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ACE-I INHIBITORY PEPTIDES FROM BOVINE HEART HYDROLYSATES

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I. INTRODUCTION

Meat industry by-products are an abundant and sustainable source of high-quality proteins and represent a valuable raw material for the development of functional food ingredients and nutraceuticals. Enzymatic hydrolysis is a widely accepted, environmentally friendly method for converting these proteins into bioactive hydrolysates, as it operates under mild conditions and eliminates the need for harsh chemicals or extreme temperatures [1]. This process facilitates the release of small peptides with demonstrated nutritional and functional potential [2]. Given these advantages, the aim of this study was to evaluate the effect of using two different enzymatic treatments on the generation of bioactive peptides from beef heart by-product, specifically peptides with angiotensin I-converting enzyme (ACE-I) inhibitory activity, which is an enzyme involved in antihypertensive mechanisms.

II. MATERIALS AND METHODS

Beef hearts were trimmed of adipose tissue and the aorta, then minced using a commercial meat grinder. Minced samples were mixed with water (1:1.5, w/v) and homogenised for 5 min using a masticator (IUL Instruments, Spain). The pH was adjusted to 6.0 prior to enzymatic treatments. PRX hydrolysate was prepared with 2% Protamex at 55 °C for 1 h, whereas PRX/PP hydrolysate was prepared with 2% Protamex at 55 °C for 1 h followed by 5 % Protana Prime for 12 h. Reactions were stopped by heating to 95 °C for 5 min and cooling in an ice bath. A control sample (C) was also prepared, without hydrolysis treatment. The degree of hydrolysis (DH) of the samples was determined according to the method described by Nielsen et al. [3]. The determination of FAAs content was carried out following the deproteinisation and derivatisation procedure described by Aristoy and Toldrá [4], and chromatographic analysis was performed according to Flores et al. [5]. The samples were also analysed by reversed-phase high-performance liquid chromatography (RP-HPLC) according to Gallego et al. [6], both for obtaining peptide profiles and for fractionation. Prior to this analysis, samples were deproteinised with ethanol (1:3, v/v), incubated overnight at 4 °C, centrifuged at 10,000 rpm for 10 min, and the supernatants were freeze-dried and reconstituted to 100 mg/mL for analysis. The ACE-I inhibition of the hydrolysates (10 mg/mL) and RP-HPLC fractions was determined according to Sentandreu and Toldrá [7]. Statistical analysis of the data was performed using OriginPro 2023b (OriginLab Corporation, Northampton, USA). One-way analysis of variance (ANOVA) and Fisher's honest significant difference (HSD) test was applied to assess statistical differences ($P < 0.05$).

III. RESULTS AND DISCUSSION

The DH achieved in each sample is presented in Figure 1A. Statistically significant differences were observed between hydrolysates, with the DH of PRX/PP being approximately 33 % higher than that of PRX. This result can be attributed to the combined action of Protamex (endopeptidase) and Protana Prime (exopeptidase), allowing for more thorough protein degradation. In fact, free amino acid profiles of the hydrolysates showed significant differences between the two enzymatic treatments. PRX/PP exhibited the highest concentration of free amino acids, with a total of 76.17 ± 3.64 mg/mL, compared to 5.567 ± 0.184 mg/mL in the PRX hydrolysate. In the PRX/PP sample, the most abundant amino acids were Glu and Leu, followed by Lys and Ala. In contrast, Gln and Leu were the most prevalent in the PRX hydrolysate, followed by Glu and Ala. However, as illustrated in Figure 1B, a higher DH does not necessarily correlate with increased biological activity. Hydrolysate PRX exhibited significantly higher ACE-inhibitory activity, with values by approximately 12% greater than those observed in sample PRX/PP.

The fractions obtained from RP-HPLC peptide separation revealed distinct patterns of ACE-inhibitory activity between the two hydrolysates. For the PRX sample (Figure 2A), the highest ACE-inhibitory activity was observed in fractions collected between 20 and 40 min, reaching 75.24 % inhibition. In contrast, the PRX/PP hydrolysate (Figure 2B) exhibited the highest ACE-inhibitory activity (58.21 %) in the early-eluting fractions, collected within the first 10 min, and containing more hydrophilic peptides. So, these results were due to differences in the composition and characteristics of the peptides, resulting from the enzymes used to obtain each of the hydrolysates.

