Meat Science 139 (2018) 192–200

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Zinc-protoporphyrin content in commercial Parma hams is affected by proteolysis index and marbling

Ricard Bou*, Mar Llauger, Jacint Arnau, Elena Fulladosa

Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Food Technology, Monells, Finca Camps i Armet s/n, 17121, Spain

ARTICLE INFO

Keywords:
Dry-cured ham
Colour
Pigments
Proteolysis
Marbling

ABSTRACT

The contents of zinc-protoporphyrin (ZnPP) and heme in twenty-four sliced Parma hams made without the addition of curing agents were determined. Expressed on a dry weight basis, ZnPP averaged 45 mg/kg and ranged from 23 to 85 mg/kg. The heme content averaged 37 mg/kg on a dry matter basis and ranged from 17 to 73 mg/kg. A Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression analyses were carried out to examine the existing correlations between these pigments and various physicochemical parameters in the final product. PCA showed the existence of associations between ZnPP, sensory redness and salt content. PLS suggests that the conversion of ZnPP from heme is facilitated in those hams with a higher proteolysis index and higher marbling.

1. Introduction

Dry-cured ham with its characteristic red colour, is a popular meat product in the Mediterranean region of Europe. This colour is typically obtained by reaction of nitric oxide, coming from curing agents (i.e. nitrate or nitrite), with muscle myoglobin to form nitrosylmyoglobin (Haldane, 1901; Hornsey, 1956). However, the main pigments in dry-cured hams produced without the addition of curing agents (e.g. *Prosciutto di Parma*) are heme and zinc-protoporphyrin IX (ZnPP) chromophores (Adamsen, Moller, Laursen, Olsen, & Skibsted, 2006; Wakamatsu et al., 2009; Wakamatsu, Nishimura, & Hattori, 2004). In ZnPP, the porphyrin moiety coordinates with Zn(II) instead of Fe(II) and is responsible for the distinctive bright, stable colour of non-nitrified Parma hams (Adamsen, Moller, Hismani, & Skibsted, 2004; Wakamatsu, Nishimura, & Hattori, 2004).

However, the formation pathway of ZnPP in meat and meat products is complex and not yet completely elucidated. Non-enzymatic reactions, endogenous enzymatic reactions and enzymatic reactions caused by typical ham bacteria may be involved (Becker, Westermann, Hansson, & Skibsted, 2012; Morita, Niu, Sakata, & Nagata, 1996; Wakamatsu, Okui, Ikeda, Nishimura, & Hattori, 2004). Despite this, the formation of ZnPP is widely believed to be of enzymatic origin mainly given that this chromophore is not formed after treating the meat thermally (Wakamatsu, Okui, et al., 2004). Ferrochelatase (Zn-chelatase, heme synthase, E.C. 4.99.1.1) is the endogenous enzyme suggested as being responsible for the formation of ZnPP in meat (Benedini, Raja, & Parolari, 2008). This enzyme is active throughout the processing time of dry-cured hams and is considered to some extent, responsible for the gradual formation of ZnPP (Adamsen, Moller, Parolari, Gabba, & Skibsted, 2006; Parolari, Aguzzoni, & Toscani, 2016; Parolari, Benedini, & Toscani, 2009). The residual activity of this enzyme, even in hams with a low water activity and a high salt concentration, would explain why many authors find a higher ZnPP content at the end of the process (Adamsen, Moller, Parolari, et al., 2006; Parolari et al., 2009, 2016). However, in addition to meat endogenous enzymes, various authors have indicated the existence of other alternative and complex mechanisms that could be involved in this pigment formation and occur simultaneously (Becker et al., 2012; Grossi, do Nascimento, Cardoso, & Skibsted, 2014; Parolari et al., 2016).

