THE EFFECT OF TENDERIZING ACIDS ON LINOLEIC ACID OXIDATION DURING MARINATION OF PORK

Peeter Toomik[1], Kristina Lepp[2], Lembit Lepasalu[2], Tõnu Püssa[1*]

[1]Department of Food Hygiene, Estonian University of Life Sciences, Kreutzwaldi 58A, 51014, Tartu, Estonia

[2]Department of Food Science and Technology, Estonian University of Life Sciences, Kreutzwaldi 58A, 51014, Tartu, Estonia

[*]Corresponding author (phone: +372-7-313810; fax: +372-7-313432; e-mail: pyssa@emu.ee)

***Abstract*—The oxidation of polyunsaturated fatty acids during pork marination in the presence of different acidifiers was investigated by measurement of thiobarbituric acid reactive substances and by liquid chromatography-mass spectroscopy. The highest degree of oxidation was observed in acetic acid and lactic acid marinades with maximum at acid concentration 0.5-1.0 per cent, whereas the oxidation of lipids was significantly suppressed by citric and ascorbic acids. Among the primary oxidation products, trihydroxy-octadecenoic acid and hydroxy-epoxy-octadecenoic acids were dominating. In all cases, the concentration of all the observed oxidation products increased nearly proportionally during the marination process.**

***Index Terms* - linoleic acid, oxidation, oxylipins, TBARS, LC-MS/MS**

I. INTRODUCTION

Semi-fabricated food products like marinated meat are becoming more and more popular. Marination is one of the best ways to give meat some more flavour and tenderize it at the same time. During marination, polyunsaturated fatty acids (PUFA) may, to some extent, be oxidized to oxylipins that have a detrimental effect on both the organoleptic properties and safety of the meat. Our aim was to clarify the effect of different most commonly used acids on the extent of thiobarbituric acid reactive substances (TBARS) and different distinct linoleic acid primary oxidation products (oxylipins) in marinated meat. A comparison of the results of these two approaches for estimating the oxidation extent of linoleic acid was also performed.

II. MATERIALS AND METHODS

Pork: Oval slices (40 of each) of the longest spinal muscle of hog with a thickness of 1 cm, exempted from lard, rind and connective tissue and supplemented with 10 g of fat tissue. Oxylipin standard of 9-hydroxy-10,12-octadecadienoic acid (9-HODE) was from Cayman Europe.

Marinades: aqueous solutions of acetic, lactic, citric acids - 4 concentrations of each, ascorbic acid (3 concentrations) and a mixture of ascorbic (0.1%) and acetic (2%) acids, each supplemented with 2 % of NaCl.

Marination: Meat slices (duplicate samples) with a fat slice were placed one by one into polyethylene bags together with a marinade (meat/marinade ratio 2:1; w/w) and kept at +4 °C for up to 9 days.

Analytical sample preparation: 2 g of a meat sample was homogenized with 4 ml of methanol, centrifuged for 10 min. at 978 × g, and then extracted twice with 2 ml of hexane. Finally, the methanolic layer was passed through a C18 SPE-column.

Chromatographic analysis: Reversed-phase liquid chromatography-tandem MS/MS (LC-MS/MS) at Agilent 1100 series LC system connected to an ion trap MS/MS device equipped with an electrospray interface (ESI) was used to identify and quantify the oxidized fatty acids (oxylipins) in the marinated meat. Column: Zorbax 300SB-C18 (2.1×150 mm; 5µm – Agilent Technologies). The content of oxylipins in the meat samples was estimated by the areas of extracted ion chromatogram peaks using a calibration curve constructed for the commercial sample of 9-hydroxyoctadecadienoic acid (9-HODE).

Thiobarbituric acid reactive substances (TBARS): Extraction method (Esterbauer, Cheeseman 1990) was used. 10 g of the marinated meat was homogenized during 1 minute at 10000 rpm in 40 ml of 4% perchloric acid containing 90 mg per 100g of BHA, and filtered through a glass fiber filter. 5 ml of filtrate and 5 ml of 0.02 M solution of thiobarbituric acid were mixed in test tubes, sealed and heated on a water bath at 80 ± 0,2 ºC for one hour. The solutions were cooled down in the water bath filled with cold water during 10 minutes and the optical density was measured at 532 nm on a spectrophotometer (Specord). The malondialdehyde (MDA) concentrations were calculated from the calibration curve built for the 1,1,3,3-tetraethoxypropane standard. All the analyses were carried out in duplicate or triplicate.

Sensory analysis: For sensory analysis, the rest of the marinated meat samples were microwaved on the 4th and 9th days in cooking-bags at 600 W for 1 minute. A small panel of assessors was formed to compare the appearance, tenderness, taste and flavor of the products.

