Importance of intrinsic parameters in relation to *in vitro* zinc protoporphyrin IX formation in different meat sources

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Abstract- This study presents the screening of eight meat sources, namely chicken, turkey, pork, lamb, beef, veal, horse and porcine liver, on seven intrinsic parameters possibly important for the formation of zinc protoporphyrin IX (Zn(II)PPIX). For each meat source, the potential for endogenous Zn(II)PPIX formation was estimated by means of a meat-based *in vitro* model. Using Partial Least Squares Regression (PLS) analysis, the Zn(II)PPIX formation was related to the intrinsic parameters screened in order to identify their relative importance. Porcine liver tissue, but also horsemeat, had the highest ability to form Zn(II)PPIX. From the measured intrinsic parameters, zinc chelatase activity showed to be the most important one to explain Zn(II)PPIX formation in different meat sources.

Key Words – horsemeat, porcine liver, zinc chelatase activity

I. INTRODUCTION

For several years, researchers have studied the formation of Zn(II)PPIX in meat, as it is considered to be an important natural colouring agent in dry cured or fermented meat products in the absence of nitrite and/or nitrate [1]. It is assumed that Zn(II)PPIX is formed from heme through the substitution of iron by zinc, whereby ferrochelatase (FECH) is considered as the main enzyme involved in the reaction pathway [2]. Until now, however, mainly pork has been used for these investigations. The goal of this research was to relate *in vitro* Zn(II)PPIX formation in eight meat sources (chicken, turkey, pork, lamb, beef, veal, horse and porcine liver) to seven intrinsic parameters (pH, initial metmyoglobin formation (IMF), metmyoglobin reduction ability (MRA), total heme, zinc chelatase activity, total iron and total zinc). FECH is known to be pH dependent. Reduction of ferric iron in metmyoglobin would promote the substitution reaction. Heme is considered as the substrate for Zn(II)PPIX formation [2]. Zinc chelatase activity includes specifically the zinc insertion reaction of FECH [3]. More heme implies the presence of more iron. However, ferrous iron is a strong inhibitor of zinc insertion, thus it is often assumed that Zn(II)PPIX is merely formed in meat products when iron availability decreases [4].

II. MATERIALS AND METHODS

Meat samples: Chicken, turkey, pork, lamb, beef, veal, horse, and porcine liver samples were purchased from a local butcher, each at three different randomly chosen times during a period of 6 months. They were collected within 48 hours after slaughter and stored at -24 °C until analysis.

Detailed descriptions of all analyses, including the investigated *intrinsic parameters*, are described in [5]. All analyses were performed in triplicate.

In vitro Zn(II)PPIX formation: A meat-based *in vitro* model was established with the addition of antibiotics (a mix of penicillin, streptomycin, gentamicin and amphotericin) to exclude the influence of microorganisms and to focus on the endogenous enzymatic pathway of Zn(II)PPIX formation. Meat homogenates were incubated anaerobically at 26°C for 7 days, subsequently extracted with 75% v/v acetone prior to Zn(II)PPIX determination via high performance liquid chromatography (HPLC) equipped with a fluorescence detector (ex./em. 420/590 nm). To check the efficiency of the antibiotics in the model, microbial count was analyzed before and after incubation.

Statistical analysis: Differences in *in vitro* formation of Zn(II)PPIX of the respective meat sources were determined by one-way analysis of variance (ANOVA) at a significance level of $P < 0.05$ and using Tukey's post-hoc tests (IBM SPSS Statistics 21.0, Chicago, USA). PLS analysis was performed for evaluating the influence of the intrinsic parameters on the *in vitro* formation of Zn(II)PPIX using meat-based models (XLStat Base, Paris, France).

III. RESULTS AND DISCUSSION

Figure 1 shows that *in vitro* Zn(II)PPIX formation was very low in chicken, turkey, pork, lamb, beef and veal. Slightly higher, although not significant, Zn(II)PPIX formation was seen in horsemeat. In contrast, significantly higher Zn(II)PPIX formation was obtained when liver tissue was used.

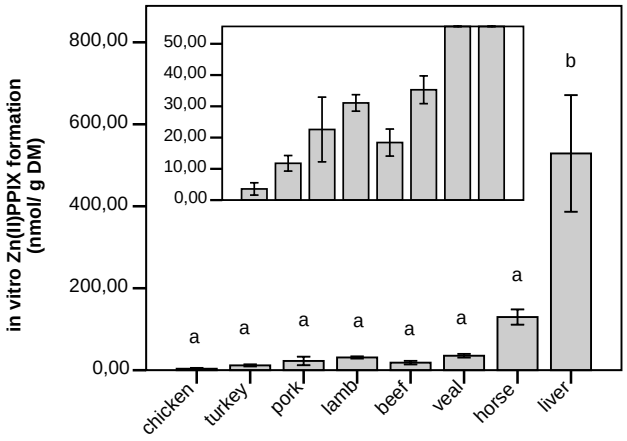

Figure 1 *In vitro* formation of Zn(II)PPIX after 7 days at 26°C (the small figure enclosed is an enlargement of the y-axis for better visibility) of eight meat sources. Data are expressed as means ± SE (n=3). Mean values indicated with the same letter are not significantly different based on Tukey's post hoc tests ($P < 0.05$)

Significant differences in intrinsic parameters between meat sources were found (data not shown).

PLS analysis revealed that 61.2% of the variation in Zn(II)PPIX formation of the different meat sources was explained by the investigated intrinsic parameters. Zinc chelatase activity, followed by total heme and total iron and zinc, were the most important factors for the formation of Zn(II)PPIX, whereas pH, IMF and MRA were of minor significance (Figure 2).

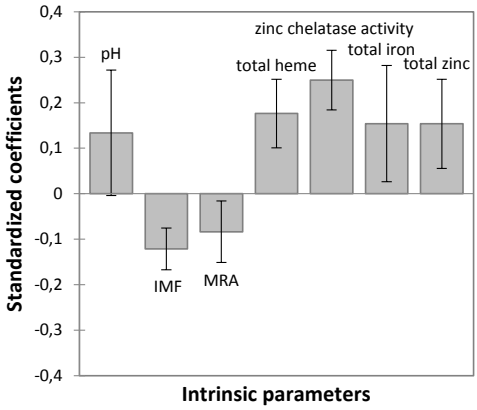

Figure 2 Standardized coefficients (CI 95%) for *in vitro* Zn(II)PPIX formation modelization assessed by PLS analysis (n = 24)

IV. CONCLUSION

The highest *in vitro* Zn(II)PPIX formation was achieved in horsemeat and liver tissue. PLS analysis revealed that zinc chelatase activity was the most important factor to explain the variation in Zn(II)PPIX formation, confirming that the endogenous enzymatic Zn(II)PPIX formation pathway occurs in meat and that it is species-dependent. More investigation is required to elucidate other influencing intrinsic parameters that were not included in this study, e.g. other divalent metal ions, for the endogenous formation of Zn(II)PPIX in meat. These findings could be important for meat industry in order to establish the production of red coloured nitrite-free meat products.

ACKNOWLEDGEMENTS

This work was performed with financial support of internal funding of KU Leuven.

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