STABILITY OF SEA BUCKTHORN BERRY POLYPHENOLS DURING COOKING OF ENRICHED SAUSAGES

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Abstract – Commercial grill sausages enriched with sea buckthorn (*Hippophae rhamnoides*) **berry puree were analyzed by liquid chromatography with UV-Vis and mass-spectrometric detection (LC-DAD-MS/MS). Thermal stability of sea buckthorn polyphenols, mostly flavonol and flavone glycosides, during heat treatment of sausages was studied. Identified polyphenols were relatively stable (yield 52-99%). Since flavonoid glycosides are partly hydrolyzed to respective aglycones, bioavailability of berry polyphenols and antioxidative capacity of enriched meat products may even rise during thermal treatment. A novel parameter for expression of linoleic acid oxidation level was used.**

Keywords: flavonoids, oxylipins, thermal processing

I. INTRODUCTION

Plant polyphenols including flavonols are powerful antioxidants that hinder the (per)oxidation of polyunsaturated fatty acids (PUFAs) and other oxidation-prone constituents of meat during processing and storage [1]. Sea buckthorn berries are a well-known source of flavonoids [2], as well as vitamins C and E, carotenoids, minerals and other bioactive compounds. Flavonoids demonstrate a wide range of biochemical and pharmacological activities including antioxidative, anti-inflammatory, antiplatelet, antithrombotic, and antiallergic effects, all beneficial for health. Sea buckthorn berries have been used in ethnopharmacy for treatment of cardiovascular disorders. Furthermore, as ingredients in meat, flavonoids are expected to help maintain the oxidant-antioxidant equilibrium in the consumers' organism. However, these expectations assume a sufficient stability of the phenolic substances in the meat matrix during thermal processing.

Liquid chromatography – tandem diode array-mass spectrometry was used to study stability of sea buckthorn berry polyphenols during cooking of sausages enriched with the berry puree. Also the concentration dynamics of oxylipins, the primary oxidation products of unconjugated linoleic (*all-cis*-9,12-octadecadienoic) acid, was investigated.

II. MATERIALS AND METHODS

Sausages: Commercial small grill sausages with 1% sea buckthorn puree in natural sheep casings were purchased from a local retail store.

Ingredients on product label: Pork (75%); lard, water, sodium chloride potato starch, soy protein; sea buckthorn puree (1%); milk protein, flavorings, acidity regulators (sodium citrate, di- and triphosphates), antioxidant (ascorbic acid), flavor enhancer (monosodium glutamate); preservative (sodium nitrite).

Thermal processing: Sausages were cooked in an oven at moderate heat (180 °C) during 15 minutes, until the skins were golden brown and crispy (inner temperature 70 °C). Weight loss of sausages during processing was 33%.

Analytical sample preparation: Sausage samples (2 g) in duplicates were extracted with 4 ml of methanol, shaken for 30 min, centrifuged, methanol layer extracted twice with hexane and passed through a C18 SPE-column.

Chromatographic analysis: LC-DAD-ion trap MS/MS Agilent 1100 series chromatograph (Agilent Technologies) with Zorbax 300SB-C18 (2.1×150 mm; 5µm) column was used.

Quantitation of polyphenols: Areas under LC-UV-Vis chromatograms (AUC) of the extracts of sausage samples at wavelengths 280 and 370 nm (AUC_{280} and AUC_{370}) were used for the study of the dynamics of total polyphenols and total flavonols, respectively. Yields of single major polyphenols were calculated from the areas under curves of respective extracted ion MS chromatograms.

Quantitation of oxylipins: Total concentrations of oxylipins were expressed as the areas under the extracted ion chromatographic (EIC) curves of the

MS^2 daughter fragment with m/z =171 (AUC_{171}). This daughter ion is characteristic and common for a majority of linoleic acid primary oxidation products, mostly products of 9-lipoxygenase catalytic process.

III. RESULTS AND DISCUSSION

Polyphenols. A number of polyphenols, primarily various glycosides of flavonols isorhamnetin and quercetin, characteristic for sea buckthorn berries [2], but also of a flavonone apigenin (Fig. 1) were identified and semiquantified in sausages before and after cooking (Table 1). However, kaempferol and myricetin glycosides, also characteristic for sea buckthorn berries [2], were not detectable in the sausages.

Most of the major polyphenols were relatively stable in the course of thermal processing. The mean yield was 62%, 78% and 81% for isorhamnetin, quercetin and apigenin glycosides, respectively. Remarkable is that whereas the content of the flavonoid glycosides as well as isorhamnetin and apigenin aglycones reduced during cooking, the content of flavonol quercetin was unvaried (yield 99%) (Table 1.) It may be caused by acid hydrolysis of quercetin glycosides at higher temperatures yielding more heat-resistant aglyconic quercetin. Aglycones are more efficient free radical scavengers and have higher bioavailability than respective glycosides [3].

