Food Chemistry 183 (2015) 283–290

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of long-term consumption of standard diets including glucose–lysine model glycated compounds on the antioxidant status of adult rats

Silvia Pastoriza [a], José Ángel Rufián-Henares [b], Cristina Delgado-Andrade [a,*]

[a] Departamento de Fisiología y Bioquímica de la Nutrición Animal (INAN), Estación Experimental del Zaidín (CSIC), Armilla, Granada, Spain
[b] Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, Granada, Spain

ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 19 February 2015
Accepted 11 March 2015
Available online 17 March 2015

Keywords:
Model Maillard compounds
Global antioxidant response
CAT
SOD
GPx
GSH

ABSTRACT

Our purpose was to evaluate the uptake of antioxidant capacity (AC) in rats fed long-term a diet containing commonly consumed Maillard reaction products (MRPs) from the glucose–lysine system. The effects on the oxidative status of liver, *biceps brachii* muscle and serum were also tested. The presence of model MRPs in the diet, especially melanoidins, led to a significantly higher intake (24.0 μmol Trolox/day), faecal excretion (0.604 μmol Trolox/day), and uptake (23.4 μmol Trolox/day) of AC, although the uptake rate remained stable compared to the control group (97.5%). Consumption of the assayed MRPs did not affect the hepatic antioxidant defence while some positive modifications, like an increase in glutathione peroxidase, were detected in muscle (29%) and serum (400%). This pointed to an improved antioxidant capacity. Despite the interesting findings for these specific MRPs, attention must be paid to the overall consumption of MRPs from different sources in a conventional diet, due to their implications in the development/advance of many disorders.

1. Introduction

The Maillard reaction (MR) takes place during the thermal processing and preservation of food and it is responsible for the formation of characteristic flavours, colours, and tastes that are desirable for consumers (Ames, 1990). Typically, it takes place between the carbonyl groups of reducing sugars and the free amino groups from amino acids, peptides and proteins, but oxidised lipids can also participate (Hodge, 1953). The huge variety of products formed during this reaction is known as Maillard reaction products (MRPs). The chemical structure and health effects of MRPs are strongly dependent on food composition or pH (Kim & Lee, 2008), as well as technological conditions, such as heating time and temperature (Rufián-Henares, Delgado-Andrade, & Morales, 2006). MRPs are a particularly complex mix of compounds with different molecular weights, including flavouring (i.e. aldehydes, ketones, pyrazines, etc.) and colouring compounds (i.e. heterocyclic compounds and melanoidins) formed at the advanced stages of the MR (Rufián-Henares, García-Villanova, & Guerra-Hernández, 2004).

The formation of MRPs leads to a decrease in the nutritional value of food due to the destruction of vitamins, loss of essential amino acids and lower protein digestibility (Seiquer et al., 2006). In addition, some MRPs with potent mutagenic and/or carcinogenic activity (i.e. acrylamide or heterocyclic aromatic amines) can be formed (Delgado-Andrade, 2014). MRPs may also be generated in biological systems by the reaction of α-dicarbonyl compounds with amino groups of endogenous proteins, the so-called advanced glycation end-products (AGEs). These AGEs can accumulate in some tissues or are present in the systemic circulation, playing a physiopathological role in cases of metabolic and degenerative disorders (Delgado-Andrade, 2014). For example, the accumulation of AGEs in bone has been shown to have a negative effect on biomechanical properties, decreasing its toughness and increasing its stiffness and therefore contributing to skeletal fragility (Roncero-Ramos et al., 2014).

On the other hand, MRPs have been also proven to have healthy properties such as antimicrobial (Rufián-Henares & de la Cueva, 2009), prebiotic (Seiquer, Rubio, Peinado, Delgado-Andrade, & Navarro, 2014) or antihypertensive activities (Rufián-Henares & Morales, 2007), among others. Moreover, one of the main beneficial effects of MRPs is their antioxidant capacity. In this sense,

* Corresponding author at: Departamento de Fisiología y Bioquímica de la Nutrición Animal, Estación Experimental del Zaidín (CSIC), Camino del Jueves, 18100 Armilla, Granada, Spain. Tel.: +34 958 572757; fax: +34 958 572753.
E-mail address: cdelgado@eez.csic.es (C. Delgado-Andrade).

http://dx.doi.org/10.1016/j.foodchem.2015.03.038
0308-8146/

studies have primarily focused on the antioxidant properties of melanoidins or brown MRPs from different foods, such as coffee, bread crust, beer, biscuits, roasted cocoa or balsamic vinegar (Pastoriza & Rufián-Henares, 2014). However, due to the complex chemical composition of foods, model MRPs are commonly used in animal studies in order to study their biological effects, since they are easily obtainable and simplify results and their interpretation. One of the most widely studied model systems is glucose–lysine, because the lysine amino acid is very sensitive to thermal treatment and highly reactive in the Maillard reaction. Consequently, the availability of lysine also decreases more than that of other amino acids (Hodge, 1953). In addition, the defined composition of the reaction medium could allow inference of some relationship between those compounds formed during the preparing of the model system and the biological activities found in the animal model.

Previous results found discordances in the antioxidant status of rats fed on diets containing bread crust or its soluble high molecular weight, soluble low molecular weight or insoluble fractions (Pastoriza, Roncero-Ramos, Rufián-Henares, & Delgado-Andrade, 2014). This could be related to the complex composition of such fractions. In this sense, the complex glycation behaviour of gluten, the main source of MRPs in bread, did not enable precise conclusions about what type of products could be responsible for the activities observed. Therefore, the aim of the present study was to evaluate the *in vivo* uptake of antioxidant capacity (AC) in rats fed on a diet containing commonly consumed MRPs obtained from a simple glucose–lysine model system, trying to correlate such uptake with the development of some specific MRPs. Additionally, we focused on understanding the effects of those compounds on the oxidative status of these animals at hepatic, muscle and blood level.

2. Materials and methods

2.1. Chemicals

The standard antioxidant used, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), reagents for enzymes determination, alpha amylase (A1031-5KU), pepsin, pancreatin and bile salts were from Sigma–Aldrich (St. Louis, MO). 2, 2′-Azobis-(3-ethylbenzothiazoline-6-sulfonic acid) for the ABTS method was obtained from Fluka Chemicals (Madrid, Spain). The rest of the chemicals used were of analytical grade and obtained from Merck (Darmstadt, Germany) unless mentioned otherwise.

