p < 0.05$ (Mann Whitney U Test) vs control. [b] $p < 0.01$ (Mann Whitney U Test) vs control.

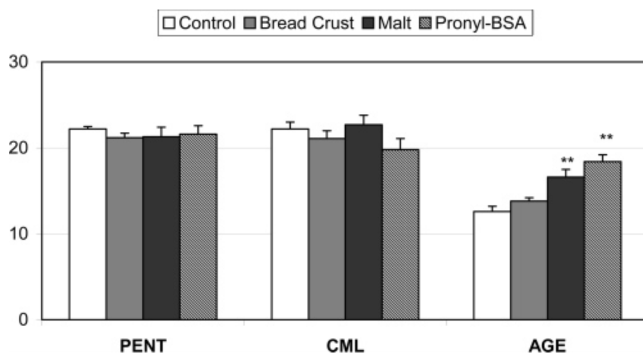

Figure 3. Contents of PENT, CML, and total AGEs in kidneys of rats after oral administration of bread crust, malt, and pronyl-BSA for 15 days; **$p < 0.01$ (Mann Whitney U Test) vs control ($n = 7$–8 per group).

and pronyl-BSA group compared to that of the control group (**Table 5**). Though the cholesterol concentrations also increased after the administration of bread crust, malt, and pronyl-BSA (**Table 5**), the increase in tocopherol equivalents was even more pronounced.

This improved antioxidant status in the plasma was also reflected by an increased antioxidant capacity and by a decrease in TBARS (**Table 5**). Administration of bread crust and pronyl-BSA increased the antioxidant capacity by 37 and 47% and decreased the contents of TBARS by 37 and 35%, respectively. Animal which were fed the malt containing diet only showed 32% lower TBARS values compared to animals of the control group.

The glycation parameter *AGE* indicated a 32 and a 46% higher load of advanced glycation end products in the kidneys after administration of the malt and pronyl-BSA containing diet, respectively (**Figure 3**). But neither the contents of PENT not those of CML increased in response to the administration of the experimental diets (**Figure 3**).

DISCUSSION

Quantitative analysis of pronyl-L-lysine in thermally browned foods revealed that the highest concentrations of that antioxidant were found in sourdough fermented bread crust as well as in medium to dark colored malts. Aimed at the in vivo verification of the antioxidant and chemopreventive activity observed for bread crust, pronylated proteins and malt in cell culture studies (*3*, *8*, *10*), those compounds for which the pronyl-lysine content is given in **Table 1** were administered to rats and key markers of chemopreventive and antioxidative effects were analyzed in biological compartments.

The dietary intake and the body weight gain throughout the feeding period were not different among the experimental groups but a higher content of non- or less-digestible compounds present in all of the experimental resulted in slightly increased feces excretion ($p > 0.05$) and in higher caecum weights ($p < 0.05$) compared to animals of the control group. Increased caecum weights, in particular, point to an altered microbial activity in the caecum which was also observed by visually elevated internal gas production leading to substantial caecum inflation.

Urine excretion did not differ among the experimental groups, but urine color in animals fed the light-brown malt diet differed by color from that of control animals. These changes in urine color clearly indicate that either MRP compounds present in the roasted malt or their metabolites, produced by intestinal microbial activity, must have been absorbed. Feeding of bread crust also changed the urine color slightly although this difference to animals of the control group did not reach the level of statistical significance.

Absorption of MRPs is the basis for any in vivo effects of which the chemopreventive and antioxidative activity were investigated in the present study. Means of the chemopreventive Phase I CCR enzyme activities in the liver and the kidneys of animals fed one of the experimental diets did not differ from those analyzed for animals fed the control diet. However, activities of the Phase II enzymes GST in the kidneys and the UDP-GT in the liver were increased after feeding the bread crust and the pronyl-BSA diet by 18 and 27%, respectively. This GST-modulating effect of bread crust is in accordance with earlier results obtained from in vitro studies on human intestinal cells (*3*, *8*) and from an animal feeding study in which bread crust was administered to rats at a five times higher dose of 25% diet for 6 weeks (*3*). The present results therefore indicate that even a low dose of 5% bread crust increases the GST activity and may contribute to the chemopreventive potential of the body. By consideration of a daily intake of about 1 kg of solid food, the addition of 5% bread crust might correspond to a total amount of about 50 g. However, the intake of excessive doses of bread crust on a daily basis cannot be recommended on the basis of the present results, as the CCR activity also tended to increase ($p > 0.05$) after bread crust consumption. Dose−response feeding trials are required in which the effects of bread crust supplementation on chemopreventive enzymes are investigated.

