STABILITY OF BERRY POLYPHENOLS DURING COOKING OF ENRICHED MARINATED PORK

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Stability of various berry and red wine polyphenols during thermal treatment of marinated enriched pork was studied by liquid chromatography with UV-Vis and mass-spectrometric detection (LC-DAD-MS/MS). A majority of the polyphenols were rather stable (yield 29-63%). Exceptions were anthocyanins, the best antioxidants, showing a yield of only 13-40%. Since flavonol glycosides are largely hydrolyzed to the respective aglycones, the bioavailability of berry polyphenols and the antioxidative capacity of enriched marinated pork may even increase during thermal treatment. A new parameter for expression of linoleic acid oxidation level, AUC_{171}, is proposed.

I. INTRODUCTION

Natural health-promoting plant polyphenols are versatile and powerful antioxidants that hinder the (per)oxidation of polyunsaturated fatty acids (PUFAs) and other oxidizable constituents of meat during processing and storage by free radical scavenging, transition metal ion chelating and oxidation enzyme inhibition (1). Furthermore, these ingredients are also expected to help maintain the oxidant-antioxidant equilibrium in the consumers' organism. The latter expectation presumes a sufficient stability of the phenolic substances in the meat matrix during thermal processing. However, information about the thermal stability of polyphenols in meat matrix is scarce.

Therefore, the aim of this study was to assess the stability of antioxidant polyphenols during cooking of marinated meat. As a meat tenderizer, citric acid was chosen for its extra antioxidative effect (2). The inhibiting effect of a part of these natural additives on the oxidation of linoleic acid during marination of pork was already published in the proceedings of the ICoMST 2008. Due to their intensive color, the classical TBARS assay was not applicable for study of the oxidation process in the anthocyanin-containing marinated meats. Instead, a liquid chromatographic-mass spectrometric (LC-MS/MS) method was developed to study the concentration dynamics of oxylipins, the primary oxidation products of unconjugated linoleic acid in the marinated meats.

II. MATERIALS AND METHODS

Pork: Slices from pork sirloin (*Musculus longissimus dorsi*) with a thickness of 1 cm and average fat content of 1.7% (40 g) were combined with 10 g of lard slices.

Berry materials and red wine: Commercial powders of bilberry (*Vaccinium myrtillus* L.), black chokeberry (*Aronia melanocarpa* [Michx.] Elliott), black currant (*Ribes nigrum* L.), rowanberry (*Sorbus aucuparia* L.) (all from Estonia) and lingonberry (*Vaccinium vitis-idaea* L.) berries from Finland, and red grape (*Vitis vinifera* L.) wine (Cabernet Sauvignon, France, 2006).

*Marinades***:** Berry powder (BP) + distilled water (W), ratio BP:W = 1:9, or wine, seasoned with 4.5% of NaCl and 0.25 % citric acid.

Marination: Duplicate samples of combined meat and lard slices were kept in polyethylene bags in a marinade with the meat/marinade ratio of about 3:2; w/w at +4 °C for up to 14 days.

Thermal processing: Marinated meats were individually wrapped into foil and cooked in an oven at 200 °C during 30 minutes. Weight losses during processing were 28-45%.

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

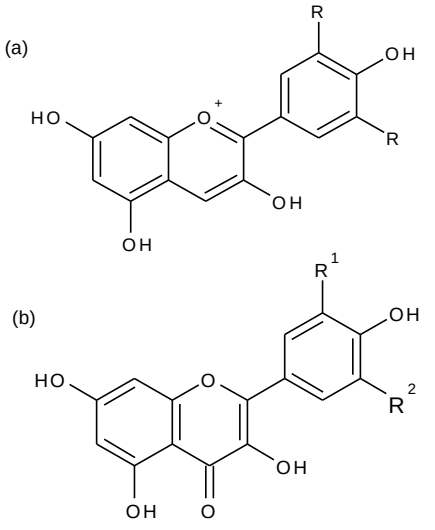
Chromatographic analysis: LC-DAD-MS/MS at Agilent 1100 series chromatograph. Column: Zorbax 300SB-C18 (2.1×150 mm; 5µm – Agilent Technologies).

Quantitation of polyphenols in meat: Areas under LC-UV-Vis chromatograms (AUC) of the extracts of meat samples at wavelengths 280, 370 and 520 nm, from which the AUC of the chromatogram of citric acid marinated pork was subtracted, were used for the study of the dynamics of total polyphenols, total flavonols and total anthocyanins during thermal treatment, respectively. Yields of single major polyphenols were estimated from the areas of the respective extracted negative ion mass chromatograms (Table 2).

Quantitation of meat oxylipins: The total concentrations of oxylipins were expressed as the areas under the extracted ion chromatographic curves of the MS^2 daughter fragment with m/z = 171 (AUC_{171}), characteristic for a majority of linoleic acid primary oxidation products, products of 9-lipoxygenase catalysis (Figure 2). Concentrations of daughter ion with m/z = 195, specific for products of 13-lipoxygenase pathway were by about an order lower.

III. RESULTS AND DISCUSSION

According to the results of chromatographic analysis, all the berry powders used contained various types of polyphenols, while anthocyanins and flavonols (Figure 1) and their glycosides are the most outstanding groups for their antioxidative effect.



Kaempferol R ^{1} = H and R ^{2} = H

Quercetin R ^{1} = OH and R ^{2} = H

Myricetin R ^{1} = OH and R ^{2} = OH

Figure 1. Molecular formula of anthocyanidins (a) and major flavonol aglycones (b).

Different anthocyanins that may appear as red, purple or blue pigments depending on the pH are responsible for the dark color of various berries and red grape wine.

