DOI 10.1002/mnfr.200600141 Mol. Nutr. Food Res. 2007, *51*, 496–504

Research Article

The potential antimutagenic and antioxidant effects of Maillard reaction products used as "natural antibrowning" agents

Karl-Heinz Wagner[1], Stefanie Reichhold[1], Karin Koschutnig[1], Sophie Chériot[2] and Catherine Billaud[2]

[1] Department of Nutritional Sciences, University of Vienna, Vienna, Austria
[2] Conservatoire National des Arts et Métiers, Chaire de Biochimie Industrielle et Agro-alimentaire, Paris, France

The aim of this study was to investigate the potential antioxidative and antimutagenic effects of Maillard reaction products (MRPs) formed from glucose or fructose and cysteine or glutathione in the Ames *Salmonella* test and the 2,2′-azobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. The mixtures were heated for 4 h 20 min or 14 h at 103 or 110°C and tested in five concentrations from 0.05 to 11 mg/plate in strains TA 98 and TA 102 in the plate incorporation assays. In order to promote the formation of mutant revertants the prooxidants hydrogenperoxide (H_2O_2) and tertiary-butyl hydroperoxide (*t*BOOH) were used in the TA 102. Tests were conducted with preincubation with (+S9) and without (−S9) metabolic activation. 5-Hydroxymethylfurfural (5-HMF) was investigated as carbonyl compound. In TA 98, no effect of the MRPs was shown. The shorter heated samples (4 h 20 min) were in general more active than the longer heated ones (14 h). Up to 1 mg/plate (1%) all the reaction mixtures remained safe, but the 5% and in particular the 11% fractions increased the number of revertants significantly for the shorter heated mixtures. The 14 h mixtures did not show any response for almost all concentrations. No significant difference in the number of revertants could be observed between the cysteine and glutathione mixtures, the fructose mixtures increased revertants number to a higher extent than the glucose mixtures only in the 4 h 20 min heated mixtures for the highest concentration (11%). The highest activity was always observed in the +S9 tests. Antioxidative effects expressed as Trolox equivalents were higher in the 4 h 20 min heated samples. When detectable, HMF concentration was found to be higher in the 14 h MRP samples. In order to use the tested mixtures as antibrowning agents for technological purpose, the concentration should not be higher than 1% and the longer heated reaction mixtures were preferred since the brown pigments seemed to be less reactive than the intermediate products.

Keywords: Antioxidant activity / Cysteine / Fructose / Glucose / Maillard reaction

Received: August 9, 2006; revised: December 10 2006; accepted: December 13, 2006

1 Introduction

The Maillard reaction, which is a series of reactions between carbohydrates and proteins, occurs in food storage at low temperature as well as during cooking conditions. The rate of Maillard reaction products (MRPs) formation accelerates with increasing temperature. MPRs account for the color and aroma of foods, but research of the last decade broadens their possible effects on health.

Highly depending on the reaction conditions, MRPs have been shown to have positive effects on gut health due to stimulating effects on health beneficial bacteria [1, 2] or binding gut harmful substances [3], exhibit antioxidative effects [4–7], they are discussed to have effects on chemoprevention [8–10] and MRPs were shown to influence the glycation reaction [11, 12]. The current available information on their mutagenic potential is heterogeneous [13]. Some papers describe MRPs as mutagenic or genotoxic [14–16],

Correspondence: Professor Karl-Heinz Wagner, Department of Nutritional Science, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria
E-mail: karl-heinz.wagner@univie.ac.at
Fax: +43-1-4277-9549

Abbreviations: ABTS, 2,2′-azobis-(3-ethylbenzothiazoline-6-sulfonic acid); **5-HMF,** 5-hydroxymethylfurfural; **MRPs,** Maillard reaction products; **PPOs,** polyphenoloxidases; ***t*BOOH,** tertiary-butyl hydroperoxide

Table 1. Preparation and HMF content of the reaction mixtures used in the study

Sugar/source of amino acid	Concentration (M/L)	Temperature (°C)	Incubation time	HMF concentration (mM)
Glucose/cysteine	0.8/0.5 M	103	14 h	nd[a)]
Glucose/cysteine	0.8/0.5 M	103	4 h 20 min	nd
Glucose/glutathione	1.0/0.15 M	110	14 h	21.3 ± 1.9
Glucose/glutathione	1.0/0.15 M	110	4 h 20 min	0.39 ± 0.04
Fructose/cysteine	0.8/0.5 M	103	14 h	nd
Fructose/cysteine	0.8/0.5 M	103	4 h 20 min	nd
Fructose/glutathione	1.0/0.15 M	110	14 h	28.4 ± 2.0
Fructose/glutathione	1.0/0.15 M	110	4 h 20 min	0.34 ± 0.04

a) nd: not detectable.

some with slight mutagenic potential [17], recent publications indicate only weak or no mutagenic or genotoxic potential [18 – 21].

One recent aspect in broadening the knowledge on MRPs was to explore the use as potential "antibrowning" agents [22 – 25]. In many edible plant products, postharvest conditions and processing induce phenolics oxidation *via* catalytic action of oxidoreductases, mainly the polyphenoloxidases (PPO). Primary oxidation products then rapidly polymerize to dark pigments, which are detrimental to food quality. MRPs formed from different sugars (pentoses, hexoses, or disaccharides) with cysteine or glutathione were shown to reduce the natural browning effects by inhibiting the PPO reaction. Under *in vitro* conditions it was shown that they were able to inactivate the PPOs, partly by chelating the copper ions of the active site of the protein.

Since this MRPs field of application is new and of special technological interest, the safety aspect of the so far investigated MRPs is of high importance.

Therefore, this study was aimed at evaluating whether the sugar – amino acids or sugar – protein reaction mixtures, which have been successfully used as antibrowning agents so far [22 – 25] are mutagenic active in the tester strains TA 98 and TA 102. Furthermore, we wanted to investigate whether the MRPs might induce a protection in the TA 102 when incubated with two oxidants, hydrogenperoxide (H_2O_2) or tertiary-butyl hydroperoxide (*t*-BuOOH).

