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IMPACT OF ULTRASOUND ON THE ENZYMATIC HYDROLYSIS OF BOVINE TRACHEAS TO GENERATE POTENTIAL ANTIDIABETIC PEPTIDES

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I. INTRODUCTION

Meat industry generates a large quantity of by-products during slaughtering and processing. These by-products are gaining attention as a promising source of protein hydrolysates rich in bioactive peptides, offering valuable opportunities for their valorisation and promoting sustainable utilization [1]. Enzymatic hydrolysis using commercial proteases remains a common method for obtaining protein hydrolysates, but ultrasound (US) technology has recently gained attention as a non-thermal and eco-friendly alternative. US is generally used to increase the extraction yields and achieve a higher degree of hydrolysis by enhancing the action of enzymes and thus the release of bioactive peptides [2], which opens doors to innovative applications. Given the current limited knowledge about the application of this technology on meat by-products, this work evaluates the effect of US on the obtention of enzymatic hydrolysates from bovine tracheas as well as on the generation of peptides capable of inhibiting α-glucosidase and dipeptidyl peptidase-IV (DPP-IV) enzymes and therefore with potential antidiabetic activity.

II. MATERIALS AND METHODS

Bovine tracheal cartilage was dried at 40 ºC and ground in a cutting mill SM 300 (Retsch GmbH, Haan, Germany). Tracheal cartilage (200 mg/mL) was subjected to different sequential hydrolysis using papain and alcalase (PAL), combination of papain and US followed by alcalase (PAUSAL), or US followed by papain and alcalase (USPAL). Hydrolyses were performed in a ball reactor (Radleys, Saffron Walden, UK), using 1% papain at 60ºC, pH 5.5 for 1 h, and 1% alcalase at 65ºC, pH 8 for 2 h. Pulsed US (20 KHz; 2 s on/ 4 s off, Ultrasonic processor VCX 500, Sonics & Materials Inc., Newtown, USA) was combined with papain for 1h, or applied for 15 min before papain treatment. A control sample (C, without hydrolysis) was also prepared. Enzymatic reactions were stopped at 95ºC for 5 min, centrifuged (10000x*g*, 10 min), and supernatants were freeze-dried. The degree of hydrolysis (DH) of the hydrolysates was evaluated following the OPA method [3]. Samples (200 mg/mL) were fractionated according to the molecular weight of peptides using centrifugal filters. The peptide profiles of the collected fractions (30–100 and <30 kDa) were obtained by reversed-phase high-performance liquid chromatography (RP-HPLC), using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, USA) and a Symmetry C18 column (5 um, 4.6 × 250 mm). Phase A was 0.1% trifluoroacetic acid (TFA) in water, phase B was 0.085% TFA in acetonitrile:water (60:40), and the gradient elution was 2% B for 5 min, and 2-45% B during 50 min at a flow rate of 1 mL/min. The peptide profile was monitored at 214 nm. Fractions (1 mg/mL) were also analysed for their potential antidiabetic activity. For that, the α-glucosidase inhibitory activity was determined by mixing 50 µL of sample with 25 µL of α-glucosidase (5 U/mL) and incubated at 37ºC for 5 min. Then, 25 µL p-NPG substrate was added and the absorbance at 405 nm was measured after 20 min incubation [4]. The DPP-IV inhibitory activity was determined using the fluorescence-based screening kit from Abcam Plc. (Cambridge, UK) according to manufacturer instructions. Results were expressed as percentage of enzyme inhibition and statistically analysed using ANOVA and Fisher's Least Significant Difference (LSD) tests ($p < 0.05$).

III. RESULTS AND DISCUSSION

Cavitation effects of US can induce changes in the structure and conformation of proteins, which could lead to their unfolding, denaturation, or partial hydrolysis [5]. Results showed around 7.5 % DH for all the hydrolysates, with no significant effect of US application favouring hydrolysis, neither in the case of a combined treatment of US and papain (PAUSAL), nor with the application of US prior to enzymatic action (USPAL). Nevertheless, when the hydrolysates were fractionated according to molecular weight, peptide profiles obtained by RP-HPLC evidenced differences in the fractions containing peptides of 30-100 KDa (Figure 1A). Sample PAL showed a higher content of more polar peptides (eluting from 10 to 20 min), whereas profiles of PAUSAL and USPAL presented minor differences. The peptide profiles of the samples < 30 KDa showed a similar pattern for all the hydrolysates, although the application of US would generate a slightly higher amount of small polar peptides (Figure 1B).

