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In vivo effects of Maillard reaction products derived from biscuits

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ABSTRACT

The antioxidant activity, antihypertensive effect and prebiotic activity of Maillard reaction products (MRPs) derived from biscuits were investigated in Wistar rats. Animals were fed the following diets for 6 weeks: control (AIN-93 diet); Asc-diet (AIN-93 diet with ascorbic acid in the drinking water); HT-B diet (containing high amount of MRP derived from biscuits) and LT-B diet (containing negligible amounts of biscuit MRP). Serum antioxidant activity (FRAP, ABTS), as well as lipid peroxidation (TBARS) were determined at the end of the experiment. Results showed that dietary MRP reduced the food efficiency, increased the antioxidant activity of serum, increased the ratio between lactic and total aerobic bacteria, increased water-holding capacity of faeces and reduced blood pressure, but did not reduce mineral absorption. Therefore, the biscuit MRP functional claims could be demonstrated by an *in vivo* study.

1. Introduction

Nowadays there is an increasing demand for healthy products in the food industry. The antioxidant micronutrients are involved in many metabolic processes and block the effects of reactive oxygen species that can lead to the destruction of cells and DNA damage (Langner & Rzeski, 2014). Although different antioxidants are currently used in the food industry, the consumer sometimes questions their safety (Rufián-Henares & Morales, 2007a). Therefore, the use of natural antioxidants, such as α-tocopherol, β-carotene or ascorbic acid is of great concern. In addition, the possible benefits of antioxidants naturally formed during cooking procedure, like the Maillard reaction products (MRPs), are of particular interest.

The Maillard reaction (MR) is a non-enzymatic browning reaction that occurs between the amino groups of amino acids and the carbonyl groups, mainly of the sugars. The MRP is a heterogeneous group that includes low molecular weight compounds, which contribute to the product flavour, as well as polymeric products, which are linked to the colour and texture of the foodstuffs (Wang, Qian, & Yao, 2011). In particular, melanoidins, formed at the final stages of the MR, have attracted much attention in recent years. Numerous investigations have reported the possible antioxidant, antihypertensive, prebiotic and antimicrobial activity that these compounds exhibit in model systems (Borrelli & Fogliano, 2005; Kitrytė, Adams, Venskutonis, & De Kimpe, 2012; Rufián-Henares & Morales, 2007a). Furthermore, some works have evaluated these effects in real systems like bakery products and coffee with excellent results: Martín et al. (2009) described the ability of biscuit melanoidins to protect human Hep G2 cells against free radical damage; the results obtained by Monente et al. (2015) strongly suggested that a high content of coffee melanoidins could inhibit the growth of Gram-negative bacteria by metal-chelating mechanisms. Moreover, Lindenmeier, Faist, and Hofmann (2002) explored the modulating activity of bread crust melanoidins in intestinal Caco-2 cells. However, the effect of these compounds derived from real systems using *in vivo* assays has been barely studied.

The two major sources of dietary melanoidins are coffee and bakery products. Recent studies by Pastoriza and Rufián-Henares (2014) showed that coffee contributed most to the antioxidant activity exerted by melanoidins followed by biscuits, while bread only showed a slight contribution. What is more, biscuits consumption has grown steadily; the annual consumption *per capita* ranges from 2.5 kg in Asian countries to 7.5 kg in the USA (Filipčev, Šimurina, & Bodroža-Solarov, 2014). In spite of this, the impact on health of a diet with high amount of biscuit melanoidins remains unstudied and requires clarification.

The aim of the present work was to investigate the *in vivo* effects of MRP on rats. In order to simulate the consumption conditions, the MRP were not isolated from the biscuits and were administered as biscuits. The antioxidant activity, prebiotic effect, antihypertensive capacity, as well as some other biomarkers, were determined in different groups of animals in order to describe the effects of MRP derived from biscuits. One rat group was fed with a

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diet partially replaced by biscuits with a high amount of MRP. To achieve accurate results, three control groups were used: a group fed only with a control diet (negative control); a second group in which ascorbic acid was added in the drinking water (positive control), and a third group fed with the control diet partially replaced by biscuits with a negligible amount of MRP. This last group was used as a "negative diet control", to rule out any possible effect linked to the biscuit ingredients.

Since the *in vivo* effects of the biscuit melanoidins were investigated, this study provides valuable information about the health benefits of MRP.

2. Materials and methods

2.1. Materials

The biscuit ingredients were: wheat flour (Favorita 000; Molinos Río de la Plata, Buenos Aires, Argentina), corn starch (Maizena, Unilever de Argentina S.A., Buenos Aires, Argentina), skim milk powder (SanCor Sunchales, Santa Fe, Argentina), high oleic sunflower oil (Propia; Lezama, Buenos Aires, Argentina), sucrose (Ledesma, Jujuy, Argentina) and baking powder (Royal, Kraft Foods, Buenos Aires, Argentina).

2.2. Biscuit preparation

Biscuits used in the experimental diets were prepared in the laboratory as described by Patrignani, Conforti, and Lupano (2014) with slight modifications. Briefly, 20 g of the high oleic sunflower oil were mixed with wheat flour (35 g), corn starch (25 g), skim milk powder (20 g), sucrose (12.5 g) and baking powder (0.9 g) in a kneading Philips Cucina mixer (Sao Paulo, Brazil) for 60 s at low speed. Then 25 ml of tap water were added in two consecutive steps, mixing each time at high speed. Finally everything was mixed for 1 min at low speed to obtain a clay-like dough. Rectangles of dough (3.3 × 5.2 × 0.3 cm) were cut and baked in an oven (White Westinghouse, W-CG18). Biscuits were processed under two different conditions: 150 °C for 25 min (high temperature biscuits) or 100 °C for 80 min (low temperature biscuits) to reach final moisture contents lower than 10%.