2 Materials and methods

2.1 Chemicals

All chemicals used were obtained from Sigma (Vienna, Austria). The *Salmonella thyphimurium* strains TA 98 and TA 102 were obtained from Discovery Partners International (San Diego, USA). The S9 liver homogenate (from Sprague – Dawley rats induced with Aroclor 1254 prepared as a KCL homogenate) was obtained from MP Biomedicals (Illkirch, France). All mutagens and other reagents were of analytical reagent grade or better and stored at −80°C if necessary. Agar no. 1 and Nutrient Broth were obtained from Oxoid/Bertoni (Vienna, Austria), Dulbecco's PBS was from PAA (Pasching, Austria).

2.2 Preparation of the reaction mixtures

Some MRP samples (glucose – cysteine (0.8/0.5 M), heated for 14 h at 103°C and glucose – glutathione (1/0.15 M) heated for 4 h 20 min at 110°C) were provided from the Conservatoire national des arts et métiers (Paris, France) and were sent to Vienna on dry ice. Other MRPs consisted of glucose resp. fructose and cysteine resp. glutathione, which were heated for 4 h 20 min or 14 h at temperatures of 103 or 110°C (Table 1). Briefly, the solutions were prepared in 10 mL of distilled water, adjusted to a pH of 3, heated as shown in Table 1, frozen at −80°C and finally freeze-dried for 33 h.

2.3 5-Hydroxymethylfurfural (5-HMF) determination

5-HMF amount present in MRPs was determined by HPLC with a Waters (Milford, MA, USA) liquid chromatograph equipped with a Model 600 pump system controller, a Model 996 photodiode array detector with Millenium 32 chromatography manager software, a 20 µL sample loop and a YMC-ODS AQ (250×4.6 mm^2 id) column from A.I.T (France), protected with a guard column of the same material. Samples were used undiluted, filtered through 0.20 µm nylon filters and injected into the HPLC. HMF was separated using water/ACN 95:5 v/v as mobile phase, at a flow of 0.8 mL/min, monitored at 285 nm and quantification was performed by calibration with external standard.

2.4 Ames test

In this study the potential of MRPs were investigated in the strains TA 98 and TA 102. TA 98 detects frame-shift mutations, whereas TA 102 detects crosslinking agents and can be reverted by mutagens that cause oxidative damage.

The preincubation assay allows a closer contact of the test compounds and the indicator strain and was applied with a preincubation period of 25 min at 37°C. In order to

investigate the antimutagenic effects, the plate incorporation assay was used. H_2O_2 (60 mmol) and *t*BOOH (1 mmol) were used to induce the formation of mutant revertants. Both test models were performed with and without metabolic activation.

2.5 S9 Mix

The S9 mix was prepared according to the recipes of Maron and Ames [26] consisting of 19.75 mL $_dH_2O$, 25 mL of PBS buffer, 0.5 mL of $MgCl_2$ (0.85 M), 0.5 mL of KCl (1.65 M) and 2 mL of NaDP (90.8 mM), 250 μL of glucose-6-phosphate (1.08 M), and 2 mL of S9.

2.6 Experimental design

The highest soluble concentration of 11% was used as the highest concentration. From each fraction, five final concentrations of 0.05; 0.1; 1.0; 5.5; and 11 mg *per* plate (≈%) were prepared with sterile, distilled water. The range of concentrations tested comprised nonphysiologically high concentrations but also very low concentrations (less than 1%), which are below the daily intake.

The tests were performed according to the protocol of Maron and Ames [26] with preincubation. The reaction mixture (100 μL), followed by 100 μL of mutagen solution, 500 μL of PBS or S9 mix (4%v/v), and then 100 μL of overnight bacterial culture were added to the test tubes. The test tubes were incubated for 30 min at 37°C on a rotary shaker. Subsequently, 2 mL of molten top agar was added to every tube which, was then vortexed and poured onto minimum glucose plates. After the agar had set they were incubated for 48 h at 37°C and the his^+-revertants were counted manually.

Three plates were counted for every concentration and each condition and each test was repeated twice ($n = 6$ in total). Each test included a positive control to confirm the reversion properties and specificity of each tester strain [27]. 2,4,7-Trinitro-9-fluorenone was used for both strains without metabolic activation and 2-aminofluorene with metabolic activation. The evaluation of "mutagenic", "promutagenic" and "antimutagenic" compounds was conducted in accordance with guidelines published by Mortelmans and Zeiger [27].

2.7 Free-radical scavenging activity determination

Trolox (Hoffman-La Roche, Vienna, Austria; 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) was used as the antioxidant standard. Trolox (2.5 mM) was prepared in PBS, pH 7.4, for use as a stock standard. Fresh working standards (0.5, 1, 1.5, and 2 mM) were prepared daily on dilution with PBS. The reaction mixture consisted of 400 μL of PBS, 10 μL of standard (20 μL sample), 20 μL of metmyoglobin (135 μM), and 400 μL of 150 μM 2,2′-azo-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), with vortexing. The reaction was started by the addition of 170 μL of 75 μM H_2O_2, the clock was set, and the contents of the tube were mixed.

MRPs were diluted in PBS buffer prior to the test. The percentage inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of Trolox for the standard reference data.

2.8 Statistical analysis and evaluation of the mutagenic experiments

All data are expressed as mean ± SD. Obtained data ($n = 6$ for each concentration used) were analyzed by one-way ANOVA since they were all normally distributed, using SPSS 11.5 for Windows. Different antimutagenic *vs.* antioxidative responses observed from the two different sugars, sources of amino acids and heating times were analyzed with the Student's *t*-test. Statistical differences were considered significant at a value of $p < 0.05$.

In addition to the statistical, a nonstatistical evaluation was also carried out. According to Mortelmans and Zeiger [27], a compound is "antimutagenic" when the relative mutagenic response fell below 50%. A compound is "promutagenic" when the relative mutagenic response increased beyond 200%. Furthermore, dose dependence must also be existent.

3 Results

The samples were tested for five different concentrations: 0.05; 0.1; 1.0; 5.5; and 11 mg *per* plate (≈%), in order to consider low, physiological conditions, and also high concentrations up to the limit of solubility.