2.2. Sample preparation

Glucose (Merck, Darmstadt, Germany) and lysine-HCl (Sigma Chemical, St. Louis, MO) were used as equimolar mixtures in unbuffered distilled water, to prepare the model system for the production of MRPs as described by Delgado-Andrade, Seiquer, Nieto, and Navarro (2004). The mixture obtained was named GL sample and used in the diets preparation.

2.3. Preparation of diets

The AIN-93G purified diet for laboratory rodents (Dyets Inc., Bethlehem, PA) was used as the Control diet. The GL sample was added to the AIN-93G diet to reach a final concentration of 3%. This diet was termed GL. The individual analysis of the GL diet revealed no modification of the overall nutrient composition compared with the Control diet (AIN-93G). The mean ± SD nutrient content of the diets was: moisture (%) 8.14 ± 0.08; energy (kcal/kg) 3845 ± 109; protein (g/kg) 176.6 ± 3.1; fat (g/kg) 78.1 ± 0.9; Ca (g/kg) 4.79 ± 0.09 and P (g/kg) 3.28 ± 0.03. The higher MRP content in the GL diet with respect to the Control was confirmed by analysing the furosine and hydroxymethylfurfural (HMF) contents, following the procedures described by Delgado-Andrade, Morales, Seiquer, and Navarro (2010). The data obtained for furosine (mean ± SD) were as follows: 28.8 ± 0.5 and 1787 ± 7.31 mg/kg diet for Control and GL diets, respectively. The results for HMF (mean ± SD) were as follows: 0.44 ± 0.06 and 5.15 ± 0.08 mg/kg diet for Control and GL diets, respectively. In addition, the amount of melanoidins present in the GL sample was also assessed as stated in Pastoriza and Rufián-Henares (2014). In parallel, the melanoidins present in the bread crust used as an ingredient in the rat diet previously assayed in our work Pastoriza et al. (2014) were quantified by the same procedure. Both types of melanoidins were isolated and characterised to compare the studies and to understand the actions observed (Table 1).

2.4. Biological assays

Twelve female weanling Wistar rats weighing 41 ± 0.50 g (mean ± SE) were divided into two groups and randomly assigned to one of the dietary treatments. The animals were housed individually in metabolic cages in an environmentally controlled room under standard conditions (temperature 20–22 °C with a 12-h light/dark cycle and 55–57% humidity). The rats had *ad libitum* access to their diets and demineralised water (Milli-Q Ultrapure Water System; Millipore Corp., Bedford, MA).

The duration of the experiment was selected to reach the adult age of the rats, when major effects would start to happen on the redox balance due to the chronic consumption of the tested diets. The experiment involved a preliminary 81-day period where solid food intake and body weight changes were monitored weekly, followed by a 7-day period in which faeces from each animal were collected daily and stored separately as a 1-week pool. The faeces were weighed, lyophilised, powdered and then homogenised. On Day 88 the animals were anaesthetised with sodium pentobarbital (5 mg per 100 g of body weight) (Abbott Laboratories, Granada, Spain) after an overnight fast. Terminal exsanguination was performed by a cannulation of the carotid artery. Blood was drawn to obtain serum and the livers and the left *triceps brachii* muscles

Table 1
Characterisation of melanoidins present in the ingredients used for the preparation of the diets. Portion of antioxidant capacity (AC) and fluorescence excreted in faeces and uptaken from the diets.[a,b]

Parameter	Ingredient	
	Bread crust[c]	GL
Melanoidins (%)	18.2 ± 1.1[a]	96.4 ± 1.7[b]
Fluorescence intensity (Units/g melanoidin)	3,704,000 ± 3872[a]	34,300,000 ± 14071[b]
Furosine (mg/g melanoidin)	0.44 ± 0.06[a]	58.6 ± 3.6[b]
HMF (mg/g melanoidin)	0.001 ± 0.000[a]	0.157 ± 0.028[b]
$TEAC_{ABTS}$ (μmol equivalents of Trolox/g melanoidin)	6.9 ± 0.8[a]	603 ± 39[b]
Faecal fluorescence excretion (units/day)	122,000 ± 398[a]	415,000 ± 502[b]
Fluorescence uptake (units/day)	1,100,320 ± 1022[a]	15,962,000 ± 10021[b]
Faecal AC excretion (μmol equivalents of Trolox/day)	462 ± 9[a]	604 ± 11[b]
AC uptake (μmol equivalents of Trolox/day)	4459 ± 371[a]	23,372 ± 898[b]

[a] Values are means ± SD, $n = 3$.
[b] Different letters within different columns indicate significant differences between groups ($p < 0.05$).
[c] This ingredient was used in the formulation of the bread crust diet applied in a previous work by our research group (Pastoriza et al., 2014) and introduced in this table to perform a comparison between both sources of melanoidins.

were removed, weighed and frozen at −80 °C until enzymatic analysis.

All management and experimental procedures carried out in this study were in strict accordance with the current European regulations (86/609 E.E.C.) regarding laboratory animals. The Bioethics Committee for Animal Experimentation at EEZ-CSIC approved the study protocol.

2.5. Determination of in vitro antioxidant activity in the experimental diets

The total antioxidant activity of the diets was evaluated determining their global antioxidant response by the GAR method. This procedure starts with an *in vitro* gastrointestinal digestion, following the technique described by Pastoriza, Delgado-Andrade, Haro, and Rufián-Henares (2011). Once finished, the bioaccessible (soluble) and the non-bioaccessible (insoluble) fraction of the diets were separated and their antioxidant activity was assessed by using the ABTS procedure (Re, Pellegrini, Proteggente, Pannala, & Rice-Evans, 1999), conducted with slight modifications as described by Rufián-Henares and Delgado-Andrade (2009). Finally, the global antioxidant response (GAR) of the diets was calculated as the sum of the antioxidant activity from their soluble fractions + the antioxidant activity trapped in the insoluble fractions.

2.6. Calculation of antioxidant activity intake and excreted with faeces

Taking into account the food intake during the last week of the experiment and the GAR values for each diet, the daily intake of AC was calculated for each dietary treatment. The AC still retained in faeces was also determined. Using the data for daily intake and AC retained in faeces in the last week of the assay, the apparent uptake of AC was calculated (ingested AC – faecal AC) as well as its apparent uptake efficiency, % $A/I = 100 \times$ (apparent uptake/ingested AC). As describes, both indexes are "apparent", so that term "apparent" will be ommited in the rest of the manuscript.