Biscuits were milled (Philips Cucina mixer, HR 7633, Sao Paulo, Brazil) and the following components were determined: protein by Kjeldahl method (AACC 46-11), total lipids by Soxhlet (AACC 30-25); total dietary fibre (AACC 32-07.01); moisture content (AACC 44-01); ash (AACC 08-03) and carbohydrates by difference. Diets were ashed in a furnace at 550 °C, dissolved in HNO_3 and the obtained solutions were used to determine Zn and Ca concentration by atomic absorption spectroscopy and Na by atomic emission spectroscopy (Perkin Elmer AAnalyst 400) using standard solutions.

Biscuit final composition was 77.8 g of carbohydrates (14.5% of lactose from milk; 67.6% of starch from flour, baking powder and corn starch and 18.1% of sucrose), 11.5 g of proteins (70% of proteins from milk and 30% from flour), 8.2 g of lipids (from high oleic sunflower oil), 2.3 g of dietary fibre, 1.72 mg of Zn, 351.7 mg of Ca and 213.4 mg of Na per 100 g of biscuit (dry basis). The moisture content of the final products was lower than 10%.

2.3. Experimental diets

Three different experimental diets were prepared according to the American Institute of Nutrition Rodent Diets Recommendations (Reeves, Nielsen, & Fahey, 1993):

- Control: AIN-93 diet.
- HT-B diet: AIN-93 diet replaced by high temperature biscuit (39.1 g of dried weight biscuit per 100 g of diet).
- LT-B diet: AIN-93 diet replaced by low temperature biscuits (39.1 of dried weight biscuit per 100 g of diet).

The composition of each diet is detailed in Table 1. All the diets were accurately prepared and supplied the same amounts of proteins, minerals, fibre, lipids, and energy (372 ± 8 kcal/100 g). Dextrin was added as a carbohydrate source to achieve 1000 g of diet.

2.4. Diet analysis

2.4.1. Sample extraction

Diets (0.2 g) were extracted in 1.5 mL of warm deionised water (45 °C). Then, the tubes were shaken vigorously for 10 min (25 g) and left for 30 min at 4 °C. After that time, the mixtures were centrifuged 10 min at 10,000*g* (5415 R; Eppendorf, Hamburg, Germany). The supernatants were collected, filtered (0.45 μm pore size) and stored at −20 °C until analysis (Morales, Martin, Açar, Arribas-Lorenzo, & Gökmen, 2009). The aqueous solutions were used for determination of the browning intensity and antioxidant activity.

2.4.2. Measurement of the browning intensity

The amount of Maillard reaction products was measured by absorbance at 420 nm (Monente et al., 2015). Appropriate dilutions (1/4) of the aqueous extracts of diets were made using distilled water, and the absorbance was measured at 420 nm using a UV-mini 1240 spectrophotometer (Shimadzu, Kyoto, Japan).

2.4.3. Antioxidant activity of diets

The antioxidant activity was measured by the FRAP assay according to the method of Benzie and Strain (1996). Briefly, 1.8 mL of freshly prepared FRAP reagent were mixed with 50 μL of the diet aqueous solution and 150 μL of distilled water. After 4 min, the sample absorbance was measured at 593 nm. A Trolox solution was used as standard. Results were expressed as μg of Trolox/mg of dried sample.

The DPPH assay was used in order to measure the free radical scavenging of 50 μL of the diet aqueous solution (1 mL final volume), as described by Brand-Williams, Cuvelier, and Berset (1995). Results were expressed as μg Trolox/mg of dried sample.

All determinations were performed in triplicate for each sample.

Table 1
Composition, A_{420} and antioxidant activity (FRAP and DPPH) of control, HT-B and LT-B diets.

Composition (g/kg of diet)*	Diet		
	Control	HT-B	LT-B
Casein (85% protein)	140	87	87
Mineral mix (AIN-93M-MX)	35	35**	35**
Vitamin mix (AIN-93-VX)	10	10	10
L-Cysteine	1.8	1.8	1.8
Soybean oil	40	7.8	7.8
Choline bitartrate (ml)	7.1	7.1	7.1
Cellulose	50	40.9	40.9
Dried biscuit (g)	–	391	391
Diet parameters			
A_{420}	0.02 ± 0.00^{c}	0.13 ± 0.00^{a}	0.03 ± 0.00^{b}
FRAP (μg Trolox/mg dried sample)	0.05 ± 0.01^{b}	0.62 ± 0.04^{a}	0.11 ± 0.05^{b}
DPPH (μg Trolox/mg dried sample)	0.20 ± 0.07^{b}	0.77 ± 0.02^{a}	0.29 ± 0.03^{b}

Results expressed as means ± SD. Values in the same row with different superscript letter are significantly different ($p \leqslant 0.05$).

* Dextrin was added as carbohydrate source to achieve 1000 g of diet.

** Composition according to AIN-93 with the necessary adjustments to ensure an adequate mount of Zn, Ca and Na.