III. RESULTS AND DISCUSSION

1. Results of sensory analysis: No significant differences were found between pork samples marinated in the presence of different acidifiers. However, there was some tendency to prefer meat marinated at lower acid concentrations.
2. Dynamics of primary oxidation products of non-conjugated linoleic acid in different acid marinades: A number of oxylipins, derivatives of linoleic (*cis, cis*-9,12-octadecadienoic) acid in the marinated porcine meat were separated by liquid chromatography and identified by comparison of the compound's MS^2-spectra with the respective spectra from literature, in the case of 9-HODE also with the MS^2 daughter ion spectrum of the commercial standard. The most abundant identified oxylipins were 9,12,13-trihydroxy-10-octadecenoic acid (9,12,13-THOD, $[M-H]^-$ = 329) and its isomers and 9-hydroxy-12,13-epoxy-10-octadecenoic acid (9-HepoD; $[M-H]^-$ = 311). The presence of 9-HODE ($[M-H]^-$ = 295) and some other minor oxylipins was also detected (Figure 1).
The concentration of the two main oxylipins increased significantly during the meat treatment with lactic or acetic acid marinades, slightly with citric acid, but remained almost unchanged during a 9-day marination with ascorbic acid. Only a slight increase occurred in acetic acid supplemented with a small amount of ascorbic acid. Hence, both citric and ascorbic acids reveal clear antioxidant properties, although most likely with different mechanisms. Ascorbic acid is a good free radical scavenger, whereas citric acid acts mainly as a transition metal chelator.
9,12,13-THOD deserves special attention as a sensitive marker of fatty acid oxidation and 9-HepoD as an epoxy-fatty acid. The presence of acids, containing an epoxy-group capable of eliciting mutagenicity and carcinogenicity is rather disquieting. The epoxy-group is usually very reactive at neutral and alkaline conditions, but obviously protected by low pH in our experimental conditions.
The concentration of leukotoxins (LTX – epoxy-octadecenoic acids) and even more toxic leukotoxin diols (LTX-diols – dihydroxyoctadecenoic acids) (Markaverich et al., 2007) remains also relatively low. Obviously, unlike mechanically deboned meat (MDM), whose oxidation is characterized by a sharp increase of their content (Püssa et al., 2009), marinated meat contains little CYP 2C9 isomer and epoxide hydrolase, which are responsible for the production of remarkable amounts of leukotoxins and leukotoxin diols from linoleic acid (Viswanathan et al., 2003).
3. The correlation between the total content of two major oxylipins, and respective TBARS values is illustrated in Figure 4. This correlation is nearly linear with some fluctuations at higher oxidation rates, starting from the TBARS value of about 0.3μMol/g. This correlation is better than the correlation between the contents of individual oxylipins and the respective TBARS values (data not shown). At lower oxidation rates where TBARS is measurable, the oxylipins are almost undetectable. In all the cases, the TBARS molar values are of the same order of magnitude as the total molar content of oxylipins**.**

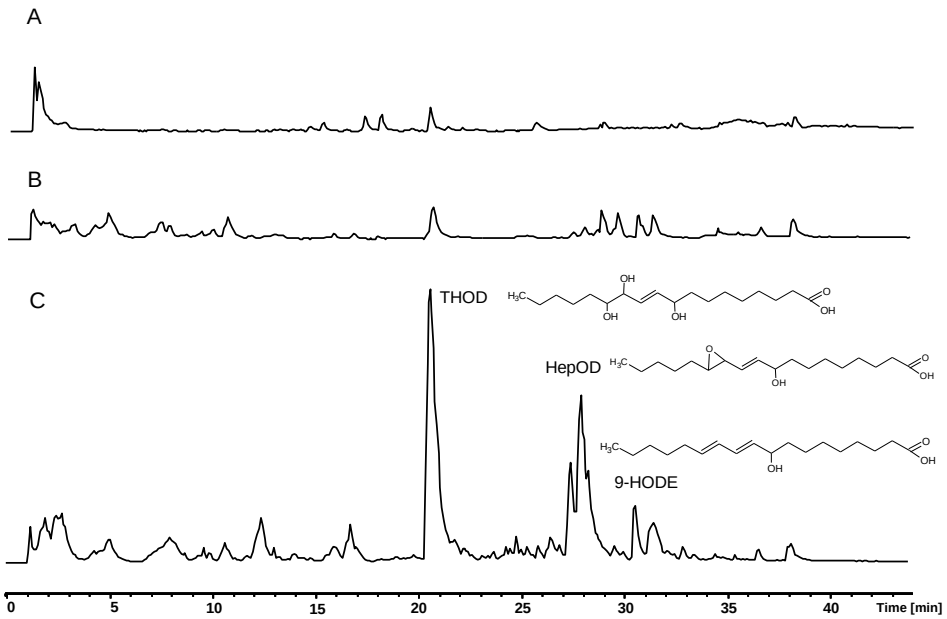

Figure 1. Examples of base peak ion chromatograms during the meat marination. A – control meat sample, kept in 4% saline during 9 days at +4 ^oC, B - pork sample, marinated during 9 days in 1% citric acid, C- pork sample, marinated during 9 days in 1% lactic acid. The vertical axis has the same scale.