2.7. Measurement of oxidative stress biomarkers in liver, muscle and serum

The analysis of the antioxidant enzymatic activity was measured in the cytosolic supernatants from liver and muscle as well in the serum. Tissues homogenates were obtained as stated in Pastoriza et al. (2014). Catalase (CAT) activity was determined following the method described by Aebi (1984), superoxide dismutase (SOD) was determined by the method of Fridovich (1975), glutathione peroxidase (GPx) activity was assessed by the technique of Flohé and Gunzler (1984) and the measurement of reduced glutathione (GSH) was carried out by colorimetric assay (QuantiChrom™ Glutathione Assay Kit; BioAssay Systems Ltd., Hayward, CA,). All oxidative stress biomarker determinations were carried out within one week at least in triplicate.

2.8. Fructosamine determination in serum

The test kit commercialised by Química Clinical Aplicada (Química Clinical Aplicada, Amposta, Spain) was used to determine the serum fructosamine concentration (μmol/L).

2.9. Statistical data analysis

All data were statistically tested by one-way analysis of variance (ANOVA), followed by Duncan's test to compare means that showed a significant variation ($p < 0.05$). Evaluation of the relationship between the different variables was carried out by computing the relevant correlation coefficient (Pearson's linear correlation). Analyses were performed using Statgraphics Plus, version 5.1, 2001 (StatPoint, Inc., Herndon, VA).

3. Results and discussion

3.1. Relationship between dietetic melanoidins content and antioxidant intake

The enrichment of a diet with different fractions of bread crust showed contradictory results in a previous paper of our group (Pastoriza et al., 2014) probably due to the complex composition of such fractions. Therefore, we decided to use a GL model system in order to find out some explanations regarding the effect of MRPs on the antioxidant balance of rats.

It is well-known that Maillard reaction is able to generate many different compounds depending on the food matrix used and the heat treatment conditions. Some of the products formed at the final steps of the Maillard reaction are melanoidins, brownish high-molecular-weight compounds that contribute largely to the overall antioxidant intake in the overall human population (Pastoriza & Rufián-Henares, 2014). The strong heat treatment used to prepare the GL sample, as well as the bread crust assayed in our previous study, could generate a large amount of melanoidins. In this sense, there were statistically significant differences ($p < 0.05$) in the amount of melanoidins (Table 1) in bread crust and the GL sample (18.2% *vs*. 96.4%, respectively). This could be related to the ease of reaction between glucose and lysine instead of the gluten glycation observed in bread crust. GL melanoidins had a higher HMF and furosine content, and also showed a ten-times higher fluorescence intensity and 100-times strongest antioxidant activity compared with bread crust melanoidins. It is known that free radical scavenging capacity of MRPs is highly correlated with fluorescence intensity (Morales & Jiménez-Pérez, 2001). Thus, different antioxidant and fluorescence balances among rats fed bread crust and those fed GL melanoidins could be expected. As stated in Table 1, for rats fed a diet enriched with bread crust the ratio fluorescence uptake/fluorescence excreted was 9.0, which was quite similar to the ratio AC uptake/AC excreted (9.7%). In the same way, for rats fed the GL model system the ratios fluorescence uptake/fluorescence excreted and AC uptake/AC excreted were 38.5 and 38.7, respectively. From a mechanistic point of view this could mean a relationship between the intake of fluorescent compounds and the intake of antioxidant capacity, which reinforces the hypothesis of Morales and Jiménez-Pérez (2001) about the relationship of fluorescence and the antioxidant activity of MRPs. A part of such fluorescent/antioxidant characteristics could be attributed to the polymeric melanoidins included within the diet by means of bread crust or GL model system.

3.2. Global antioxidant response (GAR) of diets

Total AC of experimental diets was measured by applying the GAR procedure using the $ABTS^{\cdot+}$ radical (Table 2), a method successfully used as a more physiological approach to test the total AC of different foodstuffs (Pastoriza et al., 2011). The procedure allows the separate measurement of the soluble antioxidant fraction (bioaccessible) and the insoluble one (non-bioaccessible). The final result is calculated as the sum of both portions.

Some antioxidant compounds in the diet, especially polyphenols, may be hydrolysed in the small intestine from their associated compounds, releasing new antioxidant activity *in vivo*. Moreover, they can be fermented in the colon to produce antioxidant metabolites, which could be absorbed and even go back to the gastrointestinal tract if they take part in the enterohepatic

Table 2
Global antioxidant response (GAR) measured in the experimental diets.[a,b]

Diets	Soluble antioxidant activity after *in vitro* digestion (μmol equivalents of Trolox)	Insoluble antioxidant activity after *in vitro* digestion (μmol equivalents of Trolox)	GAR[c] (μmol equivalents of Trolox/g diet)
Control	402 ± 58[a]	7.6 ± 0.8[a]	409 ± 59[a]
GL	1560 ± 110[b]	32.2 ± 2.5[b]	1592 ± 107[b]

[a] Values are means ± SD, $n = 4$ are referred to 1 g of digested diet.
[b] Different letters within a column indicate significant differences between groups ($p < 0.05$).
[c] GAR: global antioxidant response. It summarises soluble and insoluble antioxidant ability from 1 g of diet.

circulation (Cardona, Andrés-Lacueva, Tulipan, Tinahones, & Queipo-Ortuño, 2013). It must be mentioned that total polyphenols were measured in our diets (data not shown) and the amounts were negligible. Therefore, the antioxidant activity in such diets could mainly come from the presence of vitamin mix (0.7%) (Reeves, Nielsen, & Fahey, 1993), the hydrolysis of carbohydrates and proteins during the gastrointestinal process and from the added MRPs. These are compounds not supposed to become an important part of the enterohepatic circulation; it has been stated that about 30% of low molecular weight MRPs are absorbed, whereas only a very low proportion of high molecular weight MRPs are absorbed (Somoza, 2005). Thus, all these sources of antioxidant compounds present in the rat diets of this trial are considered within the GAR procedure. However, it is worthy to mention that the application of the approach proposed in this study to evaluate the uptake of antioxidant capacity using other type of diets could underestimate the antioxidant capacity available at intestinal level, due to the *in vivo* production of antioxidants and the possible enterohepatic circulation, a matter especially important in human trials but not in rats, which usually consume standard diets.

