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ORIGINAL CONTRIBUTION

Influence of bread crust-derived Maillard reaction products on phosphorus balance in rats

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Abstract
Background Maillard reaction products (MRP) improve food palatability and are linked to some positive biological actions. However, diverse negative consequences, some related to protein damage and mineral availability, have been established.
Aim of study We investigated the effects of MRP, from a bread crust diet, on phosphorus bioavailability and tissue distribution in rats to determine whether these effects are related to the molecular weight of browning products.
Methods During a study period of 88 days, rats were fed either a control diet or one of the following: with bread crust as a source of MRP, or one with its soluble high molecular weight, soluble low molecular weight or insoluble fraction (bread crust, HMW, LMW and insoluble diets, respectively). In the final week, a phosphorus balance was performed, after which the animals were sacrificed and some organs removed to analyse phosphorus content. A second balance was carried out throughout the experimental period to calculate phosphorus retention.
Results Phosphorus balance in the last week was unchanged. However, considering the whole experimental period, a trend towards improved bioavailability, significant in the HMW group, was observed. Higher phosphorus concentrations were measured in the small intestine and bone.
Conclusions The consumption of MRP derived from bread did not alter phosphorus retention, due to increased bioavailability, especially concerning HMW compounds. The overall phosphorus body content remained unchanged and there were no changes in the bone, its principal metabolic destination. However, MRP consumption markedly raised phosphorus levels at the digestive level, especially when consumed as isolate fractions. The slower rate of stomach emptying is assumed to be related to this effect.

Keywords Maillard reaction products · Phosphorus bioavailability · Tissue distribution · Bone

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Introduction

The processing of foods rich in protein and carbohydrates or fat promotes the development of the Maillard reaction and the formation of the browning compounds known as Maillard reaction products (MRP), which are often responsible for improvements in food palatability [1]. The Maillard reaction, therefore, is employed by the food industry to achieve attractive aromas, colours and flavours. High cooking temperatures and longer heating times are the most significant processing factors influencing MRP formation, and browned compounds frequently appear during heat treatments such as frying, roasting, grilling and baking [2].

Bread is still one of the most widely consumed foods worldwide, although its consumption has decreased in recent years due to changes in eating patterns and the rising number of more highly processed substitutes such as breakfast cereals [3, 4]. The application of high temperature during the baking process, together with an appropriate flour composition, facilitates the development of the Maillard reaction and the appearance of its early, advanced

and end products in different proportions, especially in the bread crust.

Consumption of MRP is associated with certain positive biological actions [5] such as antioxidant activity, the inhibition of tumour growth and antimutagenic effects [6–8], but these compounds may also provoke undesirable nutritional effects, some of which are related to protein damage [9] and mineral availability [10], since MRP behave as anionic polymers, forming stable complexes with metal cations. Ca, Mg, Fe, Cu and Zn are capable of binding to soluble and insoluble melanoidins derived from different amino acid–sugar model systems or from a real food [11–14]. The effects of MRP consumption on the absorption and bioavailability of phosphorus are not well understood, as few studies have explored the issue, in example the works of Andrieux and Sacquet [15]. Moreover, phosphorus acts as an anion, not a cation, in the biological fluids, and thus, the metal–MRP chelation hypothesis employed to explain the effects on other mineral nutrients is not valid in this case.

Phosphorus is an essential nutrient for the organism; it plays an important role in cellular physiology and skeletal mineralisation as well as in energetic molecule production and maintenance of the acid–base balance [16]. Phosphorus deficiency is uncommon since it is sufficiently abundant in natural foods, and its rate of intestinal absorption is quite high [17]. However, with increasing consumption of MRP due to changes in dietary habits, it would be very useful to study the effect of MRP consumption on phosphorus absorption and its metabolic destination. In this respect, a previous assay carried out in male adolescents fed a diet rich or poor in MRP over a period of 2 weeks showed that the fractional absorption of phosphorus is negatively affected by the presence of MRP [18]. This interesting result in humans suggests we should explore the potential long-term effects, both at digestive and organic level, for which animal experimentation is required.

