The effect of NO-synthase and/or arginine on color changes and forms of hemoglobin solutions

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Introduction: Various ideas have been proposed to substitute nitrite in meat processing while obtaining the same effect in terms of color, taste, antioxidant activity, and microbial stability. Understanding mechanisms underlying reactions of the additives with myoglobin and/or hemoglobin is a basis to propose a solution applicable in the industry. It is suspected that the pink color of fermented sausages should be contributed to the activity of Staphylococcus xylosus or Weisella bacterial strains. Those strains produce NO synthase – an enzyme responsible for NO production from arginine. The same enzyme was also detected in mammalian cells. Thanks to NO synthase NO is released from arginine, it binds to myoglobin, and forms a nitroso pigment in meat (Ras et al, 2018; Xuejun et al., 2020). The aim of this preliminary study was to investigate changes occurring in hemoglobin solutions after enzyme and/or arginine addition.

Materials and methods: Two types of 0.2% hemoglobin solutions were prepared: water (W) and HEPES buffer (pH 7.3) (B). NO synthase (iNOS -inducible form of NO synthase; Sigma-Aldrich, USA) was used for the analyses. The amount of 20 ml of each solution was mixed with: 0.5 g of arginine, 2U of enzyme; 0.5g of arginine and 2U of enzyme; 1 ml of 3% $NaNO_2$. W and B samples were used without any additive. Samples were incubated at 37°C for 30 minutes to create optimal temperature conditions for the enzyme. pH was measured in all the solutions. The color (transmittance mode) (Konica Minolta CM-3500d, Japan) and absorbance spectra of the prepared solutions were measured (BMG, Labtech, Germany) before and after incubation. Samples were then heated (30 min./95 °C) to form nitrosohemichrome, and centrifuged – the color of the pellet was measured. The pellet was mixed with acetone (Hornsey, 1956), centrifuged again, and the absorbance spectra of the filtrate were measured.

Results: pH was comparable in all buffer solutions (average 7.7) while pH of water extracts with arginine was higher. The most interesting was the color of the pellet after a thermal denaturation of myoglobin. Arginine added to hemoglobin water solution caused hemoglobin refolding (Asano et al., 2002) and no protein precipitation was observed. A subjective color evaluation indicated that the pellet from the buffer sample containing arginine and enzyme (BAE) was redder than hemoglobin in a water sample and comparable to the water sample with $NaNO_2$. This would indicate that the desired effect was obtained. However, CieLab analysis did not show that. An a* value in BAE pellet was lower than the water sample and both pellets with $NaNO_2$. Redness (a*/b*) value, on the other hand, was higher than water- $NaNO_2$ pellet (1.47 vs 0.71). The highest value was noted for buffer-$NaNO_2$ sample (1.83). The analysis of absorbance spectra showed that in the pellet samples, prepared with acetone, characteristic peaks at 540 nm and 563 nm were present in BEA and all the other samples with $NaNO_2$. The same signals were shown by Hornsey (1956) in the cured meat prepared with acetone. Other stronger signals appeared in the enzyme-treated samples, which is worth exploring.

Conclusions: The fact that only the spectrum of BAE sample was similar to all the absorbance spectra of samples with $NaNO_2$ may prove the creation of NO-hemoglobin complex. This result is very important in terms of using NO-synthase as a color-forming agent without direct usage of nitrite. Further studies must be conducted to analyze the phenomenon.

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