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Amaranth-hydrolyzate enriched cookies reduce the systolic blood pressure in spontaneously hypertensive rats

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ABSTRACT

The effect of consuming baked foods enriched with antihypertensive compounds on blood pressure remains uncertain. In this study, an alcalase-generated amaranth hydrolyzate was used to prepare cookies and their technological characteristics (expansion factor, color, and texture) and antihypertensive properties evaluated. The bioavailability of the antihypertensive compounds were assessed in Balb/c mice and the antihypertensive effects in hypertensive rats. Enriched cookies developed a more intense yellow-brown color than the non-enriched ones ($p < 0.05$). Compared to the group that received water only, the sera of mice fed with enriched cookies significantly inhibited ACE-1 activity ($p < 0.05$). Similarly, enriched cookies, but not non-enriched ones, reduced the blood pressure in hypertensive rats ($p < 0.05$). Amaranth hydrolyzates maintain their antihypertensive properties after their incorporation into a cookie recipe and could be used as an ingredient for functional foods development.

1. Introduction

The identification and purification of peptides with potential to treat human diseases has been the subject of intensive research. Purifying a peptide to high levels would not only be costly, but would also increase the risk to lose any synergic beneficial effect given by other components present in the source of the peptide of interest (Chakrabarti, Guha, & Majumder, 2018). Currently, the use of hydrolyzates as an ingredient for functional foods development is more feasible than the use of purified peptides (Sabbione, Suárez, Añón, & Scilingo, 2018). Particularly, amaranth hydrolyzates obtained with alcalase can inhibit the angiotensin 1 converting enzyme (ACE-1) activity *in vitro* (Silva-Sanchez et al., 2008; Tovar-Pérez, Guerrero-Legarreta, Farrés-González, & Soriano-Santos, 2009) and *in vivo* (Fritz, Vecchi, Rinaldi, & Añón, 2011; Ramírez-Torres et al., 2017). This inhibition property could be used to control hypertension in human beings, since ACE-1 catalyzes the reaction to produce angiotensin II, a vasopressor peptide involved in the pathophysiology of hypertension (Joel, Sutopo, Prajitno, Su, & Hsu, 2018). Although the hydrolysis conditions with alcalase to obtain an amaranth hydrolyzate with potential to inhibit ACE-1 activity *in vitro* and to reduce blood pressure in hypertensive rats have been optimized (Ramírez-Torres et al., 2017), the potential antihypertensive effects of the hydrolyzate after its incorporation into a food matrix have not been evaluated *in vivo* yet. Thus, the aim of this study was to evaluate the bioavailability and the antihypertensive properties of an optimized amaranth hydrolyzate after its incorporation into a standardized cookie recipe. Additionally, the main technological characteristics of this food product were evaluated.

Abbreviations: ACE-1, angiotensin converting enzyme 1; IC50, half maximal inhibitory concentration

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2. Materials and methods

2.1. Animals and ethical aspects

Female Balb/c mice (4 weeks old, 20–25 g) and male spontaneously hypertensive rats (8 weeks old, 200–250 g) were used for bioavailability and antihypertensive evaluations, respectively. Male hypertensive rats were chosen because male rats develop higher blood pressure than female. (Reckelhoff, Zhang, & Srivastava, 2000). Female Balb/c mice were chosen because they were easier to handle than male. Rats and mice were placed in plastic cages with stainless steel caps. The room temperature was maintained at 28 °C with 12:12 h light-dark cycles. Diet (Rodent Lab Chow 5001) and water were available *ad libitum*. The experimental protocol was in accordance with the Mexican Official Standard 062 (NOM-062-ZOO-1999). The Ethics Committee of the Academic Unit of Nutrition Sciences of the Autonomous University of Sinaloa approved the study protocol (CE-UACNYG-2015-SEP-001).

2.2. Cookies formulation

Amaranth hydrolyzate was produced as previously described (Ramírez-Torres et al., 2017). Table 1 shows the ingredients used in the elaboration of both control (AOAC, 2000) and amaranth-hydrolyzate enriched cookies. The cookies dough was circular with 6 cm diameter and 0.7 cm height.

2.3. Technological evaluations

The expansion factor was calculated according to AACC method 10-50D (AOAC, 2000). Color was measured after cooling (2 h) (Color Spectrophotometer; CM-508d, Minolta Co., Ramsey, N.J., U.S.A). a* (from green: −60, to red: +60), b (from blue: −60, to yellow: +60), and L* values (luminosity, from black: 0, to white: 100) were recorded from 7 points of the cookies' central area. The texture was expressed as hardness and was measured as the maximum force (in Newtons) needed to break the cookie using a texture analyzer (TA-XT2; Texture Technology Corp., Scardale, NY).

