Colour Stabilisation of Haem-Iron Hydrolysate Using Sodium Nitrite Alternatives

Sorivan Chhem-Kieth[1*], Jorge Ruiz-Carrascal[2]

[1]Danish Crown Ingredients, Marsvej 43, 8960 Randers, Denmark;

[2]Department of Food Science, University of Copenhagen, Rolighedsvej 30, 1958 Frederiksberg C, Denmark

*Corresponding author email: schki@danishcrown.dk

In order to investigate a potential alternative to sodium nitrite in the color stabilisation of meat products, the effects of 4-methylimidazole (4-MeI), pyrrolidine (Pyrl), and sodium nitrite (SN) on colour parameters (UV-Vis spectra and derived CIELab) of haem-iron hydrolysate (HiH) isolated from pig haemoglobin and pure haemin standard were studied. Haem-ligand complexes were kept in solution over a 15-days storage period. Considering both stability over time and haem-ligand affinity, HiH-derived samples showed more promising results than their haemin counterparts. 4-MeI showed colour changes from a 1:50 molar ratio, and a higher chromatic stability over a two weeks period. On the other hand, Pyrl exhibited a red hue from a 1:100 molar ratio onwards, and low stability after a 24h-storage period.

Key Words – Meat pigment, haemoglobin, haem ligand

I. INTRODUCTION

Porcine blood is a by-product generated in great volume in industrial abattoirs. Blood plasma obtained after the centrifugation of whole blood is used as a protein source in food and dietary supplements. The remaining fraction, consisting mainly of haemoglobin, is underutilized due to the instability of the desirable red colour, which tends to an unwanted brown hue upon oxidation of the haem-iron. Recent approaches using enzymatic hydrolysis have permitted for separation of the globin moiety and the haem group [1]. Due to the high bioavailability of haem-iron and its role in the coloration of meat products, there is a good opportunity to add value to this fraction. There have been several attempts to stabilize the colour of haemoglobin, although very limited ones used the isolated haem group, or haem-iron hydrolysates, as starting material. It is our hypothesis that known haem ligand groups such as imidazoles and N-heterocycles [2, 3] bind as strongly with HiH as they do with haem, and more extensively than with haemoglobin, due to absence of the globin moiety and associated steric hindrance.

II. MATERIALS AND METHODS

Haem-enriched extract (15% haem content) was isolated from porcine red blood cells fraction after enzymatic hydrolysis, and subsequently freeze-dried. Haemin standards were obtained from Sigma Aldrich. Formation of haem-ligand complexes was performed following a modified protocol form Shahidi *et al.* [4]. Briefly, haem extract and haemin were dissolved in 20 mM NaOH and diluted to a haem concentration of 0.1 mg/mL, before being bubbled under nitrogen gas and reduced using sodium ascorbate (1:100 molar ratio). Dissolved haem solutions were aliquoted in 2mL-tubes, and varying amount of ligands (haem-to-ligand molar ratio of 1:0 (control), 1:20, 1:50, 1:100, 1:200, 1:300) were added to the tubes, in triplicates. The ligation was conducted in the absence of oxygen and in the dark. UV-Vis spectra for the range 350 to 780 nm were recorded at the following timepoints: 3 hours, 1, 2, 8 and 15 days. The colour parameters L* (lightness), *a** (redness), and *b** (yellowness) were derived from the spectra generated according to CIE standards, using illuminant reference D65 [5]. Principal component analysis (PCA) was performed using Latentix© software (v2.12, b5749).

III. RESULTS AND DISCUSSION

Haem-iron samples exhibiting a desirable red hue characteristically display maxima in absorbance spectra over the 500-600 nm range, as shown in Figure 1a. It can be seen that at the same haem-to-ligand molar ratio, different ligands show different absorbance intensities and maxima shape. The flattening of those maxima can be associated with a loss of the red colour. Figure 1b shows the evolution of the CIE *a** (redness) value for all three ligands over time (only for samples of 1:100 molar ratio). Here, only the 4-MeI with HiH showed a red coloration at 15 days of storage. Haem-SN complexes displayed red hue at a maximum of 8 days, and haem-Pyrl complexes were stable for two days.

The primary component analysis further describes these differences. Factoring all variables of storage time and molar ratio of the ligands, the effect in colour intensity and the ligand identity become more evident (Figure 1c). Here, more red samples presenting more marked spectra maxima are farther from the important cluster of controls and chromatically unchanged samples. Three groups specific to the 3 different ligands can also be identified and, as highlighted previously, red complexes tend to lose colour and converge back to the control samples cluster over time. And as seen with the 4-MeI samples using the CIE *a** value, it is interesting to note that the colour difference between pure haemin- and HiH-ligand samples is observable for all ligands. The farthest points from the controls cluster, corresponding to a more marked spectral difference and a more intense red colour, can exclusively be associated with HiH-ligand complexes.