3.1 Mutagenicity testing

For the preincubation assay, *S. typhimurium* indicator strains TA 98 and TA 102 were employed.

In the TA 98 strain, either –S9 or +S9 the number of his^+-revertants were in the range of the simple negative control and therefore devoid of mutagenic activity.

The same was observed in the TA 102 strain without metabolic activation. However, the concentrations 11 and 5.5% of the fructose/glutathione fraction (heated for 4 h 20 min at 110°C) increased the number of revertants significantly ($p < 0.05$), but were below 200%,which was defined as the threshold for mutagenicity. Furthermore, a significant dose-related tendency has been observed with these concentrations (Fig. 1).

Interestingly, the strongest response was observed in the TA 102 +S9. Three out of eight fractions showed a significant increase in the number of his^+-revertants beyond the doubled negative control (200%) for the 5 and 11% concen-

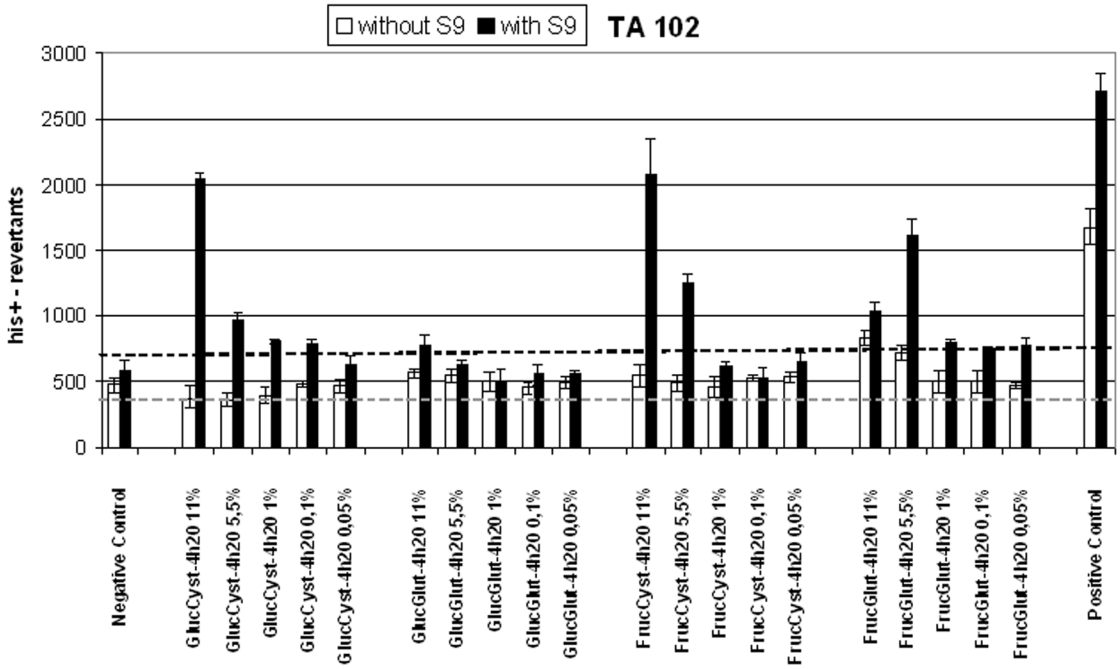

Figure 1. Number of his$^+$-revertants of the 4 h 20 min heated fractions in the preincubation assay with the strain TA 102 with (+S9) and without (−S9) metabolic activation (significances are shown in Table 2).

trations. In addition, a significant dose–response was observed. The fractions were glucose–cysteine, fructose–cysteine, and fructose–glutathione, all of them heated for 4 h 20 min. This allows the conclusion that after a short time heating some reactive intermediate substances might have been formed which were then destroyed or inactivated after longer heating.

In order to follow the formation of intermediate products, the concentration of HMF which is an intermediate decomposition product of carbohydrates was determined (Table 1). No HMF was detectable in the glucose or fructose/cysteine MRPs heated at 103°C for 4 h 20 min or 14 h. For the glutathione mixtures the HMF concentrations were: 0.39 ± 0.04 mM (fructose), 0.34 0.01 mM (glucose) for the 4 h 20 min heating time and 28.4 ± 2.0 mM (fructose), 21.3 ± 1.9 mM (glucose) for the 14 h heating time. The HMF concentrations were significantly higher ($p < 0.05$) for the longer heated samples.

3.2 Antioxidant testing in the Ames test

Possible antioxidant effects of MRPs in the absence or presence of metabolic activation was tested after stressing with H_2O_2 or *t*BOOH. In order to prove the oxidative DNA damage the *S. typhimurium* strain TA 102 was used which is highly sensitive toward ROS. Table 2 gives an overview on the obtained data in the plate incorporation assay.

3.3 Prevention of H_2O_2 induced mutagenicity by MRPs

Hydrogenperoxide possess high membrane permeability, and it is not only a reducing agent but also an oxidizing reagent and acts mainly *via* the Fenton reaction thereby generating hydroxyl radicals. Under the influence of H_2O_2 −S9 the longer heated glucose–cysteine fraction (14 h) showed a significant antimutagenic effect for the highest concentration (11%). Glucose–glutathione (4 h 20 min) and fructose–glutathione (4 h 20 min) indicated a nonsignificant protection which was shown previously for MRPs [28, 29].