The aim of this study was to investigate the effects of the consumption of MRP derived from bread crust on phosphorus bioavailability and tissue distribution, and to determine whether these effects are related to the molecular weight of browning products.

Materials and methods

Chemicals

All chemicals used were of analytical grade and were obtained from Merck (Darmstadt, Germany), unless stated otherwise. Pronase E (4,000,000 PU/g) was also purchased from this company.

Extraction of bread crust and its soluble and insoluble fractions

The bread crust was supplied by a Spanish manufacturer of cereal-derived food products. Once the attached bread crumbs had been manually removed, the sample was weighed, lyophilised, powdered and homogenised. A fraction of the bread crust was then stored at −20 °C until diet formulation. Since our aim was to study the effects of the MRP as a function of their molecular size, in order to access the MRP and melanoproteins linked to proteins, another important fraction was subjected to enzymatic hydrolysis with pronase E, an enzyme commonly applied for this purpose. The results obtained from previous studies by our research group indicated the most appropriate pronase E concentration and incubation time [19]. Briefly, 125 g of bread crust was digested with 750 mL of a 0.100 mg/mL pronase E solution (400 U/mL in 1 M phosphate buffer, pH 8.2) in stoppered test recipients at 37 °C for 72 h in a water bath under shaking. This procedure was repeated as many times as necessary to obtain enough sample for the diet formulation. After cooling, the sample was treated with 15 mL of 40% trichloroacetic acid solution (w/v) and centrifuged at 4,500*g* for 10 min at 4 °C to separate the soluble and insoluble fractions. The insoluble fraction was then weighed, lyophilised, homogenised and stored at −20 °C until used for diet formulation. The soluble fraction was subjected to ultrafiltration employing a Pellicon Ultrafiltrate cassette connected to a cassette-style tangential flow filtration device (Millipore, MA, USA) and a flow variable peristaltic pump. A Biomax polyethersulphone membrane (0.5 m^2 size, 17.8 cm width × 21 cm length, Millipore, MA, USA) with 5 kDa NMWL was used. Fractions constituted of compounds with a molecular mass higher than 5 kDa were retained (retentate, high molecular weight, HMW) and those with a lower molecular mass were filtered (filtrate, low molecular weight, LMW). Both the retentate and the filtrate were lyophilised, powdered and homogenised, and stored at −20 °C until used for diet formulation.

Preparation of diets

The AIN-93G purified diet for laboratory rodents (Dyets Inc, Bethlehem, PA) was used as the control diet [20]. The bread crust was added to the AIN-93G diet to reach a final concentration of 10%. This diet was named bread crust. In order to determine the factors responsible for the effects observed in the trial, the LMW, HMW and insoluble fractions were also individually added to the diet in the same proportion as they were present in the 10% of bread crust, calculated from the recovery of each fraction after pronase E digestion. These diets were named LMW, HMW

and insoluble, respectively. Bread crust is a sodium source, and so, to maintain the concentration of this element at adequate levels, these diets were prepared mixing appropriate proportions of AIN-93G and low-sodium AIN-93G diets, respectively. Calcium carbonate was added when necessary to reach the values originally present in the AIN-93G diet.

The individual analysis of the different diets revealed no significant modification of the overall nutrient composition, compared with the control diet (AIN-93G). The mean ± SD nutrient content of the diets was moisture (%) 7.9 ± 0.4, protein (g/kg) 168.4 ± 4.0, fat (g/kg) 77.9 ± 1.6, Na (g/kg) 1.34 ± 0.02 and P (g/kg) 3.22 ± 0.08. Moisture, protein and P content were analysed as described in AOAC [21], while Na was measured by flame atomic absorption spectroscopy using lithium chloride to avoid interferences.

