LEUKOTOXIN-DIOLS IN MECHANICALLY DEBONED MEAT

Tõnu Püssa, Peeter Toomik, Piret Raudsepp, Dea Anton

Estonian University of Life Sciences, Department of Food Science and Hygiene, Kreutzwaldi 58A, Tartu, Estonia, **Email: pyssa@emu.ee**

www.emu.ee
Eesti Maaülikool
Estonian University of Life Sciences
Veterinaarmeditsiini ja loomakasvatuse instituut
Institute of Veterinary Medicine and Animal Sciences

World Congress on Oils and Fats
& 28th ISF Congress
27 - 30 September 2009 • Sydney Australia

Introduction

Mechanically deboned meat (MDM) is a food material, obtained by the 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 lipids (about 15-25% of the total weight) than any of HDMs (1-2%) of the same animal that may originate from both the bone marrow and the bone tissue.

Due to a high concentration of polyunsaturated fatty acids (PUFA), which may cause adverse physiological effects via their primary (oxylipins) as well as secondary (aldehydes, ketones) (per)oxidation products, as well as several factors activating lipid oxidation enzymes, **MDM is considered to be a potentially harmful product**.

Introduction

Among the oxylipins are two potentially toxic oxidation products of linoleic acid - 9,10-dihydroxy-12-octadecenoic acid (9,10-DiHOME, $[M-H]^-$ = 313, also known as **leukotoxin diol** (**LTX-diol**) and 12,13-dihydroxy-9-octadecenoic acid (**isoleukotoxin-diol** – **isoLTX-diol**).

The acute toxicity of the endogenous LTX-diols is well characterised (Zheng, et al., 2001).
It was recently found that **exogenous LTX-diols disrupt the endocrine function** in female rats (Markaverich et al., 2007).
LTX-diols have exhibited also a mitogenic activity and stimulated human breast cancer cell proliferation *in vitro* (Markaverich et al., 2005).

We have studied the primary oxidation products of different MDMs by liquid chromatography with mass selective (MS/MS) detection and identified over 10 various oxylipins, including LTX-diols.

Experimental

Chicken, turkey and porcine MDMs were kept during 6 days at +4-6 °C. 2 g of meat material was extracted by 4 ml of methanol by shaking during 30 min and centrifuged at 3200 x g for 10 min. The supernatant was extracted twice with 2 ml of hexane to remove fat. The hexane phases were discarded and the methanol phase passed through a C18-SPE column.

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 XCT (Agilent Technologies) working in the negative ionization mode in the m/z interval of 100-1000 amu.

For more detailed description of experimental procedures, see: Püssa et al., 2009

