IMPROVEMENT OF ZINC-PROTOPORPHYRIN FORMATION YIELD AND ITS SOLUBILITY IN PORCINE LIVERS BY ADDING ZINC ACETATE AND HEMOGLOBIN

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I. INTRODUCTION

The Zn-protoporphyrin pigment (ZnPP) has gained interest as it could be used as a colouring in various meat products in the absence of nitrifying agents [1]. Its formation implies the insertion of Zn instead of Fe into the heme moiety in which the enzyme ferrochelatase may play a crucial role [2, 3]. Given that porcine livers have a high activity of this enzyme, they have been used to obtain a new colouring ingredient based on the formation of this pigment that may help avoid the use of nitrite and/or nitrate in meat products. However, this ingredient remains in the insoluble protein fraction, limiting its potential application to comminuted meat products. The potential of this ingredient may be improved by increasing ZnPP formation yield and its solubility as this will facilitate their inclusion in a wide range of meat products. This study is aimed at improving the formation of ZnPP by adding Zn acetate and haemolyzed blood and examining their effects on soluble ZnPP.

II. MATERIALS AND METHODS

Porcine livers were homogenized in distilled water to obtain 20% and 50% liver homogenates (1:4 and 1:1 w/w, respectively) in which ascorbic acid and acetic acid were added to a final concentration of 1000 mg/kg and 0.25 mg/kg, respectively. Depending on the experiment, Zn acetate and different amounts of haemolyzed red blood cell (RBC) fraction were added to the homogenates and incubated for 24 h at 45 °C under anaerobic conditions. RBC was obtained by centrifuging blood at 2530x*g* at 5 °C for 15 min and decanting the supernatant fraction (plasma). RBC was haemolyzed by adding Milli-Q water (1:1, v/v). ZnPP was measured as described elsewhere [3]. Soluble ZnPP was measured in the supernatant after centrifuging at 38,900x*g* at 4ºC for 30 min. Series of ANOVA were used to determine the existence of significant differences. Tukey's HSD test was used to separate means. Differences at $P < 0.05$ were considered statistically significant.

III. RESULTS AND DISCUSSION

The formation of ZnPP in 20% liver homogenates after the anaerobic incubation at 45 C for 24 h increases with the addition of Zn acetate at 100, 200, and 300 μM and decreases at 400 μM (data not shown). Figure 1 shows the formation of ZnPP in 20% liver homogenates with and without the addition of 200 μM of Zn acetate as affected by the addition of RBC. It can be observed that the formation of ZnPP was increased with the addition of RBC with the level of addition up to a maximum (5% and 7.5% without and with the addition of Zn acetate, respectively) and then decreased. In the presence of Zn acetate, the formation of ZnPP reached a maximum of (91 ± 0.4 mg/kg) when RBC was added at 5% and compared with the control (51 ± 0.2 mg/kg). The amount of soluble ZnPP followed a similar pattern to that of total ZnPP and at 5% RBC reached 13 ± 1.5 mg/kg whereas at 0% RBC the amount of soluble ZnPP was 3 ± 0.1 mg/kg. The amount of water in the liver homogenates was reduced until obtaining 50% liver homogenates. Then, the effect of the addition of 200 μM Zn acetate and 5% RBC and their combination in 50% liver homogenates was studied. In these conditions, the addition of 5%

RBC increased ($P < 0.01$) the formation of ZnPP (149 ± 3.1 and 154 ± 1.5 mg/kg, alone and with Zn acetate, respectively) whereas the addition of Zn acetate led to similar amounts of ZnPP to that of the control (114 ± 2.6 mg/kg and 108 ± 4.5 mg/kg for Zn acetate addition and control, respectively). Moreover, the content in soluble ZnPP was higher ($P < 0.01$) when RBC and Zn acetate were combined (23 ± 0.1 mg/kg) in comparison to the control (9 ± 0.3 mg/kg), and the only addition of RBC, and Zn acetate (12 ± 1.0 and 13 ± 1.0 mg/kg, respectively).

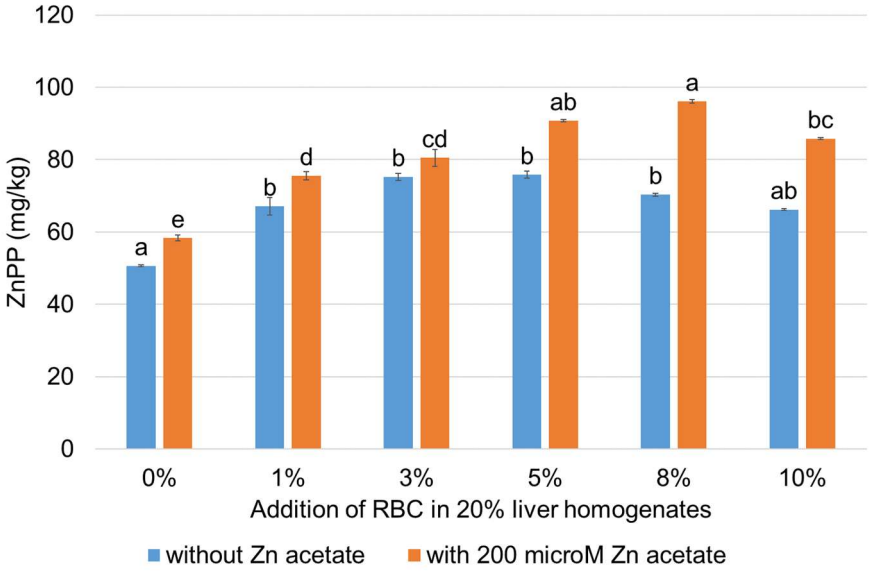

Figure 1. Zn-protoporphyrin content in 20% liver homogenates as a function of red blood cell (RBC) fraction. Different letters (a-d) within the same Zn acetate conditions denote significant differences at ($P < 0.01$)

IV. CONCLUSION

The addition of Zn acetate maximizes the formation of ZnPP in 20% liver homogenates whereas RBC can serve to maximize ZnPP formation regardless of the concentration of liver in the homogenate. The formation of total and soluble ZnPP in 50% liver homogenates can be improved with the combined addition of RBC and Zn acetate, which in the case of soluble ZnPP is 2.5-fold the amount of the control.

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