# HEME PROTEIN AND FREE IONIC IRON INFLUENCE THE ODOUR-ACTIVE VOLATILES AND ODOUR OF COOKED BEEF

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## I. INTRODUCTION

Improving the flavour of beef is a continued goal of the beef industry. The flavour of cooked beef is largely dependent on the formation and release of odour-active volatiles, which could be influenced by heme proteins, including myoglobin, hemoglobin, and free ionic iron. Both heme proteins and free ionic iron could potentially influence odour-active volatiles, such as aldehydes and alcohols, by facilitating their formation in lipid oxidation. Also, heme proteins have been shown to directly bind volatiles and inhibit their release *in vitro* [1]. We hence hypothesised that heme protein and free ionic iron can influence the odour of cooked beef through altering the formation and release of odour-active volatiles.

## II. MATERIALS AND METHODS

To test our hypothesis, we spiked ground beef *semitendinosus* with hemoglobin and free ionic iron at two different levels (Level 1, 2 mg hemoglobin or 6.74 µg iron/g patty; level 2, 4 mg hemoglobin or 13.48 µg iron/g patty). At each level, the hemoglobin contained an approximately equivalent amount of iron to the free ionic iron. We prepared the samples in four separate batches, samples in each treatment × batch were prepared and analysed in triplicate. The spiked ground beef was formed into patties, vacuum-packed, and stored at -20 °C until further analysis. The patties were then cooked for 5 min 20 s from frozen until the internal temperature reached 70 ± 5 °C. The volatiles in cooked patties were measured using gas chromatography-mass spectrometry (GCMS). The odour of cooked patties was measured in a sniff test using a trained consumer panel (n = 15) in two separate sessions. The five odour attributes used in the sniff test were odour liking, odour intensity, grilled beef, overall meatiness, livery, and oxidised. In statistical analysis, the volatile and odour data were analysed using analysis of variance in Genstat (19th Edition, VSN International, Hemel Hempstead, UK). The relationship between odour and volatiles in cooked patties was analysed using partial least-square regression in the caret package in R (3.6.1, R Foundation for Statistical Computing, Vienna, Austria).

## III. RESULTS AND DISCUSSION

The results of our study shows that both heme protein and free ionic iron can influence the volatiles in cooked beef (Table 1). Spiking heme protein at level 1 reduced the concentration of almost all linear aldehydes such as octanal and E-2-octenal ($P < 0.05$ for all). This could be explained by the molecular binding between heme proteins and linear aldehydes. In contrast, spiking heme protein at level 1 increased concentrations of Maillard- and Strecker-derived volatiles including methylbutanals and pyrazines possibly by enhancing the Maillard reaciton during cooking ($P < 0.05$ for all). Spiking free ionic iron at level 2 increased the concentrations of 1-octen-3-ol and akylfurans but reduced the concentrations of many linear aldehydes ($P < 0.05$ for all). The effect of free ionic iron on 1-octen-3-ol could be explained by the prooxidative effect of free iron [2]. The decrease in linear aldehydes and increase in akylfruans could be explained by the further oxidation of aldehydes to akylfurans [3]. The results of the sensory assessment show that spiking free ionic iron at level 2 increased the livery and oxidised odour of cooked beef ($P < 0.05$ for both), whereas other odour attributes were not affected

by spiking (P > 0.05 for all). The results of partial least-square regression shows that the livery and oxidised odours increased with the concentrations of 1-octen-3-ol and akylfurans (P < 0.05 for both). In addtion, the livery odour increased with linear aldehydes but decreased when the concentrations of pyrazines and methylbutanals increased (P < 0.05 for all). The results indicate that free ionic iron increased the oxidised and livery odour of beef through facilitating the formation of lipid-derived volatiles. Heme protein could potentially reduce the livery odour of beef by inhibiting the release of linear aldehydes and increasing the formation of Maillard- and Strecker-derived volatiles. However, this speculation needs to be confirmed in further studies.

Table 1 Effect of heme protein and free ionic iron on volatiles (mg/kg patty) and odours of cooked beef patties

| Treatments | Control | Level 1[a] | | Level 2 | | SED[b] | P-value |
|---|---|---|---|---|---|---|---|
| | | Heme | Fe | Heme | Fe | | |
| Volatiles | | | | | | | |
| Octanal | 1.09 | 0.63 | 0.89 | 1.08 | 0.65 | 0.150 | 0.0005 |
| E-2-Octenal | 2.36 | 1.37 | 1.50 | 2.23 | 1.29 | 0.260 | <0.001 |
| 3-Methylbutanal | 0.28 | 0.35 | 0.27 | 0.31 | 0.27 | 0.026 | <0.001 |
| 3-Ethyl-2,5-dimethylpyrazine | 0.26 | 0.37 | 0.22 | 0.30 | 0.29 | 0.038 | 0.008 |
| 1-Octen-3-ol | 0.84 | 0.95 | 2.50 | 1.03 | 2.33 | 0.250 | <0.001 |
| 2-Ethylfuran | 0.07 | 0.06 | 0.11 | 0.08 | 0.10 | 0.009 | <0.001 |
| Odours | | | | | | | |
| Livery | 41.5 | 36.7 | 51.0 | 46.2 | 52.1 | 5.16 | 0.018 |
| Oxidised | 35.3 | 36.5 | 43.8 | 39.2 | 51.6 | 4.45 | 0.011 |

[a]Level: Level 1, patties spiked with 2 mg hemoglobin/g or 6.74 µg iron/g; level 2, patties spiked with 4 mg hemoglobin/g or 13.48 µg iron/g. At each level, the hemoglobin contained approximately equal amount of iron to the free ionic iron.
[b]SED: Standard error of difference.

## IV. CONCLUSION

Our study shows that free ionic iron could negatively influence the odour of cooked beef by facilitating the formation of lipid-derived volatiles. In contrast, heme protein has the potential to reduce the livery off-flavour of beef by inhibiting the release of lipid-derived volatiles and increasing Maillard- or Strecker-derived volatiles.

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