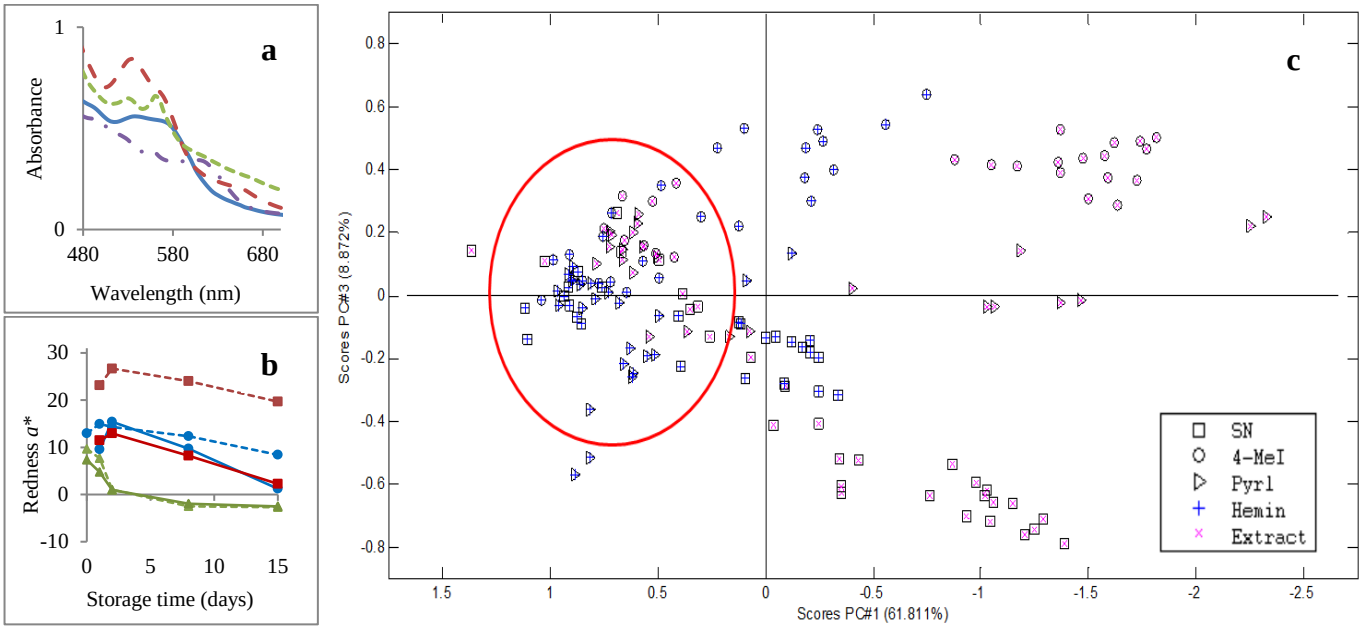

Figure 1. (a) Absorbance spectra for HiH-Ligand complexes (1:100 molar ratio and 3h timepoint). Solid line, SN; Long dashed line, 4-MeI; Short dashed line, Pyrl; Dash-pointed line, No ligand; (b) Calculated *a** values over storage time (1:100 molar ratio); ● SN; ■ 4-MeI;▲ Pyrl; —Hemin; --- HiH; (c) Primary Component Analysis for averaged triplicates of samples, PC#1 (61.8%) vs. PC#3 (8.9%). Circle encompasses the cluster of non-red samples.

IV. CONCLUSION

In the aim of finding alternatives to the use of sodium nitrite in the chromatic stabilisation of meat products, this work shows the increased haem binding affinity of 4-MeI, and its stabilising effect on the red colour. As the hydrolysed haem extract exhibited a more intense red hue at equal storage time and ligand concentration for all samples, it appears that the presence of protein hydrolysates improves the haem-ligand binding affinity. Further research will follow to explain the binding discrepancies between the two haem sources.

ACKNOWLEDGEMENTS

The present research has been funded by Innovation Fund Denmark under the grant #4135-00133B. The authors thank Erik T. Hansen from Danish Crown Ingredient for his help. Jorge Ruiz-Carrascal thanks Norma & Frode S. Foundation for its support.

REFERENCES

1. Wismer-Pedersen, J. (1988). Use of Haemoglobin in Foods – A Review. Meat Science 24: 31-45.
2. Akoyunoglou, J.-H. A., Olcott, H. S. & Brown, W. D. (1963). Ferrihemochrome and Ferrohemochrome Formation with Amino Acids, Amino Acid Esters, Pyridine Derivatives, and Related Compound. Biochemistry 2 (5): 1033-1041
3. Gallagher, W.A. & W.B. Elliott (1967). Alkaline Haematin and Nitrogenous Ligands. Biochemical Journal 108: 131-136.
4. Shahidi, F., Rubin, L. J., Diosady, L. L. & Chew, V. (1984). Preparation of Dinitrosyl Ferrohemochrome from Hemin and Sodium Nitrite. Canadian Institute of Food Science and Technology Journal 17 (1): 33-37.
5. CIE (2004). Technical Report: Colorimetry (3rd ed.). CIE 15.3:2004, Commission Internationale de l'Eclairage, Vienna.