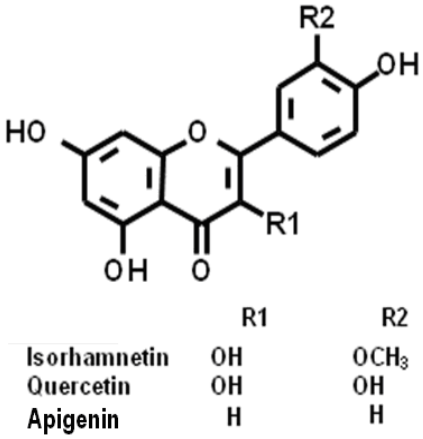

Figure 1. Molecular formula of flavonoid aglycones.

Table 1. Content of the major polyphenols, measured as areas under the curves (AUC) of respective extracted ion chromatograms (EIC) before and after cooking of sausages and yields of single polyphenols.

	Isorhamnetin	Isorhamnetin rhamno glucoside	Isorhamnetin rutinoside	Isorhamnetin glucoside	Isorhamnetin rhamnoside	Quercetin	Quercetin glucoside	Quer-cetin rhamnoside	Quercetin rutino-side	Apigenin	Apigenin glucoside	Apigenin acetyl-gluco-side
Before	7699	5728	3792	11494	3509	2266	2337	998	700	50347	7868	4674
After	5320	3359	2496	6624	2624	2244	2054	525	557	29689	6431	3672
Yield (%)	69	59	66	58	75	99	88	53	80	59	82	79

Table 2. Content of three major oxylipins, total content of oxylipins, phenolics and flavonols before and after cooking of sausages and respective yields.

	THODE isomers m/z = 329	DHOME isomers m/z = 313	9-HoDE m/z = 295	Total oxylipins AUC_{171}	Total phenolics AUC_{280}	Total flavonols AUC_{370}
Before	20534	6417	5082	1055	10055	169
After	20376	7429	8695	1018	6939	153
Yield (%)	99	116	171	97	69	91

Oxylipins. A number of oxylipins, primary oxidation products of linoleic acid were identified and quantified in the sausages (Figure 2).

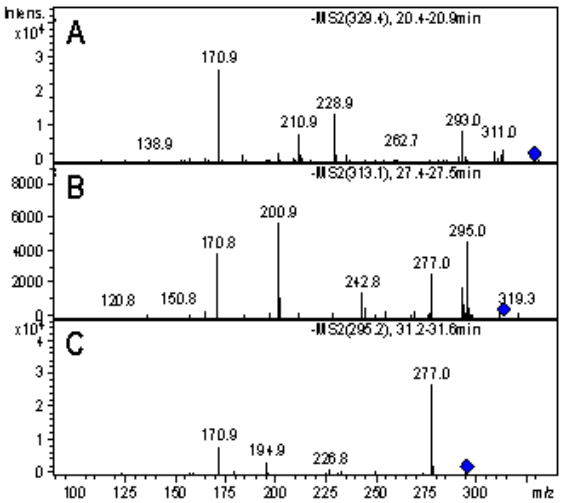

Figure 2. MS^2 fragmentation spectra of oxylipins 9,12,13-THODE (A); 9,10-DHOME (B) and 9-HODE (C). All the spectra include a daughter ion with negative m/z = 171 atom mass units (amu)

The most abundant were: 9,12,13-trihydroxy-10-octadecenoic acid (9,12,13-THODE and other isomers; m/z = 329 amu); 9,10-dihydroxy-12-octadecenoic acid (9,10-DHOME; leukotoxin diol) and 12,13-dihydroxy-9-octadecenoic acid (12,13-DHOME; isoleukotoxin diol; m/z = 313 amu), and 9-hydroxy-10,12-octadecadienoic acid (9-HODE; m/z = 295 amu)

Since the total list of linoleic acid oxylipins in studied sausages is rather long, total relative concentrations were calculated from area under chromatographic curve (AUC) of MS^2 daughter ion with m/z = 171 amu, common for a majority of products of linoleic acid oxidation

Concentration of 9-HoDE in sausages rises during cooking (Table 2). Consequently, content of antioxidants in these commercial sausages is not sufficient to entirely inhibit linoleic acid oxidation. Concentration of potentially toxic leukotoxin diols (DHOMEs) was remarkable, but still notably lower, both before and after cooking of sausages than in highly oxidized mechanically deboned meats [4].

Part of the oxylipins originate from sea buckthorn, where linoleic acid content is up to 1% of the wet weight of berries [5].

Ascorbic acid and sodium citrate, as ingredients of the sausages, may also take part in inhibiting of linoleic acid oxidation [6].

IV. CONCLUSION

Polyphenols of sea buckthorn berries, added to sausages, have remarkable thermal stability (70% in average) and, in principle, can function as natural antioxidants in human organism. Concentration of various linoleic acid oxylipins is also reduced during cooking. Added sea buckthorn puree improved colour, texture and nutritional properties of these meat products, the sausages were juicy, had pleasant fruity taste and a specific yellowish colour. Previously, 9,12,13-THODE and 9-HepoDE were proposed by our research group as markers of fatty acid oxidation level in meat instead of the classical TBARS value that may underestimate the degree of lipid oxidation in meat [6]. Hereby we used a novel marker of lipid oxidation in foods containing linoleic acid as the most oxidizable polyunsaturated fatty acid.

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