PE2.04 Headspace volatiles of oxidized food proteins 299.00

Mario Estévez (1) mario.estevez@helsinki.fi, S Ventanas(2), M Heinonen (1)
(1)University of Helsinki, Finland
(2)University of Extremadura, Spain

Abstract— To investigate the formation of volatile compounds from oxidizing food proteins was the main purpose of the present study. Myofibrillar proteins, α-lactalbumin, BSA and soy proteins were suspended (20 mg/mL) in 15mM PIPES buffer (pH=6) containing 0.6M sodium chloride. Protein suspensions (30 mL) were oxidized (10 µM FeCl3, 0.1 mM ascorbic acid, 1 mM H2O2) while constantly stirred and kept in a oven at 37ºC for 14 days. At sampling times (days 1, 4, 7, 10 and 14), oxidized proteins were analyzed for headspace volatiles using solid-phase microextraction coupled to gas-chromatography and mass spectrometry (SPME-GC-MS). In order to evaluate the role of oxidative reactions on volatiles formation, protein suspensions were also analyzed for protein carbonyls using the DNPH method. Strecker aldehydes, namely, acetaldehyde, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde were the most abundant volatile components of oxidized proteins. The relative amount of Strecker aldehydes increased concurrently with the oxidation of proteins suggesting the timely relationship between oxidative reactions and volatiles formation. In fact, a positive correlation was found between certain Strecker aldehydes and protein oxidation measurements. In the absence of oxidizing lipids, it is plausible to consider that protein carbonyls might have reacted with non-modified amino acids to yield volatile compounds through the Strecker degradation pathway. Results from the present study originally highlight that food proteins could act as relevant sources of odor-active volatile compounds without the collaboration of oxidizing lipids. The role played by protein carbonyls in the Strecker degradation of amino acids is supported by upcoming results.

Author M.E. is with the Department of Applied Chemistry and Microbiology (Food Chemistry Section), University of Helsinki, 00014, Helsinki. Finland.. Current affiliation of M. E.: Department of Animal Production and Food Science (Food Technology Section), University of Extremadura, 10071, Cáceres, Spain (corresponding author phone: +34927257122; fax: +34927257110; e-mail: mariovet@unex.es). Author S.V. is with the Department of Animal Production and Food Science (Food Technology Section), University of Extremadura, 10071, Cáceres, Spain. Autrhor M.H. is with the Department of Applied Chemistry and Microbiology (Food Chemistry Section), University of Helsinki, 00014, Helsinki. Finland.

Index Terms - protein oxidation, semialdehydes, SPME-GC-MS, Strecker aldehydes, volatiles.

I. INTRODUCTION

VOLATILE compounds are responsible for the odor and flavor of foods. The generation of volatile compounds in muscle foods has been largely studied because of the role of flavor in the overall acceptability these products [1]. Certain volatile compounds, however, contribute to unpleasant odors and are used as indicators of oxidative and spoilage deterioration of foods. Lipids are considered the main source of volatile compounds in meat systems [1]. Handling, processing and storage of meat and meat products enhance lipid hydrolysis and oxidation leading to the formation of a diversity of oxidation products including volatile compounds from diverse chemical families such as acids, aliphatic aldehydes, ketones and alcohols [2]. As odor-active compounds, some of them display low detection thresholds and contribute defined aromatic notes to muscle foods [1]. Muscle proteins are considered to play a secondary role as proteins form the matrix from which lipid-derived volatiles are usually released. The Strecker degradation of amino acids is, however, a relevant route for the formation of volatile compounds – Strecker aldehydes – in which proteins play a major role. The chemical mechanism involves the condensation of the amino group with a carbonyl group which eventually triggers the transamination and decarboxylation from the original amino acid [3]. Dicarbonyls derived from Maillard reaction readily react with amino acids for the formation of the corresponding Strecker aldehydes [3]. Considering the scarce amount of reducing sugars in muscle foods, carbonyl compounds derived from lipid oxidation are more likely involved in the Strecker degradation of amino acids from muscle proteins [4]. Therefore, it is generally accepted that the formation of Strecker aldehydes in meat products necessarily requires the presence of early Maillard products or oxidizing lipids. The role of protein oxidation in the