2.4. IC50 evaluation

The IC50 was determined using an ACE-1 activity assay kit (ACE kit-WST-Dojindo Molecular Technologies, Inc.) following the manufacturer's instructions. The amaranth hydrolyzate's protein concentration was determined using the BCA assay (BCA assay, PierceTM Thermo Scientific, Rockford, IL, US).

Table 1
Dough ingredients and technological characteristics of the cookies.

		Control cookie	Enriched cookie (Amaranth hydrolyzate)	p value
Dough ingredients				
Wheat (*Triticum aestivum*) flour (medium force flour)		47.6%	45.3%	–
Hydrolyzate		0.0%	4.6%	–
Butter		13.5%	12.9%	–
Sugar		27.5%	26.2%	–
Salt		0.5%	0.5%	–
Sodium bicarbonate		0.5%	0.5%	–
Water		3.4%	3.2%	–
Dextrose		7.0%	6.6%	–
Technological characteristics of cookies (n = 6)				
Expansion factor		5.87 ± 0.03	5.89 ± 0.02	$p > 0.05$
Color	a*	3.12 ± 0.64	1.22 ± 0.79	$p < 0.05$
	b*	20.49 ± 0.45	19.51 ± 1.39	$p > 0.05$
	L*	63.8 ± 2.24	63.2 ± 2.18	$p > 0.05$
Hardness (N)		165.5 ± 18.3	119.6 ± 25.5	$p < 0.05$

2.5. Bioavailability assay

Fifteen mice were randomly assigned into three groups (n = 5 each). (1) Amaranth-hydrolyzate enriched cookie (2.4 g of hydrolyzate/kg of body weight; ~2 g of cookie per mice) (Ramírez-Torres et al., 2017); (2) ACE-I inhibitor (Captopril in water 25 mg/kg of body weight) (Ramírez-Torres et al., 2017); (3) Water group (300 µL). Captopril and water were administered intragastrically. The cookies were eaten freely after 12 h fasting. Blood samples were drawn from the tail vein before and after treatments (0, 5, 30, 60 and 120 min) and serum samples collected for further ACE-1 activity assays. For cookie ingestion, the animals were individually housed and 2gr of cookie were placed on the floor of the cage. A time period of 2 h was enough for the animals to eat the 2gr piece of cookie. The post-treatment evaluations started after this time period. All evaluations where performed in triplicate using 20 µL of serum as the source of ACE-1 inhibitory compounds.

2.6. Effect on blood pressure

Twenty-eight hypertensive rats were randomly assigned into four groups (n = 7 each). (1) Amaranth-hydrolyzate enriched cookie (10.0 g/animal, ensuring 1.2 g of hydrolyzate/Kg of body weight) (Fritz et al., 2011; Ramírez-Torres et al., 2017); (2) Control cookie (10.0 g/animal); (3) ACE-I inhibitor (Captopril in water 25 mg/kg body weight) (Isogai, Kameyama, Iso, & Yoshino, 1998; Zhou et al., 2014; Zhou, Yiming, Ma, Mamat, & Umar, 2015); (4) Water group. All treatments were administered as described above. Blood pressure was measured at: 0, 1, 2, 3, 4, 5, 6, and 7 h post-treatment (CODA tail cuff blood pressure measurement instrument; Kent Scientific, Torrington, CT, USA).

2.7. Statistical analysis

Analyses of variance were carried out using GraphPad Prism version 7.0 (Kruskal-Wallis and Dunn test). A p value < 0.05 was considered statistically significant.

3. Results

3.1. Technological characteristics.

The expansion factor was similar between cookies (Table 1). Among color parameters, the a* value was higher in control cookies than in amaranth-hydrolyzate enriched ones ($p < 0.05$) (Table 1). The hardness (texture) was higher in control cookies than in the amaranth-hydrolyzate enriched ones ($p < 0.05$) (Table 1).

3.2. IC50 determination

The IC50 value of the hydrolyzate was 0.0296 mg/mL (Fig. 1). The highest ACE-1 inhibition activity (99.0% inhibition) was reached at 4.86 mg/mL (Fig. 1).

3.3. Bioavailability evaluation

The serum samples from mice fed with the amaranth-hydrolyzate enriched cookies showed capacity to inhibit the ACE-1 activity from the first evaluation at time 5 min until the last evaluation at time 120 min ($p < 0.05$, compared to the water group) (Fig. 2). Similarly, the serum samples from mice treated with captopril showed capacity to inhibit the ACE-1 activity until the time of 60 min ($p < 0.05$, compared to the water group), but at the time 120 min the ACE-1 activity was similar to the activity found in the water group ($p > 0.05$) (Fig. 2).