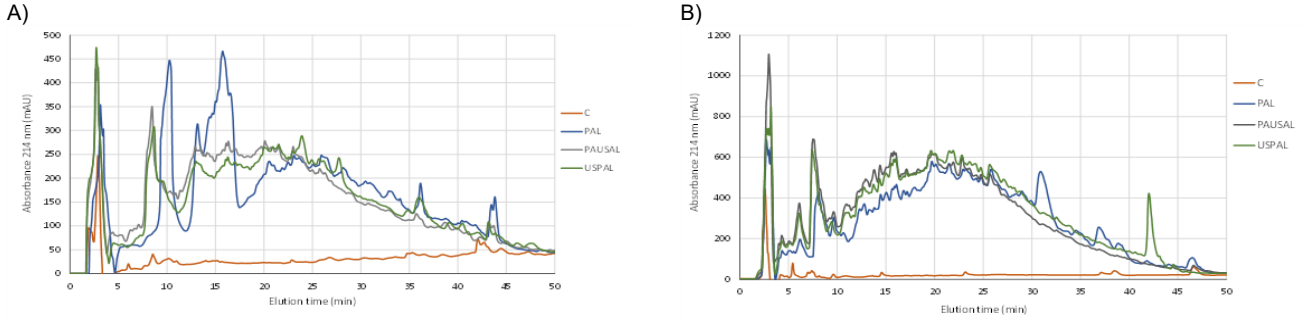


Figure 1. Peptide profiles obtained by RP-HPLC of fractions A) 30–100 KDa and B) <30 kDa of the tracheal hydrolysates.

As observed in Figure 2A, the α-glucosidase inhibitory activity increased by approximately 15 % in the hydrolysates compared to the control. US improved the generation of peptides < 30 KDa with α-glucosidase inhibition, with USPAL reaching values of 22.5 %. However, US had no significant effect on fractions 30-100 KDa, as all the hydrolysates presented values close to 20 % inhibition. On the other hand, low DPP-IV inhibitory activity was observed for all samples (Figure 2B). Fractions 30-100 KDa of the hydrolysates presented higher values than the control, while in fractions < 30 KDa, PAUSAL presented the highest activity (13.3 %) and PAL the lowest value (2.4 %), which suggests that the combination of US and papain would favour the generation of DPP-IV inhibitory peptides.

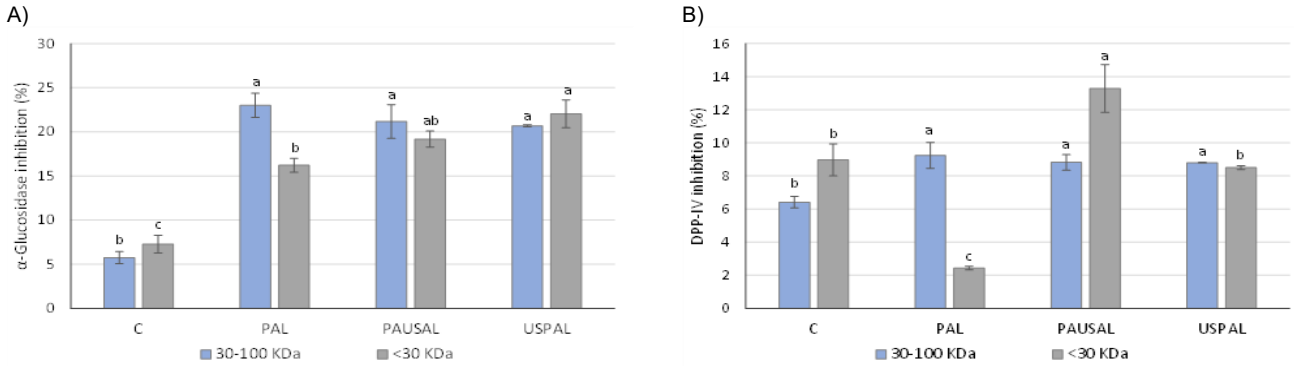


Figure 2. A) α-Glucosidase inhibition and B) DPP-IV inhibition of the fractionated tracheal hydrolysates.

IV. CONCLUSION

The results of this study evidenced the impact of US when combined with enzymatic hydrolysis to generate small peptides (< 30 KDa) from bovine tracheal cartilage. Both the combination of papain and US previous to hydrolysis with alcalase as well as the application of US prior to sequential hydrolysis generated peptides that presented inhibitory activity against α-glucosidase and DPP-IV. Additional studies are needed to identify the peptide sequences responsible for the observed bioactivity, but these results demonstrated the antidiabetic potential of this by-product.

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