The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

Natural brewing peptides with enhanced inhibitory effect towards Angiotensin-Converting Enzyme

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

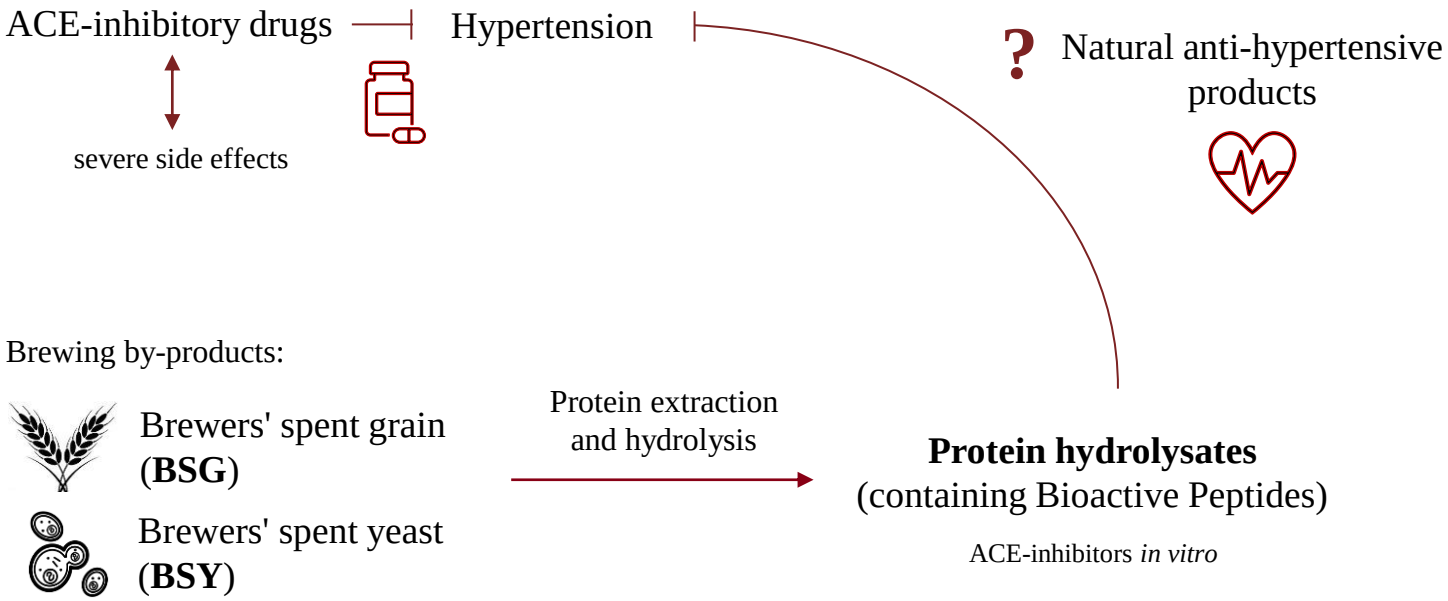
Rita Ribeiro-Oliveira [1,*], Zita E Martins [2], Miguel Ângelo Faria [2], Joana Beatriz Sousa [1], Isabel MPLVO Ferreira [2], and Carmen Diniz [1,*]

[1] LAQV/REQUIMTE, Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, 4050-313, Porto, Portugal
[2] LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, 4050-313, Porto, Portugal.

* Corresponding author: up201303483@edu.ff.up.pt (R.R-O.); cdiniz@ff.up.pt (C.D.)