The highest MRP content in the prepared diets, with respect to the control diet, was established by analysing the furosine and hydroxymethylfurfural (HMF) contents following the procedures described by Delgado-Andrade et al. [22]. The data obtained for furosine (mean ± SD) were as follows: 28.8 ± 0.5, 49.5 ± 0.3, 39.7 ± 1.4, 39.4 ± 1.0 and 34.7 ± 0.8 mg/kg diet for control, bread crust, LMW, HMW and insoluble diets, respectively. The results for HMF (mean ± SD) were as follows: 0.44 ± 0.06, 4.26 ± 0.02, 0.47 ± 0.04, 0.47 ± 0.01 and 0.89 ± 0.01 mg/kg diet for control, bread crust, LMW, HMW and insoluble diets, respectively.

Biological assays

Seventy weanling Wistar rats weighing 41.02 ± 0.16 g (mean ± SE) supplied by Charles River Laboratories, Spain, S.A., were used in the study. Sixty rats were randomly distributed into five groups (12 animals per group), and each group was assigned to one of the dietary treatments. The animals were individually housed in metabolic cages in an environmentally controlled room under standard conditions (temperature: 20–22 °C with a 12 h light–dark cycle and 55–70% humidity). The rats had ad libitum access to their diets and demineralised water (Milli-Q Ultrapure Water System, Millipore Corps., Bedford, MA, USA). The remaining ten animals were sacrified by anaesthesia overdose at day 0, and their initial phosphorus body content was analysed.

The animals were fed the different diets for 88 days. Excluding the insoluble group, in which the accidental death of four animals limited the number of trials, two different balances were carried out during the experimental period. The phosphorus balance for the entire experimental period, termed the 'Global balance', was calculated from the difference between the final phosphorus body contents of each animal and the average initial content of the element (102.0 ± 2.5 mg P). Phosphorus intake was monitored during this period. Six animals from each group were sacrificed by anaesthesia overdose on day 88 to calculate their final phosphorus body content. None of their organs were extracted. The animals in the insoluble group were excluded from this global balance.

In the last week of the experimental period (days 82–88), another phosphorus balance was performed on all the animals. This test involved a preliminary 81-day period during which solid food intake and body weight changes were monitored weekly, followed by a 7-day period in which a phosphorus balance was performed. In this last week, faeces and urine from each animal were collected daily and stored separately as a 1-week pool. The faeces were weighed, lyophilised and homogenised. The urine was collected on 0.5% HCl (v/v), filtered (Whatman Filter Paper No. 40, ashless, Whatman, England) and diluted to an appropriate volume. To control for possible environmental contamination during the collection of urine and faeces, empty cages were manipulated in the same way as those used for the animals. On day 88, after an overnight fast, the remaining six animals in each group were anaesthetised with sodium pentobarbital (5 mg per 100 g of body weight) (Abbott Laboratories, Granada, Spain) and terminal exsanguination was performed by a cannulation of the carotid artery. Blood was drawn to obtain serum, and the liver, right kidney, spleen, small intestine and right femur were removed, weighed and frozen at −80 °C until phosphorus analysis.

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

Analytical techniques

To determine the global balance, the whole cadavers were weighed, lyophilised and homogenised. The liver, spleen, kidney, small intestine and femur of the other six animals were dry-ashed in a muffle furnace (Selecta, Mod.366, Barcelona, Spain) at 450 °C, and the white ashes obtained were dissolved with $HCl/HNO_3/H_2O$ (1:1:2). Aliquots of the remaining samples (urine, faeces, diets and whole cadavers) were completely digested by the addition of concentrated HNO_3, $HClO_4$ and by heating at high temperatures (210–220 °C) in a sand beaker. All samples were diluted with Milli-Q water to an appropriate volume for phosphorus measurement. Total phosphorus was determined colorimetrically at 820 nm in a spectrophotometer (Shimadzu UV-1700, Model TCC-240A, Columbia, USA) by the vanadomolybdate procedure [21].