As shown in various meat models, the enzymatic formation of ZnPP can be affected by several factors. For instance, Wakamatsu, Okui, Hayashi, Nishimura, and Hattori (2007) reported that the presence of oxygen decreases the formation of ZnPP in meat solutions. In fresh meat extracts, Benedini et al. (2008) reported that the enzyme is temperature-dependent with an increase in activity from 10 °C to 37 °C. The same authors indicated that the formation of ZnPP is favoured by the presence of high amounts of sodium chloride. However, elevated sodium chloride contents have been also reported to limit the formation of ZnPP in meat systems (Adamsen, Moller, Parolari, et al., 2006; Becker et al., 2012). In porcine heart extracts, the formation of ZnPP is favoured at pH between 5.5 and 6.0 (Ishikawa et al., 2006, 2007). According to Chau, Ishigaki, Kataoka, and Taketani (2010) the enzyme is able to remove the iron atom from porphyrin in the latter pH range whereas the zinc insertion is favoured at neutral or basic pH (7.5–8.0).

* Corresponding author.
E-mail address: ricard.bou@irta.cat (R. Bou).

https://doi.org/10.1016/j.meatsci.2018.01.027
Received 1 June 2017; Received in revised form 12 January 2018; Accepted 30 January 2018
Available online 01 February 2018
0309-1740/

The latter authors also reported that the presence of fatty acids and phospholipids affect ferrochelatase activity. It is also worth mentioning that myoglobin suffers significant modifications during ham maturation which have been reported to facilitate the substitution of the heme iron by zinc (Adamsen, Moller, Parolari, et al., 2006). Furthermore, Paganelli et al. (2016) reported that heme moiety transmetallation is enhanced by a partial proteolysis of the globin which facilitates Zn-chelatase activity.

However, in dry-cured hams, it has been found that the formation of ZnPP occurs even when they are manufactured at 4 °C, thus suggesting that non-enzymatic processes could also be involved given that at this temperature the activity of the enzyme is limited (Parolari et al., 2016). Therefore, a number of endogenous and exogenous factors that have not yet been completely elucidated could have an effect on the formation of ZnPP in Parma hams (Grossi et al., 2014; Paganelli et al., 2016). Although several factors affecting ZnPP have been studied in *in vitro* models, data are scarce on typical ZnPP contents in dry-cured Parma ham and studies dealing with the factors involved in the formation of this pigment in hams are still necessary (De Maere et al., 2014; Parolari et al., 2009; Wakamatsu, Uemura, et al., 2009). Therefore, it is important to gain a better understanding of the factors that contribute to this pigment content because they determine the characteristic colour of non-nitrified dry-cured hams. The formation of ZnPP is also interesting because of its increased stability in comparison with the pigments obtained from nitrified hams (Adamsen et al., 2004; Durek, Bolling, Knorr, Schwagele, & Schluter, 2012).

Besides, a better knowledge about elements that could influence the formation of ZnPP may help to develop strategies for obtaining a more intense and homogeneous colour in non-nitrified dry-cured meat products. The aim of this work was to report typical ZnPP and heme contents in commercial sliced non-nitrified Parma ham. In addition, the relationships between ZnPP and heme, and various physicochemical characteristics (proximate composition, proteolysis index, water activity, NaCl content and pH) plus sensory redness were studied.

2. Material and methods

2.1. Acquisition and processing of Parma ham samples

Twenty-four packages of PDO Parma sliced dry-cured ham obtained from six different producers were used and each package of ham came from a different batch. The hams were manufactured in compliance with the Parma ham production guidelines (European Commission, 2013) with an elaboration time ranging between 12 and 20 months. It must be mentioned that during Parma ham elaboration no nitrite or nitrate are added.

High quality images from the first slice of each package were acquired as described in Section 2.4 in order to evaluate intramuscular fat. Subsequently, subcutaneous and intermuscular fat were discarded from all the slices. The lean part of the slice, which included *Semimembranosus*, *Semitendinosus* and *Biceps femoris* muscles, was minced and homogenized for further physicochemical analysis. Homogenized samples were aliquoted and kept vacuum packed in aluminium bags at −80 °C to further determine ZnPP and heme content.