U.PORTO FACULDADE DE FARMÁCIA UNIVERSIDADE DO PORTO requimte rede de química e tecnologia LAQV

Natural brewing peptides with enhanced inhibitory effect towards Angiotensin-Converting Enzyme



ACE = Angiotensin-converting enzyme

Abstract: Angiotensin-converting enzyme (ACE) inhibitors are anti-hypertensive drugs associated with several side effects. Natural compounds, namely bioactive peptides from brewing by-products – brewers' spent grain (BSG) and yeast (BSY) – are promising alternatives since they can inhibit ACE *in vitro* and are less likely to cause severe side effects while maintaining the therapeutic efficacy. However, the impact in peptides' bioavailability after oral administration have not been assessed so far. Thus, the aim of this study was to understand *in vitro* the impact of the oral route on the effectiveness of BSG/BSY peptides as ACE inhibitors. Extracted BSG/BSY proteins were hydrolysed and sequentially subjected to simulated gastrointestinal digestion (INFOGEST), intestinal absorption and liver metabolism (co-culture of Caco-2 and HepG2 cells). MTT assay was used to assess BSG/BSY peptides safeness. The ACE-inhibitory potential of initial and final products (BSY, BSG and a mixture 50:50 - MIX) at identical concentration (0,857mg/mL) was measured (fluorometric assay) and compared with Captopril (1µM, a clinically used ACE-inhibitory drug). Simulation of oral administration increased brewing peptides' ACE-inhibitory capacity. When comparing the final peptides with captopril, BSY demonstrated identical potency, while BSG showed 22% greater efficacy, and the new tested product MIX presented 30% higher inhibition. In conclusion, the current study shows that BSG, BSY and MIX natural peptides derived from the brewing industry enhance their bioactive properties as ACE-inhibitors after oral administration, validating the usefulness of these peptides to reduce the risk, ameliorate or treat primary hypertension.

Keywords: Bioactive peptides; Brewer's spent grain; Brewer's spent yeast; Hypertension.

Introduction

Currently, there is an emergent search for **natural compounds** with less side effects than the traditional **anti-hypertensive drugs**, such as **angiotensin-converting enzyme (ACE) inhibitors**, while maintaining the therapeutic efficacy.

Bioactive peptides from brewing by-products, brewers' spent grain (BSG) and yeast (BSY), are promising alternatives since they can inhibit ACE *in vitro*. However, the hypotensive potential reported so far does not take into account possible changes in the active peptides' bioavailability/metabolism after **oral administration**, which is the most common route of administration used to manage hypertension.

? Can oral administration modify the ACE-inhibitory effect of brewing peptides and thus, conditioning the therapeutic outcome?

? Does a mixture (MIX) of BSG and BSY peptides improve the bioactivity regarding the individual protein hydrolysates?

? Which brewing protein hydrolysates presents higher antihypertensive potential to be pursued as a putative supplement or nutraceutical or even as an anti-hypertensive drug?

Results and discussion

0.86 mg/mL was chosen as the most adequate concentration to pursue and accomplish the proposed goals

Cytotoxicity evaluation (MTT assay):

