Berry polyphenols as inhibitors of fatty acid oxidation during marination of pork

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Abstract

The effect of various berry powders on the oxidation rate of polyunsaturated fatty acids (PUFA) in the course of marination of pork with citric acid at +4 °C was studied during 14 days. Due to the intensive colour of the marinated meats, the classical 2-thiobarbituric acid reactive substances (TBARS) assay is not applicable in the case of most berries. Instead, the PUFAs oxidation products – oxylipins were identified and quantified by the liquid chromatography-mass selective detection (LC-MS^2). The berry powders contain also their own oxylipins, markers of their oxidation. The antioxidant efficiency of berry powders increases in the order: lingonberry<sea buckthorn<black chokeberry<bilberry<black currant, as demonstrated by the reduction of the increase during marination of the concentration of 9,12,13-trihydroxy-10-octadecenoic acid (9,12,13-THODE), a novel marker of PUFAs oxidation, in comparison with marination with citric acid alone. Most of the berry polyphenols, except anthocyanins, are well preserved during marination of pork at +4 °C during 14 days as established by LC-DAD-MS^2. They can function as natural antioxidants for a consumer of the marinated meat. Every berry powder provides pork with a specific colour and flavour.

Introduction

During marination of meat in citric acid marinade, the polyunsaturated fatty acids (PUFA) are oxidized both chemically and enzymatically, producing more or less unsavoury and hazardous reaction products - oxylipins. Our main objective was to clarify at what extent this oxidation can be inhibited by different berry powders containing health-promoting antioxidant polyphenols. Due to their intensive colour, the classical TBARS assay is not applicable to the estimation of the oxidation level of the dark berry-meat compositions. Estimation of the concentration dynamics of PUFAs oxidation products - oxylipins was used instead of that.

Materials and methods

Pork: A slice of the longest spinal muscle (*m. longissimus dorsi*) of hog with a thickness of 1 cm and mean fat content of 1.7 %, exempted from lard (40 g) and combined with 10 g of lard slice, two parallel samples.

Berry powders: Commercial powders of bilberry (*Vaccinium myrtillus* L.), lingonberry (*Vaccinium vitis-idaea*), black chokeberry (*Aronia melanocarpa*), black currant (*Ribes nigrum*) and sea buckthorn (*Hippophae rhamnoides*).

Marinades: Berry powder (BP) + distilled water (W), ratio BP:W =1:9, supplemented with 4.5% of NaCl and acidified with 0.08 % citric acid. As reference, distilled water, supplemented with NaCl and citric acid was used.

Marination: Combined meat and lard slices were kept in polyethylene bags in a marinade (meat/marinade ratio 2:1; w/w) at +4 °C up to 14 days.

Analytical sample preparation: A marinated sample (2g) was extracted with 4 ml of methanol, centrifuged, methanol layer extracted twice with hexane and the methanol layer passed through a C18 SPE-column.

Chromatographic analysis: LC-DAD-ESI/MS^2 at Agilent 1100 series liquid chromatograph. Column: Zorbax 300SB-C18 (2.1×150 mm; 5µm – Agilent Technologies).

Quantitation of oxylipins: Commercial 9-hydroxy-10,12-octadecadienoic acid (9-HODE) was used to build the calibration curve in coordinate system: MS-peak height versus concentration of the oxylipin (ng/ml).

Relative quantitation of polyphenols absorbed by meat: Areas under chromatograms (AUC) of the extracts of meat samples at wavelengths 280 and 520 nm, from which the AUC of the chromatogram of the same day sample of pork, marinated with citric acid has been subtracted, were used for the study of the dynamics of total polyphenols and anthocyanins in meat, respectively.

Results and discussion

A number of oxylipins, oxidation products of the conjugated linoleic (*cis, cis*-9,12-octadecadienoic) acid, most of them having a negative fragment ion with m/z = 171, were identified and quantified in the pork, depending on the marination mixture by LC-MS/MS. The most abundant and omnipresent ones were (Figures 1 and 2):

- 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)

Their concentration significantly increases during treatment of pork with citric acid saline, but much less during marination in the presence of any of the berry powder supplements (Figure 3).

Concentration of total polyphenols increases in the case of all the studied berries until day 7, after that it decreases in most cases (Figure 4A). In the case of anthocyanins, all berries are characterized by remarkable decrease of their concentration after day 7 (Figure 4B). This phenomenon shows the weightiest role of anthocyanins in the defence against oxidation of pork being marinated.