Results - LC-MS/MS of MDM-s

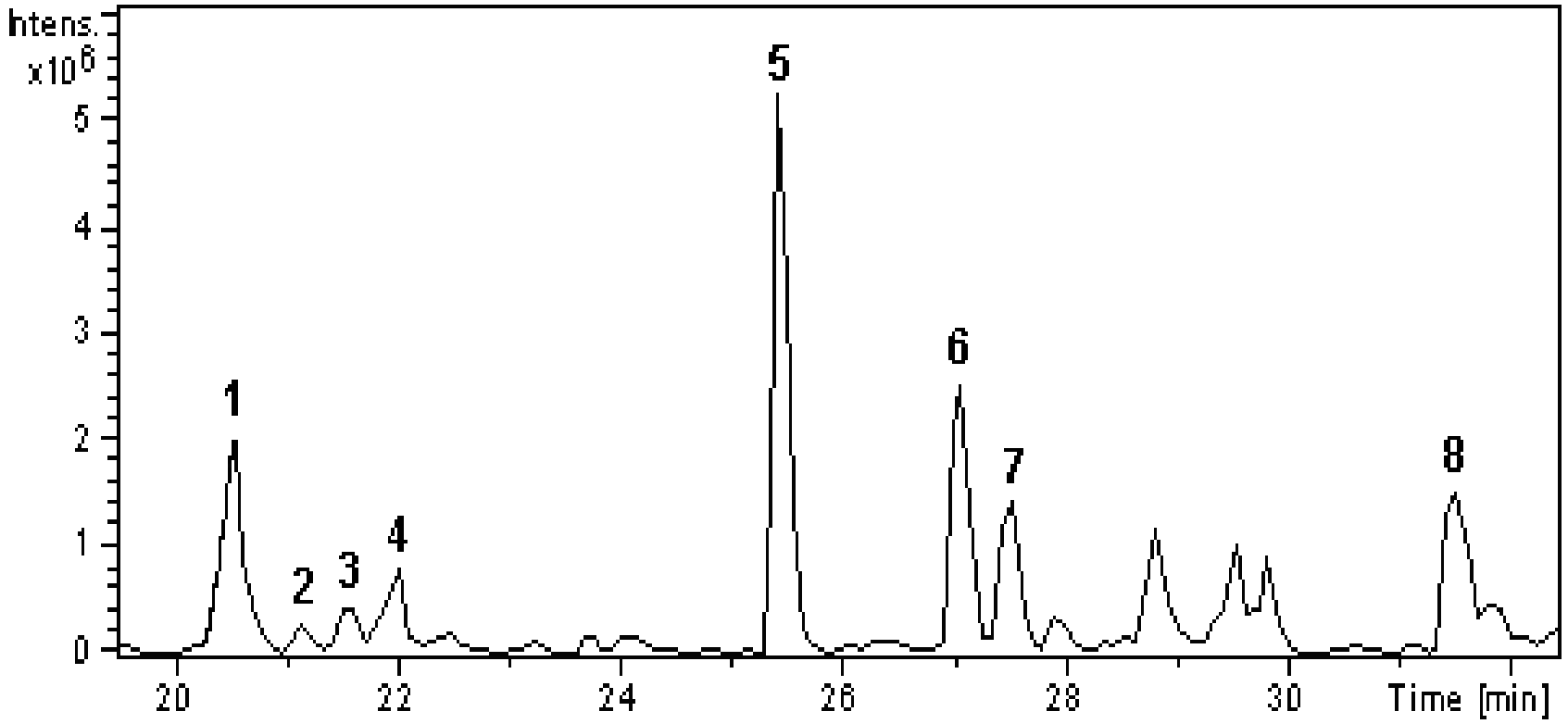
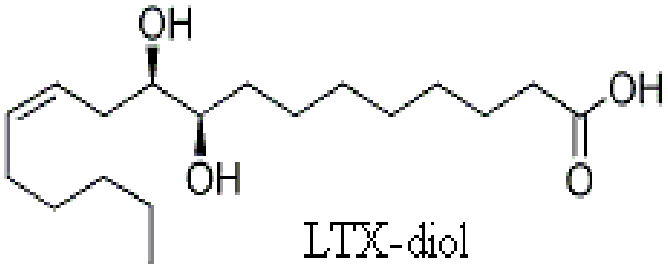


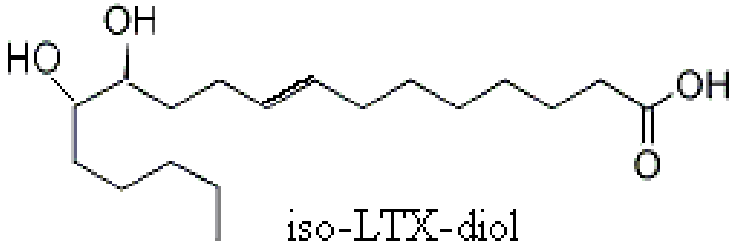
Figure 1. MS-base peak chromatogram of chicken MDM oxylipins. 1-4 - isomers of trihydroxyoctadecenoic acid (THODE); 5. mixture of 13-hydroxy-9-oxo- and 13-oxo-9-hydroxy-10-octadecenoic acids; 6. **iso-LTX-diol; 7. LTX-diol**; 8. mixture of 9- and 13-hydroxyoctadecenoic acids (HODE)

Results- identification and quantitation of LTX-diols

LTX-diol and iso-LTX-diol were identified by comparison of their MS^2 daughter ion spectra with both the fragmentation spectra of LTX-diols reported in literature and of the respective commercial standard (Figure 2) and quantified using the calibration curve of a commercial standard of LTX-diol (Cayman Europe, Tallinn, Estonia).