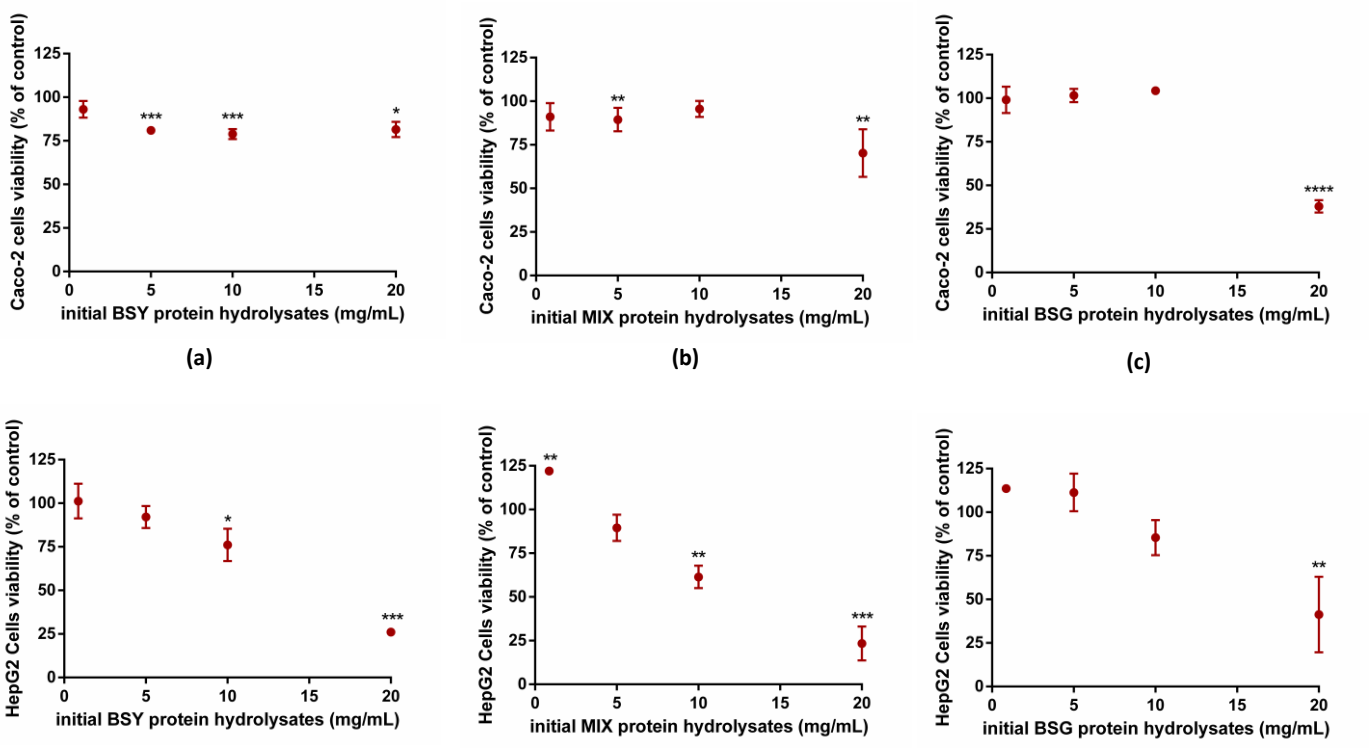


Figure 1. Caco-2 and HepG2 cell viability after incubation with brewing protein hydrolysates. Cells were exposed to (a) BSY; (b) MIX; (c) BSG, for 24 h. Results are presented as % of control (cells without treatment) and mean ± SD values of 3 independent experiments. Significant differences of cells viability from control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ (One-way ANOVA followed by post hoc Dunnett's multiple comparisons t-test).

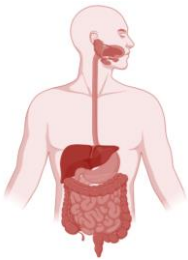
Results and discussion

The ACE-inhibitory capability of brewing protein hydrolysates is positively influenced by oral administration

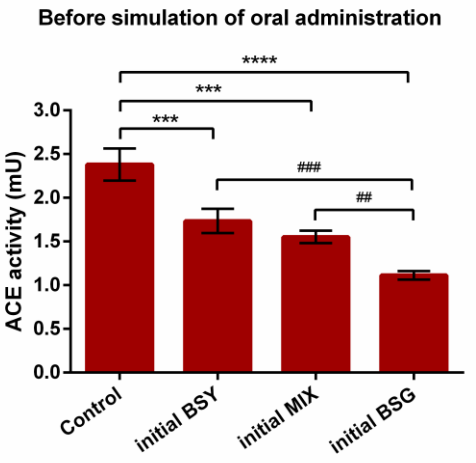
MIX and BSG protein hydrolysates display the best bioactivity

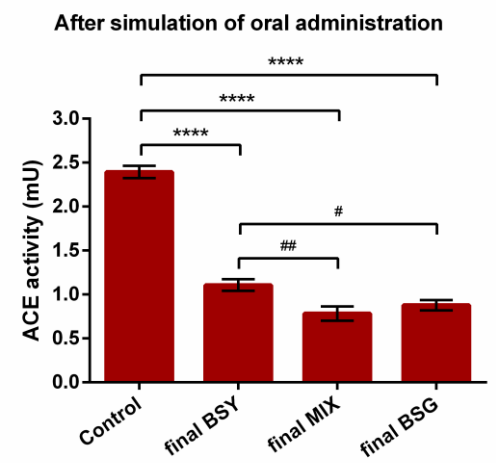
Impact of oral administration on ACE-inhibitory capacity:

oral route simulation



gastrointestinal digestion (INFOGEST) [1]
+
intestinal absorption + liver metabolism (co-culture of Caco-2 and HepG2 cells) [2,3]