Important increases were detected in the soluble and insoluble antioxidant activities after *in vitro* digestion of the GL diet, compared with the Control one (1560 *vs.* 402 μmol equivalents of Trolox/g diet) (Table 2). Since the GAR value is obtained by addition of both portions, this parameter exhibited a significant higher result in the GL diet. The only difference between both dietary treatments was the inclusion of MRPs coming from the heated glucose–lysine model system in the case of the GL diet, therefore differences among their AC must be attributed to the presence of those compounds. In this line, Seiquer et al. (2008) reported that a diet rich in Maillard reaction products, designed for healthy male adolescents, exhibited higher radical-scavenging activity measured by the DPPH method than a diet low in those products. Only one previous study, also coming from our research group, has reported the suitability of the GAR method to evaluate the global AC of experimental diets for rodents (Pastoriza et al., 2014). In that case, meals including both MRPs and glycated compounds from bread crust were tested, revealing that the presence of those products did not lead to a substantially higher AC compared with the control diet. It is worthy to mention that the average GAR value detected in those diets was 368 μmol equivalents of Trolox/g diet, while a level of 1592 μmol equivalents of Trolox/g was measured in the GL diet of the present trial. This difference is probably due to the different origins for the MRPs, naturally formed in the case of bread crust while strongly promoted under laboratory conditions in the case of glucose–lysine model system.

3.3. Food intake and body weight

Although there were slight variations, the food consumption was stable between diets during the whole experimental period and the balance week (Table 3). There is no consensus on the effects of MRPs on food intake. The consumption of high *vs.* low MRP diet by healthy subjects induced an increase in the food intake (Birlouez-Aragon et al., 2010), which could be attributed to the pleasant aroma and flavour, as well as improvement in palatability developed by MRPs. However, animal assays have revealed controversial conclusions. The study of Hofmann et al. (2002) depicted stable food consumption after feeding mice diets containing high or low MRP levels, while Delgado-Andrade (2002) reported decreases in the food intake by administrating the same browning products to rats as those generated in this trial (glucose–lysine model system heated at 150 °C for 90 min). The discrepancy between these results and the data here presented could be attributed to the age of the rats, which were younger in the former study (therefore with lower capacity of adaptation to the diet). This statement is supported by the fact that, in the present trial, animals belonging to the GL group did not show any statistically significant decrease in their global food intake with respect to the Control group (1189 *vs.* 1324 g of food intake for GL and Control groups, respectively), although a tendency was observed. However, during the balance week (last one) the food consumption of the GL group was a little bit higher ($p > 0.05$) than in the Control group (14.6 *vs.* 13.2 g food per day, respectively), evidencing that an adaptation to the diet took place during the advance of the experiment.

The mentioned trend to decrease the global food intake was manifested in a lower final weight of animals belonging to the GL group, but without reaching statistical relevance (Table 3). A similar animals weight pattern was previously observed by Pastoriza et al. (2014) after feeding rats diets including 10% bread crust or its soluble fractions for three months. However, Sebekova et al. (2005) observed an increase in body weight when rats consumed a diet containing 25% of bread crust but not when the proportion included was 5%.

Table 3
Food intake and daily input of antioxidant capacity (AC) in the organism after feeding rats different diets.[a,b]

	Parameter	Diets	
		Control	GL
Food intake	Global intake (g)	1324 ± 34[a]	1189 ± 51[a]
	Intake in the balance week (g/day)	13.2 ± 1.0[a]	14.6 ± 1.9[a]
	Final weight (g)	244 ± 5[a]	220 ± 9[a]
AC	Intake (mmol equivalents of Trolox/day)	5.40 ± 0.398[a]	24.0 ± 0.888[b]
	Faeces (mmol equivalents of Trolox/day)	0.136 ± 0.007[a]	0.604 ± 0.011[b]
	Uptake (mmol equivalents of Trolox/day)	5.26 ± 0.392[a]	23.4 ± 0.898[b]
	Uptake efficiency (%)	97.5 ± 0.1[a]	97.5 ± 0.1[a]

[a] Values are means ± SE, $n = 6$.
[b] Different letters among column indicate significant differences between groups ($p < 0.05$).

3.4. Daily input of antioxidant capacity in the organism

Taking into account the previous results obtained from experimental diets containing MRPs from bread crust and its AC apparent uptake (Pastoriza et al., 2014), we decided to perform a parallel study focusing on a particular type of MRPs: those generated in a glucose–lysine model system, as a major source of Maillard-derived compounds in the diet. The calculations were based on the application of the GAR procedure to the diets, in order to establish the total AC ingested and the AC still present in faeces. This approach is adequate in our experimental conditions, since the antioxidant sources in the rat diets are controlled (AIN93-G diet added with model MRPs). Moreover, this procedure also considered the outstanding action of the intestinal microbiota, releasing those antioxidant compounds trapped in the undigested portion of the diets.

In spite of the stability in the food intake of the last week (Table 3), the animals fed the GL diet ingested more than four times the daily AC of the Control group (Table 3) due to the higher GAR value detected in the GL diet (Table 2). As expected, the AC detected in faeces of these animals was also greater, but due to the high AC intake, a very high apparent uptake of AC was shown in the GL group (23.4 *vs.* 5.26 mmol equivalents of Trolox/day for GL and Control groups, respectively). These data suggest that many of the compounds generated in the heated glucose–lysine model system had the correct size and structure to allow their absorption *in vivo*. Surprisingly, the uptake efficiency was exactly the same in both dietary treatments (97.5%). In our previous study focused on the antioxidant balance after long-term consumption of diets including bread crust glycated compounds by adult rats, lower AC uptakes and uptake efficiencies were reported (≈90%), pointing out that the MRPs originating in a complex food matrix, the bread crust, had a worse availability at intestinal level (Pastoriza et al., 2014). The higher ratio of AC uptake/AC excretion in the GL diet means a favoured absorption compared to the bread crust diet. This could be explained taking into account that the melanoproteins formed in bread crust are mainly composed of glycated gluten, which could be hardly digested and/or absorbed compared to those melanoidins formed in the GL model system. In addition, the formation of melanoidins in the GL model system is specially favoured contrary to the bread crust. Finally, the higher antioxidant capacity uptake of those rats fed on the diet enriched in GL could be related with the higher AC of the GL melanoidins compared with those fed a diet enriched in bread crust.