Pronyl-BSA has not been administered to rats before, but it was identified to be the key component found in bread crust responsible for the induction of chemopreventive enzymes.

Phase II enzymes in human intestinal cells (*8*). In the present study, administration of a pronylated protein representative for the concentration of 62.2 mg/kg pronylated-lysine in bread crust resulted in an increased Phase II UDP-GT activity by 27% ($p < 0.05$), whereas the 14% increase of the GST activity did not reach the level of significance.

Feeding of malt did not result in a modulated Phase I or II enzyme activity, although water-soluble malt extracts separated into different molecular weight fractions by gel permeation chromatography did show enzyme-modulating effects in human intestinal cells after different exposure times (*10*). Passing the intestinal tract might result in microbial degradation products of the roasted malt which do not modulate these enzyme activities in the liver and the kidneys after absorption. The increased caecum volumes observed in animals fed the malt diet might support this hypothesis. Another explanation for the less pronounced effect of the malt containing diet on the selected Phase II chemopreventive enzymes might be that the concentra-

tion of pronyl-lysine in this diet was about one tenth of that in the bread crust diet.

This hypothesis is also supported by the results reported on the antioxidative defense parameters analyzed in the plasma. The total antioxidant activity was not effected by the administration of the malt containing diet but increased in animals of the bread crust group. On the other hand, the contents of tocopherol equivalents and TBARS were different in animals of the malt diet from those of controls. This result unequivocally points to an absorption of antioxidatively active malt components, such as pronyl-lysine, or their metabolites.

Elevated plasma tocopherol contents were also observed for animals that were fed the bread crust and the pronyl-BSA diet. Although any direct effect on tocopherol metabolism by one of the experimental diets does seem highly speculative, the increase in total cholesterol in all three experimental groups gives rise to the explanation of an enhanced transport capacity of tocopherols by cholesterol (*30*). A rise in plasma cholesterol has already been observed in subtotally nephrectomized rats after administration of 5% bread crust for six weeks (*31*). But the mechanism by which feeding bread crust result in enhanced plasma cholesterol levels needs to be investigated in future studies.

In the present study, feeding of the bread crust and the pronyl-BSA containing diet resulted in an enhanced total antioxidant capacity and in lowered TBARS values in the plasma, also indicating the absorption of antioxidant components. These results are in agreement with those of an in vitro study, in which pronyl-lysine was identified to be the key antioxidant present in bread crust (*8*). It is noteworthy here that the dose of pronyl-BSA administered in the present study reflected that present in the bread crust, meaning that the antioxidant effect of bread crust in vivo is largely determined by its content in pronylated proteins.

Besides the desired increase in the chemopreventive and antioxidative potential by MRP-containing foods, an increase in endogenously circulating AGEs is hypothesized due to structural analogy of both chemical classes. Indeed, this hypothesis has been proven by elevated contents of AGEs in the kidneys of animals fed the malt and the pronyl-BSA diet, whereas the slight increase after feeding the bread crust diet did not reach the level of significance. Although these results are conceivably harmful due to putative activation of their cellular receptor RAGE (*17*) and a subsequent release of reactive oxygen species (ROS) (*14*, *15*, *16*), no systemic effect of an elevated load of ROS has been found. In contrast, all of the parameters of antioxidant capacity analyzed in the plasma supported an improved antioxidant status. The healthy organism apparently is able to handle dietary MRPs in such a way that a moderate accumulation of AGEs in the kidneys does not result in pro-oxidative reactions indicated by respective parameters in the plasma. In the present study, the main systemic effects of dietary malt, bread crust, and pronyl-BSA were, for the first time, demonstrated to be the enhanced antioxidant capacity and the increase in Phase II chemopreventive enzymes. As both mechanisms play a key role in maintaining health, further studies are needed to understand the molecular mechanisms of action.

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