Pools of faeces, urine and diet were used as an internal control to assess precision. The inter-assay coefficient of variation was 1.80% in faeces, 1.08% in urine and 2.07% in the diet. Milk powder (certified reference material CRM 063; Community Bureau of Reference, Brussels, Belgium) was used to quantify accuracy, yielding a value of 13.47 ± 0.04 mg/g (mean ± SD; certified value: 13.49 ± 0.10 mg/g) for phosphorus.

All glassware and polyethylene sample bottles were washed with 10 N nitric acid, and milli-Q water was used throughout the study.

The following indices were calculated using the data for phosphorus intake and faecal and urinary excretion obtained in the last week of the assay: apparent absorption (ingested P − faecal P), apparent retention or balance (apparent absorption − urinary P), apparent absorption efficiency or fractional absorption (%A/I) = apparent absorption/ingested P × 100, apparent retention efficiency (%R/A) = apparent retention/apparent absorption × 100, and bioavailability (%R/I) = apparent retention/ingested P × 100. Since all the indices were calculated in apparent form, henceforth, the term 'apparent' will be omitted.

The parameters calculated for the global balance were global retention (final P body content − initial P body content) and global %R/I (global retention/total P intake × 100).

Statistical analysis

All data were statistically tested by one-way analysis of the variance (ANOVA), followed by Duncan's test to compare means that showed a significant variation ($P < 0.05$). Analyses were performed using Statgraphics Plus, version 5.1, 2001. The relationship between the different variables was evaluated by computing the relevant correlation coefficient (Pearson's linear correlation) at the $P < 0.05$ confidence level.

Results

The phosphorus balance data for the last week of the assay are presented in Table 1. Phosphorus intake during the balance week decreased significantly (13%) in the group fed the insoluble diet, and a downward trend was also observed in the HMW group.

The total faecal excretion of phosphorus was significantly lower in the HMW ($P = 0.0015$) and insoluble ($P = 0.0129$) groups, whereas in the other experimental groups, this value was similar to that presented by the control group. The absorption remained unchanged among the five groups fed the experimental diets, ranging from 20.7 mg/d (HMW group) to 17.9 mg/d (insoluble group), without reaching significant differences. Urinary phosphorus tended to decrease in all groups with respect to the control group but was only significant in the bread crust ($P = 0.0006$) and insoluble ($P = 0.0252$) groups. No significant differences were found in phosphorus retention among any of the animals given the bread crust diet or its fractions (Table 1).

Consumption of diets containing bread crust led to higher values of phosphorus fractional absorption (A/I%) and bioavailability (R/I%) with respect to the controls, although they were only statistically significant in the HMW group (Fig. 1). Similarly, phosphorus retention efficiency (R/A%) tended to improve after the ingestion of all MRP diets, with increases ranging from 8.5 to 17.6%; the values for the bread crust group were significantly higher than those of the controls (Fig. 1).

The global balance reflected a profile similar to that of the balance for the final week (Fig. 2), with phosphorus intake over the whole experimental period tending to be lower in the animals given MRP diets, but with only the HMW group presenting statistically significant differences ($P = 0.0375$). Body phosphorus content and overall retention remained unchanged among the groups, but phosphorus bioavailability was significantly higher in the HMW group than in the control group.

Data corresponding to the final body weight of animals, phosphorus content and concentration in the organs are shown in Table 2. Regarding the weight of the organs, no significant differences were found in the liver, spleen and small intestine. In the weight of the kidney, a general reduction of around 10% was observed in all the experimental groups fed bread derivatives, but this was only

Table 1 Phosphorus last week balance in rats fed the different diets

Diets	Intake	Faeces	Urine (mg/d)	Absorption	Retention
Control	46.3 ± 1.5^{a}	26.5 ± 0.9^{a}	6.41 ± 0.45^{a}	19.8 ± 1.1	13.4 ± 1.2
Bread crust	45.7 ± 0.8^{a}	26.1 ± 0.7^{a}	4.23 ± 0.31^{b}	19.6 ± 0.9	15.4 ± 0.8
LMW	46.3 ± 1.1^{a}	25.7 ± 0.8^{a}	5.69 ± 0.18^{ac}	20.7 ± 0.7	15.0 ± 0.8
HMW	42.8 ± 1.0^{ab}	22.1 ± 0.8^{b}	5.69 ± 0.40^{ac}	20.7 ± 0.9	15.0 ± 0.9
Insoluble	40.5 ± 1.4^{b}	22.7 ± 0.9^{b}	4.74 ± 0.50^{bc}	17.9 ± 1.3	13.1 ± 1.1

