Analyses of the white film on fast produced dry-cured loin

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I. INTRODUCTION

During dry-cured ham production, substances with low solubility can precipitate, e. g. on the cut, as individual crystals or as a white film affecting the appearance [1]. Caused by the low water solubility, once formed, crystalized substances can not necessarily be washed off. In several studies the crystals have been analyzed, using e. g. gel chromatography [1], microscopy, electrophoresis [2], or HPLC [3]. These studies identified the amino acid tyrosine as one of the main components but also other aromatic amino acids, such as phenylalanine and methionine [2]. As the mentioned methods are targeted in terms of the substance to be analyzed, our aim is that the combination of the non-targeted methods digital microscopy (DigM), energy-dispersive X-ray (EDX), scanning electron microscope (SEM), and Raman spectroscopy (Raman) enables an open search for the identification of the substances precipitating on the cut.

II. MATERIALS AND METHODS

Two days p.m. 6 pork loins were frozen, thawed on day 4 p.m., salted on day 5 with 3.6 % nitrite curing salt with 0.5 % $NaNO_2$, 0.7 % Tari Mix (ICL BK Giulini GmbH, Ladenburg, DE) and 0.02 % starter culture RPW consisting of *Staphylococcus xylosus*, *Staphylococcus carnosus* and *Debaryomyces hansenii* (AVO-Werke August Beisse GmbH, Belm, DE) and stored at 2 °C. After 14 days, meat was rinsed with cold water, stored again at 2 °C on a grid and after 3 days placed in a ripening chamber (KR 1 · 100 / E, Autotherm Ludwig Brümmendorf GmbH & Co KG, Waxweiler, DE) hold at 22 °C and 86 % relative humidity (rh) for 36 h, followed by 20 min smoking at 20 °C, and ripening for 24 h at 16 °C and 80 % rh. Then, drying took place at 15 °C and 75 % rh. After 22 days of ripening, meat was vacuumized and stored at 2 °C. After 14 days, meat was cut in half to obtain a fresh cut and vacuumized again. The resulting surface was monitored weekly for the appearance of crystals. One loin was used to test the scheme for analyses of the white film and its crystals. The chosen loin (SELF) lost 44.7 % of its weight and had a pH of 5.52. To verify analyses, an industrial produced ham was analyzed in the same way and used as a control (CON). For analyses, a digital microscope (VHX-7000, Keyence, Osaka, JPN), an EDX with SEM (JSM 6460 LV, JEOL, Akishima, JPN), and a Raman (alpha300, WiTec, Ulm, DE) was used. Sample were analyzed with each method after the following steps: drying, washing with hexane, washing with water.

III. RESULTS AND DISCUSSION

Figure 1 presents the steps of analyses of the surface. The photo reveals differences in the density and distribution of the white film: white film of CON was distributed evenly on the whole surface, whereas white film on SELF was randomly distributed with a less white film in the middle of the loin. The less pronounced white film in the middle of SELF could be caused by a too short ripening time indicating an uneven weight loss, which could become even after additional storage time. With the DigM the crystals forming the white film became visible showing that the white film on both, SELF and CON, consists of small crystals with app. 0.1 mm in length. However, color and morphology of the crystal varies as crystals on SELF look like NaCl crystals, are transparent and rectangular with clear borders although crystals overlap. On the contrary, crystals on CON are whitish, dense, round with blurred borders, and do not overlap. These differences are also obvious in the SEM images, which clearly show differences in the morphology of the crystals. Crystals of CON are jaggy and show edges differentiating one from each other. Crystals on SELF also show clear edges, but crystals do overlap. Microscopy indicates that CON crystals might have grown next to each other, whereas SELF crystals do also grow on each other. However, even if the appearance of the crystals between CON and SELF vary, it is assumed that the crystals contain mostly one pure precipitated substance, which might differ between CON and SELF.

