PE4.69 Proxidative properties of monocarboxy myoglobin in systems with added polyunsaturated fatty acids 249.00

Jon Volden (1) jon.volden@umb.no, GA Bjørlykke(1), O Sørheim 2, B Egelandsdal 1 E Slinde 3
(1)Norwegian University of Life Sciences, Norway
(2)Nofima Mat, Norway
(3)Institute of Marine Research, Norway

***Abstract*— A model system investigating the interaction between the protein myoglobin (Mb) and polyunsaturated fatty acids (PUFA) has been established directly in headspace (HS) vials. The volatile degradation products were characterized by HS gas chromatography and mass-spectrometry (GC-MS). Myoglobin with bound carbon monoxide (MbCO) was more stable towards degradation than metmyoglobin (metMb) at pH values similar to those found in meat. MbCO did not promote oxidation of PUFA to the same extent as metMb. The model system presented is easily adaptable to a wide range of different proteins, as well as other health related chemical constituents, holding an expedient potential studying many different parameters**.

J. Volden, G. A. Bjørlykke and Bjørg Egelandsdal are with the Department of Chemistry, Biotechnology and Food Science at the Norwegian University of Life Sciences, N-1432 Aas, Norway (corresponding author: phone: +476-496-5870; fax: +476-496-5901; e-mail: jon.volden@umb.no; gry.bjorlykke@student.umb.no; bjorg.egelandsdal@umb.no).

O. Sørheim is with Nofima Mat, Osloveien 1, N-1430 Aas, Norway (e-mail: oddvin.sorheim@nofima.no).

E. Slinde is with the Institute of Marine Research, P.O. Box 1870, Nordnes, N-5817 Bergen, Norway (Erik.slinde@imr.no).

***Index Terms* —**
GC/MS, Headspace, Myoglobin, Polyunsaturated Lipids, Volatiles.

I. INTRODUCTION

An intake of fatty fish containing polyunsaturated fatty acids (PUFA), has been associated with an increased well-being and decreased risk of several adverse conditions *(1, 2)*. Incorporation of PUFA in terrestrial animals is attempted through feeding in order to make the meat and meat products more healthy. Eicosapentaenoic (EPA) and docosahexanenoic acids (DHA) are the most interesting fatty acids in this respect being precursors to prostaglandins which are affecting blood vessel dilation/constriction, blood clotting and inflammation *(3)*. However, PUFA are prone to oxidation dependent on the degree of saturation and chain-length *(4)*. Especially myoglobin and its iron within the heme group give raise to numerous oxidation products dependent on red/ox state and type of ligands bound to the sixth coordination site. In this connection packaging in high oxygen atmosphere leads to high amounts of oxidation products. Decimation of PUFA can lead to a less optimized product regarding the health-related perspective. Also, the oxidation products are perceived at very low concentrations leading to unsatisfactory quality.

The aims of this study have been two-fold:

One: Establishing a model system consisting of salmon oil and myoglobin directly in vials suitable for dynamic single headspace analysis. The underlying idea was to construct a system in which degradation products between various PUFA and proteins can be monitored. A method like this would be promising for monitoring a wide range of different systems and parameters.

Two: Studying the degradation of MbCO at pH 7.4 (pH in muscle), 6.2 and 5.6 (final post mortem pH in salmon and pork) and 5.0 (pH found in fermented salami). This was done in order to determine how MbCO degraded at different pH values of relevance for meat products. Furthermore, the degradation products were believed to have different effects on the oxidation of PUFA and other proteins in meat products.

II. MATERIALS AND METHODS

Chemicals

$Na_2S_2O_4$ and $NaNO_2$ were obtained from BDH Chemicals ltd. (Poole, UK). KH_2PO_4, K_2HPO_4 and $K_3[Fe(CN)_6]$ were from Merck KGaA (Darmstadt, Germany). Equine myoglobin (Mb) was purchased from Sigma Chemicals Corp. (St. Louis, MO). Helium and CO gas was supplied from Yara International ASA (Oslo, Norway). All chemicals and gases used were of analytical grade.