Our results show also the necessity of measuring intrinsic oxylipin content in the berry powders used. This number, depending on the conditions of powder preparation, may substantially contribute to the overall content of oxylipins in the marinated meat (data not shown).

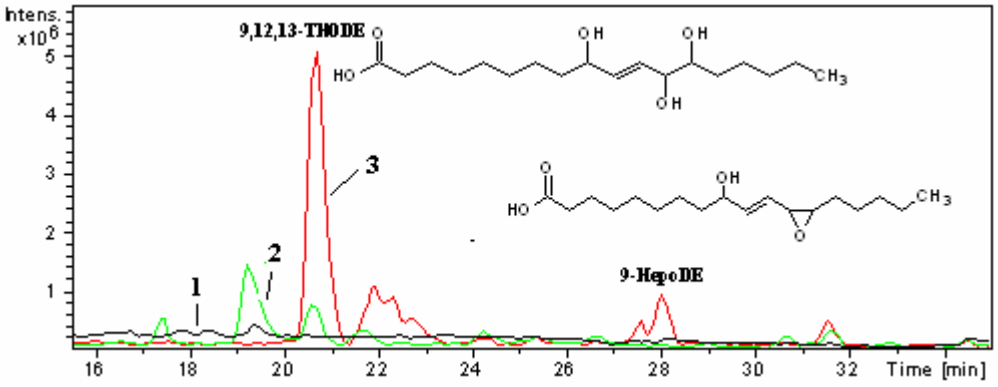

Figure 1. LC-MS base peak chromatograms of pork, marinated in 0.08 % citric acid – day 1 (curve 1) and day 14 (curve 3) and in citric acid supplemented with sea buckthorn berry powder – day 14 (curve 2).

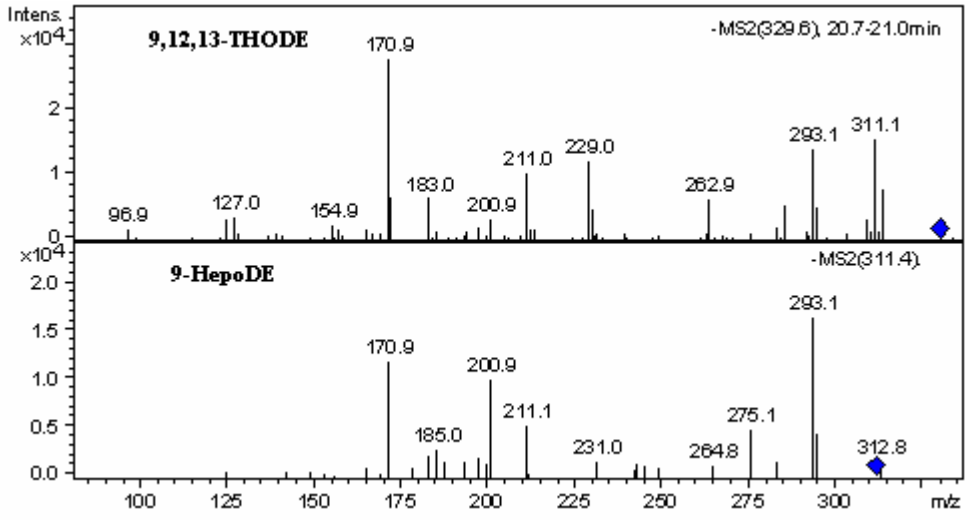

Figure 2. MS^2 spectra of the two main oxylipins, formed during pork marination. Both spectra are characterized by a daughter ion with m/z = 171, belonging to the $^-OOC(CH_2)_7CH\text{-}OH$ moiety (Lee et al., 2003).

