# USE OF BILBERRY SAUCE FOR INHIBITION OF FATTY ACID OXIDATION DURING MARINATION OF PORK

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## 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 to tenderize it at the same time. During marination the polyunsaturated fatty acids (PUFA) may oxidize and produce hazardous reaction products. Our aim was to clarify whether this oxidation can be inhibited by a berry sauce containing large amounts of health-promoting antioxidant polyphenols. Due to the intensive color, the classical 2-thiobarbituric acid (TBARS) assay is not applicable in the case of the bilberry-meat compositions.

## Materials and Methods

Pork: Oval slices of the longest spinal muscle of hog with a thickness of 1 cm and mean fat content of 1.7 %, exempted from lard, rind and connective tissue, were prepared. Berries: Frozen ripe berries of bilberry *Vaccinium myrtillus* L., grown in Estonia. Bilberry marinade: Berries (B) + destilled water (W), ratio B:W = 2:1, 1:1 and 1:2, NaCl (5%). For comparison water and citric acid marinade – 0.22% of acid (both containing 5% NaCl) were used. Fatty acid standard of 9-hydroxy-10,12-octadecadienoic acid (9-HODE) was purchased from Cayman Europe.

Marination: Meat slices (duplicate samples) were placed one by one into polyethylene bags together with marinade (meat/marinade ratio 2:1; w/w) and kept at +4 °C up to 7 days. The marinated meat was microwaved in a cooking-bag by 600 W during 1 minute. Analytical sample preparation: 2 g of a meat sample was extracted with 4 ml of methanol, centrifuged for 10 min. at 978 × g, extracted twice with 2 ml of hexane and the methanol layer passed through C18 SPE-column. Chromatographic analysis: Reversed-phase liquid chromatography-tandem MS/MS (LC-MS/MS) at Agilent 1100 series LC system connected with an ion trap MS/MS equipped with an electrospray interface (ESI) was used to identify and quantify the oxidated 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 using a calibration curve constructed for the commercial sample of 9-HODE

## Results and discussion

Since the organoleptic analysis of the microwaved marinated meat in the preliminary test revealed the optimal B:W ratio in the marinade to be 1:1, the main experiment was carried out using this ratio of the components. The taste of the meat marinated with B:W = 2:1 was too sour and the weight loss of the meat samples was higher.

A number of oxylipins, derivatives of linoleic (*cis, cis*-9,12-octadecadienoic) acid, were identified in the marinated meat by comparison of the compound's $MS^2$-spectrum with the respective spectrum 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-THODE, $[M-H]^-$ = 329), 9-hydroxy-12,13-epoxy-10-octadecenoic acid (9-HepoDE; $[M-H]^-$ = 311) and 9-HODE ($[M-H]^-$ = 295) (Table 1 and Figure 1). Their concentration increases significantly during meat treatment with citric acid marinade, but stays unchanged during the 7-day marination with bilberry sauce (Figures 1 and 2). 9,12,13-THODE deserves special attention as a sensitive marker of fatty acid oxidation and 9-HepoDE as an epoxy-fatty acid. Especially disquieting is the presence of the acids, containing an epoxy-group, capable of eliciting mutagenicity and carcinogenicity.

**Table 1**. Identification of meat oxylipins by LC-MS/MS

| Retention time (min) (Fig.1) | Parent ion $[M-H]^-$ | Daughter ions (in the descending order of intensity) | Compound | Literature |
|---|---|---|---|---|
| 21.8 | 329 | 171;229;211;293;311;139 | 9,12,13-THODE | Kim et al.,2002 |
| 29.6 | 311 | 293;171;201;275;211;197 | 9-HepoDE | Oliw et al., 2006 |
| 33.4 | 295 | 277;171;195;173 | 9-HODE | Lee et al., 2003 |

