ANTIOXIDANT AND SENSORY PROPERTIES OF ITALIAN TYPICAL DRY-CURED HAM AS RELATED TO SALT AND PROTEOLYSIS VALUES

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Abstract – In this study, a group of 19-month old Italian typical dry-cured hams were taken from a batch processed with two levels of salt addition (standard and reduced), to obtain a wide variability in the proteolysis degree. Nine samples were chosen to fit three possible proteolysis levels, named low, intermediate and high, respectively. Samples belonging to the low proteolysis group corresponded to standard salt (NaCl=6.6% in whole slice), while intermediate and high proteolysis samples derived from reduced salt (NaCl=4.2% in whole slice) level. Two radical probe tests were applied, namely DPPH and FRAP, to assess the antioxidant activity stemming from the interaction between the freeze-dried dry-cured ham samples and a solution of coloured radical probe. An increase in DPPH was found in dry-cured hams with intermediate and high proteolysis, while no variation in FRAP occurred in connection to proteolysis level. Samples with reduced salt and intermediate proteolysis level met nutritional improvement and sensory requirements.

Key Words – meat products, protein breakdown, DPPH

I. INTRODUCTION

Many biochemical changes take place during the processing of Italian dry cured ham leading to the unique characteristics of the product. Among them, proteolysis process, resulting in the dynamic generation of peptides and free amino acids, is affected by meat traits, processing conditions and salt amount [1, 2]. Due to the strong relationship between high intake of dietary salt (sodium) and serious health problems, salt reduction is an on-going process within dry-cured ham industry [3, 4]. Salt, which is a multifunctional ingredient in dry-cured ham elaboration, has a key role for both quality and safety. NaCl content affects proteolytic activity and, therefore, texture and flavour of final outcome. However, proteolysis could lead to the accumulation of biologically active compounds; in the last decade, several studies ascribed antioxidant properties to identified proteolysis products of dry-cured ham [5, 6].

The aim of the present study is to investigate antioxidant capacity and sensory profile of typical Italian dry-cured hams, processed with different salt additions to induce an enhanced variability in final proteolysis.

II. MATERIALS AND METHODS

A batch of 60 fresh hams were salted with conventional and reduced salt amounts [7]: processed hams came from the same slaughter house and slaughtering session, fell within a narrow range of weight (12.9 - 13.9 kg) and pH_{24h} (5.55 - 5.90) in *semimembranosus* muscle. After 19-months processing time, deboned dry-cured hams were cut at the knee level to obtain two sections (Fig.1), which were vacuum packaged and kept under refrigeration until analysis; both the *biceps femoris* muscle (BF), and the whole slice deprived of the external fat, underwent analyses. Proteolysis index (PI), was measured as percent non-protein soluble nitrogen after treatment of the aqueous extract with 10% trichloracetic acid [8]. PI values from BF were distributed into terziles (three groups with the same abundance), to define the low, intermediate and high proteolysis levels within the batch. Next, three dry-cured hams for each PI level, were randomly selected for further analyses. Moisture, protein [9], and NaCl, obtained by chloride potentiometric titration of the aqueous extract with Titrando 809 Metrohm Ltd (Herisau, Switzerland), were expressed in grams per 100 grams wet samples.

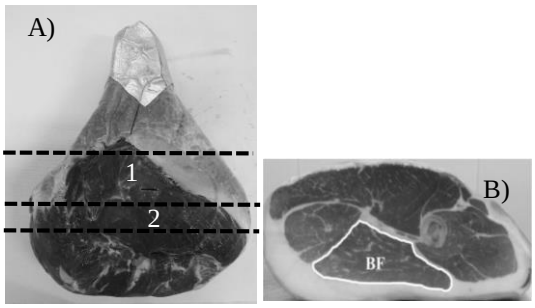

Figure 1. Ham sampling. A) portions 1 and 2 were used for PI, basic and sensory analyses, and antioxidant activities, respectively. B) highlighted section of *biceps femoris* muscle.

Antioxidant activities were measured on freeze-dried meat samples, according to the Quencher procedure [10]. Ten (± 1.0) mg of samples were tested with DPPH· solution (2, 2-diphenyl-1-picrylhydrazil 0.1mM) in water/methanol (50:50, v/v) mixture and with a FRAP reagent (10 mM TPTZ in 40 mM HCl /20mM $FeCl_3 \cdot 6H_2O$/0.3M acetate buffer, pH 3.6, 1:1:10, v/v/v), colored radicals. The measurements were performed at 517 and 593 nm, respectively, after 60, 90 and 120 min at room temperature, in the dark. The antioxidant activities were expressed as millimoles Trolox equivalent Antioxidant Capacity (TEAC) and Antioxidant Power (TEAP) per kilogram dry weight, by means of a dose-response curve for Trolox, using equations (1a), (1b), (2).