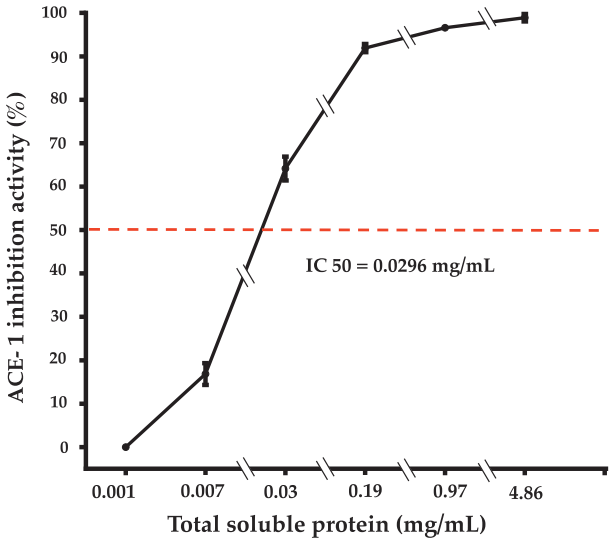

Fig. 1. IC50 determination of the optimized amaranth hydrolyzate.

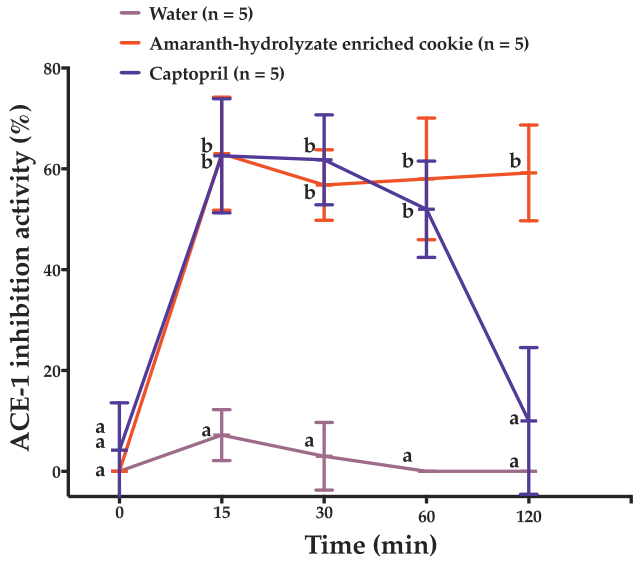

Fig. 2. Bioavailability of antihypertensive compounds present in the amaranth hydrolyzate measured as a percentage of ACE-I inhibition. Sera from mice treated with captopril (25 mg/kg body weight), amaranth-hydrolyzate enriched cookie (2 g; 1.2 g/kg body weight), and water (1.5 mL) are shown. Different letters mean statistical difference ($p < 0.05$).

3.4. Antihypertensive properties

Overall, after 3 h of started the treatments, the hypertensive rats fed with amaranth-hydrolyzate enriched cookies significantly reduced the blood pressure compared to the water and control cookie groups ($p < 0.05$) (Fig. 3). Amaranth-hydrolyzate enriched cookies and captopril showed similar antihypertensive effects ($p > 0.05$) (Fig. 3), which were sustained for 6 and 7 h, respectively ($p < 0.05$ compared to control cookie and water groups). However, captopril showed a more efficient antihypertensive effect than enriched cookies (Fig. 3).

4. Discussion

The incorporation of bioactive compounds into food matrices could alter both the bioavailability of the compounds and the technological characteristics of the final food product. In the present study, these two parameters were evaluated in a ready to eat baked product enriched

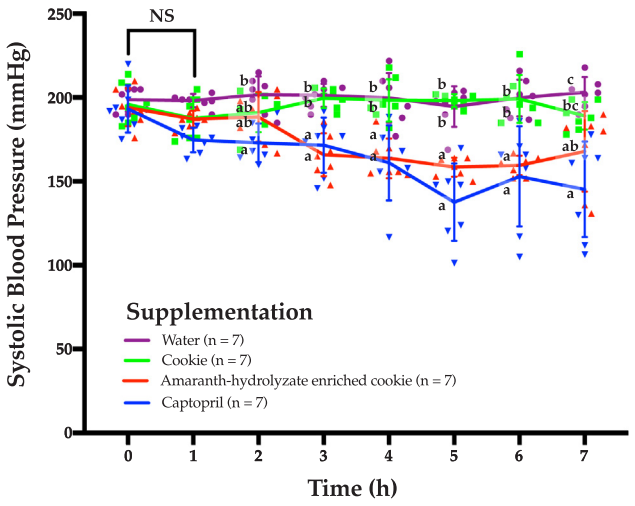

Fig. 3. Systolic blood pressure in spontaneously hypertensive rats after treatment with control cookie (10 g), water (1.5 mL), amaranth-hydrolyzate enriched cookie (10 g; 1.2 g of the hydrolyzate/kg body weight), and captopril (25 mg/kg body weight). Different letters mean statistical difference ($p < 0.05$). NS; **non-significant** difference.

