DYNAMICS OF SUN-DRIED TOMATO FRUIT POLYPHENOLS AND OXYLIPINS DURING COOKING OF ENRICHED GRILL SAUSAGES

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Abstract – Concentration dynamics of various tomato (*Solanum lycopersicum L*) **fruit polyphenols, mostly flavonoid glycosides, as well as meat linoleic acid oxidation products (oxylipins) was studied in the course of heat treatment of commercial sausages enriched with sun-dried tomato by liquid chromatography with UV-Vis and mass-spectrometric detection. Polyphenol glycosides were relatively stable, with yields between 62 and 100%. Chlorogenic and dicaffeoylquinic acids, abundantly present in tomato fruits, were not detectable already in the raw sausages. Usability of a novel parameter (AUC_{171}) for characterization of linoleic acid oxidation level in meat products was confirmed.**

Key Words – phenolics, oxylipins, thermal stability, antioxidants

I. INTRODUCTION

Plant polyphenols as versatile and powerful antioxidants remarkably inhibit the oxidation of polyunsaturated fatty acids (PUFAs) and other oxidizable constituents of meat products during processing and storage [1]. Tomato fruits are a well-known source of various polyphenols, particularly phenolic acids and flavonoids [2] that demonstrate a wide range of biochemical and pharmacological effects including antioxidative effects. Furthermore, these ingredients are also expected to help maintain the oxidant-antioxidant equilibrium in the consumers' organism. This expectation presumes sufficient stability of the phenolic substances in the meat matrix during thermal processing.

Liquid chromatography-mass spectrometry (LC-MS/MS) was used to study the yield of tomato polyphenols in the cooking process of sausages enriched with sun-dried tomatoes. Concentration dynamics of oxylipins, the primary oxidation products of unconjugated linoleic (*all-cis*-9,12-octadecadienoic) acid as a possible marker of antioxidant capacity of tomato components, was also studied.

II MATERIALS AND METHODS

Sausages: Commercial grill sausages supplemented with sun-dried tomatoes in sheep casings.

Ingredients at the package label: Pork (63%), beef (6%), pork rind, tomato (1.5%), spices, flavorings, starch, sodium chloride, glucose, antioxidant (sodium ascorbate), stabilizer (diphosphate), acidity regulators (sodium acetate, acidic acid), preservative: sodium nitrite.

Cooking: Sausages were cooked in an oven at 180 °C during 15 minutes. Weight loss during processing was 19 %, content of various compounds was corrected taking into account the weight loss.

***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 at Agilent 1100 series chromatograph with negative ionization. Column: Zorbax 300SB-C18 (2.1×150 mm; 5µm – Agilent Technologies).

***Quantitation of polyphenols*:** Areas under LC-UV-Vis chromatograms (AUC) of the extracts of sausage samples at wavelengths 280 and 355 nm (AUC_{280nm} and AUC_{355nm}, respectively) were used for the study of the dynamics of total polyphenols and total flavonols, respectively, in the course of thermal treatment. Yields of single identified polyphenols were calculated using the areas under curves of respective extracted ion MS chromatograms (EIC).

***Quantitation of meat oxylipins*:** Concentration of three most abundant oxylipins was calculated by the areas of respective extracted ion chromatographic (EIC) curves. Total concentrations of oxylipins were expressed in mg/kg using the areas under the EIC curves of the MS^2 daughter fragment with m/z =171 (AUC_{171}), characteristic for a majority of linoleic acid

oxylipins, products of 9-lipoxygenase catalytic process (Figure 2) as well as non- enzymatic oxidation. 9-HODE was always used as the standard compound.

III RESULTS AND DISCUSSION

Polyphenols A number of polyphenols, primarily various glycosides of quercetin and other flavonols, characteristic for tomato fruits [2] were identified and quantified in sausages before and after cooking (Table 1).

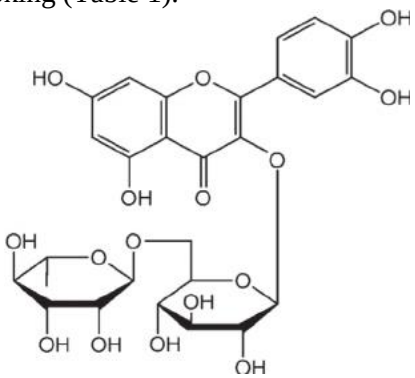

Fig.1. Quercetin rutinoside (rutin), one of the most abundant polyphenols in tomato fruit.

Most of the polyphenols were relatively stable in the course of thermal processing. The mean yield varied between 75 and 100% for quercetin glycosides, less stable were glycosides of kaempferol and apigenin. Naringenin was the only measurable flavonoid aglycone in the sausages, both before and after thermal treatment. Chlorogenic and dicaffeoylquinic acids, abundantly present in tomato fruits of various varieties [2], were not detectable in the raw sausages, where caffeic acid hexoside was found in traces. Evidently, these polyphenols were destroyed already during drying of tomatoes.
Remarkable is that whereas the concentration of most flavonol glycosides is reduced during cooking, the content of aglyconic naringenin was stabile (100 %). Polyphenol aglycones are more efficient free radical scavengers and have higher bioavailability than the initial glycosides [3].