[1] Brodkorb, A. *et al. Nat. Protoc.* **14**, 991–1014 (2019); [2] Choi, S. H. *et al. Mater. Sci. Eng. C* **24**, 333–339 (2004); [3] Hubatsch, I. *et al. Nat. Protoc.* **2**, 2111–2119 (2007).

Figure 2. Impact of natural peptides derived from brewing by-products on ACE activity. Effect of 0.87 mg/mL of initial and final BSG, BSY and MIX protein hydrolysates on ACE activity. ACE without treatment was used a control. Values are mean ± SD from 3 independent experiments (duplicates) in each group. Significant differences of ACE activity from control: *** $p < 0.001$ and **** $p < 0.0001$ (One-way ANOVA followed by post hoc Dunnett's multiple comparisons t-test). Significant differences of ACE activity were found between groups: # $p < 0.05$, ## $p < 0.01$ and ### < 0.001 (One-way ANOVA followed by post hoc Tukey's multiple comparisons t-test).

Results and discussion

Effectivity of brewing protein hydrolysates as potential antihypertensive compounds:

Compare with the effect elicited by **captopril** (at 1 µM dissolved in the same buffer as samples), a conventional anti-hypertensive drug that present ACE-inhibitory capacity

Table 1. ACE-inhibitory capacity exhibited by brewing protein hydrolysates compared to an anti-hypertensive drug

	ACE activity as % of captopril (1 µM)	
	Initial protein hydrolysates	**Final protein hydrolysates**
BSY	126.8±10.1 **	98.0±5.6
MIX	113.4±5.2	69.3±7.2 **
BSG	81.4±3.6 *	77.7±5.2 **

Influence of natural peptides derived from brewing by-products on ACE activity presented as % of captopril. Brewer's spent grain (BSG), brewer's spent yeast (BSY) and 50:50 mixture of BSY:BSG (MIX) protein hydrolysates before (initial) and after (final) simulated oral administration were tested at 0.86 mg/mL (assuming all hydrolysates were absorbed by Caco-2 cells). Significant differences of ACE activity against Captopril: * $p < 0.05$ and ** $p < 0.01$ (One-way ANOVA followed by post hoc Dunnett's multiple comparisons t-test).

Oral administration improves the ACE-inhibitory capacity of brewing protein hydrolysates

All final brewing protein hydrolysates were as good or more effective than captopril (1 µM)

MIX and BSG protein hydrolysates display the best bioactivity

Conclusions

- ✓ BSG, BSY and MIX protein hydrolysates suffer changes during oral administration enhancing their capacity as ACE-inhibitors.

- ✓ This study corroborates the usefulness of brewing peptides to reduce the risk, ameliorate or treat primary hypertension since the final products presented similar or higher ACE-inhibitory capacity than captopril.

- ✓ **BSG and MIX protein hydrolysates** seem to be the best brewing products to pursue in order to develop natural anti-hypertensive products that could be marked as food ingredients, supplements, nutraceuticals or even drugs.

Acknowledgments

This research was supported by FEDER funds and National Funds (FCT/MEC, Fundação para a Ciência e Tecnologia and Ministério da Educação e Ciência) PTDC/OCE-ETA/32567/2017 and UIDB/50006/2020.

Rita Ribeiro-Oliveira thanks the Portuguese Foundation for Science and Technology (FCT) for the Ph.D. grant SFRH/BD/146243/2019, funded by the European Social Fund of the European Union and national funds FCT/MCTES through the Norte's Regional Operational Programme.