However, the addition of +S9 influenced the MRPs and the system and the antioxidative behavior shifted toward prooxidative effects. In particular, the shorter heated reaction mixtures induced the revertant growth at the high concentrations. Up to 1% no strong effect was observed, but the shorter heated 5.5 and 11% mixtures increased the revertants growth significantly ($p < 0.05$). More or less each fraction exceeded the 200% threshold; in particular, the cysteine-based mixtures increased the number of revertants up to 400%, which is strong evidence for mutagenic effects at these higher concentrations since they were higher than the positive control too. Figure 2 shows an effect for the 14 h heated glucose–cysteine mixture. Furthermore, a significant concentration dependent increase was observed from 1 mg/plate (Fig. 3), which is an additional sign of

Table 2. Overview of all obtained data (numbers of his$^+$-revertants: mean ± SD) in the plate incorporation assay with the TA 102 strain

	Concentration (mg/plate)	TA 102+*t*BOOH (revertants/plate −S9)	TA 102 + *t*BOOH (revertants/plate +S9)	TA 102 + H_2O_2 (revertants/plate −S9)	TA 102 + H_2O_2 (revertants/plate +S9)
Negative control	0	549 ± 120	1194 ± 150	1109 ± 138	653 ± 56
GlucCyst, 14 h	11	486 ± 34	1704 ± 609[a)]	463 ± 51[a)]	1457 ± 97[a)]
	5.5	515 ± 91	1872 ± 102[a)]	1094 ± 187	997 ± 86[a)]
	1	643 ± 67	1614 ± 43[a)]	1419 ± 113[a)]	588 ± 97
	0.1	674 ± 116	1563 ± 42[a)]	1323 ± 113[a)]	530 ± 53
	0.05	684 ± 106	1556 ± 166[a)]	1324 ± 155[a)]	618 ± 58
GlucCyst, 4 h 20 min	11	716 ± 140	3756 ± 142[a,b)]	1363 ± 442[a)]	5152 ± 132[a,b)]
	5.5	547 ± 93	2107 ± 174[a)]	1545 ± 182[a)]	3371 ± 266[a,b)]
	1	641 ± 63	1593 ± 118[a)]	1378 ± 84[a)]	1114 ± 39[a)]
	0.1	565 ± 63	1531 ± 40[a)]	1399 ± 115[a)]	883 ± 100[a)]
	0.05	537 ± 50	1513 ± 29[a)]	1381 ± 179[a)]	914 ± 50[a)]
GlucGlut, 14 h	11	473 ± 53	1384 ± 185	983 ± 233	1139 ± 92[a)]
	5.5	574 ± 62	1402 ± 204[a)]	1392 ± 97[a)]	1112 ± 87[a)]
	1	566 ± 69	1249 ± 106	1290 ± 116	962 ± 54[a)]
	0.1	633 ± 96	1257 ± 84	1174 ± 129	865 ± 44[a)]
	0.05	608 ± 91	946 ± 26	1283 ± 82[a)]	903 ± 30[a)]
GlucGlut, 4 h 20 min	11	726 ± 138	3027 ± 466[a,b)]	844 ± 135[a)]	1257 ± 25[a)]
	5.5	670 ± 67	2432 ± 179[a,c)]	999 ± 140	770 ± 25[a)]
	1	647 ± 42	1592 ± 95[a)]	1168 ± 71	579 ± 62
	0.1	710 ± 53	1524 ± 68[a)]	1403 ± 176[a)]	572 ± 52
	0.05	683 ± 109	1363 ± 104[a)]	1270 ± 177	790 ± 14[a)]
FrucCyst, 14 h	11	521 ± 57	1785 ± 81[a)]	1113 ± 116	1011 ± 74[a)]
	5.5	561 ± 58	1452 ± 124[a)]	1348 ± 244	760 ± 90
	1	664 ± 52	1159 ± 141	1355 ± 80	590 ± 8
	0.1	707 ± 71	1432 ± 70[a)]	1390 ± 146	697 ± 14
	0.05	741 ± 94	1538 ± 130[a)]	1352 ± 155	726 ± 40
FrucCyst, 4 h 20 min	11	1023 ± 264[a)]	3201 ± 314[a,c)]	1518 ± 86[a)]	4625 ± 341[a,b)]
	5.5	692 ± 85	2040 ± 78[a)]	1363 ± 243	3361 ± 401[a,b)]
	1	603 ± 47	1074 ± 38	1339 ± 95	1189 ± 152[a)]
	0.1	637 ± 90	1066 ± 98	1316 ± 129	880 ± 57[a)]
	0.05	585 ± 71	1202 ± 65	1202 ± 122	580 ± 60
FrucGlut, 14 h	11	596 ± 82	1092 ± 68	1200 ± 171	1127 ± 191[a)]
	5.5	566 ± 41	1046 ± 52	1336 ± 118	1057 ± 37[a)]
	1	595 ± 85	987 ± 61	1221 ± 184	835 ± 111[a)]
	0.1	624 ± 35	999 ± 73	1236 ± 100	861 ± 37[a)]
	0.05	598 ± 49	955 ± 47	1301 ± 87	775 ± 72
FrucGlut, 4 h 20 min	11	1719 ± 166[a)]	4410 ± 219[b)]	891 ± 181	2577 ± 221[a,c)]
	5.5	856 ± 56[a)]	2849 ± 59[a,c)]	1163 ± 160	1037 ± 95[a)]
	1	676 ± 77	1544 ± 42[a)]	1114 ± 175	765 ± 57
	0.1	749 ± 78	1602 ± 151[a)]	1201 ± 149	782 ± 57
	0.05	659 ± 112	1516 ± 50[a)]	1323 ± 127	704 ± 25
Positive control	0	2163 ± 91	2600 ± 305	1848 ± 151	2268 ± 32

a) $p < 0.05$ to negative control.
b) Higher than positive control: $p < 0.05$.
c) No difference from positive control.

mutagenic effects. Thus the data show that in the absence of metabolic activation the different reaction mixtures were neutral or antioxidative, while in the presence of S9 the behavior changed toward increase in revertants.

