

POTENTIALLY TOXIC LINOLEIC ACID OXIDATION PRIMARY PRODUCTS IN COMMINUTED POULTRY MEAT

T. Püssa and P. Toomik

Department of Food Hygiene, Estonian University of Life Sciences, Kreutzwaldi St. 58A, 51014, Tartu, Estonia.

Abstract – Comminuted fat-rich meat products such as mechanically deboned meat (MDM), are, in principle, in the absence of antioxidants, extremely (per)oxidizable. Numerous oxygenated fatty acids (oxylipins) are formed during the first steps of the oxidation. The purpose of this study was to compare by liquid chromatography-mass spectrometry (LC-MS/MS) the content of leukotoxin diols (LTX-diols), potentially toxic primary products of free linoleic acid oxidation, in poultry MDM, in hand-deboned poultry meats (HDM), and in commercial meat products supplemented with poultry MDM. The MDMs show high contents (up to 60 ppm) of leukotoxin diols after 1-week storage in a fridge. Approximately the same summary content of LTX-diols was established in some commercial meat products containing MDM. Such a content of LTX-diols may turn out to be chronically toxic in the case of daily consumption of 100-200 g of MDM during long periods. The content of LTX-diols in the aged poultry HDMs is substantially lower. Consequently, supplementation of meat products with low-quality oxidized MDM may cause a significant rise in the content of oxylipins, including LTX-diols in the final products (sausages etc.), unless the rancidification is minimized by proper handling of MDM.

Key Words – leukotoxin diols, mechanically deboned meat, LC-MS/MS.

I. INTRODUCTION

Fat-rich comminuted meat such as mechanically deboned meat (MDM), or minced meat are, in the absence of antioxidants, extremely (per)oxidizable both by chemical (free radical) and enzymatic pathways. MDM is a food material, obtained by application of mechanical force (pressure and/or shear) to animal bones or poultry carcasses from which the bulk of meat (hand deboned meat – HDM) has been manually removed. MDM contains substantially more fat (about 15-25% of the total weight) and free polyunsaturated fatty acids (PUFAs) originating from the bone marrow

and bone tissue than any of HDM-s (containing 1-2% of fat) of the same animal (Table 1).

Due to a high concentration of polyunsaturated fatty acids (PUFA), especially linoleic acid, which may cause adverse physiological effects via their (per)oxidation products, and several factors activating lipid oxidation enzymes, MDM is considered to be a potentially harmful product.

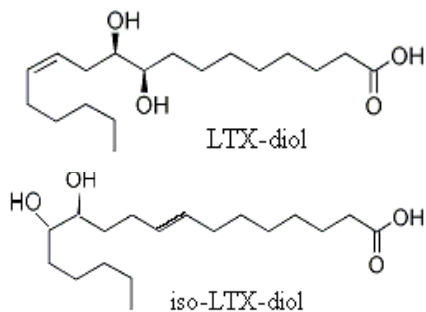
Table 1. Total fat content (%) and fatty acid composition (%) of different meats, estimated by gas chromatographic method according to [6]. The numbers are the mean values for two batches.

Type of meat	HDM		MDM	
	chicken	turkey	chicken	turkey
Total fat (%)	1.4 ± 0.2	2.1 ± 0.3	14.7±0.6	20.3±0.6
Saturated fatty acids	28.4±0.8	33.8±0.8	29.7±1.2	31.2±1.4
MUFAs	42.5±0.6	37.8±1.0	48.6±1.0	47.2±1.6
PUFAs,	29.2±0.7	28.4±0.6	21.5±1.0	21.3±0.8
including linoleic	24.3±0.6	22.5±0.5	17.8±0.8	18.4±0.8
α-linolenic	1.3±0.1	0.8±0.1	2.7±0.2	2.3±0.2
arachidonic	1.8±0.2	3.2±0.2	0.4±0.05	0.3±0.02

It is well known that the volatile secondary (end) products of PUFAs oxidation, thiobarbituric acid reactive substances (TBARS) such as malondialdehyde express mutagenicity reacting with DNA to form adducts to deoxyguanosine and deoxyadenosine [1]. Other reactive carbonyls, i.e. degradation products of oxylipins, are also toxic. Little is known about the content, dynamics, and toxicity of the oxygenated fatty acids (oxylipins), the primary products of PUFAs oxidation. We have studied the oxylipins derived from linoleic acid in meat, and identified over 10 various compounds by liquid chromatography hyphenated with mass selective (MS/MS)

detection [2]. Among them, special attention has to be paid to 9,10-dihydroxy-12-octadecenoic acid (9,10-DiHOME, known also as leukotoxin diol (LTX-diol)) and 12,13-dihydroxy-9-octadecenoic acid (isoleukotoxin-diol – isoLTX-diol).