Myoglobin forms

Metmyoglobin (metMb) was made using 50 mM pH 5.0, 5.6, 6.2 and 7.4 phosphate buffers, oxidized with potassium hexacyanoferrate(III), filtered and purified using Econo-Pac® 10DG column (Bio-Rad Laboratories, Hercules, CA). Sodium dithionite was used to obtain deoxymyoglobin before flushing with CO for 30 s producing MbCO. Solutions were scanned from 650 to 340 nm at 1 nm intervals using a Hitachi U-2000 Double-Beam UV/Vis Spectrophotometer (Hitachi Ltd., Tokyo, Japan). Concentrations of metMb were determined using ξ 188 at 408 nm *(5)*. The relative degradation of MbCO was determined by measuring the change in the absorption peak at 580 nm subtracting light scatter and other absorption by only using the peak value as such.

Model system

The system was established directly in 20 ml headspace sample vials. 0.2 ml salmon oil was added to 1.8 ml metmyoglobin (metMb), sealed and left for 24 and 48 h at 25 ˚C with continuous stirring.

Headspace analysis

Sampling of volatiles was carried out using a Teledyne Tekmar HT3™ Static/Dynamic Headspace System (Teledyne Tekmar, Mason, OH) using a sample preheating time of 5 min, helium flow of 50 ml/min for 10 min, trapping the analytes on a 24 cm Tenax® GR 60/80 mesh size (Supelco Analytical, Bellefonte, PA) at 25 ˚C. Software version was HT3 Teklink Ver. 1.2.1104 (Teledyne Tekmar). Desorption was performed at 280 ˚C with a gas flow of 75 ml/min for 5 min and the GC-transfer line temperature was 100 ˚C.

Gas chromatography/mass-spectrometry

Analyte separation was achieved using a 6890N Network GC System (Agilent Technologies, Waldbronn, Germany) fitted with a DB-WAXETR 30 m × 0.25 mm × 0.50 µm capillary column (Agilent Technologies) with 1 ml/min helium as carrier gas. Temperature program was: 30 ˚C for 10 min, then 1 ˚C/min to 40 ˚C, 3 ˚C/min to 70 ˚C and 6.5 ˚C/min to 230 ˚C followed by 5 min hold-time.

Detection was obtained using a 5975 Inert XL Mass Selective Detector (Agilent Technologies) in electron ionization mode (70 eV) with ion source temperature at 200 ˚C scanning continuously the range 33 to 300 m/z. The GC/MS used MSD ChemStation D.02.00.275 software (Agilent Technologies). The analytes were identified using NIST MS Search 2.0, retention times and single reference compounds.

III. RESULTS AND DISCUSSION

The presented model in this study has measured degradation products formed from, amongst others, EPA and DHA in the presence of MbCO and metMb. The development of volatile degradation products from the mixture of salmon oil and metMb and MbCO is illustrated in Figure 1. Volatiles identified are shown in Table 1 and correspond to previous reports on PUFA degradation products *(4, 6, 7)*. Some of the most prominent volatiles were 1-penten-3-ol, propanal, 2-ethylfuran and 2,4-heptadienal. The cumulative volatile amounts were found in greater quantities in the samples containing Mb^{3+}.

Stability measurements over eight days of MbCO at 6 ˚C and 20 ˚C are shown in Figure 2 and 3, respectively. MbCO is more stable at lower than higher temperatures. The relative stability of MbCO decreased as the pH was lowered. Slinde studied the dissociation of heme from horse metMb, and in agreement with what we observed, found that heme dissociated at lower pH values, and that a complete detachment of the heme group was found around pH 5 *(8)*. Sørheim et al. also reported a decline in absorbance for MbCO at pH 4.7 after 20 h roomtemperature storage *(9)*. Horse metHb was found to be more stable than metMb at lower pH values, but also for this protein a complete detachment of the heme groups from the protein was found below pH 5. It is a general experience that oxidized Mb promote lipid oxidation, and the oxidative properties of reduced Mb is less and even lower when a ligand such as CO is bound to the sixth position of iron in the heme moiety. This can also be seen from Figure 1, where the development of oxidation products was higher for metMb than MbCO.