3.4 Prevention of *t*BOOH-induced mutagenicity by MRPs

As different mechanisms of oxidative DNA damage exist, *t*BOOH was used as second oxidant. It is known as an initiator of lipid peroxidation and leads to the formation of alkoxyl and alkyl radicals [30]. With this compound no antioxidative effects were observed regardless of the experimental conditions. In the plate incorporation assay, without S9 fructose–cysteine (4 h 20 min) and fructose–glutathione (4 h 20 min) showed a significant increase for the 11% concentration ($p < 0.05$) (Fig. 4), but only the highest concentration of fructose–glutathione (4 h 20 min) exceeded the threshold level of 200%. With S9, all 4 h 20 min heated fractions (11%) exceeded the doubled negative control, for glutathione the 5% fraction reached this level too, and the 5% cysteine mixtures were at the border-

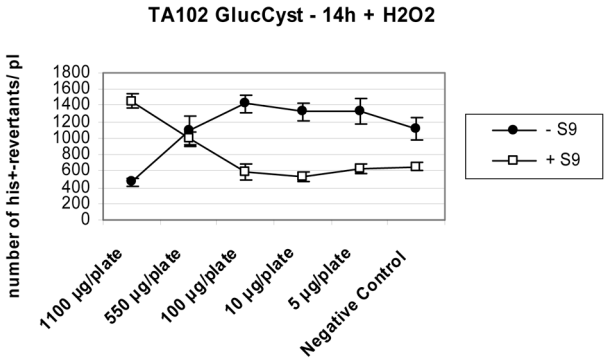

Figure 2. Changes in the number of his$^+$-revertants of glucose – cysteine (14 h) in the plate incorporation assay with (+S9) and without (–S9) metabolic activation with H_2O_2 as the mutagen 1100 µg/plate ≠ 550 ≠ 100 = 10 = 5 ($p < 0.05$); +S9 ≠ –S9 (1100, 100, 10, 5; $p < 0.05$).

line to the 200% level. Moreover, a significant dose–response was observed. The 14 h heated samples induced neither prooxidative nor antioxidative effects.

3.5 Free radical scavenging activity

Figure 5 shows the result of the ABTS assay, which is based on the assessment of the reducing potential of a sample. Similar to the results obtained in the Ames test, the shorter heated samples were more active and showed a significantly higher antioxidant potential ($p < 0.05$) than the darker 14 h heated samples. This was not based on the function of browning, since the intermediate products of the Maillard reaction are brighter and only the long heated end products are brown pigments with higher browning activity (detailed results not shown).

4 Discussion

Mutagenic as well as antimutagenic properties of MRPs have already been investigated in several studies. Both Glösl *et al.* [18] with the *S. typhimurium* strains TA 98 and TA 102 and Taylor *et al.* [21] with the strains TA 98 and TA 100 tested glucose–glycine systems in the Ames test. No mutagenic effects were assessed. Antimutagenic tendencies were observed by Yen *et al.* [31], and found that the glucose samples seemed to be more reactive than the fructose samples. Brands *et al.* [17] showed that heated sugar-casein systems showed different mutagenic properties.

In most of the studies published [17, 32], a significant increase in revertants when tested for –S9 was turned into a reduction when tested for +S9 , which indicated a deactivation by the liver enzymes. In the present investigations we found the opposite effect, +S9 increased the revertant growth significantly at the higher concentrations. The liver homogenate S9 contains phase I oxidative enzymes including cytochrome P450 that catalyzes the formation of alkyl and alkoxyl radicals from organic hydroperoxides *via* a Fenton-like reaction [33]. Theoretically, the presence of greater amounts of cytochrome P450's in the incubation would increase the initial rate and number of free radicals produced [34], which could increase DNA damage and mutations. Furthermore, a different affinity for the reaction

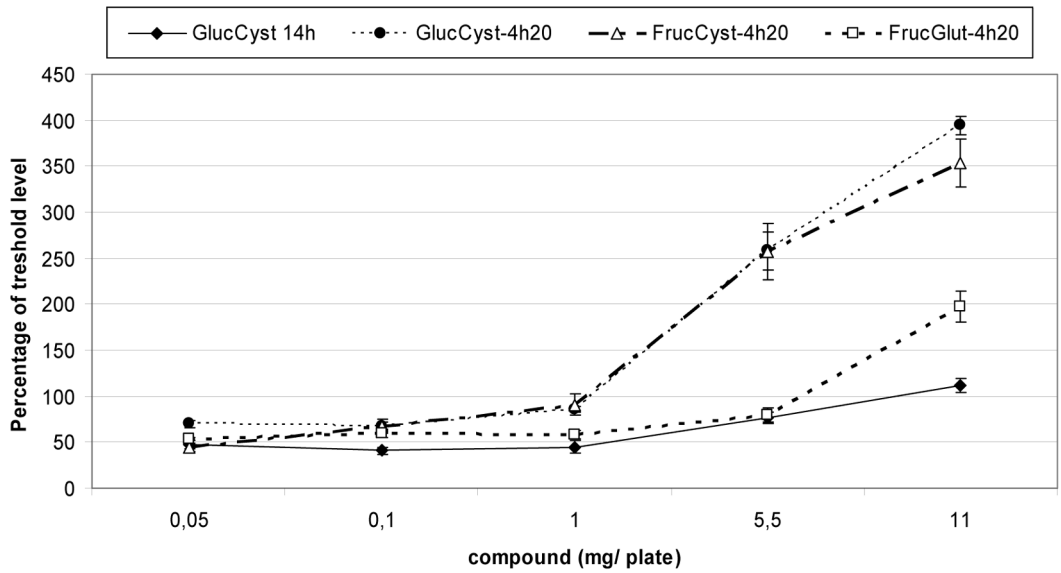

Figure 3. Changes in the number of his$^+$-revertants as percentage of the threshold level (+200 or +100%) in the plate incorporation assay with metabolic activation and with H_2O_2 as mutagen. 11 mg/plate > 5.5 > 1 = 0.1 = 0.05 ($p < 0.05$); 5.5 mg/plate: FrucCyst-4 h 20 min = GlucCyst-4 h 20 min > FrucGlut-4 h 20 min = GlucCyst-14 h ($p < 0.05$) 11 mg/plate: FrucCyst-4 h 20 min = GlucCyst-4 h 20 min > FrucGlut-4 h 20 min > GlucCyst-14 h ($p < 0.05$).