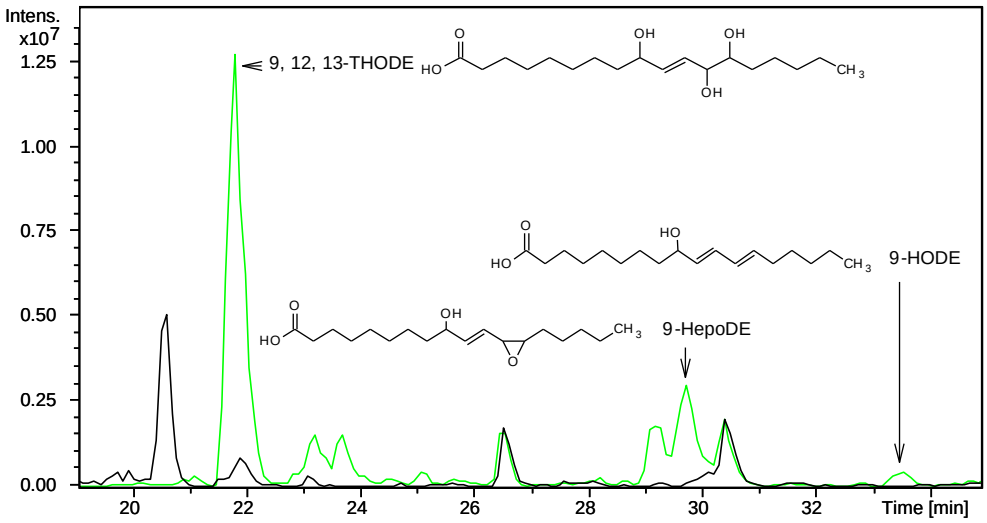


**Figure 1.** LC-MS/MS base peak chromatograms (BPC) of marinated pork methanol extract. Chromatograms: pork marinated with citric acid (light line) and with bilberry sauce (dark line) - both during 7 days.

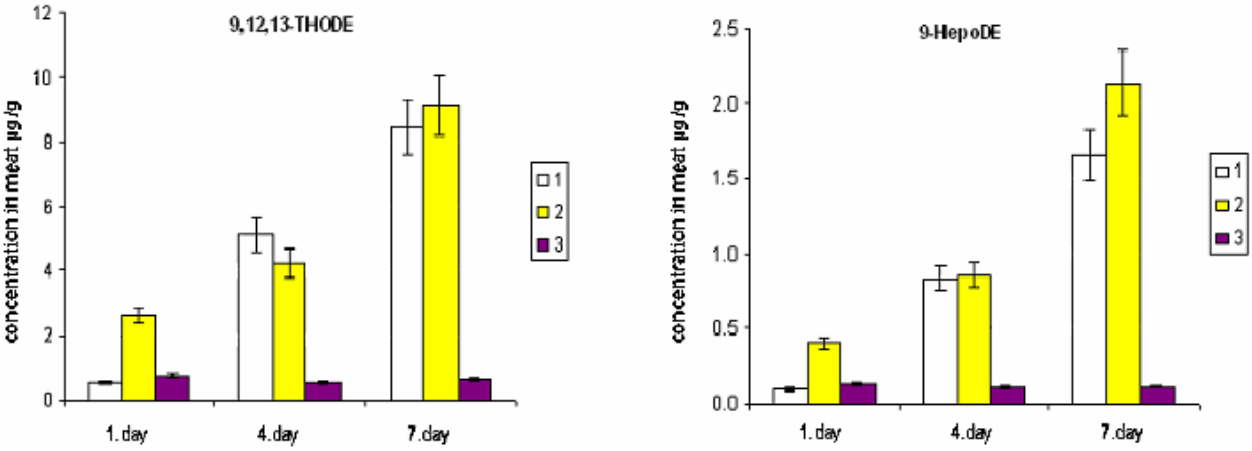


**Figure 2.** Concentration dynamics of the two main oxylipins in raw meat in the course of marination. Compositions: 1- meat+destilled water +NaCl; 2- meat+citric acid marinade; 3- meat+bilberry marinade

## Conclusions

Bilberry sauce that supplies meat with health-promoting polyphenol antioxidants, completely inhibits the oxidation of linoleic acid during marination of pork. The increase of the concentration of 9,12,13-THODE during processing and storage might serve as a marker of fatty acid oxidation in meat instead of TBARS.

## References

1. 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.
2. 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.
3. 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.