Table 1. Content and yield of major single polyphenols in mg/kg before and after cooking of sausages.

Polyphenol	Quercetin rhamnoside hexoside	Quercetin rutinoside	Quercetin hexoside	Quercetin rhamnoside	Kaempferol pentoside hexoside	Naringenin hexoside	Naringenin	Apigenin pentoside hexoside	Apigenin hexoside
Before	0.4	4.4	0.2	1.1	3.1	0.2	0.4	8.1	0.7
After	0.3	3.4	0.2	0.9	2.6	0.15	0.4	5.0	0.6
Yield%	**75**	**77**	**100**	**82**	**84**	**75**	**100**	**62**	**86**

Table 2. Content of major oxylipins (mg/kg), total oxylipins (mg/kg), total polyphenols and total flavonols before and after thermal processing of sausages and respective yields.

	THODE isomers	DHOME isomers	9-HODE	Total oxylipins	Total phenolics AUC_{280nm}	Total flavonols AUC_{370nm}
Before	8.8	0.82	4.2	9.4	12332	1874
After	8.2	0.91	2.4	6.2	14106	1844
Yield %	**93**	**111**	**57**	**66**	**107**	**95**

Oxylipins A number of oxylipins, primary oxidation products of linoleic acid were identified and semiquantified in the sausages.
The most abundant were: 9,12,13-trihydroxy-10-octadecenoic acid (9,12,13-THODE and other isomers; m/z = 329); 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) and 9-hydroxy-10,12-octadecadienoic acid (9-

HODE; m/z = 295) (Figure 2).

Since the list of linoleic acid oxylipins in studied sausages is much longer, total oxylipin concentrations were calculated using areas under chromatographic curve (AUC) of MS^2 daughter ion with m/z = 171, common fragment for a majority of products of linoleic acid oxidation (Figure 2). 9-HODE was used as the standard compound for this quantitation.

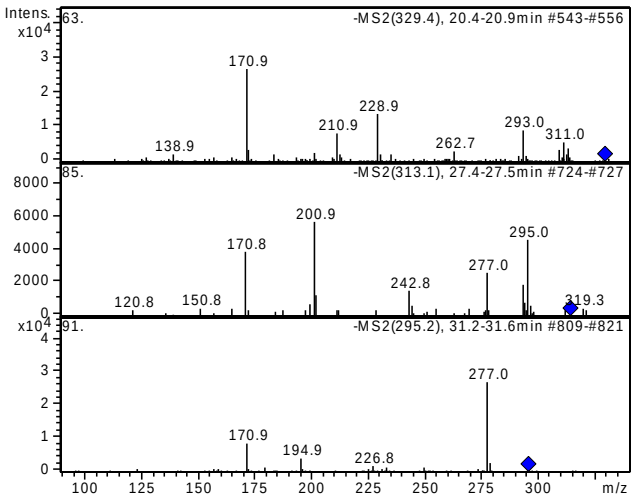

Fig. 2. MS^2 fragmentation spectra of oxylipins 9,12,13-THODE (m/z = 329.4), 9,10-DHOME (leukotoxin diol; m/z = 313.1) and 9-HODE (m/z = 295.0).

Concentration of oxylipins in sausages is generally decreasing during cooking (Table 2). Consequently, content of antioxidants in these commercial sausages is sufficient to almost entirely inhibit linoleic acid oxidation. Only concentration of potentially toxic leukotoxin diols (DHOME isomers) is slightly increasing. However, it is still significantly lower, both before and after cooking of sausages than in highly oxidized mechanically deboned meats (MDM) [4]. A small portion of the oxylipins may originate from dried tomato fruits, where linoleic acid content is up to 1% of total weight [5].

Concerning sensoric properties, the sausages studied were juicy and had pleasant taste and specific nice colour.

IV. CONCLUSION

Polyphenols of tomato fruit, added to sausages, have remarkable thermal stability and, in principle, can function as natural antioxidants in human organism. Total content of various linoleic acid oxylipins is reduced during cooking.

Previously, area under the extracted ion chromatographic (EIC) curve of the MS^2 daughter fragment ion with m/z = 171 (AUC_{171}), characteristic for a majority of linoleic acid primary oxidation products, mostly products of 9-lipoxygenase catalytic process was proposed as a marker of linoleic acid oxidation level in meat products instead of the classical TBARS value that may underestimate the degree of lipid oxidation in meat and in sausages, supplemented with plant antioxidants [6]. Hereby we confirm AUC_{171} as a more universal parameter for determination of oxidation level of fatty acids in meat products containing linoleic acid as a highly oxidizable polyunsaturated fatty acid.

ACKNOWLEDGEMENT

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