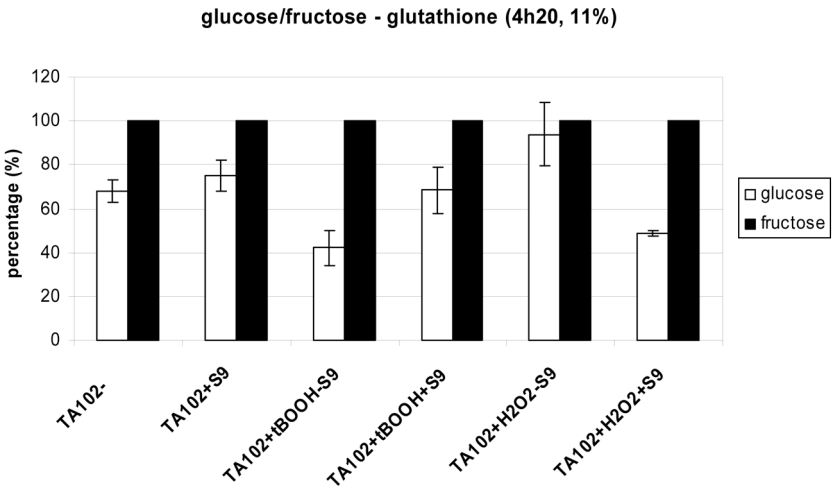

Figure 4. Formation of his$^+$-revertants of glucose–glutathione (4 h 20 min, 11%) compared with fructose–glutathione (4 h 20 min, 11%) in the experiments with strain TA 102. The numbers of his$^+$-revertants of the fructose samples were set to 100%. glucose < fructose (except H_2O_2-S9), $p < 0.05$.

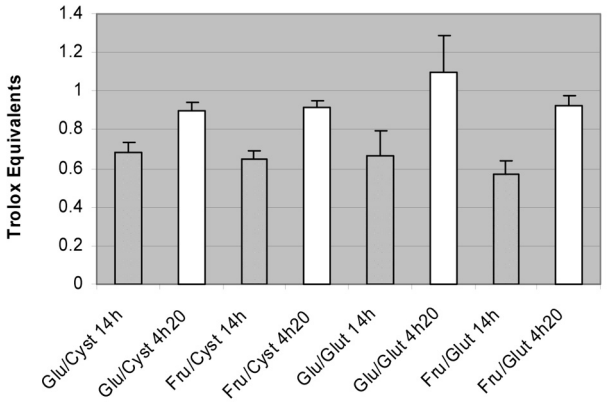

Figure 5. Free radical scavenging activity of glucose/fructose–cysteine/glutathione MRPs heated for 4 h 20 min or 14 h expressed as Trolox equivalents (mM). 14 h < 4 h 20 min ($p < 0.05$).

mixtures, in particular for the higher concentrations, could be the reason for preventing them from entering the bacteria, where reactive oxygen species can exert their mutagenic effects [34–36]. It is well known that one main mode of action of MRPs is their chelating effect toward different substances like iron, copper, or low molecular weight compounds in general [30, 37–39].

4.1 Glucose *versus* fructose and cysteine *versus* glutathione reactivity

In the literature, MRPs with a protein as the source of amino acid appear to be more reactive [40, 41]. In this investigation, glutathione mixtures seemed to be similar to the cysteine based mixtures for the long heating period. For the shorter heating time in combination with both sugars the cysteine-based mixture was more reactive for TA 102/+S9/H_2O_2, for the TA 102/+S9/*t*BOOH the glutathione mixture was more reactive for fructose and similar with glucose.

Regarding the influence of the sugar on the reactivity of the MRPs, inconsistent statements are found in the literature. Some [17, 31, 42] argue that ketoses are more reactive, whereas others [28, 29] consider aldoses as the more active ones. In the present study, no significant difference between the sugars glucose and fructose was observed in general, except in combination with glutathione (4 h 20 min). At this condition, the fructose samples led to higher numbers of his$^+$-revertants in all tests with TA 102 compared to the glucose analogues. However, in the experiments with H_2O_2 without S9 no significant difference between glucose and fructose were found (Fig. 4).

4.2 Impact of the incubation time

In general, it can be said that 4 h 20 min heated fractions appear to be more reactive than those heated for 14 h. It is well known that the products of early stage Maillard reaction are the so-called "Amadori-products" whereas higher temperatures and/or longer time periods lead to the formation of the advanced brown pigments, the melanoidins [43]. HMF as one of these intermediate products was higher in the longer heated, less reactive samples. Mutagenic and prooxidative properties in the Ames test were only observed within the short time treated samples, in the preincubation assay as well as in the plate incorporation assay with and without metabolic activation with the exception of glucose–cysteine (14 h) in the plate incorporation assay with S9 and H_2O_2. These results indicate that other primary or

intermediate products of the Maillard reaction may be involved in the mutagenic activity of these MRPs. As already discussed, the reactive substances of the short term heating period were degraded and less active brown pigments were formed. In several studies, a decrease in the antioxidative potential was observed during the early stage of the Maillard reaction, which increased with the heating time [44, 45]. In the ABTS test the shorter heated reaction mixtures appeared to be more active, similar to the Ames test data.

4.3 Mutagenic effects in respect to the shown inhibition of the polyphenoloxidase

Several studies regarding the potent inhibition of the polyphenoloxidase dependent enzymatic browning reaction in different foods through MRPs have already been investigated [46 – 48]. Among others, glucose *versus* fructose and cysteine *versus* glutathione, heated for 2 – 39 h at different degrees have been tested. In general it can be said that samples heated either at 103°C for 14 h or at 110°C for times ranging from 4 to 7 h are optimal inhibitors, whereas glucose – cysteine and glucose – glutathione fractions are stronger inhibitors than their fructose analogues. Regarding the source of amino acid, the authors assume that glutathione has a better inhibition potential than cysteine. Furthermore, it was shown that the longer the heating time (> 2 h) and the higher the heating temperature (> 90°C) the stronger was the inhibition of the polyphenoloxidase activity [46 – 48].

Referring to our investigation, up to 1% there is no negative effect shown for both heating times, but the results particularly at higher concentrations raise several concerns on the use of the mixtures.

However, considering the further findings of the French investigations [25] glucose – cysteine and glucose – glutathione are found to be very potent, even at low concentrations (0.25 M; 285-fold diluted), under the assay conditions. As all fractions used in this investigation were shown to be safe at this low concentration level, all of them could be employed, but higher concentrations raise concerns on their use and further investigations are needed.

