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ZINC-PROTOPORPHYRIN DOES NOT AFFECT *RED RING* OCCURRENCE IN DRY-CURED HAM

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I. INTRODUCTION

The main pigments in dry-cured hams are nitrosyl heme (NO-H) and zinc-protoporphyrin (ZnPP). The former is formed as a consequence of the reaction of nitric oxide (derived from added nitrite) with myoglobin and hemoglobin [1]. The latter is typically formed in uncured dry hams, such as Parma hams, likely due to the endogenous enzymatic activity of the ferrochelatase which takes part in the process of the iron removal from the porphyrin ring to form protoporphyrin IX (PPIX) and subsequent zinc ion insertion to form ZnPP. A defect commonly found in dry cured hams is the so called “red ring” that occurs when the outer part of the ham develops a colour which is visually different to the colour in the centre, with almost no transition zone between both areas (Figure 1). This defect is attributed to an insufficient formation of the NO-H in the centre and it is favoured by meat pH below 5.60. This pH range is also optimal for the formation of ZnPP, which competes with the formation of NO-H [2, 3]. The objective of this work was to study the evolution of pigments to evaluate whether the formation of ZnPP affects the occurrence of this defect.



Figure 1: Red ring in a dry-cured ham

II. MATERIALS AND METHODS

Manufacture of hams

A total of 18 hams obtained from 9 commercial carcasses were selected from a local slaughterhouse at 24 h post-mortem (12 ± 1.0 Kg in weight, $pH_{24} \leq 5.60$ in *Semimembranosus* muscle). The hams were assigned to a certain processing time, according to the experimental design shown in table 1.

Table 1: Experimental design

Processing time:	30 days	75 days	270 days
Hams assigned to each processing time:[a]	1a, 2a, 3a, 4a, 5a, 6a	1b, 2b, 3b, 7a, 8a, 9a	4b, 5b, 6b, 7b, 8b, 9b

[a]Hams with the same number (but different letter) come from the same carcass.

The 3 groups were balanced by ham pH_{24h} and weight. After 24 h of storage at 2–3 °C, they were rubbed with a mixture of 5 g of fine salt and 0.80 g of $NaNO_2$ per Kg of ham before being pile salted with coarse salt for 10-11 days. Then, the hams were washed with cold water to remove salt on surface and hung in a drying room with the conditions of 90 days at 4-8 °C and relative humidity (RH) 70–80 %; 150 d at 11-13°C RH 60–70 %, and 30 d at 26-27 °C RH 50-60 %. The hams were taken for analyses at the end of the assigned processing time.

Sample preparation Transversal cuts were made to obtain a slice 30-mm thick at a point 4 cm from the aitch bone towards the hoof. The skin and subcutaneous fat were removed, and the slices were vacuum-packed, stored at -18°C and moved to a cold chamber the day before the analyses to let them thaw. Two zones were defined as follows: internal zone, as the inner area of the slice where muscles present an uniform colour different to the external part; external zone, as the part of the slice left after removing both the internal zone and the area delimited 4 mm around the internal zone, bearing the typical colour given by NO-H. Once thawed, each zone was minced separately before being analysed.

Chemical Analyses: The chloride content was determined according to the ISO protocol 1841-2 using a 785 DMP Titrino potentiometric titrator (Metrohm AG, Herisau, Switzerland) and expressed as the NaCl content. The

moisture was determined by drying at 103 ± 2 °C until a constant weight was reached (AOAC, 2000). NO-H, ZnPP, and PPIX were quantified by HPLC separation and UV-Vis detection after being extracted with aqueous acetone, as described elsewhere [1]. The results were given as mg/Kg desalted matter.

Statistical analysis: An analysis of variance (ANOVA) was carried out to evaluate the effect of processing time on the concentrations of NO-H, ZnPP and PPIX in each zone (SAS Institute, Cary, North Carolina, USA). The model included the processing time and the carcass as fixed effects. The means for each processing time were obtained by the least square means procedure of the same software.

III. RESULTS AND DISCUSSION

All hams developed a red ring after 30 days, and its relative area did not change (results not shown). The behaviour of the NO-H over time was different in each area (Table 2). On the external zone, no significant changes were observed between 30 and 75 d, but afterwards there was a significant decrease ($p<0.05$), which was probably due to oxidative degradation [4]. In the internal zone, there was a significant increase ($p<0.05$) between 75 and 270 d ($p<0.05$), indicating that that nitric oxide was able to diffuse into inner parts with longer processing time.

The concentration of ZnPP started at values below 6,2 mg/kg at 30 d and increased over time in the 2 zones, although the greatest increase was produced in the internal zone, especially between 70 and 270 d. In this area, the concentration was always higher than in the external one. The concentration of PPIX followed a behaviour similar to that of ZnPP, but with lower values. The fact that both pigments were lower in the external zone could be explained by the inhibitory effect of nitric oxide on the activity of ferrochelatase [5]. All hams showed a red ring after 30 days, when the content of ZnPP was very low, therefore, the development of the red ring seems to be independent from the formation of ZnPP and PPIX.

Table 2: Least square means and root mean square error (RMSE) of pigments[1]

	EXTERNAL				INTERNAL			
Pigment	**30 d**	**75 d**	**270 d**	**RMSE**	**30 d**	**75 d**	**270 d**	**RMSE**
NO-H	180^{a}	172^{a}	125^{b}	22.9	20^{b}	4^{b}	77^{a}	19.3
ZnPP	0.3^{b}	0.2^{b}	2.7^{a}	0.63	6.2^{b}	9.9^{b}	34.2^{a}	5.73
PPIX	$<0.1^{b}$	$<0.1^{b}$	0.5^{a}	0.17	5.2^{ab}	2.7^{b}	7.5^{a}	1.99

[1] Results are expressed as mg/Kg desalted dry matter. NO-H are expressed as hemin.[a, b] Different letters in the same row (a–b) within the same zone indicate significant differences ($p \leq 0.05$).

IV. CONCLUSION

Although the formation of zinc-protoporphyrin competes with the formation of nitrosyl heme, the development of the red ring is not related with the formation of zinc-protoporphyrin.

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