development of food odor and flavor is mostly unknown. The lack of knowledge of precise chemical mechanisms and oxidation products are main responsible for this situation. However, novel results recently published in relation to the detection of specific protein carbonyl compounds from food proteins [5] provide a suitable background for performing further studies aimed to shed light on the role of protein oxidation in volatiles generation. The formation of volatile compounds during in vitro oxidation of food proteins is investigated in the present study.

II. MATERIALS AND METHODS

Materials All chemicals were supplied by J.T Baker (Deventer, Holland), Riedel de-häen, Sigma Aldrich (Steinheim, Germany) and Extrasynthese (Germany, France). Porcine longissimus dorsi muscle was purchased in a local supermarket in Helsinki. Myofibrillar proteins were extracted from the porcine muscle following a method described elsewhere [6]. Methods Extraction and in vitro oxidation of muscle proteins Myofibrillar proteins, α-lactalbumin, soy protein and BSA were suspended (20 mg/mL) in 15mM PIPES buffer (pH=6) containing 0.6M sodium chloride. Protein suspensions (30 mL) were dispensed in sealed vials and oxidized (10 μM FeCl3, 0.1 mM ascorbic acid, 1 mM H2O2) while constantly stirred and kept in a oven at 37ºC for 14 days. Sampling was carried out at days 1, 4, 7, 10 and 14 for analyses. SPME of volatiles The SPME fiber, coated with a divinylbenzene-carboxen-poly(dimethylxilosane) (DVB/CAR/PDMS) 50/30μm, was preconditioned prior analysis at 220ºC during 45 min. The headspace (HS) sampling and the subsequent GC-MS analysis was performed following a method previously described [2]. 1 g of frankfurter was placed in 2.5 mL vials and the SPME fiber was exposed to the headspace of the pâté while the sample equilibrated during 30 minutes immersed in water at 50ºC. Volatile compounds were positively identified by comparing their linear retention indexes (LRI) with those from standard compounds. Chromatographic areas from MS are provided as area units (AU). Protein carbonyls - DNPH method Total protein carbonyls were quantified in protein suspensions at sampling times according to the method described elsewhere [5]. The amount of carbonyls was expressed as nmol of carbonyl per mg of protein using an absorption coefficient of 21.0 nM-1 cm-1 at 370 nm for protein hydrazones. Experimental design and statistical analysis All suspensions were made three times and the analysis performed in duplicate (n=6). The Analysis of Variance from SPSS for Windows ver. 6.1 was used in order to assess differences between protein suspensions. T-student tests were performed when ANOVA found significant differences. Pearson correlations were also calculated in order to establish relationships between parameters. Statistical significance was set at $p<0.05$.

III. RESULTS AND DISCUSSION

The analysis of the headspace from oxidized food proteins revealed that Strecker aldehydes were the most abundant volatile compounds. Some of them, namely, ethanal, methylpropanal, 3-methylbutanal, 2-methylbutanal and 2-phenylethanal were positively identified and listed in Table 1. Each Strecker aldehyde derives from the degradation of an specific amino acid and contribute defined aroma notes. The Strecker aldehydes reported in the present paper are common volatile components of a wide range of meat products [2,4]. In particular, 3-methylbutanal and 2-methylbutanal which derive from the Strecker degradation of leucine and isoleucine, respectively, have been highlighted as relevant aroma contributors in meat products [1] and labelled as ripening markers in dry-cured products [4]. The evolution of the total amount of Strecker aldehydes in the protein suspensions during the oxidation assay is displayed in Figure 1. The relative amount of Strecker aldehydes slightly increased during the first 7 days whereas a very intense increase was observed by the end of the oxidation assay. Significant differences were found between protein suspensions as the formation of Strecker aldehydes was more intense in BSA and myofibrillar proteins than in α-lactalbumin while soy proteins showed the lowest amount of Strecker aldehydes. The progress of the protein oxidation, measured by means of protein hydrazones (Figure 2) suggest that the formation of Strecker aldehydes in protein suspensions could be related to the susceptibility of these protein to undergo oxidative degradation. The differences between groups for the total amount of protein carbonyls are consistent with those previously reported for the relative amount of Strecker aldehydes. The different susceptibility of these proteins towards oxidative reactions were discussed in a previous paper [5]. The present results suggest that carbonyl compounds derived from protein oxidation might be involved in the Strecker degradation on non-