4.4 Conclusion

Glucose – cysteine (14 h) turned out to be most reactive, in particular, in the presence of H_2O_2. Without metabolic activation this sample showed antioxidative properties, whereas +S9 was prooxidative. The other 14 h incubated fractions were less active.

In contrast, all 4 h 20 min heated samples were strongly reactive; however, at additions of less than 1%, the reaction mixtures – which were shown in this concentration range to inhibit the polyphenoloxidase oxidation – seem safe when considering comprehensively the Ames test. High concentrations (> 1%) should not be used.

5 References

[1] Ames, B. N., Lee, F. D., Durston, W. E., An improved bacterial test system for the detection and classification of mutagens and carcinogens, *Proc. Natl. Acad. Sci. USA* 1973, *70*, 782 – 786.

[2] Borrelli, R. C., Mennella, C., Barba, F., Russo, M., *et al*., Characterization of coloured compounds obtained by enzymatic extraction of bakery products, *Food Chem. Toxicol.* 2003, *41*, 1367 – 1374.

[3] Solyakov, A., Skog, K., Jägerstad, M., Binding of mutagenic/carcinogenic heterocyclic amines to MRPs under stimulated gastrointestinal conditions, in: Fogliano, V., Henle, T. (Eds.), *Melanoidins in Food and Health*, EC Directorate for Research, Germany 2002, Vol. 3, pp. 195 – 197.

[4] Delgado-Andrade, C., Morales, F. J., Unraveling the contribution of melanoidins to the antioxidant activity of coffee brews, *J. Agric. Food Chem.* 2005, *53*, 1403 – 1407.

[5] Lindenmeier, M., Faist, V., Hofmann, T., Structural and functional characterization of pronyl-lysine, a novel protein modification in bread crust melanoidins showing *in vitro* antioxidative and phase I/II enzyme modulating activity, *J. Agric. Food Chem.* 2002, *50*, 6997 – 7006.

[6] Morales, F. J., Jimenez-Perez, S., Peroxyl radical scavenging activity of melanoidins in aqueous systems, *Eur. Food Res. Technol.* 2004, *218*, 515 – 520.

[7] Wagner, K.-H., Derkits, S., Herr, M., Schuh, W., Elmadfa, I., Antioxidative potential of melanoidins isolated from a roasted glucose-glycine model, *Food Chem.* 2002, *78*, 375 – 382.

[8] Wenzel, E., Tasto, S., Erbersdobler, H. F., Faist, V., Effect of heat-treated proteins on selected parameters of the biotransformation system in the rat, *Ann. Nutr. Metabol.* 2002, *46*, 9 – 16.

[9] Somoza, V., Lindenmeier, M., Wenzel, E., Frank, O., *et al.*, Activity-guided identification of a chemopreventive compound in coffee beverage using *in vitro* and *in vivo* techniques, *J. Agric. Food Chem.* 2003, *51*, 6861 – 6869.

[10] Faist, V., Lindenmeier, M., Geisler, C., Erbersdobler, H. F., Hofmann, T., Influence of molecular weight fractions isolated from roasted malt on the enzyme activities of NADPH-cytochrome c-reductase and glutathione-S-transferase in Caco-2 cells, *J. Agric. Food Chem.* 2002, *50*, 602 – 606.

[11] Argirova, M. D., Breipohl, W., Glycated proteins can enhance photooxidative stress in aged and diabetic lenses, *Free Radic. Res.* 2002, *36*, 1251 – 1259.

[12] Zill, H., Bek, S., Hofmann, T., Huber, J. *et al.*, RAGE-mediated MAPK activation by food-derived AGE and non-AGE products, *Biochem. Biophys. Res. Commun.* 2003, *300*, 311 – 315.

[13] Friedman, M., Food browning and its prevention: An overview, *Adv. Exp. Med. Biol.* 2005, *561*, 135 – 156.

[14] Spingarn, N. E., Garvie-Gould, C. T., Slocum, L. A., Formation of mutagens in sugar-amino acid model systems, *J. Agric. Food Chem.* 1983, *31*, 3601 – 3604.

[15] Kim, S. B., Kim, L. S., Yeum, D. M., Park, Y. H., Mutagenicity of Maillard reaction products from D-glucose-amino acid mixtures and possible roles of active oxygen in the mutagenicity, *Mutat. Res.* 1991, *254*, 65 – 69.

[16] Lee, H., Lin, M. Y., Hao, N. J., Effects of Maillard reaction products on mutagen formation in boiled pork juice, *Mutagenesis* 1995, *10*, 179 – 183.

[17] Brands, C. M., Alink, G. M., Van Boekel, M. A., Jongen, W. M., Mutagenicity of heated sugar-casein systems: Effect of the Maillard reaction, *J. Agric. Food Chem.* 2002, *48*, 2271 – 2275.

[18] Glösl, S., Wagner, K.-H., Draxler, A., Kaniak, M., *et al.*, Genotoxicity and mutagenicity of melanoidins isolated from a roasted glucose-glycine model in human lymphocyte cultures, intestinal Caco-2 cells and in the *Salmonella typhimurium* strains TA98 and TA102 applying the Ames test, *Food Chem. Toxicol.* 2004, *42*, 1487 – 1495.

[19] Marko, D., Habermeyer, M., Kemeny, M., Weyand, U., Niederberger, E., Frank, O., Hofmann, T., Maillard reaction products modulating the growth of human tumor cells *in vitro*, *Chem. Res. Toxicol.* 2003, *16*, 48 – 55.

[20] Pintauro, S. J., Lucchina, L. A., Effects of Maillard browned egg albumin on drug-metabolizing enzyme systems in the rat, *Food Chem. Toxicol*. 1987, *25*, 369 – 372.

[21] Taylor, J. L. S., Demyttenaere, J. C. R., Abbaspour Tehrani, K., Olave, C. A., *et al.*, Genotoxicity of melanoidin fractions derived from a standard glucose/glycine model, *J. Agric. Food Chem.* 2004, *52*, 318 – 323.

[22] Roux, E., Billaud, C., Maraschin, C., Brun-Mérimée, S., Nicolas, J., Inhibitory effect of unheated and heated D-glucose, D-fructose and L-cysteine solutions and Maillard reaction products model systems on polyphenoloxidase from apple. II. Kinetic study and mechanism of inhibition, *Food Chem.* 2003, *81*, 51 – 60.