3.5. In vivo antioxidant status after long-term consumption of experimental diets

The main aim of this experiment was to investigate whether MRPs derived from the glucose–lysine model system, a pool of compounds frequently consumed in our usual diet, have some antioxidant effect *in vivo* and therefore a possible health benefit. With that purpose, absorption of MRPs is a prerequisite to exert any *in vivo* effects and metabolic transit. Studies performed mainly on rats have evidenced that at least 30% of low molecular weight compounds are absorbed, whereas high molecular weight compounds are absorbed to a much lesser extent (Somoza, 2005) and probably after intestinal transformations (Homma & Fujimaki, 1981).

Among others, the enzymes CAT, SOD and GPx are important tools available in cells and tissues to fight against reactive oxygen species (ROS) generated during the oxidative metabolism (Ueda et al., 1998). The CAT activity of tissues varies greatly, being important in liver and kidney, and more residual in muscles and connective tissue (Aebi, 1984). Along with this, the CAT activity detected in the liver of rats after being fed different diets was substantially higher than in the muscle and serum (Figs. 1–3, respectively).

GPx plays a crucial role in the intracellular quenching of cell-damaging peroxide species and the effective recovery of the GSH stock (Ueda et al., 1998). Enhanced activity of GPx is a cell requirement to face the generation of ROS induced by prooxidant compounds (Alía, Ramos, Mateos, Bravo, & Goya, 2006). As described for CAT data, in the present trial SOD and GPx were quantitatively higher in the hepatic tissue than in muscle or serum (Figs. 1–3, respectively), which is in agreement with the important detoxificant and metabolic role of the liver. Finally, GSH depletion reflects intracellular oxidation, whereas an increase in its level could be expected to prepare the cell against potential oxidative damage (Alía, Ramos, Mateos, Bravo, & Goya, 2005).

Focusing on the liver (Fig. 1), the intake of the GL diet did not induce any significant modification in the CAT, SOD or GPx activities compared with the control group. Levels of GSH were also unaffected by consumption of model MRPs. Therefore, the products here assayed seem to be well detoxified in the liver, without affecting its antioxidant balance. Even so, an inverse correlation was manifested between the AC uptake and the SOD levels ($r = -0.8506$; $p = 0.0318$), pointing out that the presence of model MRPs in the organism consume a major portion of this compound used to measure antioxidant activity. Opposite to these results, our previous study established that the ingestion of bread crust glycated compounds induced a clear increase in the CAT and GPx activities, as well as higher levels of reducing power (GSH) within the cell, revealing a positive action of those specific compounds at this level (Pastoriza et al., 2014).

CAT, SOD, GPx and GSH were also tested in the left *triceps brachii* muscle of the rats (Fig. 2), where oxidative status was substantially different and less uniform. GPx significantly increased after MRPs consumption and a clear reduction, although quantitatively less important, was reported in the case of SOD. Kono and Fridovich (1982) reported that the lower SOD activity could raise the levels of anion superoxide and lead to certain inhibition of CAT, which could be linked to the finding of the minor CAT activity detected in this tissue. Moreover, SOD activity has been demonstrated to be inhibited by methylglyoxal (Choudhary, Chandra, & Kale, 1997), an α-dicarbonyl compound probably present in the GL diet and able to contribute to the *in vivo* genesis of AGEs. Levels of GPx kept high after the intake of the GL diet, pointing that the actions of the CAT and SOD enzymes with the participation of the GSH molecule were enough to repell the oxidative damage caused to the muscle. Data found in the muscle indicate that this tissue was submitted to a higher oxidative stress than the liver as a consequence of the MRPs consumption, but still maintained the ability to perform an adequate antioxidant defence.

A deeper effect on the oxidative status of this muscle was obtained in rats fed on bread crust glycated compounds (Pastoriza et al., 2014). In that study the different oxidative status of the muscle compared with the liver was supported by the high fructosamine levels in the serum of animals fed glycated compounds from bread crust. Serum fructosamine is considered an index of glycaemic control and its increase is a sign of elevated blood glucose concentration for a sustained period (Ng, Thai, Lui, & Koay, 1989). Its suitability for our studies is due to the recent demonstration that diet-induced insulin-resistant mice maintained high levels of blood glucose (149 *vs.* 211 mg/dL for control and experimental groups, respectively), evidence of a state of oxidative stress in the skeletal muscle which resulted in mitochondrial dysfunction (Bonnard et al., 2008). Opposite to the results of our previous study, in the present trial the fructosamine levels of serum were unaffected by the consumption of model MRPs (mean ± SE: 32.2 ± 6.9 and 39.8 ± 5.5 μmol/L, for the Control and the GL group, respectively; $p > 0.05$) a fact that also corroborates

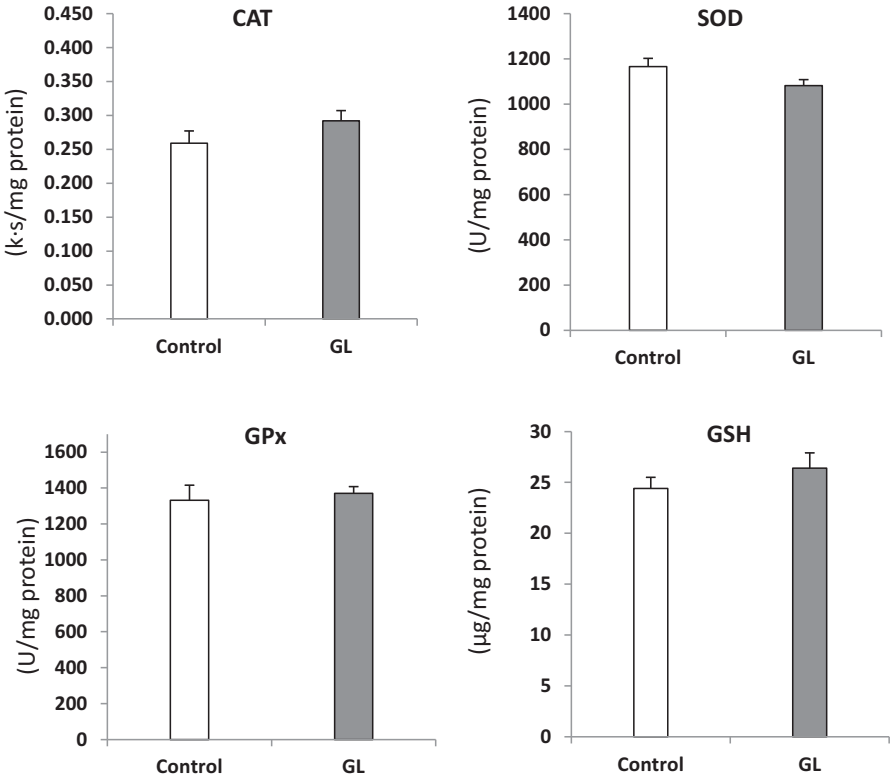

Fig. 1. Parameters related to oxidative stress analysed in liver after consumption of experimental diets. Values are means ± SE, $n = 6$. No significant differences were found between groups ($p > 0.05$).