$$Inhibition(\%) = (Abs_b - Abs_s)/Abs_b \text{x} 100 \quad (1a)$$

$$TEAC_{DPPH}\left(\frac{mmol\ Trolox\ Eq.}{kg\ dry\ weight}\right) = \frac{\%\ inhibition}{s*w} \text{x} 10 \quad (1b)$$

$$TEAP_{FRAP}\left(\frac{mmol\ Trolox\ Eq.}{kg\ dry\ weight}\right) = \frac{Abs\ (593nm)}{s*w} \text{x} 10 \quad (2)$$

Abs_s and Abs_b were the absorbances of the radical solution with and without the sample, *s* was the slope of Trolox calibration curve for DPPH and FRAP, *w* was the weight (mg) of freeze-dried sample, 10 is the multiplying factor to express the activity as mmol Trolox Eq./ Kg of dry sample.

Assayed dry-cured hams were sensory evaluated by an eight-member panel: visual appearance (redness, colour homogeneity and brightness), texture (hardness and pastiness), odour and taste (cured, off-tastes, off-odours and saltiness) attributes were scored using a non-structured 0-9 intensity scale (0 = not detected; 9 = maximum perception). The sensory ratings were averaged over the panel and the means from twice-replicated sessions were calculated [11].

Data analysis was performed by SPSS package ver. 21.0 (SPSS inc., Chicago, USA). 'Frequency' and 'Select cases' procedures were run for PI percentile distribution and random selection of dry-cured hams, respectively. 'One-way analysis of variance (ANOVA)' and 'Least Significant Difference (LSD)' procedures were used to compare data from BF and whole slice, and between groups originating from PI levels.

III. RESULTS AND DISCUSSION

Matured dry-cured hams showed the expected variation in PI (mean value $_{BF}$ = 32.5, std. dev. = 2.9): the ranges in terziles were 28-30 (low), 31-34 (intermediate), and 35-39 (high). Nine dry-cured hams (three samples / PI level $_{BF}$ were randomly selected), were assayed for salt and moisture content (Table 1), confirming salt amount as a major source of PI variation.

BF had a higher PI (32.7 ± 3.8) than the whole slice (28.4 ± 2.9), ($P<0.05$). The intermediate and high PI values were associated with a reduced salt content both in BF and in slice, even if PI variability in the latter is less pronounced (Table 1).

The tested PI has been previously reported as positively related to free amino acids and to some peptides in the range 200-8500 Da [1]. Recent studies demonstrated that several proteolysis products of dry-cured ham revealed an antioxidant activity against radical probes including DPPH and FRAP. Some peptides and amino acids have an antioxidant action due to their ability to scavenge free radicals and chelate pro-oxidative metals [5, 6]. In this respect, salt reduction in dry-cured ham could be a tool for increasing the occurrence of antioxidant molecules deriving from proteolysis. TEAC of the freeze-dried samples from dry-cured hams, determined by DPPH· radical probe, were presented in Fig. 2 and expressed as mmoles of Trolox / Kg dry weight. Three incubations of 60, 90 and 120 minutes were compared to evaluate the

time required to allow the reaction between soluble active molecules and the radical solution. The TEAC values obtained in the DPPH assay increased up to 120 minute end-point (Fig. 2).

Table 1. Mean values ± std. dev. for moisture and salt (g/100 g wet sample), of *biceps femoris* muscle (BF) and whole slice of selected dry-cured hams as related to Proteolysis Index (PI) level.

	PI level		
	Low	Intermediate	High
BF			
PI	**28.7 ± 0.8**	**32.1 ± 0.8**	**37.2 ± 1.7**
Moisture	57.5 ± 1.1	57.9 ± 1.2	57.8 ± 1.2
NaCl	7.52 ± 0.33	4.78 ± 0.09	4.54 ± 0.18
Slice			
PI	**25.5 ± 0.6**	**28.9 ± 3.2**	**30.7 ± 1.5**
Moisture	51.5 ± 0.31	52.8 ± 1.7	53.1 ± 2.0
NaCl	6.62 ± 0.38	4.33 ± 0.05	4.16 ± 0.09

The DPPH· scavenging capacity was higher in BF (11.6 mmol Trolox Eq./Kg d.w.) than in the whole slice (10.7 mmol Trolox Eq./Kg d.w) ($P<0.05$), in agreement with the higher PI detected in BF. In both cases, TEAC values increased ($P<0.05$) according to PI levels (Fig. 2A, B); due to the negative relationship between PI and salt, reduced amounts of NaCl in dry-cured hams were associated to an enhanced TEAC.

FRAP test was completed within 90 min [10], but variations in FRAP values were irrespective of PI levels (data not reported). FRAP assay is based on the reduction of ferric ion (as Fe^{3+}-TPTZ complex) to ferrous form at low pH, conditions differing from those occurring in dry-cured ham muscles (pH ≈ 6.0).