LTX-diol



iso-LTX-diol

Results – MS2 spectra of LTX-diols

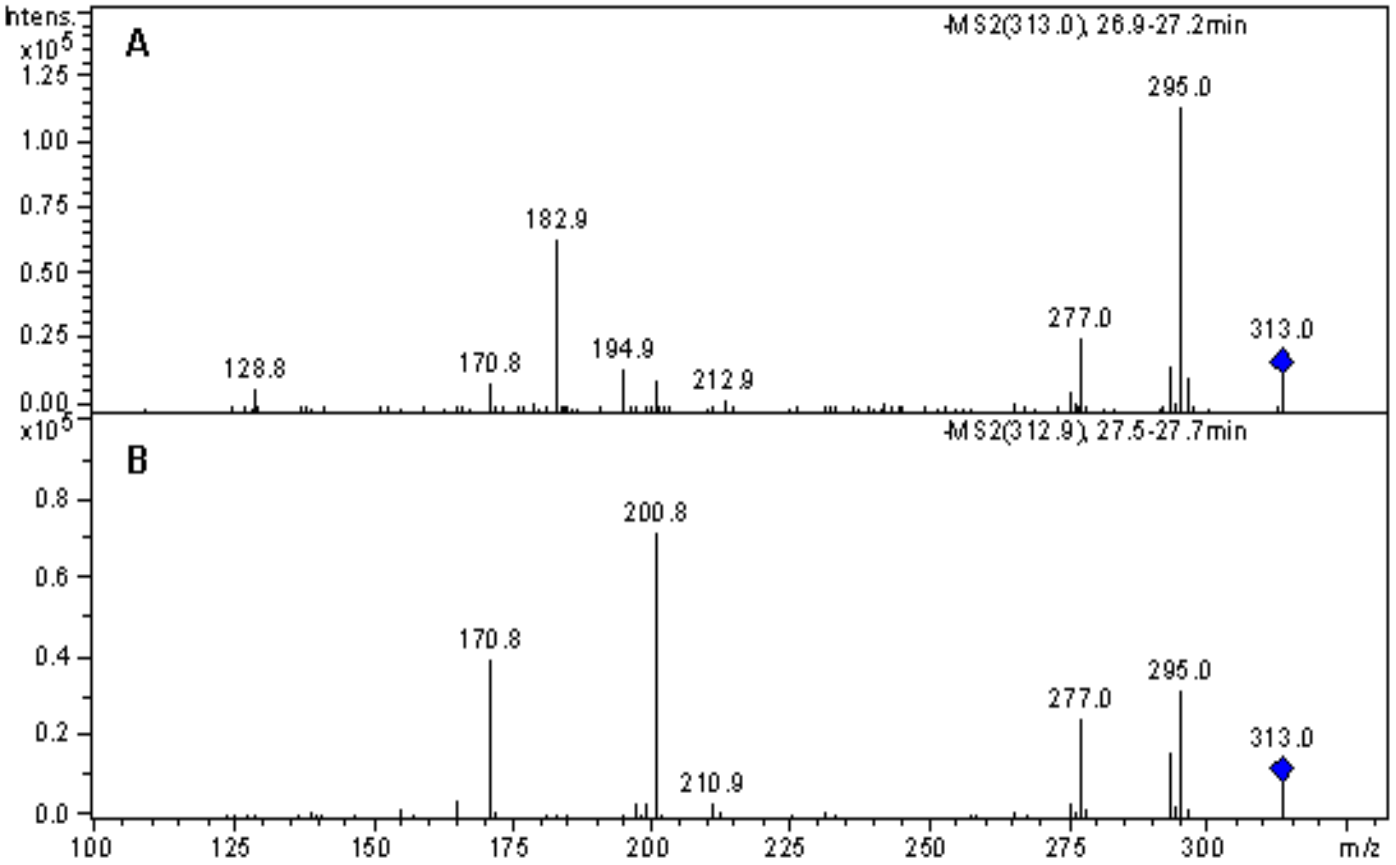


Figure 2. MS^2 spectra of iso-LTX-diol (A) and LTX-diol (B), including characteristic for most of linoleic acid oxylipins fragment – $OOC(CH_2)_7CH\text{-}OH$ with m/z = 171 amu