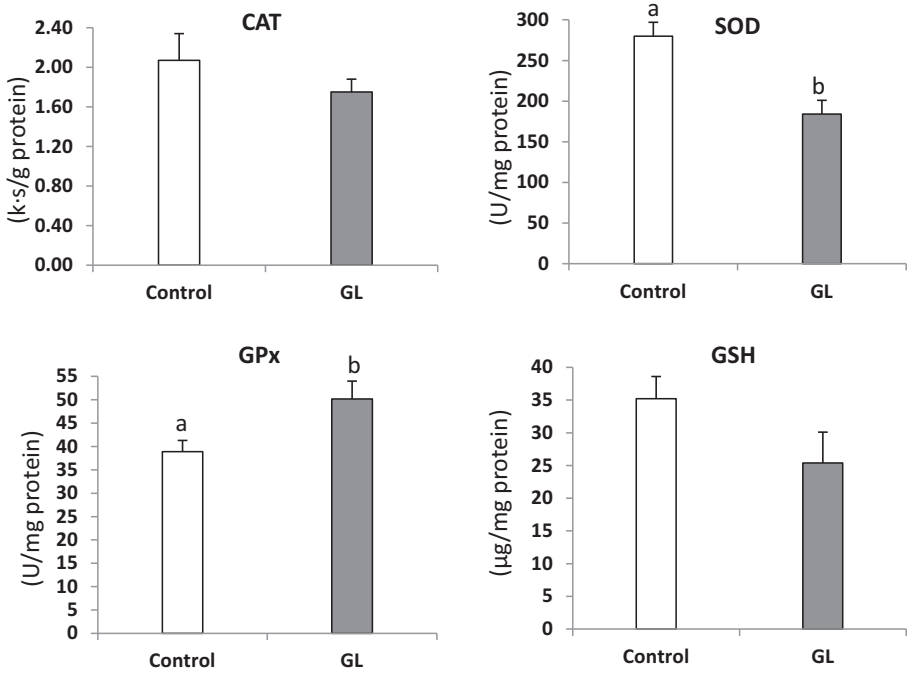

Fig. 2. Parameters related to oxidative stress analysed in *triceps brachii* muscle after consumption of experimental diets. Values are means ± SE, $n = 6$. Different letters indicate significant differences between groups ($p < 0.05$).

that the muscle was not submitted to an excessive oxidative environment.

Serum was also evaluated in order to find out differences in the activities of CAT, SOD, GPx and GSH. In this fluid, besides the observation of non-significant variations in some of the parameters analysed, a clear and significant increase in the GPx activity was evidenced after the intake of the GL diet. This change suggests a better antioxidant capacity of serum to perform the antiradical defence as a consequence of the model MRPs consumption, a fact reinforced by the finding of a high and positive relationship

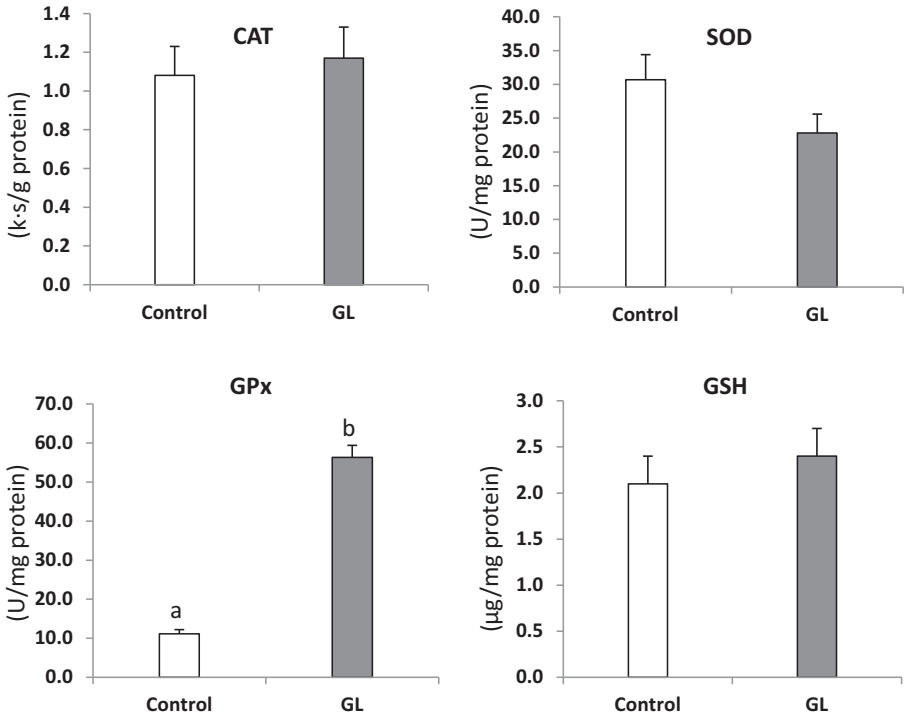

Fig. 3. Parameters related to oxidative stress analysed in serum after consumption of experimental diets. Values are means ± SE, $n = 6$. Different letters indicate significant differences between groups ($p < 0.05$).

between the uptake of AC from the diet and the GPx activity in serum ($r = 0.9518$; $p = 0.0034$).