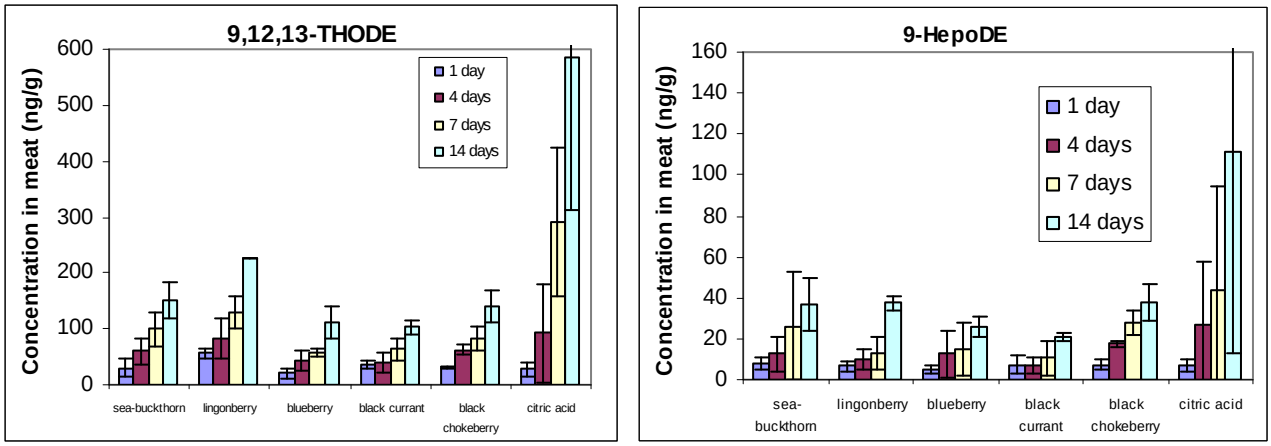

Figure 3. Concentration dynamics of 9,12,13-THODE, $[M-H]^-$ = 329 and 9-HepoDE; $[M-H]^-$ = 311 in pork during marination in citric acid solution, supplemented with different berry powders.

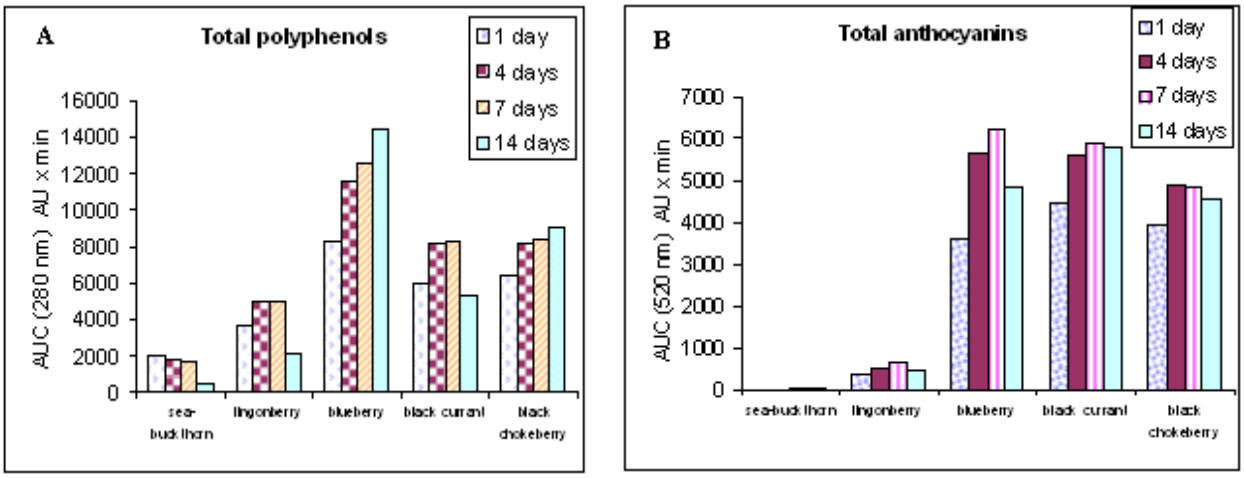

Figure 4. Dynamics of the content of total polyphenols (A) and anthocyanins (B) in the pork during marination with different berry powders.

Conclusions

- Berry powders that simultaneously supply meat with health-promoting polyphenol antioxidants, substantially inhibit the oxidation of linoleic acid during marination of pork in the presence of citric acid.
- Disquieting is the increase of the concentration of 9-HepoDE bearing an epoxy group, capable of eliciting mutagenicity and carcinogenicity.
- The concentration of omnipresent 9,12,13-THODE might serve as a marker of fatty acid oxidation in meat (especially in coloured products) along with/instead of the classical TBARS value.

Acknowledgements

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Reference

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