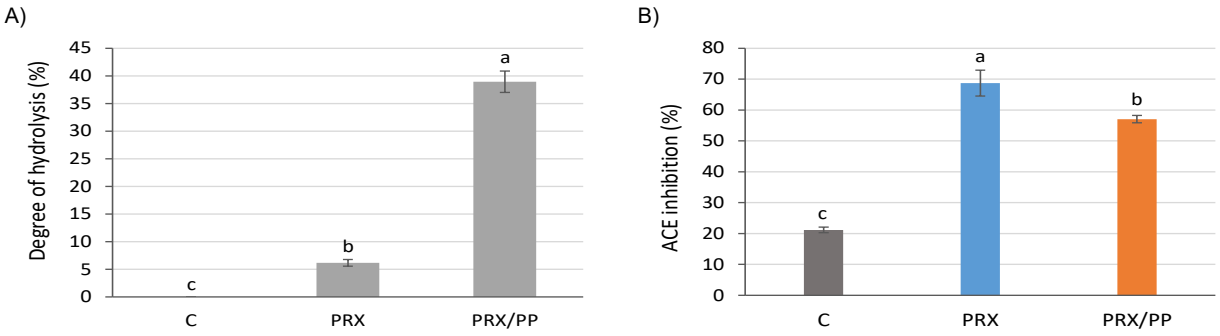


Figure 1. A) Degree of hydrolysis and B) ACE-I inhibitory activity of the samples.

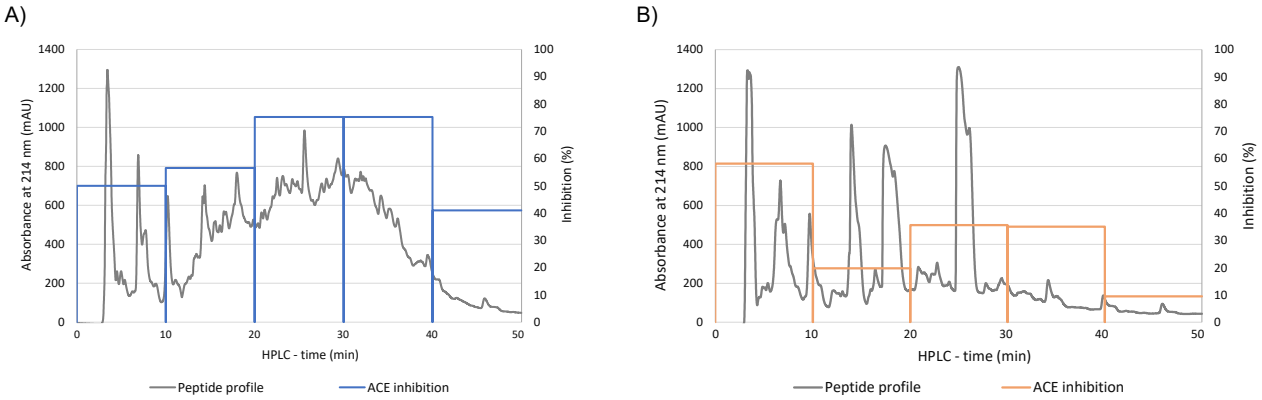


Figure 2. RP-HPLC profile and ACE-I inhibitory activities of the obtained fractions of A) PRX and B) PRX/PP hydrolysates.

IV. CONCLUSION

This study demonstrated that the combination of enzymes (PRX/PP) significantly enhanced the DH in beef heart hydrolysates as well as the release of free amino acids, but PRX exhibited superior ACE-I inhibition. RP-HPLC fractionation confirmed that PRX retained the most potent ACE-I inhibitory peptides in the mid-eluting range (20–40 min), while PRX/PP activity was concentrated in early fractions (0–10 min), containing more hydrophilic peptides. Despite the characterisation of the specific peptide sequences responsible for ACE-inhibition is necessary, these findings highlight the importance of enzyme selection and hydrolysis conditions in targeting specific bioactivities.

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