Antioxidative and antihypertensive peptides in Iberian dry-cured ham

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Abstract— **The aim of the work was to isolate peptides with antioxidative and antihypertensive effect from Iberian ham generated during the process. Extracts from dry cured (n=5) and Iberian dry cured hams (n=5) were obtained and the antioxidative and antihypertensive effect was determined by the Metal Chelating Assay and the ACE-Inhibition Test, respectively. The chelating effect percentage of extracts from Iberian hams were significantly higher (P < 0.05) than those of extracts from dry cured hams (66% vs 54%) while no significant differences were found between groups in the antihypertensive effect.**

Keywords— **Iberian ham, antioxidative peptide, antihypertensive peptide**

I. INTRODUCTION

Iberian dry-cured ham is a traditional food product with strong presence in markets in the Mediterranean area and represents an enormous economic importance for the industry in this area. Despite of the great palatability of this product, other considerations such as aspects related to health and wellbeing are increasingly important factors in consumer decisions. In this sense, consumer concerns related to this product are the quality of the fat and the presence of sodium [1], [2]. Some epidemiological and human intervention studies indicate that eating ham can produce beneficial effects with regard to cardiovascular disorders, however more scientific evidence is needed in order to assess the function that ham can perform in relation to health [3].

It is known that meat proteins are a common source of bioactive peptides [4]. Proteolytic degradation of proteins is one of the most important phenomena occurring during the long process of Iberian dry cured ham [5]. Studies on the final products of proteolysis in ham have approved various low and medium weight peptides, oligopeptides and free amino acids [6]. However, there are no investigations about the functionality and bioactivity of these compounds in this traditional product. In this sense, low molecular weight compounds with antioxidant and antihypertensive effect have been isolated in protein extracts from cured fermented sausages [7], [8]. Therefore, studies proving the presence of antioxidative and antihypertensive peptides in dry cured Iberian ham are needed to demonstrate that this traditional product may be healthy and could be included as a regular component of the diet.

II. MATERIAL AND METHODS

A. Samples

This study was carried out using samples of Iberian dry-cured ham (n=5) and dry-cured ham (n=5) which were purchased in a local supermarket. Samples were minced, vacuum packed, and stored at -80 °C until analysis.

B. Methods

Low molecular weight (LMW) (< 3kDa) compounds from ham were extracted following the method developed by Bauchart et al. [9], with some modifications. 2.5 g of sample were homogenized in 12.5 ml of perchloric acid (50 ml/l) in centrifuge tubes for 2 min on ice. The homogenate was centrifuged at 10.000 g for 12 min at 4 °C and the supernatant was collected and filtered using a cellulose acetate filter of 0.2 μm pore size. The extracts were then neutralized (pH 7.0) using potassium hydroxide (KOH). The resulting salt was eliminated using a cellulose acetate filter of 0.1 μm size pore filter. The supernatant was submitted to ultrafiltration with 3 kDa cut-off at 4000 g for 30 min. The ham extracts were finally lyophilized and stored at – 20 °C until analysis. Freeze-dried extracts were dissolved in miliQ water in order to determine the antioxidant and antihypertensive activities.

Metal chelating assay (MQA) of the extract was determined using a ferrous ion chelating assay [10]. A 800 µl test sample (10 mg/ml ham extract) was added with 10 µl of 2 mmol/l $FeCl_2$ and 20 µL of 5 mmol L-1 ferrozine. The mixture was vortexed and kept at room temperature for 10 min prior to measuring absorbance at 562 nm. Chelating effect was calculated as follows:

Chelating effect (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$,

where A_{sample} is the absorbance of the test sample and Acontrol is the absorbance of distilled water

The ACE-inhibitory activity of the samples was measured using the spectrophotometric assay of Ong y Shah [11], with some modifications. Each assay mixture contained 200 ml HHL solution (3.8 mM HHL, 0,01 M sodium borate buffer, 0,3 M NaCl, pH 8.3), 2 mU ACE and 52,5 µl sample solution (10 mg in 1 ml of distilled water). After 30 min of incubation at 37ºC, the hippuric acid was extracted with 1.7 ml ethyl acetate. The mixture was centrifuged and 1.5 ml of the organic phase (ethyl acetate) was transferred to a fresh test tube and evaporated to dryness on a water bath for 15 min at 100ºC. The residue containing hippuric acid was dissolved in 1 ml deionised water and the solution was measured using a visible spectrophotometer at 228 nm against deionised water as a blank. The percentage inhibition was calculated as follows: ACE-inhibition (%) = [1- (A - C) / (B - D)] x 100, where A is the absorbance with ACE, HHL and ACE-inhibitory sample, B is the absorbance with ACE and HHL without ACE-inhibitory sample, C is the absorbance with HHL and ACE-inhibitory sample without ACE and D is the absorbance with HHL without ACE and ACE-inhibitory sample.

Results were analysed using an ANOVA test using the GLM procedure of SPSS 15.0 (SPSS Institute Inc., Cary, NC). The level of significance was set to $P<0.05$.

III. RESULTS AND DISCUSSION

Values obtained for the antioxidative and antihypertensive effects from ham extracts are showed in table 1.

Table 1. Chelating effect and ACE-inhibition percentages of dry-cured and Iberian dry-cured ham extracts (n=5)

	Chelating effect (%)	**ACE-inhibition (%)**
Ham	54,43±1,26	27,89±15,94
Iberian ham	66,20±3,01	24,29±10,06
$\mathbf{P_{batch}}$	P<0,05	ns

Results are expressed as means ± standard deviations.

Antioxidant activity expressed as chelating effect was found in ham extracts (from 54 to 66 %), being significant higher in Iberian ham samples (P<0,05). Other authors have also demonstrated antioxidant effect of protein extracts from another traditional product such as pork fermented sausage after ripening [7], [8], [12]. They suggested that this antioxidant activity in sausage extracts could be ascribed to the action of proteolysis products that are formed during manufacturing. In this sense, myofibrillar and sarcoplasmic proteins from cured ham are degraded during the process, with the consequent formation of small peptides and free amino acids (FAAs) [6], that could be the responsible of the antioxidant properties. In relation to this, other studies have also demonstrated the relationship between antioxidant effect and the levels of FAAs and small peptides of protein hydrolysates [13]. On the other hand, the higher antioxidant activity found in extracts from Iberian cured ham compared to the one found in cured ham could be caused by the long ripening period occurring in Iberian ham (more than two years) in comparison to the other type of ham (6-8 months). Therefore, this long process could favour proteolysis and the generation of antioxidative low molecular compounds in Iberian ham.

When antihypertensive effect was studied, similar percentage of ACE-inhibition was found in protein extracts of both ham samples, cured and Iberian cured hams (table 1). Vaštag et al. [7] showed higher values of ACE-inhibition activity in extracts of fermented sausages after 90 days of ripening (70%, approximately). It seems that the long ripening period occurring in ham could cause an extensive degradation of peptides released from meat proteins, therefore increasing FAAs and very small peptides with no

antihypertensive activity. These results are in agreement with other ones showing that the level of free amino acids and very small peptides increases during ripening [6], [14]. On the other hand, this could be also the reason why no differences between batches were found.

IV. CONCLUSIONS

Particular traditional process of Iberian dry-cured ham causes higher antioxidative activity in this product in comparison to dry-cured ham due to the generation of a greater quantity of low molecular weight compounds.

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