Results – LTX-diols content in MDM

The LTX-diols content in MDMs is in the range of 20-50 ppm (Figure 3), that in the case of a human daily consumption of, for example, 100 g of MDM, corresponds to a daily dose 0.03-0.08 mg of LTX-diols per kg bw.

The last number is only about twice as low as the LOAEL for female rats.

Supplementation of meat products with MDM to reduce their price causes a significant rise of oxylipins, including LTX-diols, content in the product (sausages), unless the rancidification is inhibited by natural antioxidants such as polyphenols of sea buckthorn (*Hippophae rhamnoides*) berries or other antioxidant mixtures of plant origin.

Results – total content of LTX-diols

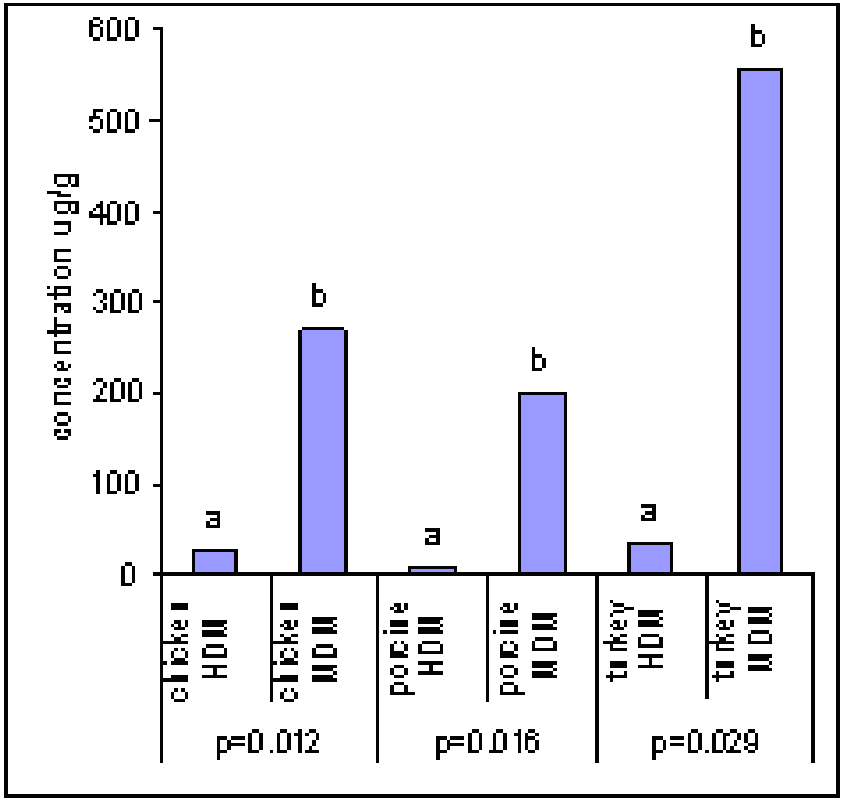


Figure 3. Summary concentration of LTX-diols after 6-day storage of meats at +4-6 °C. Initial concentration of the toxic oxylipins in MDMs was by 20-40% lower.

Literature

Markaverich et al., (2005). *Environ Health Perspect*. 113, 1698-1704
Markaverich et al., (2007). *Environ Health Perspect*. 115, 702-708
Püssa et al., (2009). *J. Food Composition and Analysis*, 22, 307-314
Zheng et al. (2001). *Amer.J.Respiratory Cell and Mol. Biol*., 25, 434-438