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PHOTOMETRIC DETERMINATION OF THE TYROSINE CONTENT IN DRY-CURED HAM SHOWING WHITE FILM

F. Stehle[1*], M. Gibis[1], K. Herrmann[1], J. Weiss[1]

[1] University of Hohenheim, Stuttgart, Germany.

*Corresponding author email: florian.stehle@uni-hohenheim.de

I. INTRODUCTION

A white film may occasionally form on dry-cured ham, often misinterpreted by consumers as microbiological contamination. The film results from proteolysis of meat proteins during production, but its precise composition remains undetermined. However, the exact composition has not yet been determined. The amino acid tyrosine has been identified as the main component, but it is not certain whether this is present free or bound in peptides. Furthermore, it is not clear if coating-forming substances diffuse from the inside of the ham to the surface.

II. MATERIALS AND METHODS

The tyrosine content of 3 hams showing white film was determined with a modified Lowry assay based on the method of Bosse [1]. The ham pieces were split in half. From one half, slices with a thickness of 1 mm were taken from the surface and 1, 5 and 10 mm below. Samples were punched out with a circular probe (diameter of 2.5 cm). The other half was vacuum-packed and subjected to the same procedure after 3 months of storage at 2 °C. The samples were homogenized in 20 mL of 3.3 % (w/w) TCA for 1 min at 10,000 rpm using an Ultra Turrax (SilentCrusher M, Heidolph Scientific Products GmbH, Schwabach, Germany). The resulting solutions were filtered through a folded filter and a syringe filter (0.45 µm, Cellulose Chromafil, Carl Roth GmbH & Co. KG, Karlsruhe, Germany). The results were referred to the dry matter content (DM) of the whole hams and statistically analyzed using a two-way ANOVA on ranks after transformation. Film samples were scraped off from 5 different hams and freeze-dried overnight. 10 mg were stirred in 10 mL 3.3 % TCA for 1 h. After filtration, the assay was done according to the method, but once without addition of $CuSO_4$. All measurements were conducted in duplicate. Light microscopic images of the film were taken with a digital camera (AxioCam ICc 3, Carl Zeiss, Jena, Germany) attached to an Axio Scope.A1 (Carl Zeiss, Jena, Germany) at 10x magnification before and after the freeze-drying process.

III. RESULTS AND DISCUSSION

The intensity of the white film on the surface did increase during storage (Figure 1A and B). The tyrosine content on the surface of the ham was significantly higher compared to the inside ($p<0.05$) and tended to increase during storage ($p<0.62$) (Figure 2). At the positions inside the ham, the tyrosine content did not change during storage. In contrast to the formation process of the efflorescence on the surface of dry fermented sausages, no diffusion gradient was formed between the inside and the surface of the ham [2]. The low temperature during storage decreased the proteolytic activity and no tyrosine was set free. Since an intensification of the white film occurs nevertheless, it can be assumed that the formation of the coating is not directly related to the proteolytic activity. The formation might be caused by the crystallization of the degradation products of proteolysis, which are metabolized earlier during the production process of the ham.

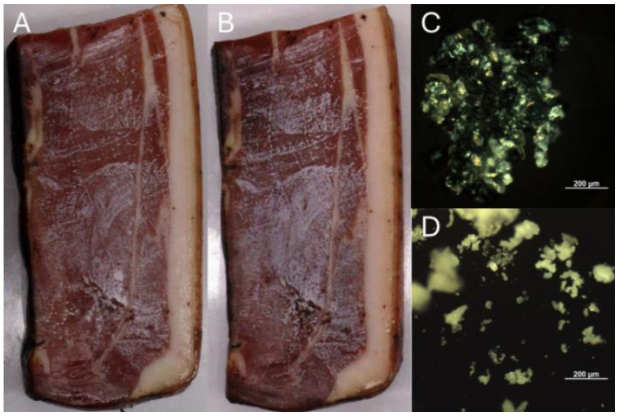


Figure 1. Pictures of a ham sample showing white film before (A) and after the storage at 2 °C for 3 months (B). Light microscopy images using polarized light of the scraped-off film were taken before (C) and after freeze-drying (D). The bar indicates a length of 200 µm.

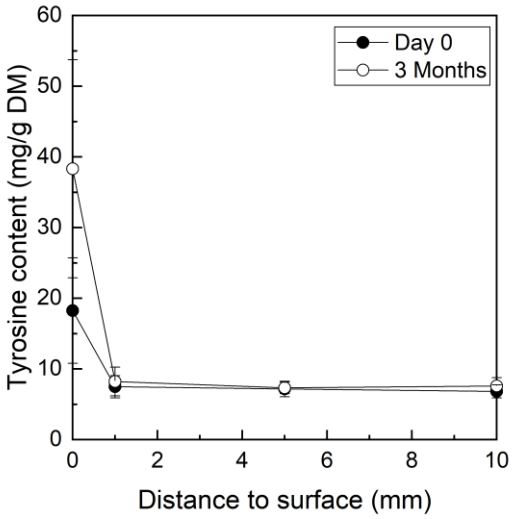


Figure 2. Concentration of tyrosine-containing peptides referred to DM at different positions before and after the storage at 2 °C for 3 months. Error bars indicate the standard deviation of the mean value (n=3). The DM content of the hams was 53,2 ± 3.1 g/100 g.

The freeze-drying of the film affected the polarization of light by the crystals (Figure 1C and D). Whether this change is caused by the removal of free water or by altering the crystalline structure of potential hydrate crystals, present in the film, has to be verified in further experiments.

The photometric determination of the tyrosine content in the film showed no differences when performed with addition of $CuSO_4$ or without. It is known, that the addition of Cu^{2+} during the assay increased the sensitivity for the determination of proteins [3]. This indicates that the tyrosine in the film was mainly present as a free amino acid or bound in small peptides, so the presence of Cu^{2+} did not affect the measurement results. The content of free amino acids in the film could be determined more precisely using liquid chromatography. However, the photometric method used was very suitable for analyzing the processes in dry-cured ham during the formation of white film due to its rapid feasibility. The sample composition was influenced by interfering factors, such as fat or muscle protein, which were also taken from the surface of the hams when scraping off the film. However, care should be taken during purification steps, as components of the crystal could also be removed.

Table 1 – Measured concentration of phenolic compounds in the white film removed from dry-cured ham, determined with a modified Lowry assay, with addition of $CuSO_4$ and without. Extinction was measured at 540 nm.

	With addition of $CuSO_4$	Without $CuSO_4$
Tyrosine concentration (mg/g)	106.2 ± 34.6	105.0 ± 36.1

IV. CONCLUSION

The content of tyrosine or tyrosine-containing peptides did not increase inside the ham during the storage. There was no diffusion from the inside of the ham to the surface during the storage time of 3 months. However, the intensity of the white film and the tyrosine content on the surface increased. The film consisted to a significant extent of tyrosine, which was present as a free amino acid or bound in small peptides.

ACKNOWLEDGEMENTS

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