The main body of studies about the actions of MRPs or glycated compounds on oxidative stress have been developed in cell culture and they usually show the protective effects of specific compounds of the Maillard reaction. Thus, the ability of coffee (final MRPs) of different molecular masses to protect human HepG2 cells against oxidative stress has been established (Goya, Delgado-Andrade, Rufián-Henares, Bravo, & Morales, 2007). In the same line, previous findings of our research group showed that incubation of Caco-2 cells with solutions including MRPs from the same glucose–lysine model system and H_2O_2 as oxidising agent, decreased lipid oxidation and maintained the antioxidant enzyme activity (Ruiz-Roca, Delgado-Andrade, Navarro, & Seiquer, 2011).

However, only a few *in vivo* assays have been performed to link the effects of MRPs or glycated compounds consumption with the effects on the enzymatic antioxidant defence. The results of the present investigation are in line with data found by Somoza et al. (2005). These authors demonstrated that feeding rats with a diet containing bread crust, malt or a pronylated albumin increased the antioxidant activity of plasma (measured by the inhibitory effect on linoleic acid peroxidation), decreased the plasma TBARS levels and raised the levels of chemopreventive enzymes in the liver and kidneys. Coffee consumption in humans (five cups per day for 1 week), probably the highest melanoidins source in the human diet, increases the plasma content of the antioxidant glutathione, but with no changes in the hydroperoxide levels (Espósito et al., 2003). Moreover, Correa et al. (2012) established that the consumption of medium-light and medium roasted coffee by healthy volunteers increased the CAT, SOD and GPx activity in erythrocytes. Seiquer et al. (2008) also documented that the consumption of a white diet (free as possible in MRPs) or a brown diet (rich in MRPs) in a group of healthy male adolescents did not modify the *in vivo* antioxidant markers of damage or defence. However, the treatment of biological samples with external oxidants revealed higher values of AC after consumption of the MRPs-rich diet.

In summary, the approach set up by of our research team in a previous study to estimate the uptake efficiency of the AC from standard chows is applied to a diet rich in model MRPs. The method, based on the GAR procedure, shows adequate suitability since the antioxidant structures present in the experimental diet essentially come from the heated glucose–lysine model system, compounds not supposed to become an important part of the enterohepatic circulation. The presence of the model MRPs in the diet highly increases the intake and uptake of AC in the organism, causing no significant damage in the antioxidant defence at hepatic or muscle level, and even improving the antioxidant capacity of blood. Despite these results, in order to establish the global impact of the consumption of MRPs or glycated compounds on health, not only the effects on the antioxidant balance should be considered but also other systemic effects related with cardiovascular pathologies, mineral metabolism or bone status.

Acknowledgements

This research was supported by a project and a "Ramón y Cajal" postdoctoral contract of the Spanish Ministry of Economy and Competitiveness and the postdoctoral scholarship of S. Pastoriza from Instituto Danone (Spain).

References

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology, 150*, 121–127.
Alía, M., Ramos, S., Mateos, R., Bravo, L., & Goya, L. (2005). Response of the antioxidant defense system to t-butyl hydroperoxide and hydrogen peroxide in a human hepatoma cell line (HepG2). *Journal of Biochemistry and Molecular Toxicology, 19*, 119–128.
Alía, M., Ramos, S., Mateos, R., Bravo, L., & Goya, L. (2006). Quercetin protects human hepatoma cell line (HepG2) against oxidative stress induced by tert-butyl hydroperoxide. *Toxicology and Applied Pharmacology, 212*, 110–118.
Ames, J. M. (1990). Control of the Maillard reaction in food systems. *Trends in Food Science and Technology, 1*, 150–154.
Bonnard, C., Durand, A., Peyrol, S., Chanseaume, E., Chauvin, M. A., Morio, B., et al. (2008). Mitochondrial dysfunction results from oxidative stress in the skeletal