Values are mean ± SE, $n = 12$ except in insoluble group ($n = 8$)

Different letters within a column indicate significant differences between groups ($P < 0.05$) (one-way ANOVA, followed by Duncan test)

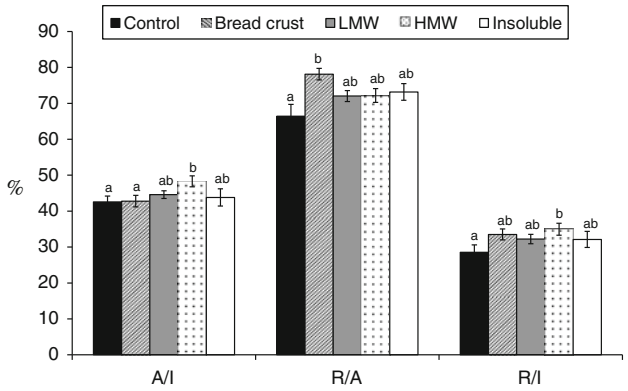

Fig. 1 Phosphorus biological indices in rats fed the different diets (mean ± SE, $n = 12$, except in the insoluble group, $n = 8$). *Different letters* within an index indicate significant differences between groups ($P < 0.05$) (one-way ANOVA, followed by Duncan test)

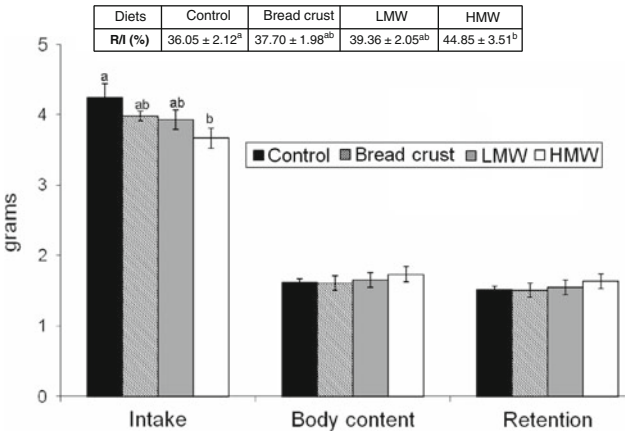

Fig. 2 Phosphorus global balance in rats fed the different diets (mean ± SE, $n = 6$). *Different letters* within an index indicate significant differences between groups ($P < 0.05$) (one-way ANOVA, followed by Duncan test)

statistically significant in the bread crust group (12%). Femur weight decreased in all the animals fed diets containing bread crust or its fractions.

After consumption of MRP, phosphorus content and concentration in the liver and kidney did not differ in any group studied. The amount of phosphorus deposited in the spleen was significantly lower in the bread crust group than in the control group, and a downward trend was observed in the groups that consumed fractions derived from bread. The splenic phosphorus concentration in the bread crust group was also lower than in all other groups except the LMW group, which showed a tendency to decrease.

Phosphorus content in the small intestine increased greatly in all animals, compared with the controls, ranging from 4.29 mg P in the control group to 12.98 mg P in the LMW group, which presented the highest values. Intestinal phosphorus concentration had a similar behaviour pattern, with the LMW group showing the most pronounced increase (296% compared with the control group).

Finally, phosphorus content in the femur did not change in any of the groups, although the mineral concentration increased in all animals fed diets containing bread crust or its fractions, due to the lower weight of the bones.

Discussion

Browning products are known to affect mineral availability in different ways, and several studies have been conducted of metal cations [11, 12, 23–25]. Nevertheless, few investigations have focused on phosphorus, and so, this study was intended to enhance our understanding of the effects of MRP on phosphorus bioavailability.

