Optimitzation of ZnPP-forming conditions in porcine livers using response surface methodology (RSM)

Ricard Bou[1], Mar Lauger[1], Michela Albano[2], Elvira Tenorio[1]

[1] Food Safety and Functionality, Institute of Agrifood Research and Technology (IRTA), Monells (Girona)
[2] Food Quality and Technology, Monells (Girona)

Introduction: The main red and stable pigment in non-nitrified dry-cured hams is Zn-protoporphyrin IX (ZnPP) (Wakamatsu et al., 2004). This pigment has gained interest as it could be used as coloring agent in various meat products in the absence of nitrifying agents. Its formation implies the insertion of Zn(II) instead of Fe(II) into the heme moiety and it seems that the enzyme ferroquelatase (FECH) plays a crucial role in its formation (Bou et al., 2020; Chau et al., 2011). Given that porcine livers have high activity of this enzyme, they have been used to obtain a new ingredient with coloring capacity that may help to avoid or minimize the use of nitrite and/or nitrate in meat products.

This study aimed to optimize the formation of the red and stable pigment ZnPP in porcine livers with the purpose to obtain coloring ingredients. In addition to this, microbial growth needs to be controlled to guarantee its potential food grade.

Materials and methods: Porcine livers (n=4) collected from commercial slaughterhouses were used. Pork livers were homogenized in distilled water (final liver concentration of 20%) and with a final concentration of 1000 mg/kg of ascorbic acid and acetic acid at 0.25 mg/kg. The formation of the pigment Zinc-protoporphyrin was optimized using the response surface methodology (RSM) based on central composite design (CCD) with 17 experimental conditions that were replicated. These conditions involved different pH (4.2, 4.4, 4.8, 5.2 and 5.4), incubation temperatures (25°C, 32°C, 40°C, 49°C and 55°C), and incubation periods under anaerobic conditions ranging from 3 to 30 hours. In this experiment both total viable counts (log CFU/mL) and ZnPP formation (Bou et al., 2020) were measured.

Results: The response surface model determined that the maximum conditions for the formation of ZnPP pigment using porcine livers is very dependent on pH with a maximum at pH 4.8 and at an incubation temperature ranging between 40 and 50°C. Moreover, it is possible to control the microbiological growth reaching the level of ≤ 3 log CFU using acetic acid and in the absence of antibiotics. These findings are relevant because it allows to obtain ZnPP pigment during 24h of incubation guarantying not only the optimal pigment formation but also to maintain the microbiological safety conditions during the process.

Conclusions: This study shows that it is possible to form the coloring pigment ZnPP in porcine homogenates while controlling microbial growth. Therefore, it seems possible to develop livers extracts that can be used as potential coloring ingredients in the development of meat products without the addition of nitrifying agents.

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Literature:

Bou, R., Llauger, M., Arnau, J., Olmos, A., & Fulladosa, E. (2020). Effects of post mortem pH and salting time on Zinc-protoporphyrin content in nitrite-free Serrano dry-cured hams. Food Research International, 133:109156

Chau, T. T., Ishigaki, M., Kataoka, T., & Taketani, S. (2011). Ferrochelatase Catalyzes the Formation of Zn-protoporphyrin of Dry-Cured Ham via the Conversion Reaction from Heme in Meat. J. Agric. Food Chem, 59, 12238–12245.

Wakamatsu, J., Nishimura, T., & Hattori, A. (2004). A Zn-porphyrin complex contributes to bright red color in Parma ham. Meat Science, 67(1), 95–100.