modified amino acids. The lack of reducing sugars and oxidizing lipids in the protein suspensions makes the above hypothesis a plausible mechanism. In fact, the intense formation of Strecker aldehydes is concomitant with the slight decrease of protein carbonyls by the end of the assay. Like this, a higher susceptibility to oxidative degradation and a more severe carbonyl production leads to a more intense formation of Strecker aldehydes. The timely interaction between protein oxidation and volatiles generation is supported by the significant positive correlation found between the protein oxidation measurement and the total amount of Strecker aldehydes (0.75; $p<0.001$). Carbonyls derived from protein oxidation have never been considered to be involved in the Strecker degradation of amino acids. Actually, the exact chemical structures of protein carbonyls formed in foods have been mostly unknown. Recently, two carbonyl compounds (α-aminoadipic semialdehyde and γ-glutamic semialdehyde, AAS and GGS, respectively) have been originally deteted in oxidized food proteins [5]. AAS derives from the oxidative deamination of lysine while GGS is formed from proline and arginine oxidation. According to the proposal shown in Figure 3, these semialdehydes could react with amino acids to yield the corresponding Strecker aldehydes (the reaction between AAS and leucine is shown as an example). Ongoing experiments aimed to confirm the reaction between protein semialdehydes and amino acids (unplublished data) support the proposal previously reported. The volatile profiles were also different between protein suspensions (Table 2). Compared to the other protein systems, BSA had significantly higher levels of branched aldehydes whereas myofibrillar proteins showed the highest amount of 2-phenylethanal. These differences should be attributed to a variety of factors, including the tertiary structure of the proteins, their size, their amino acid composition and sequence, and the distribution of amino acids on the protein structure. In this sense, some amino acids might be more abundant or more accesible for reacting than others in a particular protein which largely determines the volatiles profile.

IV. CONCLUSION

The recent detection of specific carbonyl compounds derived from protein oxidation emphasize the occurrence of novel routes for the formation of Strecker aldehydes. According to the present results, protein oxidation could play a relevant role in the formation of Strecker aldehydes through the reaction between semialdehydes derived from oxidized amino acids and non-modified amino acids. This route could be particularly significant in meat products subjected to intense proteolysis and oxidative reactions (i.e. dry-cured products). ACKNOWLEDGEMENT M.E. thanks the European Commission for the economical support from the Marie Curie Intra-European Fellowship. Technical support from Miikka Olin, Kirsti Risunen and Maija Ylinen is also acknowledged.

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TABLES AND FIGURES

Table 1. Strecker aldehydes found in the headspace of oxidized food proteins.

Amino acid precursor	Strecker aldehyde	Structure	Aroma description	Odor threshold value (µg/l; water)
Ala	Ethanal		Sharp, penetrating fruity	10
Val	Methylpropanal		Malty	1
Leu	3-Methylbutanal		Malty	0.2
Ile	2-Methylbutanal		Malty	2
Phe	2-Phenylethanal		Flowery, honey-like	2

Figure 1. Evolution of total Strecker aldehydes (AU) during oxidation of food protein suspensions.