MRP from bread crust included in the diets led to decreased food intake over the whole experimental period, especially in the HMW and insoluble groups (1311.6 ± 35.4 g for the control group vs. 1159.1 ± 27.5 g and 1050.1 ± 24.9 g for the HMW and the insoluble groups, respectively). This decreased level of food intake was responsible for the reduction in ingested phosphorus during the entire assay (Table 1) and in the last week of balance (Fig. 2). Many authors have described changes in food intake after the consumption of MRP. Furniss et al. [26] detected reductions when rats were fed a glucose/casein mixture heated to 60 °C. Sarria et al. [27] obtained similar results with liquid infant formulas containing Maillard derivatives. The findings of Delgado-Andrade [28] comparing the effects of the consumption of glucose/lysine heated mixtures for 30 and 90 min suggested that the most advanced MRP, browner and more insoluble were responsible for the most pronounced decline in food intake. Abu-Dweih et al. [29] consider that the influence of MRP on intake depends on the degree of toasting, and that decreases in food consumption are related to increased browning. The results observed in the present assay would be in line with this suggestion, since the phosphorus ingested by the HMW group in both balances decreased by around 8% with respect to the control group (Fig. 2), and a 14% decline was observed in the insoluble group during the last week of balance (Table 1). The diets of both groups are those that contained isolated compounds of higher molecular weight, and therefore, they are the most browning diets. As a result of the low intake of phosphorus in the HMW and insoluble groups, faecal excretion decreased significantly in these groups, to maintain the absorption. In fact, ingested and faecal phosphorus were positively correlated ($r = 0.6635$; $P = 0.000$). Few studies have been made of the influence of MRP on the digestive process of phosphorus, and they have been mainly developed in model systems. Thus, Delgado-Andrade [28]

Table 2 Body weight and phosphorus content and concentration in organs of rats fed the different diets

Sample	Control	Bread crust	LMW	HMW	Insoluble
Body weight (g)	247.6 ± 5.1[a]	234.7 ± 4.4[ab]	236.3 ± 7.2[ab]	227.5 ± 5.8[b]	220.7 ± 4.0[b]
Liver					
Weight (g)	6.15 ± 0.29	6.05 ± 0.23	6.30 ± 0.27	6.00 ± 0.21	5.81 ± 0.17
P (mg)	11.04 ± 0.87	10.20 ± 1.18	9.56 ± 0.95	10.45 ± 0.34	9.15 ± 0.45
P (mg/g)	1.82 ± 0.17	1.69 ± 0.18	1.54 ± 0.18	1.75 ± 0.09	1.58 ± 0.07
Kidney					
Weight (g)	0.77 ± 0.03[a]	0.68 ± 0.01[b]	0.71 ± 0.03[ab]	0.71 ± 0.03[ab]	0.69 ± 0.03[ab]
P (mg)	1.32 ± 0.07	1.14 ± 0.02	1.24 ± 0.05	1.25 ± 0.05	1.24 ± 0.07
P (mg/g)	1.72 ± 0.05	1.69 ± 0.04	1.74 ± 0.04	1.75 ± 0.03	1.78 ± 0.05
Spleen					
Weight (g)	0.50 ± 0.02	0.49 ± 0.02	0.48 ± 0.03	0.49 ± 0.03	0.46 ± 0.03
P (mg)	0.86 ± 0.06[a]	0.65 ± 0.07[b]	0.74 ± 0.04[ab]	0.79 ± 0.04[ab]	0.76 ± 0.05[ab]
P (mg/g)	1.72 ± 0.09[a]	1.33 ± 0.13[b]	1.55 ± 0.10[ab]	1.62 ± 0.10[a]	1.66 ± 0.05[a]
Small intestine					
Weight (g)	4.72 ± 0.21	4.58 ± 0.19	4.95 ± 0.30	4.37 ± 0.19	4.60 ± 0.11
P (mg)	4.29 ± 0.58[a]	7.23 ± 0.26[b]	12.98 ± 0.56[c]	10.17 ± 0.73[d]	11.35 ± 0.37[d]
P (mg/g)	0.90 ± 0.10[a]	1.59 ± 0.07[b]	2.66 ± 0.15[c]	2.32 ± 0.08[d]	2.47 ± 0.08[cd]
Femur					
Weight (g)	0.59 ± 0.04[a]	0.51 ± 0.02[b]	0.51 ± 0.02[b]	0.51 ± 0.02[b]	0.49 ± 0.01[b]
P (mg)	54.0 ± 2.8	51.7 ± 1.7	53.1 ± 3.0	53.1 ± 1.8	52.5 ± 1.2
P (mg/g)	92.7 ± 4.5[a]	101.9 ± 1.9[b]	104.3 ± 1.7[b]	104.1 ± 1.4[b]	107.1 ± 1.3[b]