The measurement of thiobarbituric acid-reactive species (TBARS) used to determine oxidation, was based on be a reaction between PUFA oxidation products and thiobarbituric acid. However, not all aldehydes produced by oxidation reactions contribute to the TBARS value *(7)*. We believe that some other product seen in our GC/MS analyze may contribute to the TBARS value, and these might be identified in the near future.

IV. CONCLUSION

An adaptable system monitoring volatile oxidation products from PUFA-proteins interactions have been established. Myoglobin with iron in oxidation state can

lead to more lipid oxidation compared to the same specie associated with CO. 1-penten-3-ol was one of the main oxidation products found in a model system of salmon oil and horse Mb. Larger quantities of volatiles were found in the samples containing Mb^{3+}. MetMb can be regarded as promotor of lipid oxidation, and the oxidation rate is dependent on the pH value.

The stability investigation of MbCO shows a higher stability at lower temperatures. Also, the relative stability of MbCO decreased with decreasing pH. In addition horse metHb were more stable than horse metMb at lower pH values, still, complete detachment of the heme groups was found at pH values below 5.

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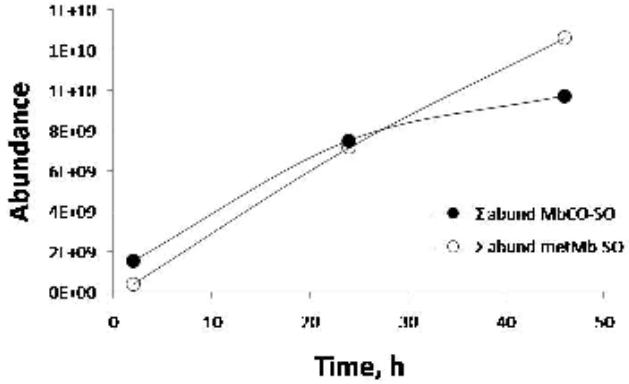

Figure 1: Cumulative abundance-time profile for the volatiles in the model system headspace.

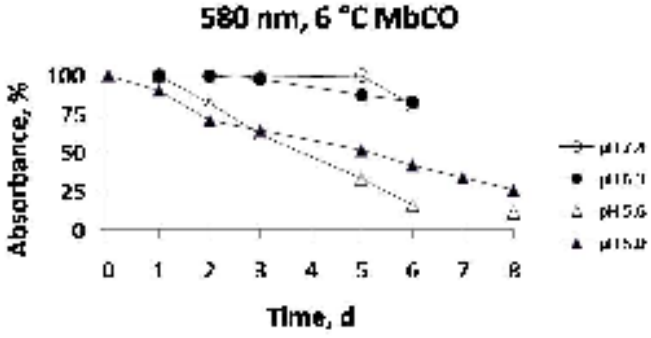

Figure 2: Relative absorbance of MbCO at 6 ˚C over time.

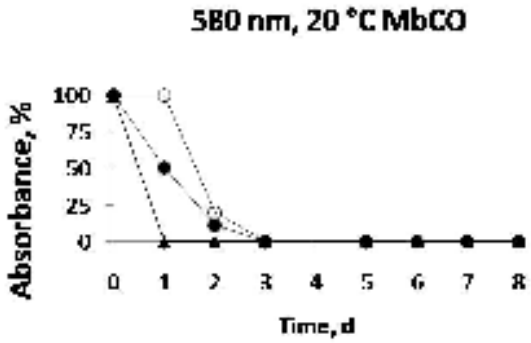

Figure 3: Relative absorbance of MbCO at 20 ˚C over time.

Table 1: Main volatile oxidation products found in the headspace of the model system.

Compound	NIST (%)	RT (min)
propanal	76	4,0
2-propenal	91	5,4
butanal	47	6,4
2-ethylfuran	70	10,7
pentanal	64	12,5
1-penten-3-one	92	16,3
2-butenal	54	18,2
2,3-pentanedione	93	21,3
hexanal	64	22,3
2-pentenal	30	26,5
1-penten-3-ol	58	30,5
2-pentylfuran	65	31,7
2-hexenal	29	32,1
trans-2,[2-pentenyl]furan	55	34,8
2,heptenal	46	35,8
2-penten-1-ol	69	36,7
2-octenal	42	38,4
2,6-hexadienal	40	38,5
2,4-heptadienal	55	40,4