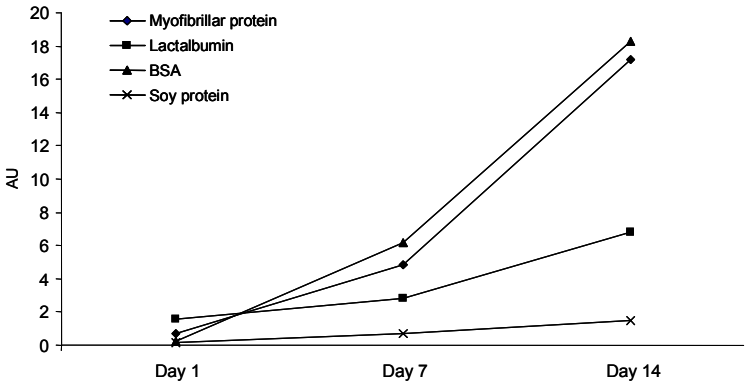

Figure 2. Evolution of total protein carbonyls (mM carbonyls/mg protein) during oxidation of food protein suspensions.

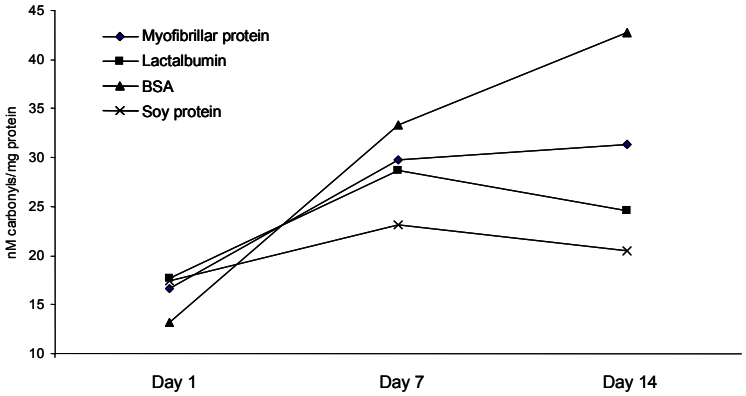

Figure 3. Formation of 3-methylbutanal from the reaction between α-aminoadipic semialdehyde and leucine.

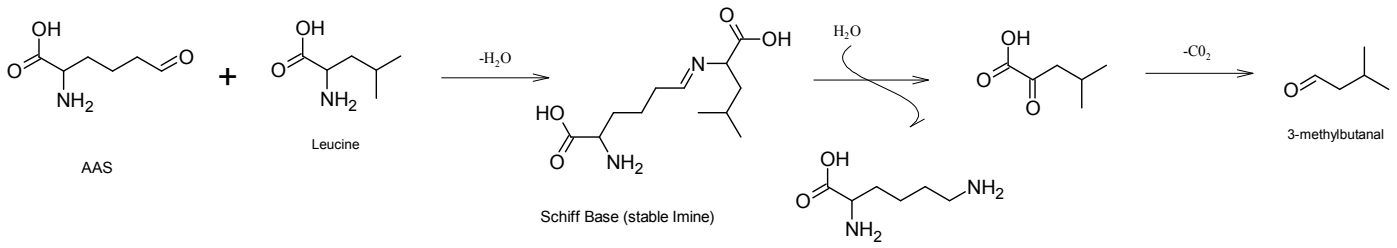

Table 2. Strecker aldehydes (mean ± standard deviation) in the headspace of different protein suspensions at day 14.

	MP	LAC	BSA	SOY
ethanal	2.26b±0.83	1.38c±0.32	3.03ab±0.64	4.63a±1.17
2-methylpropanal	2.03b±0.43	1.01c±0.28	6.14a±2.07	0.56d±0.10
2-methylbutanal	0.76b±0.18	0.83b±0.36	2.46a±0.30	0.36c±0.07
3-methylbutanal	0.85b±0.19	1.40b±0.67	2.58a±0.45	0.29c±0.07
2-phenylethanal	11.31a±4.30	2.20c±0.97	4.10b±1.04	0.00d±0.00

Means with different letters resulted significantly different in ANOVA test.