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Salt And Proteolysis Regulate Potential Antioxidant And Ace-Inhibitory Activities Of Parma Ham (#606)

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Introduction

Some peptides generated during dry-cured ham maturation displayed an Angiotensin-I Converting Enzyme (ACE) inhibitory activity in vitro and anti-hypertensive properties in vivo. ACE enzyme is one of the major regulators of blood pressure. Antioxidant activities have been also demonstrated for specific peptides naturally released during meat processing. A considerable amount of peptides and free amino acids are generated by means of the proteolytic process during Parma ham maturation. Proteolysis is influenced by meat quality (e.g. pH, enzymes activities, muscle type), and by processing parameters (e.g. salt amount, time of ripening).

The aim of this work is to evaluate in vitro the DPPH antioxidant and ACE-inhibitory activities of Parma hams differing for salt content, proteolysis degree, peptide profile, and free amino acids content.

Methods

Twenty 19 months-old Parma hams, provided by one manufacturing plant were deboned to remove biceps femoris muscle. Samples were stored under vacuum at freezing temperature until analysis.

Chloride content was measured according to *ISO 1841-2 (1996)*, using Titrando 809 (Metrohm Ltd Herisau, Switzerland) and expressed as g NaCl/100g sample. Proteolysis index (PI) was calculated as the ratio between non-protein nitrogen content after precipitation with 5% of trichloroacetic acid and total nitrogen content *(Careri et al. J. Food Sci. 1993, 158, 968-972)*. Total free amino acids (FAA) were determined through extraction with HCl 0,1 N, precipitation of proteins with ethanol *(Zhu et al. Meat Sci. 2014, 96,783-789)*, and spectrophotometric measure after derivatisation with o-Phthalaldehyde *(Panasiuk et al. Food Chem. 1998, 62,363-367)*. The same extracts were analysed for DPPH radical scavenging activity *(Lewin & Popov. J. Bioch. Bioph. Methods. 1994, 28, 277-282)* and for ACE-inhibitory activity (*Nejati et al. Food Sci. Tech. 2013, 51, 183-189)*, using FAPGG (N-[3-(2-Furyl) acryloyl]-phe-Gly-Gly) as substrate and Captopril® as reference inhibitor. Peptide fractions (MW < 10 kDa) were analysed using UHPLC–ESI-MS (*Paolella et al. Food Res. Int. 2015, 67, 136-144)*.

Descriptive statistics and correlations (Pearson's correlation coefficient) were performed using SPSS Statistics vers.21.0.

Results

The analytical parameters of Parma hams are shown in Table 1, correlation coefficients are reported in Tables 2 and 3.

Peptide fractions displayed a noticeable variability. DPPH is positively related to PI, FAA, and peptides except the fraction with 4000 Da < MW < 10 kDa. Peptides with 400 Da < MW < 4000 Da showed a positive relationship with ACE-inhibitory activity. The highest correlation coefficients were obtained for peptides with MW < 1000 Da, MW < 4000 Da, and FAA. A protracted maturation time, enabling the release of peptide fraction with MW < 400 Da or FAA could result in a decrease of ACE-inhibitory activity. According to previous studies on Parma ham, the generation of assayed proteolysis markers is negatively influenced by the salt content (Table 3).

Therefore, the antioxidant and ACE-inhibitory activities in dry cured hams with low salt content could be enhanced.Table 1. Summary statistics of assayed parameters

		Min.	Max.	Mean	CV%
NaCl	g/100 g	4.36	7.89	5.58	20.6
PI	%	27.8	39.0	32.5	10.7
MW < 10 kDA	area peptide/area internal standard Phe-Phe	nd	1.65	0.97	50.5
MW < 4000 Da	0.29	2.44	1.42	43.7	
MW < 1000 Da	0.15	1.59	0.62	59.7	
MW < 400 Da	5.98	16.0	9.84	28.6	
FAA	mmol Leucine Eq./g	0.29	0.51	0.40	15.0
DPPH[a]	mmol Trolox Eq./g	1.29	1.86	1.63	9.8
ACE[b]	% inhibition/Captopril®	2.22	159.3	77.9	54.8

[a]. DPPH radical scavenging (antioxidant) activity

[b]. ACE inhibitory activity is corrected according to the activity of the reference Captopril® ($5x10^{9}$M), to minimize the different activity of the substrate

nd: not detectedTable 2. Correlation coefficient between DPPH and ACE-inhibitory activities and proteolysis markers

		Peptide fractions, MW				
	PI	< 10 kDA	< 4000 Da	< 1000 Da	< 400 Da	FAA
DPPH	**0.69**	ns	**0.72**	**0.76**	**0.58**	**0.78**
ACE	0.52	ns	**0.70**	**0.65**	ns	0.51

Italic = P<0.05; bold = P<0.01; ns = not significantTable 3. Correlation coefficient between salt content and proteolysis markers

		Peptide fractions, MW				
	PI	< 10 kDa	< 4000 Da	< 1000 Da	< 400 Da	FAA
NaCl	**- 0.68**	- 0.51	**- 0.69**	**- 0.58**	- 0.49	**- 0.66**

Italic = P<0.05; bold = P<0.01; ns = not significant

Notes

Conclusion

Salt is confirmed as a key factor regulating proteolysis phenomena in dry-cured ham. Salt reduction could positively contribute to the antioxidant and antihypertensive properties of the proteolysis products. The obtained results could provide new markers for nutritional quality of Parma ham.

Acknowledgement

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Notes