Values are mean ± SE, $n = 6$ except in insoluble group ($n = 8$)

Different letters within a row indicate significant differences between groups ($P < 0.05$) (one-way ANOVA, followed by Duncan test)

observed the same effect using MRP obtained from a glucose/methionine mixture heated to 150 °C for 30 and 90 min and a glucose/lysine mixture heated to the same temperature for 30 min. Although views on this question are not unanimous, the fractional absorption of phosphorus does not seem to be affected by the presence of browned products. These results are to be expected, since the chelation effect of MRP on metal cations does not occur with this element.

In the same line, the fractional absorption of phosphorus did not decrease following the consumption of MRP from bread crust or its fractions; on the contrary, it tended to rise or even increase significantly in the HMW group with respect to the control and bread crust groups (Fig. 1). Although the possibility of an adaptive response associated with the lowest intake cannot be discarded completely, it should be remembered that the fractions with different molecular weights were obtained by the digestion of bread crust with pronase and that these fractions were then digested again in the gastrointestinal tract with the diet. New compounds could have been released by the action of enzymes, gastric juices and bacterial fermentation after the two digestions. If some of these soluble and high molecular weight compounds included phosphorus in their structures, this could provide a vehicle facilitating the absorption of the element. These compounds could also be present in bread crust, but perhaps not appearing in the same form as remained after digestion with pronase. In addition, these compounds are not alone in the bread crust, but are accompanied by LMW compounds and other food components that had not undergone any previous digestion. Accordingly, the fractional absorption of phosphorus could be modulated by the conjoint presence of different components in a different form from that found when the HMW compounds are isolated.

The kidney is the main organ involved in regulating the metabolism of phosphorus, and the presence of MRP appeared to decrease urinary phosphorus (Table 1). The insoluble compounds may be the main factors responsible for this, since the animals which eliminated less phosphorus in the urine were those that consumed the bread crust and insoluble diets in which these compounds were present. This decrease might be associated with lower nonsignificant values of absorption in both groups. As was to be expected, urinary phosphorus was positively correlated with the absorption ($r = 0.2669$, $P = 0.0468$). Delgado-Andrade [28] also described a decrease in urinary excretion in rats fed diets containing MRP from glucose/lysine and glucose/methionine model systems. Results from the same research group have shown a trend towards

decreased urinary excretion of phosphorus in male adolescents who consume a diet rich in MRP [18].

Despite the changes in the urinary excretion of phosphorus, or perhaps because of them, the retention of this element remained unaltered during the final week of the trial and throughout the experimental period, and so, body phosphorus content was maintained (Figs. 1, 2). Other authors, too, have reported unchanged phosphorus retention after feeding rats with MPR from a glucose–glycine model system [30].

Therefore, the animals consuming diets containing MPR from bread, by adjusting the efficiency of digestive (A/I%) and metabolic (R/A%) processes, were able to maintain body phosphorus content. The improvement in these two efficiencies was reflected in a tendency towards increased bioavailability over the whole assay, although this was only significant in the HMW group. In trials performed with MRP from glucose–lysine and glucose–methionine model systems, an increase in phosphorus bioavailability has also been observed [28].

