**Supplementary Material**

**Article title: Broken rice as a potential functional ingredient with inhibitory activity of Renin and ACE**

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## MATERIALS AND METHODS

### Antihypertensive properties

#### *Preparation of* ACE *crude extract (ACEce) from rabbit lungs*

ACE crude extract (ACEce) was obtained from rabbit lungs donated by Cátedra de Producción Animal, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Argentina.

Lungs were obtained at healthy adult animal slaughter, immediately frozen in liquid nitrogen and stored at -80°C. Lungs were weighed and a cold borate buffer containing 0.25 mol/L sucrose and 20% glycerol w/v pH 8.3 (extraction buffer ACE) was added in a 1:3 solvent/tissue ratio. The protease inhibitor phenylmethylsulfonyl fluoride 0.1 mol/L was diluted in 2-propanol and added at a 1:1000 ratio to the extraction buffer. Lung pieces were kept over an ice-water bath and homogenized at maximum speed using an Ultraturrax (T-25 Janke & Kunkel, IKA Labortechnik, Staufen, Germany) and centrifuged in a Beckman-Coulter centrifuge at 5000 *g* for 10 min and 4°C. The supernatant extract was aliquoted and stored at -80°C until use.

#### *ACE inhibitory assay*

The inhibitory assay was done as it was described in the manuscript, but 25 µl of a dilution of ACEce instead of 25 µl of commercial ACE was used.

## RESULTS AND DISCUSSION

### *Biological activities: ACE inhibition capacity*

A comparative study was performed between the commercial enzyme and a crude extract obtained from rabbit lung (ACEce). The rice hydrolysate (RPH) was used as inhibitor. Once the behavior of both enzymatic sources was known, the inhibition studies carried out with the fractions separated by gel filtration and RP-HPLC were performed only using ACEce. Figure 1 shows inhibition curves testing both enzymes with RPH.

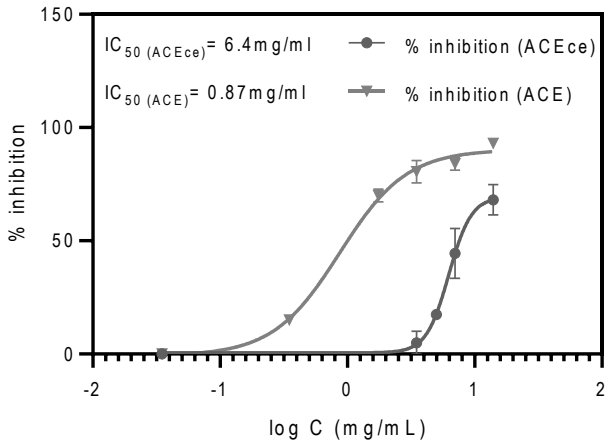


**Fig 1**. RPH $IC_{50}$ determination using ACE and ACEce. $IC_{50}$ values for both enzymes are inserted in the figure.

RPH inhibited ACE and ACEce in a dose-response manner ($IC_{50}$ =0.87 mg/ml, $IC_{50}$ = 6.2 mg/mL, respectively). However, the $IC_{50}$ value for ACEce was approximately 7 times higher than that obtained for commercial ACE. Both enzymes were tested in our research laboratory using captopril as inhibitor [1] and a behavior similar to RPH was found. ACEce enzymatic activity exhibited 60% of inhibition capacity with captopril (1.75 μM), compared to the commercial one at identical concentration conditions, commercial ACE reached 90-95% inhibition. This could be due to a different composition of the medium in which enzymes were originally found, while one is a commercial enzyme; the other is a crude extract. The presence of inhibitory substrates in the crude enzyme extract (ACEce) or the presence of different metabolites that would act as a barrier, hindering the arrival of peptides inhibitors/captopril to the active site, make their activities not comparable. Moreover, ACE inhibition may include interactions with inhibitors in sub-sites of the enzyme that are located outside the catalytic site [2]. A possible way to solve this problem could be to subject the ACEce to a sequenced purification. Considering these results, ACEce underestimate the inhibitory capacity due to an enzymatic protective behavior.

Inhibition percentages obtained using ACEce are not comparable between trials or with data reported by other authors since they underestimate the real value. However, these values are useful to screening different factions activity within the same assay performed with the same enzyme dilution.

**LITERATURE CITED**

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