The acute toxicity of the endogenous leukotoxin diols is well characterised [3], furthermore, it was recently found that the exogenous LTX-diols disrupt the endocrine function in female rats in chronic toxicity experiments [4]. LTX-diols have exerted also mitogenic activity and stimulated human breast cancer cell proliferation *in vitro* [5].



II MATERIALS AND METHODS

Chicken and turkey MDMs were prepared at a local meat processing plant, both in two batches and frozen separately. After defrosting at the laboratory, the samples were kept for 6 days at the fridge temperature (+4-6 °C) to promote oxidation. 2 g of each meat material (two duplicate analytical samples) was extracted with 4 ml of methanol by shaking during 30 min, and centrifuged at $3200 \times g$ for 10 min. The supernatant was extracted twice with 2 ml of hexane and the methanol phase was passed through a C18-SPE column. The same scheme without aging was used for preparation of samples of the commercial meat products.

Compounds were separated by HPLC on a reversed-phase Zorbax 300SB-C18 column (gradient of 0.1% aqueous formic acid and acetonitrile), and identified and quantified by electrospray ionization tandem mass-spectrometry (ESI-MS/MS), using 1100 series LC/MSD Trap (Agilent Technologies) in the negative ion mode. Major oxylipins, including LTX-diols, were identified by a comparison of their MS² daughter ion spectra (Figure 2) with the published fragmentation spectra and spectra of respective commercial standards. LTX-diols were quantified

using calibration curve of commercial standard of LTX-diol (Cayman Europe, Tallinn, Estonia).

III RESULTS AND DISCUSSION

An example of the MS-base peak chromatogram of MDM after long-term storage at the fridge temperature is presented in Figure 1.

The summary content of LTX-diols in MDMs was found to be in the range of 30-55 ppm (Figure 3) that in the case of a human daily consumption of, for example, 100 g of MDM, corresponds to a daily dose of about 0.03-0.08 mg of LTX-diols per kg bw. The last number is only about two times lower than the lowest observed adverse effect level (LOAEL) of leukotoxin diols for female rats.

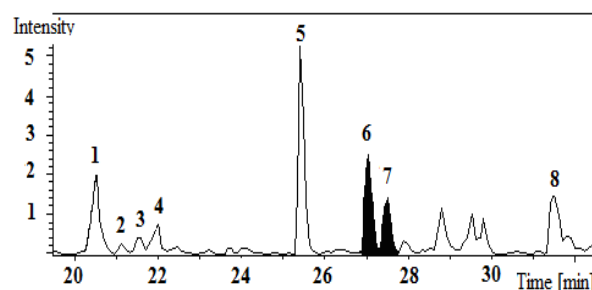


Figure 1. MS-base peak chromatogram of chicken MDM oxylipins. 1-4 - isomers of trihydroxyoctadecenoic acid; $m/z = 329$; 5. mixture of 13-hydroxy-9-oxo- and 13-oxo-9-hydroxy-10-octadecenoic acids; $m/z = 311$; 6. **iso-LTX-diol**, and 7. **LTX-diol**; $m/z = 313$; 8. mixture of 9- and 13- hydroxyoctadecenoic acids, $m/z = 295$ atom mass units (amu).

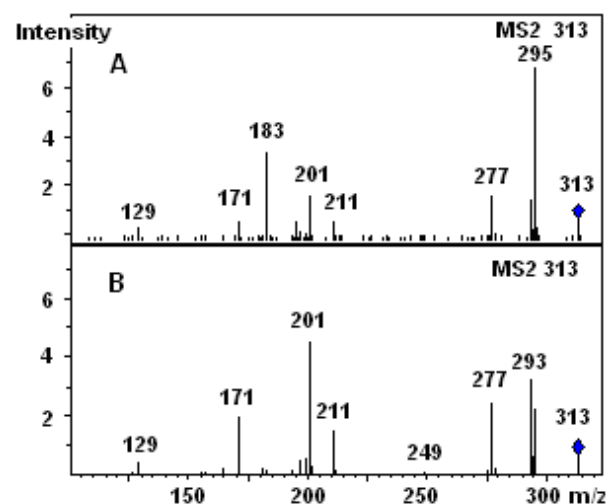


Figure 2. MS² spectra of iso-LTX-diol (A) and LTX-diol (B) ($m/z=313$). Watch the characteristic for most of linoleic acid oxylipins fragment with $m/z = 171$ amu.