[23] Brun-Mérimée, S., Billaud, C., Louarme, L., Nicolas, J., Effect of glutathione and Maillard reaction products prepared from glucose or fructose with glutathione on polyphenoloxidase from apple. II. Kinetic study and mechanism of inhibition, *Food Chem.* 2004, *84*, 235 – 241.

[24] Billaud, C., Maraschin, C., Chow, Y. N., Chériot, S., *et al.*, Maillard reaction products as "natural antibrowning" agents in fruit and vegetable technology, *Mol. Nutr. Food Res.* 2005, *49*, 656 – 662.

[25] Billaud, C., Maraschin, C., Peyrat-Maillard, M. N., Nicolas, J., Maillard reaction products derived from thiol compounds as inhibitors of enzymatic browning of fruits and vegetable. Structure – activity relationship, *Ann. NY Acad. Sci.* 2005, *1043*, 876 – 885.

[26] Maron, D., Ames, B. N., Revised methods for the *Salmonella* mutagenicity test, *Mut. Res.* 1983, *113*, 173 – 215.

[27] Mortelmans, K., Zeiger, E., The Ames *Salmonella*/microsome mutagenicity assay, *Mutat. Res.* 2000, *455*, 29 – 60.

[28] Jing, H., Kitts, D. D., Chemical and biochemical properties of casein-sugar Maillard reaction products, *Food Chem. Toxicol.* 2002, *40*, 1007 – 1015.

[29] Kitts, D. D., Hu, C., Biological and chemical assessment of antioxidant activity of sugar-lysine model Maillard reaction products, *Ann. NY Acad. Sci.* 2005, *1043*, 501 – 512.

[30] Grey, C. E., Adlercreutz, P., Ability of antioxidants to prevent oxidative mutations in *Salmonella typhimurium* TA 102, *Mut. Res.* 2003, *527*, 27 – 36.

[31] Yen, G. C., Tsai, L. C., Lii, J. D., Antimutagenic effect of Maillard browning products obtained from amino acids and sugars, *Food Chem. Toxicol.* 1992, *30*, 127 – 132.

[32] Kitts, D. D., Wu, C. H., Strich, H. F., Powrie, W. D., Effect of glucose-lysin Maillard reaction products on bacterial and mammalian cell mutagenesis, *J. Agric. Food Chem.* 1993, *41*, 2353 – 2358.

[33] White, R. E., Coon, M. J., Oxygen activation by cytochrome P-450, *Ann. Rev. Biochem.* 1980, *49*, 315 – 356.

[34] Minotti, G., Borrello, S., Palombini, G., Galeotti, T., Cytochrome P-450 deficiency and resistance to t-butyl hydroperoxide of hepatoma microsomal lipid peroxidation, *Biochim. Biophys. Acta.* 1986, *876*, 220 – 225.

[35] Epe, B., Hegler, J., Wild, D., Identification of ultimate DNA damaging oxygen species, *Environ. Health Perspect*. 1990, *88*, 111 – 115.

[36] Hwang, J. M., Tseng, T. H., Tsai, Y. Y., Lee, H. J., *et al.*, Protective effects of baicalein on tert-butyl hydroperoxide-induced hepatic toxicity in rat hepatocytes, *J. Biomed. Sci.* 2005, *12*, 389 – 397.

[37] Wijewickreme, A. N., Kitts, D. D., Modulation of metal-induced cytotoxicity by maillard reaction products isolated from coffee brew, *J. Toxicol. Environ. Health Part A* 1998, *55*, 151 – 160.

[38] Morales, F. J., Fernández-Fraguas, C., Iron-binding ability of melanoidins from food and model systems, *Food Chem.* 2005, *90*, 821 – 827.

[39] Matmaroh, K., Benjakul, S., Tanaka, M., Effect of reactant concentrations on the Maillard reaction in a fructose-glycine model system and the inhibition of black tiger shrimp polyphenoloxidase, *Food Chem.* 2006, *98*, 1 – 8.

[40] Glatt, H., Mutagenicity of glutathione and cystein in Ames test, *Science* 1983, *220*, 961 – 963.

[41] Friedman, M., Food browning and its prevention: An overview, *J. Agric. Food Chem.* 1996, *44*, 631 – 653.

[42] Brands, C. M., van Boekel, M. A., Reactions of monosaccharides during heating of sugar-casein systems: Building of reaction network model, *J. Agric. Food Chem.* 2001, *49*, 4667 – 4675.

[43] Somoza, V., Five years of research on health risks and benefits of Maillard reaction products: An update, *Mol. Nutr. Food Res.* 2005, *49*, 663 – 672.

[44] Anese, M., Nicoli, M. C., Antioxidant properties of ready-to-drink coffee brews, *J. Agric. Food Chem.* 2003, *51*, 942 – 946.

[45] Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., Lericic, C. R., Review of nonenzymatic browning and antioxidant capacity in processed foods, *Trends Food. Sci. Technol.* 2001, *11*, 340 – 346.

[46] Billaud, C., Brun-Mérimée, S., Louarme, L., Nicolas, J., Effect of glutathione and Maillard reaction products prepared from glucose or fructose with glutathione on polyphenoloxidase from apple-I: Enzymatic browning and enzyme activity inhibition, *Food Chem.* 2004, *84*, 223 – 233.

[47] Billaud, C., Maraschin, C., Nicolas, J., Inhibition of polyphenoloxidase from apple by Maillard reaction products prepared from glucose or fructose with L-cysteine under various conditions of pH and temperature, *Lebensm. Wiss. Technol.* 2004, *37*, 69 – 78.

[48] Billaud, C., Roux, E., Brun-Mérimée, S., Maraschin, C., Nicolas, J., Inhibitory effect of unheated D-glucose, D-fructose and L-cysteine solutions and Maillard reaction product model systems on polyphenoloxidase from apple-I: Enzymatic browning and enzyme activity inhibition using spectrophotometric and polarographic methods, *Food Chem.* 2003, *19 81*, 35 – 50.