PI increase and salt reduction, in dry-cured hams obtained from established processing conditions, could be positive traits from the nutritional point of view; on the other hand, sensory properties could be affected by inappropriate PI and salt levels [11, 12]. Dry-cured hams assayed for TEAC and TEAP underwent descriptive sensory analysis to check for drawbacks in sensory profile attributable to PI and salt variations.

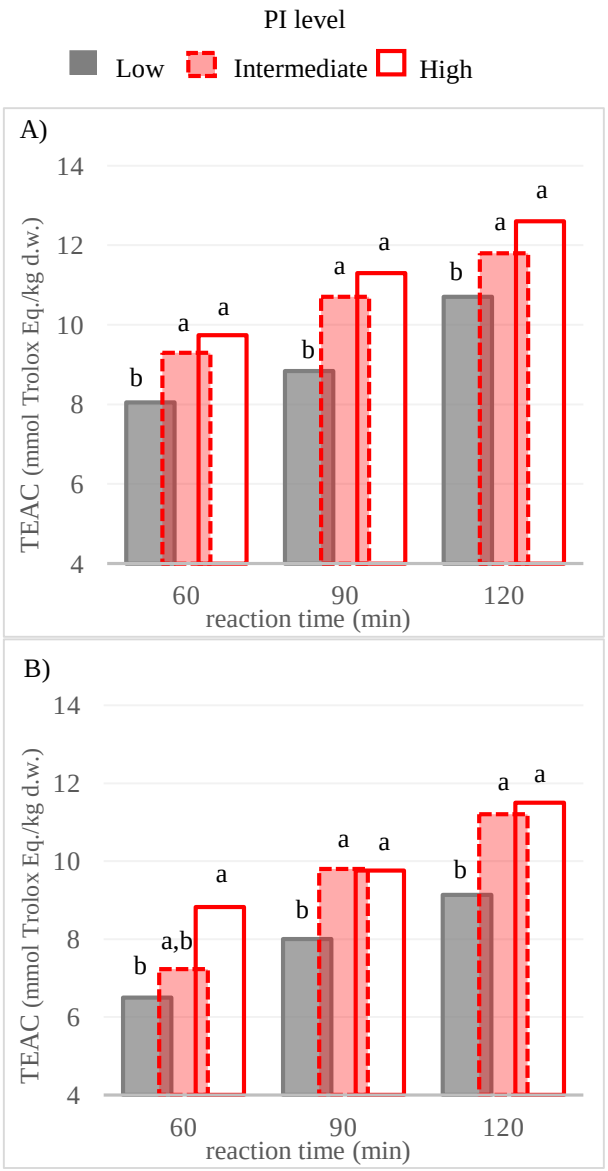

Figure 2. TEAC of *biceps femoris* muscle (A) and whole slice (B) from dry-cured ham as related to Proteolysis index (PI) level. Within each reaction time, different letter denote significant difference between PI levels ($P<0.05$).

Mean ratings for sensory descriptors are shown in Fig. 3. As expected, "pastiness" score increased in dry-cured hams with high PI and reduced salt, while "saltiness" and "hardness" were more perceived in low PI and standard salt samples. The profile of dry-cured hams with intermediate PI and reduced salt is promising from the nutritional and sensory point of view.

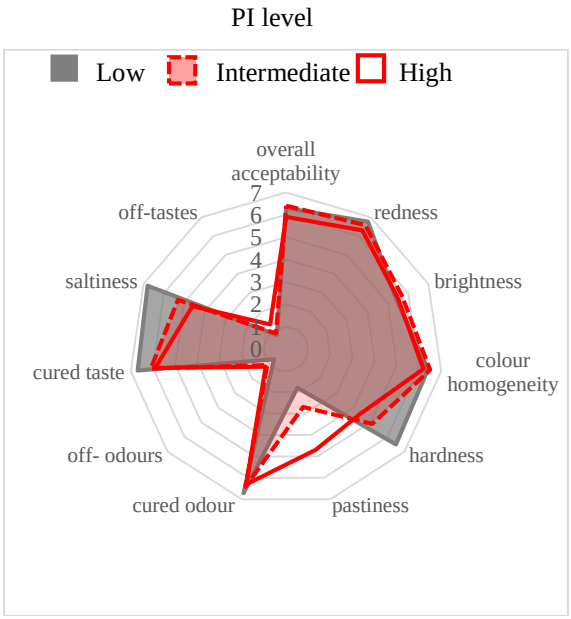

Figure 3. Sensory profile (mean scores) for dry cured ham as related to Proteolysis Index (PI) level.

IV. CONCLUSION

The study confirmed the relationship between salt and proteolysis in dry-cured hams derived from similar processing conditions (raw matter, manufacturing practices and duration). Through proteolysis enhancement, the reduced salt content promoted an increase in DPPH antioxidant activity, attributable to some free amino acids and peptides to be identified. Further research is in progress to manage proteolysis pattern toward antioxidant compounds generation preserving the sensory quality of dry-cured ham.

ACKNOWLEDGEMENTS

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