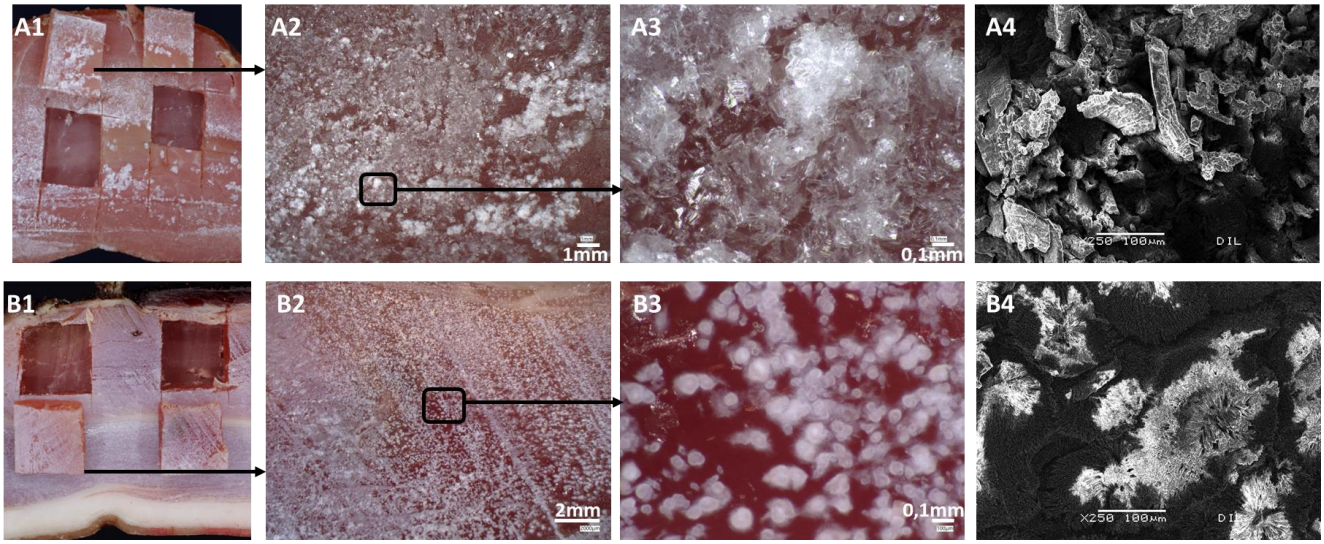

Figure 1. A: Self-produced dry-cured loin and B: control dry-cured ham, made form the leg: 1: photographic image, 2 and 3: digital microscopy with 20x and 200x magnification (the length of the bar indicates A2: 1 mm, B2: 2 mm, A3 and B3: 0,1 mm), and 4: scanning electron microscopy after washing with hexane (the length of the bar indicates 100 µm).

EDX of CON sample, which was dried and washed with hexane and water, had 10.13 ± 0.59 $\%_{mass}$ N, whereby SELF had 26.05 ± 2.67 $\%_{mass}$ N (n = 3). The N content in combination with the appearance of the crystalized substances, especially on CON, could lead to the conclusion of the presence of free, crystalized amino acids such as tyrosine [1, 2, 4]. Thus, Raman was used on CON and its database showed a probability of 76.55 to 87.92 % (n = 6) for *D*-tyrosine, which supports earlier studies [1, 3]. Since the Raman database of six positions of SELF indicated a high probability for different acids and oils, further analyses, such as X-ray diffractometry [4], and/or purification steps are needed to identify the substances.

IV. CONCLUSION

With the use of imaging methods, such as DigM, first insights of the white film on the cut of dry-cured meat can be gained. EDX in combination with SEM allows to check elemental composition and the morphology of the crystals on different positions to receive information about possible structures, such as if NaCl or amino acids are present. Despite the higher N content of SELF, Raman showed that tyrosine might not have been precipitate on the surface of SELF, but of CON. This indicates that further studies are needed to analyze dry-cured meat of 1) varying muscles, 2) different production processes, and 3) varying storage durations to check which factors influence the formation of crystals as a white film and what the receiving crystals are made of. To conclude, the aim was reached since the use of multiple non-targeted analytical methods enabled the identification of the white film made of small crystals on the cut of CON. However, as the identification of SELF crystals was not possible, the analytical procedure needs to be further adapted.

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