A number of flavonoid group polyphenols classified mostly as flavonols and anthocyanins were identified, and semiquantified in the marinated meat before and after cooking in case of every natural supplement (Tables 1 and 2). It can be stated that most of these compounds are comparatively stable during rather severe thermal processing of meat (overall yield 29-63%). The same is valid for a group of flavonols and their glycosides (overall yield 41-76%), except in the case of red grape wine, when respective yield is only 21%. It is remarkable that whereas the concentration of most flavonol glycosides is significantly reduced during thermal processing (Table 2), the content of flavonol aglycones is mostly increased (with a yield of up to 272 %). This phenomenon can be partly explained by acid hydrolysis of flavonol glycosides at higher temperatures yielding relatively heat-resistant aglycones (quercetin, myricetin, iso-rhamnetin etc.). These aglycones are also more efficient free radical scavengers and have a higher bioavailability in the gastro-intestinal tract than the initial glycosides (glucosides, galactosides, arabinosides, glucuronides and so forth) (3, 4). Whether thermal processing actually leads to an increase in *in vivo* antioxidative capacity and, hence, strengthening of health-promoting effects of marinated meat, needs further studies. However, this unexpected result may be also partly caused by incomplete extraction of specific polyphenols from thermally non-treated meat matrix. Situation that anthocyanins, glycosides of anthocyanidins such as cyanidin, delphinidin, malvidin etc have a low yield (8-21%) may be linked to their highest antioxidativity among the polyphenols (3) as well as intrinsic lability of their molecules (5).

Concentrations of various flavonol aglycone oxydimers that are shown to be formed during flavonol oxidation by free radical mechanism (6) were below limits of detection and identification.

A number of oxylipins, oxidation products of the unconjugated linoleic (*all-cis*-9,12-octadecadienoic) acid, were identified and quantified in the pork by LC-MS/MS.

The most abundant oxylipins were (Figure 2): 9,12,13-trihydroxy-10-octadecenoic acid (9,12,13-THODE; m/z = 329) and 9-hydroxy-12,13-epoxy-10-octadecenoic acid (9-HepoDE; m/z = 311). The concentration of potentially toxic dihydroxy-octadecenoic acids (leukotoxin diols), characteristic for highly oxidized mechanically deboned meats (7), was very low both before and after cooking of marinated meat.

The concentration of oxylipins in marinated meat decreases during thermal processing (Table 1 – yields of AUC_{171}).

Since the spectrum of oxylipins of linoleic (*cis*, *cis*-9,12-octadecadienoic) acid is rather broad, there are problems with establishing their total content in meat samples. Therefore, the quantitation of oxylipins was performed by common for a majority of oxylipins MS^2 daughter ion with m/z = 171, products of linoleic acid oxidation reaction, catalyzed by 9-lipoxygenase pathway (Figure 2).

A small sensorial panel considered all the cooked marinated meats as interesting specifically tasting food items.

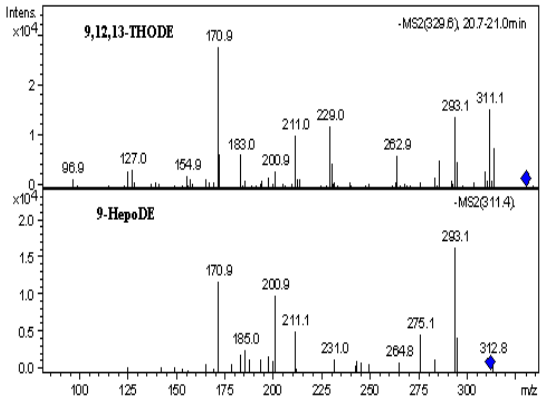


Figure 2. MS^2 spectra of two major oxylipins, formed during pork marination. Both spectra are characterized by daughter ion with negative m/z = 171.

Table 1. Yield of total phenolics, total flavonols and total anthocyanins during cooking of marinated pork, totals before cooking are in absorption units (AU).

	Total phenolics AUC_{280}	Yield after cooking %	Total flavonols and their glycosides AUC_{370}	Yield after cooking %	Total anthocyanins AUC_{520}	Yield after cooking %	Yield of AUC_{171} %
Bilberry	14264	56	1871	56	6663	21	70
Black currant	10827	53	1642	75	5765	18	52
Chokeberry	8983	29	1895	41	4683	20	20
Lingonberry	7584	63	1059	60	436	15	76
Rowanberry	6040	54	1252	76	754	19	34
Red wine	6095	40	670	21	991	8	32

Table 2. Yield of individual flavonols (%) during cooking of marinated pork.

	Flavonol glycosides				Flavonol aglycones		
	Quercetin hexosides/ dihexoside	Quercetin glucuronide	Quercetin rutinoside	Quercetin pentosides	Quercetin	Myricetin	iso-rhamnetin/ kaempferol
Bilberry	94	55	-	138	250	272	153
Black currant	50	-	32	-	92	94	161
Chokeberry	36/40	-	22	42	113	-	-
Lingonberry	51	-	-	53	85	-	-
Rowanberry	68/44	-	24	-	176	140	160/228
Red wine	24	24	-	-	37	-	105/36

IV. CONCLUSION

Polyphenols from different berry powders, added to pork marinades, are remarkable for their thermal stability (50% in average) and, hence, can really function as natural

antioxidants in human organism. Predictably, the weakest group is represented by anthocyanins. The concentration of various linoleic acid oxylipins is also reduced during cooking.

Previously, it was proposed that the total concentration of 9,12,13-THODE and 9-HepoDE might serve as a marker of fatty acid oxidation level in meat (especially in colored products) instead of the classical TBARS value that may underestimate the degree of lipid oxidation in meat (2). Hereby we can add AUC_{271} as an even more universal parameter for this marker in food products containing linoleic acid as the main oxidizable fatty acid.

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