2.2. Reagents

Chlorohemin (hemin) from porcine was from Panreac (Barcelona, Spain) whereas protoporhyrin IX and ZnPP were from Sigma-Aldrich (Madrid, Spain). Ethyl acetate, acetic acid and methanol were of a suitable grade for instrumental analysis. Other reagents were of ACS grade.

2.3. Physicochemical determinations

Protein content was calculated by multiplying by a factor of 6.25 total nitrogen which was determined via Kjeldahl digestion (AOAC, 2000). Non-protein nitrogen content was determined by precipitation of proteins with trichloroacetic acid followed by determination of the total nitrogen (Careri et al., 1993). Proteolysis index was determined as a percentage of the ratio between non-protein nitrogen and total nitrogen.

Fat content was determined according to the ISO 1443:1973 protocol (ISO, 2016). Water content (moisture) was determined by drying at 103 ± 2 °C until a constant weight was reached (AOAC, 2000). The water content of the samples on defatted basis was also calculated from the chemical composition of the ham (Moisture = g H_2O/(100 g sample − g fat)). Water activity (a_w) was measured at 25 °C ± 0.3 °C with a Novasina AW SPRINT – TH 500 instrument (Axair Ltd., Pfäffikon, Switzerland). Chloride content was determined according to ISO 1841-2:1996 protocol using a potentiometric titrator 785 DMP Titrino (Metrohm AG, Herisau, Switzerland) and expressed as NaCl content. The NaCl content on dry matter basis (NaCl DM = g NaCl/(100 g sample − g H_2O)) and on defatted dry mater basis (NaCl, defatted DM = g NaCl/(100 g sample − g H_2O − g fat)) was also calculated. Determination of pH was performed by means of a S40 SevenMulti (Mettler-Toledo S.A.E., Barcelona, Spain) and an Inlab Solids Pro (Mettler-Toledo S.A.E.) probe. All analyses were done in triplicate.

2.4. Visual estimation of intramuscular fat (marbling)

In order to estimate the visual intramuscular fat content, high quality images were acquired using a photographic system that included a calibrated digital camera Canon EOS 50D with a picture resolution of 15.1 megapixels and an objective Canon EF-S 18–200 mm f/3.5–5.6 IS. White balance was carried out with a white card (Lastolite) in order to electronically adjust the colour reproduction without showing colour dominants. The camera was connected to a PC into which the images with RAW format were uploaded. Dry-cured ham slices were positioned below the camera lens and an image of the entire slice surface was taken. Capture One Pro software (Phase One A/S Inc., Frederiksberg, Denmark) was used to carry out the white balance of the RAW images and digitalize them to 667 × 1000 pixels resulting in a .tif file with 16 bits colour and 4 MB. This was considered to be high enough in quality for computer image analysis.

Visual intramuscular fat of the entire slice was segmented using the procedures previously described elsewhere (Muñoz, Rubio-Celorio, Garcia-Gil, Guardia, & Fulladosa, 2015; Santos-Garcés, Muñoz, Gou, Garcia-Gil, & Fulladosa, 2014). In brief, Matlab scripts written in-house were used for segmentation of visual subcutaneous, intermuscular and intramuscular fat using edge detection based on the discrete Fourier transform. The total area of the slice, the subcutaneous fat area of the slice, the intermuscular fat area and visual intramuscular fat area were segmented and the number of pixels for each one was determined. The percentage of intramuscular area related to the total area of the slice was calculated.

2.5. Sensory analysis: Redness

Sensory analysis of the samples was carried out by eight trained panelists according to the ISO 8586-2:2012 protocol (ISO, 2016). Visual appearance of red colour (redness) was evaluated in the entire slice using an unstructured scale from 0 (very low) to 10 (very high).

2.6. Determination of zinc-protoporphyrin

ZnPP was quantitatively extracted in subdued light conditions with ethyl acetate/acetic acid solvent mixture (4:1, v/v) as described elsewhere with minor changes (Wakamatsu, Odagiri, Nishimura, & Hattori, 2009). The chromatographic elution of this extract as described in the determination of heme iron showed traces or the absence of demetallated protoporphyrin in our samples (data not shown). Hence, the