with an optimized amaranth hydrolyzate with antihypertensive properties (Ramírez-Torres et al., 2017). The results show that the incorporation of the amaranth hydrolyzate into a standardized cookie recipe has no impact on the dimensions of the product in terms of diameter and height (Bhat, Hamdani, & Masoodi, 2018), as the product's expansion factor was not affected. Similarly, the enriched cookies' parameters b* and L* remained practically equal to the non-enriched ones. However, the a* parameter decreases indicating a brown color development. This can be attributed to Maillard reactions, which requires reducing sugars and free amino acids to take place (Morales & Jiménez-Pérez, 2001). The hydrolyzate utilized was obtained after an exhaustive digestion of amaranth proteins with alcalase and can be the source of free amino acids facilitating the Maillard reactions. The loss of hardness (texture) in the enriched cookies was expected as the addition of extra ingredients into a standardized cookie recipe can disrupt the gluten network normally constructed in baked food products (Dapčević Hadnađev, Torbica, & Hadnađev, 2013; Sarabhai, Indrani, Vijaykrishnaraj, Kumar, & Prabhasankar, 2015). Due to the loss of texture could impact on the overall acceptability of food products (Mudgil, Barak, & Khatkar, 2017), further sensory evaluations of the enriched cookies should be carried out and, if necessary, strategies for improving texture implemented.

The ACE-1 activity assays showed that the IC50 value of the amaranth hydrolyzate utilized in the present study was lower than values reported by others who utilized the same enzyme and source of protein, but less exhaustive hydrolysis conditions (Fritz et al., 2011). The main ACE-1 inhibitory peptides encompass from di to pentapeptides and exhaustive hydrolysis conditions can improve their production. In line with previous studies (Ramírez-Torres et al., 2017), our results demonstrate that mice serum samples collected after the oral administration of the hydrolyzate can inhibit the ACE-1 activity, but this time the hydrolyzate was incorporated into a cookie recipe and the mice freely ate the cookies. Based on *in vitro* studies (Grootaert et al., 2017), administering the hydrolyzate in a food matrix can help to maintain inhibited the ACE-1 activity for a longer period of time than administering the hydrolyzate alone. This is attributed to a reduced absorption of the antihypertensive compounds, but an increased absorption time of them (Grootaert et al., 2017).

Although the ACE-1 activity inhibition at serum level was loss after two hours of started the treatment with captopril, similar to previous studies (Duchin, Singhvi, Willard, Migdalof, & McKinstry, 1982), the *in*

vivo antihypertensive effects remained for at least seven hours. Captopril readily form reversible complexes with some plasma proteins, which can act as reservoirs of the drug and explain the short half-life of free captopril in serum (Duchin, McKinstry, Cohen, & Migdalof, 1988). Notably, the ingestion of the amaranth-hydrolyzate enriched cookies significantly reduced the blood pressure in hypertensive rats. Other studies have shown that amaranth cookies hydrolyzates obtained through simulated gastrointestinal digestion can inhibit ACE-1 *in vitro* yielding IC50 values around 0.23 mg/mL of protein (Sabbione et al., 2018). The present study demonstrates *in vivo* that the incorporation of an amaranth hydrolyzate with an IC50 value of 0.029 mg/mL of protein into a wheat flour-based cookie can reduce the blood pressure. Other wheat-based products hardly reduce the high blood pressure in spontaneously hypertensive rats without the incorporation of the hydrolyzate (Valdez-Meza et al., 2019). The antihypertensive effect of the enriched cookies started after two hours of their ingestion and was sustained for five hours. In previous studies, a similar trend was observed after the oral administration of the hydrolyzate alone (Ramírez-Torres et al., 2017). Importantly, the vehicle's baking process did not affect the *in vivo* antihypertensive properties of the amaranth hydrolyzate highlighting that the hydrolyzate could be utilized in a wide range of products, from beverages to baked goods. This helps to food technologists when selecting the vehicle that best suit the incorporation of the hydrolyzate in order to obtain acceptable sensorial characteristics. Overall, these findings support the use of an amaranth hydrolyzate with antihypertensive properties *in vivo* for functional foods development.

5. Conclusions

The incorporation of an amaranth hydrolyzate with antihypertensive properties into a standardized cookie recipe decreases the cookies' hardness. However, the amaranth-hydrolyzate enriched cookies efficiently reduce the systolic blood pressure in spontaneously hypertensive rats.

Ethics statements file

The experimental protocol was in accordance with the Mexican Official Standard 062 (NOM-062-ZOO-1999), the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The Ethics Committee of the Academic Unit of Nutrition Sciences of the Autonomous University of Sinaloa approved the study protocol (CE-UACNYG-2015-SEP-001).

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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