muscle of diet-induced insulin-resistant mice. *Journal of Clinical Investigation, 118*, 789–800.
Birlouez-Aragon, I., Saavedra, G., Tessier, F. J., Galinier, A., Ait-Ameur, L., Lacoste, F., et al. (2010). A diet based on high-heat-treated foods promotes risk factors for diabetes mellitus and cardiovascular diseases. *American Journal of Clinical Nutrition, 91*, 1220–1226.
Cardona, F., Andrés-Lacueva, C., Tulipan, S., Tinahones, F. J., & Queipo-Ortuño, M. I. (2013). Benefits of polyphenols on gut microbiota and implications in human health. *The Journal of Nutritional Biochemistry, 24*, 1415–1422.
Choudhary, D., Chandra, D., & Kale, R. K. (1997). Influence of methylglyoxal on antioxidant enzymes and oxidative damage. *Toxicology Letters, 93*, 141–152.
Correa, T. A. F., Monteiro, M. P., Mendes, T. M. N., Oliveira, D. M., Rogero, M. M., Benites, C. I., et al. (2012). Medium light and medium roast paper-filtered coffee increased antioxidant capacity in healthy volunteers: results of a randomized trial. *Plant Foods for Human Nutrition, 67*, 277–282.
Delgado-Andrade, C. (2002). *Reacción de Maillard. Influencia sobre la biodisponibilidad mineral*. University of Granada. Tesis doctoral.
Delgado-Andrade, C. (2014). Maillard reaction products: Some considerations on their health effects. *Clinical Chemistry and Laboratory Medicine, 52*, 53–60.
Delgado-Andrade, C., Morales, F. J., Seiquer, I., & Navarro, M. P. (2010). Maillard reaction products profile and intake from Spanish typical dishes. *Food Research International, 43*, 1304–1311.
Delgado-Andrade, C., Seiquer, I., Nieto, R., & Navarro, M. P. (2004). Effects of heated glucose–lysine and glucose–methionine model-systems on mineral solubility. *Food Chemistry, 87*, 329–337.
Espósito, F., Morisco, F., Verde, V., Ritieni, A., Alezio, A., Caporaso, N., et al. (2003). Moderate coffee consumption increases plasma glutathione but not homocysteine in healthy subjects. *Alimentary Pharmacology and Therapeutics Aims, 17*, 595–601.
Flohé, L., & Gunzler, W. A. (1984). Assays of glutathione peroxidase. *Methods in Enzymology, 105*, 114–121.
Fridovich, I. (1975). Superoxide dismutase. *Annual Review of Biochemistry, 11*, 147–159.
Goya, L., Delgado-Andrade, C., Rufián-Henares, J. A., Bravo, L., & Morales, F. J. (2007). Effect of coffee melanoidin on human hepatoma HepG2 cells. Protection against oxidative stress induced by tert-butylhydroperoxide. *Molecular Nutrition & Food Research, 51*, 536–545.
Hodge, J. E. (1953). Chemistry of browning reactions in model systems. *Journal of Agricultural and Food Chemistry, 1*, 928–943.
Hofmann, S. M., Dong, H.-J., Li, Z., Cai, W., Altomonte, J., Thung, S. N., et al. (2002). Improved insulin sensitivity is associated with restricted intake of dietary glycoxidation products in the db/db mouse. *Diabetes, 51*, 2082–2089.
Homma, S., & Fujimaki, M. (1981). Growth response of rats fed a diet containing non-dialyzable melanoidin. *Progress in Food and Nutrition Science, 5*, 209–216.
Kim, J., & Lee, Y. (2008). Effect of reaction pH on enolization and racemization reactions of glucose and fructose on heating with amino acid enantiomers and formation of melanoidins as result of the Maillard reaction. *Journal of Agricultural and Food Chemistry, 108*, 582–592.
Kono, Y., & Fridovich, I. (1982). Superoxide radical inhibits catalase. *Journal of Biological Chemistry, 257*, 5751–5754.
Morales, F. J., & Jiménez-Pérez, S. (2001). Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. *Food Chemistry, 72*, 119–125.
Ng, W. Y., Thai, A. C., Lui, K. F., & Koay, E. S. (1989). Serum fructosamine determination as an index of glycaemic control: comparison between an automated and manual method, and the effect of serum storage. *Annals of the Academy of Medicine Singapore, 18*, 352–356.
Pastoriza, S., Delgado-Andrade, C., Haro, A., & Rufián-Henares, J. A. (2011). A physiologic approach to test the global antioxidant response of foods. The GAR method. *Food Chemistry, 129*, 1926–1932.
Pastoriza, S., Roncero-Ramos, I., Rufián-Henares, J. A., & Delgado-Andrade, C. (2014). Antioxidant balance after long-term consumption of standard diets including bread crust glycated compounds by adult rats. *Food Research International, 64*, 106–113.
Pastoriza, S., & Rufián-Henares, J. A. (2014). Contribution of melanoidins to the antioxidant capacity of the Spanish diet. *Food Chemistry, 164*, 438–445.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.
Reeves, P. G., Nielsen, F. H., & Fahey, G. C. (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition, 123*, 1939–1951.
Roncero-Ramos, I., Niquet-Léridon, C., Strauch, C., Monnier, V. M., Tessier, F. J., Navarro, M. P., et al. (2014). An advanced glycation end products (AGE)-Rich diet promotes N-E-Carboxymethyl-lysine accumulation in the cardiac tissue and tendons of rats. *Journal of Agricultural and Food Chemistry, 62*, 6001–6006.
Rufián-Henares, J. A., & de la Cueva, S. (2009). Antimicrobial activity of coffee melanoidins – A study of their metal-chelating properties. *Journal of Agricultural and Food Chemistry, 57*, 432–438.
Rufián-Henares, J. A., & Delgado-Andrade, C. (2009). Effect of digestive process on Maillard reaction indexes and antioxidant properties of breakfast cereals. *Food Research International, 42*, 394–400.
Rufián-Henares, J. A., Delgado-Andrade, C., & Morales, F. J. (2006). Relationship between acrylamide and thermal-processing indexes in commercial breakfast cereals: A survey of Spanish breakfast cereals. *Molecular Nutrition and Food Research, 50*, 756–762.
Rufián-Henares, J. A., García-Villanova, B., & Guerra-Hernández, E. (2004). Generation of furosine and color in infant/enteral formula-resembling systems. *Journal of Agricultural and Food Chemistry, 52*, 5354–5358.
Rufián-Henares, J. A., & Morales, F. J. (2007). Angiotensin-I converting enzyme inhibitory of coffee melanoidins. *Journal of Agricultural and Food Chemistry, 55*, 10016–10021.
Ruiz-Roca, B., Delgado-Andrade, C., Navarro, M. P., & Seiquer, I. (2011). Effects of Maillard reaction products from glucose–lysine model systems on oxidative stress markers and against oxidative induction by hydrogen peroxide in Caco-2 cells. *Journal of Food and Nutrition Research, 50*, 237–248.
Sebekova, K., Hofmann, T., Boor, P., Ulická, O. G., Erbersdobler, H. F., Baynes, J. W., et al. (2005). Renal effects of oral Maillard reaction product load in the form of bread crusts in healthy and subtotally nephrectomized rats. *Annals of the New York Academy of Science, 1043*, 482–491.
Seiquer, I., Díaz-Alguacil, J., Delgado-Andrade, C., López-Frías, M., Muñoz Hoyos, A., Galdo, G., et al. (2006). Diets rich in Maillard reaction products affect protein digestibility in adolescent males aged 11–14. *American Journal of Clinical Nutrition, 83*, 1082–1088.
Seiquer, I., Rubio, L. A., Peinado, M. J., Delgado-Andrade, C., & Navarro, M. P. (2014). Maillard reaction products modulate gut microbiota composition in adolescents. *Molecular Nutrition and Food Research, 58*, 1552–1560.
Seiquer, I., Ruiz-Roca, B., Mesías, M., Muñoz-Hoyos, A., Galdó, G., Ochoa, J., et al. (2008). The antioxidant effect of a diet rich in Maillard reaction products is attenuated after consumption by healthy male adolescents. In vitro and in vivo comparative study. *Journal of the Science and Food Agriculture, 88*, 1245–1252.
Somoza, V. (2005). Five years of research on health risk and benefits of Maillard reaction products: An update. *Molecular Nutrition and Food Research, 49*, 663–672.
Somoza, V., Wenzel, E., Lindenmeier, M., Grothe, D., Erbersdobler, H., & Hofmann, T. (2005). Influence of feeding malt, bread crust, and a pronylated protein on the activity of chemopreventive enzymes and antioxidative defense parameters in vivo. *Journal of Agricultural and Food Chemistry, 53*, 8176–8182.
Ueda, Y., Miyata, T., Hashimoto, T., Yamada, H., Izuhara, Y., Sakai, H., et al. (1998). Implication of altered redox regulation by antioxidant enzymes in the increased plasma pentosidine, an advanced glycation end product, in uremia. *Biochemical and Biophysical Research Communications, 245*, 785–790.