A set of commercial comminuted meat products, comprising various sausages, frankfurters, barbecue sausages, pates, *bratwürste*, purchased at supermarkets in Tartu was also studied by LC-MS/MS. In 4 samples out of 30, the summary content of leukotoxin-diols exceeded 30 ppm, the highest being 65 ppm. In all of these products, except one, the content of either chicken or turkey MDM (up to 74 %) was declared. On the other hand, in one item (frankfurters) where the content of MDM (37%) was also declared, the summary content of LTX-diols was as low as 6 ppm. It means that the occurrence of MDM in a comminuted meat product should not automatically mean a high concentration of toxic LTX-diols as well as other oxylipins in this processed meat product.

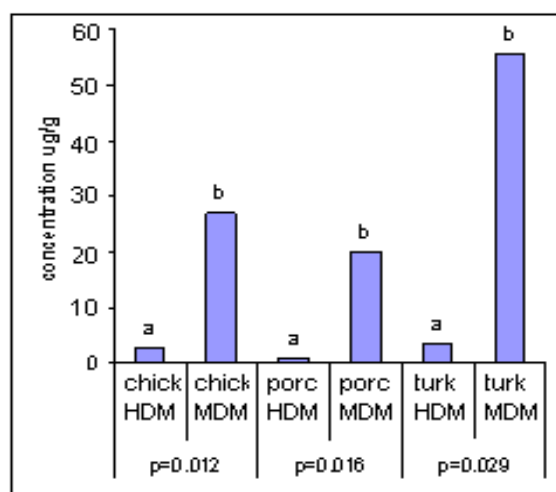


Figure 3. Summary concentration of LTX-diols after 6-day storage of chicken (chick), porcine (porc), and turkey (turk) MDMs and HDMs at +4-6 °C.

IV CONCLUSION

Supplementation of meat products with mechanically deboned meat may cause a significant rise in the content of oxylipins, including potentially toxic leukotoxin-diols, in the products (sausages), unless the rancidification is minimized by proper handling of the MDM.

The achieved levels of LTX-diols may evoke adverse effects in the consumer's body in the case of daily consumption of respective meat products during long periods.

REFERENCES

1. Marnett, L.J. (1999). Lipid peroxidation – DNA damage by malondialdehyde, *Mutation Research*, 8, 83-95.
2. Püssa, T., Raudsepp, O., Toomik, P., Pällin, R., Mäeorg, U., Kuusik, S., Soidla, R., Rei, M. (2009). A study of oxidation products of free polyunsaturated fatty acids in mechanically deboned meat, *J. Food Composition and Analysis*, 22, 307-314.
3. Zheng, J., Plopper, C.G., Lakritz, J., Storms, D.H. & Hammock, B.D. (2001). Leukotoxin-diol. A putative toxic mediator involved in acute respiratory distress syndrome. *Amer. J. Respiratory Cell and Mol. Biol.*, 25, 434-438
4. Markaverich, B.M., Crowley, J.R., Alejandro, M.A., Shoulars, K., Casajuna, N., Mani, S., Reyna, A. & Sharp, J. (2005). Leukotoxin diols from ground corn cob bedding disrupt estrous cyclicity in rats and stimulate MCF-7 breast cancer cell proliferation. *Environmental Health Perspectives*, 113, 1698-1704.
5. Markaverich, B.M., Alejandro, M., Thompson, T., Mani, S., Reyna, A., Portillo, W., Sharp, J., Turk, J. & Crowley, J.R. (2007). Tetrahydrofurandiols (THF-diols), leukotoxin diols (LTX-diols), and endocrine disruption in rats, *Environmental Health Perspectives*, 115, 702-708.
6. Christie, W.W. (1989). *Gas chromatography and Lipids, A Practical Guide*. The Oily Press, Ayr, UK.

