# Nitrosylmyoglobin and Zn-protoporphyrin in Iberian dry cured loins formulated with reduced amounts of nitrate/nitrite: Evolution during drying and refrigerated storage after high hydrostatic pressure processing.

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**Introduction:** Colour is a key factor influencing consumer acceptance of meat products. One of the main functions of nitrite is the formation of nitrosylmioglobin (NOMb) that confers the typical red colour to cured meats. In uncured meat products, the pigment responsible for the red colour is zinc protoporphyrin IX (ZnPP) (Wakamatsu et al., 2010). Despite both nitrites and nitrates are authorised as food additives in the European Union, nitrite has been questioned from a food safety standpoint. These concerns triggered the meat industry to search for alternatives to produce nitrite-free meats that could maintain the colour characteristics of cured meat products. In this sense, using clean label processes as high-hydrostatic pressure (HHP) to extend the shelf-life and assure food safety in ready-to-eat meat products is an attractive preservation technique (Bolumar et al., 2021). This study aimed to evaluate changes in NOMb and ZnPP in Iberian dry-cured loins formulated with reduced levels of NO2-/NO3- during the drying process and their evolution after HHP treatment.

**Material and methods:** Four batches were produced with decreasing amounts of sodium nitrite/potassium nitrate (NOx) added: 1) 150NOx (150 mg/ kg NaNO2 + 150 mg/ kg KNO3), 2) 75NOx (75 mg/ kg), 3) 37.5NOx (37.5 mg/ kg) and 4) 0NOx (0 mg/ kg). Experiment 1. Dry-cured loins were sampled at 10, 20, 40, and 80 of the drying. Experiment 2. At the end of processing, samples were portioned and divided in Control and High-Hydrostatic-Pressure (HHP) and were vacuum packed. Loin samples were pressurised in a semi-industrial hydrostatic pressure unit at 600 MPa - 7 min and, stored for 240 days at 4ºC and sampled at days 0, 30, 60, 120, and 240.

The NOMb content was determined spectrophotometrically at 540 nm according to Hornsey, (1956). The ZnPP was determined by fluorescence spectroscopy (λexc 415 and λem 590 nm), according to Bou et al., (2018).

The UV-vis spectra (λ 350-650 nm) of NOMb extracts and the fluorescence spectra (λexc 415 λem 470-700 nm) of ZnPP extracts were recorded.

A one-way analysis of variance (ANOVA) was used for Experiment 1 and a two-way ANOVA with interaction for Experiment 2. Tukey's test was used to compare means.

**Results:** The NOMb content increased throughout the drying process in all batches, even in the batch addition of curing salts. On all samplings, 0NOx loins showed significantly lower NOMb contents than 37.5, 75, and 150NOx counterparts, which did not differ significantly from each other. ZnPP was detected only in 0NOx loins, and their contents increased with the time of drying.

Treatment with HHP did not affect NOMb or ZnPP contents. NOMb formation increased throughout refrigerated storage. In the 0NOx samples, the ZnPP content did not change throughout storage, whereas ZnPP was not detected in the cured samples. During the first 60 days of storage, the NOMb content was significantly higher in the 150NOx samples than in the 0NOx counterparts. From day 120 to the end of storage, no differences were found between batches.

The UV-vis spectra of cured and uncured loins differed. Cured loin extracts showed peaks at 393, 472, 539, and 560 nm, while 0NOx loins initially showed peaks at 415, 545, and 583 nm, and a peak at 393 nm increased over storage after HHP treatment. The fluorescent spectra showed peaks at 588 and 641 nm in 0NOx samples contrasting with the absence of peaks in NOx loins.

**Conclusion:** In conclusion, removal of curing salts significantly decreased NOMb and increased ZnPP contents in dry loins. Reductions up to 37.5 mg/kg NOx did not affect NOMb content compared to loins with 150 mg/kg NOx. In addition, HHP can be used without affecting the content of both pigments.

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