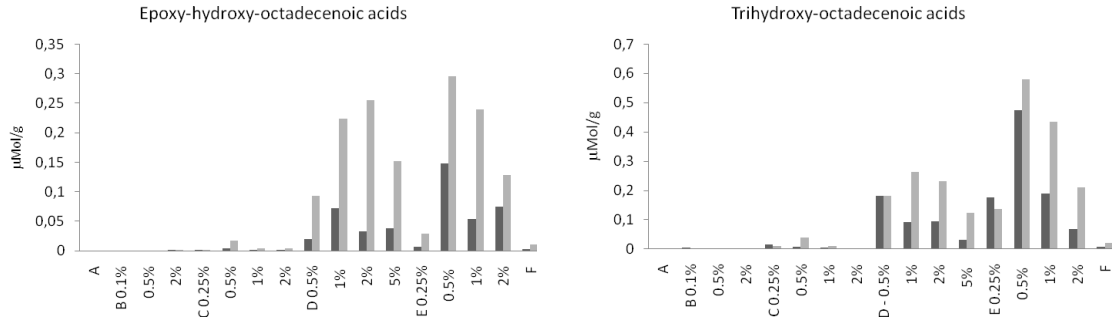

Figure 2 Formation of oxylipins in different marinades: A – control (see Fig. 1), B – ascorbic acid, C – citric acid, D – acetic acid, E – lactic acid, F – mixture of acetic (2%) and ascorbic (0.1%) acids. Graph bars: dark gray – day 4, light gray - day 9.

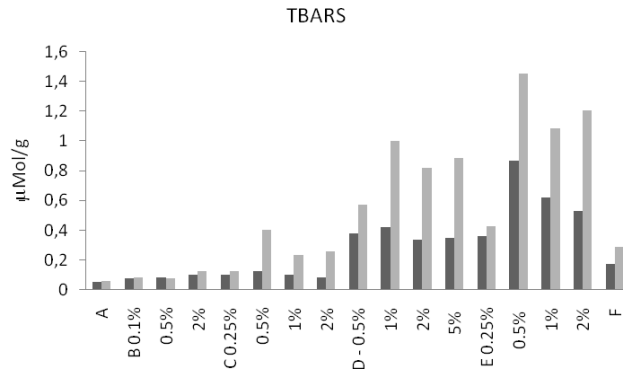

Figure 3. Formation of thiobarbituric acid reactive substances in different marinades. For details see Figure 2.

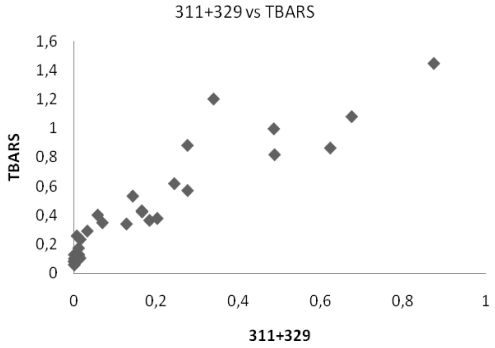

Figure 4. Relation between TBARS and the total content of two major oxylipins (THOD and HepoD)

IV. CONCLUSION

Concerning the inhibition of fatty acids peroxidation, ascorbic and citric acids are the preferred meat acidifiers and tenderizers, if no supplementary antioxidants are used. The oxidation rates are dependent on acid concentration with a maximum at 0.5-1.0% of any of the tested acids. We have also demonstrated that the measurement of the total concentration of oxylipins by liquid chromatography-mass spectroscopy could be a good alternative to the quantitation of secondary oxidation products by TBARS for estimating the peroxidation of polyunsaturated fatty acid in meat.

REFERENCES

Esterbauer, K.H., Cheeseman, K. H. (1990). Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal In: *Methods of Enzymology*, 407-421

Kim, H., Jang, Y.-S., and Hou, C.T. (2002). Effect of metal ions on the production of isomeric 9,10,13 (9,12,13)-trihydroxy-11*E* (10*E*)-octadecenoic acid from linoleic acid by *Pseudomonas aeruginosa* PR3. *Enzyme and Microbial Technology*, 30, 752-757.

Lee, S.H., Williams, M.V., DuBois, R. and Blair, I.A. (2003). Targeted lipidomics using electron capture-atmospheric pressure chemical ionization mass spectrometry. *Rapid Communications in Mass Spectrometry*, 17, 2168-2176.

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.

Oliw, E.H., Garscha, U., Nilsson, T. and Cristea, M. (2006). Payne rearrangement during analysis of epoxyalcohols of linoleic and α-linolenic acids by normal phase liquid chromatography with tandem mass-spectrometry. *Analytical Biochemistry*, 354, 111-126.

Püssa, T., Raudsepp, P., 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, *Journal of Food Composition and Analysis*, 22, 307-314

Viswanathan, S., Hammock, B.D., Newman, J.W., Meerarani, P., Toborek, M., Hennig, B. (2003). Involvement of CYP 2C9 in mediating the proinflammatory effects of linoleic acid in vascular endothelial cells, *Journal of the American College of Nutrition*, 22, 502-510.