Reductions in body weight have been associated with the consumption of MRP from model systems or food [31, 32], as occurred in the rats in the present assay, although the effect in the latter was less pronounced. The organs with lower weight losses correspond to the smaller animals. Thus, the weights of the kidney and femur were lower and correlated with the weight of the rats at the end of the experimental period, while liver, spleen and small intestine weights remained unchanged (Table 2). Thus, the hypertrophy described in organs such as the kidney, spleen and liver [32, 33] and related to the intake of MRP does not seem to have occurred in the present assay. This could be expected since the MRP used in this study were derived from bread and were not added in large amounts.

The content and concentration of phosphorus in the kidney and liver did not vary among the different groups, as reported by Delgado-Andrade [28] with respect to the consumption of MRP developed in a glucose–lysine-heated model system, although these organs have been identified as an MRP target in the organism. However, Somoza et al. [34] did not observe any accumulation of advanced glycation end products (AGEs) in these organs after feeding rats a diet including 5% bread crust.

Even though a general downward trend was observed in the content and concentration of phosphorus in the spleen, lower values of this mineral in the bread crust group are believed to be associated with a specific MRP effect, such as a conjoint action of all compounds, of both high and low molecular weight. Therefore, this outcome is most apparent in animals consuming the bread crust diet, in which all components are present.

The most surprising findings in this assay are related to the high amount and concentration of phosphorus present in the small intestine in some of the animals, reaching a value three times higher than in the controls. No data in this respect have been published previously, but it could be explained by a slower digestive process arising from MRP consumption, which would increase the concentration of nutrient in the intestine waiting to be absorbed. Larger and more indigestible particles are known to be retained in the stomach for longer periods [35], and this situation could equally apply to the presence of MRP, as they are less digestible [36], especially those with higher molecular weight. Thus, Kimiagar et al. [37] described a slower rate of stomach emptying in rats fed with Maillard browned egg albumin. Rèrat et al. [36] showed that the presence of fructoselysine, an early MRP, in pig diets delayed digestive processes, and so, the compound did not appear in portal blood until almost 4 h after the food intake, while free lysine appeared after just 1 h. On the other hand, Somoza et al. [38] reported that bread crust consumption in rats increased the appearance of non- or less-digestible compounds in faeces. In consequence, the high phosphorus concentration may be due to the element forming part of the non-absorbable compounds still present in the small intestine. The physiological consequences of these phosphorus levels are unknown and require further study, especially concerning the effects on gut microbiota, since it has been reported that high amounts of calcium phosphate in the diet have a trophic action on the intestinal microflora [39].

In recent years, AGEs, considered to be MRP endogenous, have been related with several pathologies, some associated with the bone, such as osteoporosis [40], lower osteoblastic activity [41], modifications in bone density [42], etc. Therefore, changes in bone mineral that might affect the phosphorus content could be expected. Nevertheless, after the ingestion of 10% bread crust, femoral phosphorus was not modified and the increase in its concentration was due to the lower weight of the bones. Calcium content in the femur, and phosphorus content, remained unchanged, and so, it seems that the bone mineral is not affected by the consumption of MRP from bread crust or from its fractions, but rather these compounds might exert a specific influence in the organic phase of bone.

In summary, the presence of MRP from bread in a balanced diet, although it decreases food intake and hence phosphorus consumption, does not alter its retention, due to improved bioavailability, especially when animals consume compounds that are of higher molecular weight and insoluble. Nevertheless, it is unknown how the organism could respond to such a deficit through improved bioavailability if the diet is not optimal. The global content of phosphorus remained unchanged in all animals, and there were no changes in its major metabolic destination: the

bone. Thus, MRP from bread crust, independently of their molecular weights, do not quantitatively modify bone mineralisation, and since calcium was not impaired, the relationship between AGEs and osteoporosis [40] may not be mediated by changes in the mineral phase of bone.

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Conflict of interest The authors declare there is no conflict of interest.

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