NITRITE AS A POTENT AND VERSATILE ANTIOXIDANT ADDITIVE IN MINCED PORK

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I INTRODUCTION

Free polyunsaturated fatty acids (PUFA), particularly linoleic acid (LA, 18:2n6) are prone to (per)oxidation by chemical, enzymatic and combined routes [1]. During oxidation, various toxic compounds, including leukotoxin diols and aldehydes are formed that substantially reduce quality and safety of meat products. Oxidation starts with formation of acid hydroperoxides and continues in two main phases, comprising a number of steps and enzymes. Throughout the first phase the hydrocarbon backbone of LA remains intact and oxygen atom(s) are linked to double bond(s) to sequentially form various oxylipins. During the second phase, aldehydes and other toxic evil-smelling compounds are formed [2]. It is highly desirable to find antioxidants that are able to depress oxidation of PUFA-s, both in fresh and cooked meat, regardless of the mechanism. A big number of plants have been explored for this purpose with rather promising results [3].
Our aim was to untangle the oxidation network in order to find complexes of natural substances capable to slow down the key reactions of both phases of LA oxidation. Selected plant powders [4] and their most promising blends as well as single polyphenolic antioxidants (gallic acid, rutin) and sodium nitrite were used. Nitrites as excellent antibacterials, widely used for curing of meat, have a poor reputation as parent compounds of *N*-nitrosoamines, potential rodent carcinogens. Simultaneously, nitrites are attributed cardiovascular benefits through nitric oxide radicals [5**]** and about 90% of nitrites in our diet is of leafy plant origin.

II MATERIALS AND METHODS

Meat: Minced pork with 27.2% of fat; PUFA-s 3.51%; of which LA 3.0%, was used.
***Supplements*:** Powders of roots and petioles of Siberian rhubarb, tomatoes, berries and leaves of black currant, berries of chokeberry and honeysuckle were mixed with minced pork either singly (2%) or in blends (1%+1%). Sodium chloride (1%); sodium nitrite (150 mg kg^{-1}) + sodium chloride (1%); rutin and gallic acid (both 80 mg kg^{-1}) were also used as additives. Half of every mixture was cooked at day 0. All samples were kept in the refrigerator at 4…6 °C and were analysed at definite days.
***Analytical sample preparation*:** Samples (2 g) of minced pork 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-ion trap MS/MS, Agilent 1100 series chromatograph with negative ionization. Column: Zorbax 300SB-C18 (2.1×150 mm; 5µm; Agilent Technologies).
***Quantitation of meat oxidation markers*:** Total oxylipin contents (TOC 171) were expressed in arbitrary units (AU) using areas under the extracted ion chromatograms of the MS^2 daughter fragment with *m/z* =171 (AUC_{171}), a common fragment of LA oxylipins formed by 9-lipoxygenase catalyzed route of oxidation as well as by the chemical (radical) mechanism [6]. Malondialdehyde content (MDA) was estimated spectrophotometrically by measuring concentration of thiobarbituric acid reactive substances (TBARS).

III RESULTS AND DISCUSSION

All studied additives, except NaCl, inhibited formation of oxylipins in minced pork (Figure 1), although black currant leaf, tomato and honeysuckle berry powders had already initially relatively high contents of oxylipins. In the samples of raw pork, where enzymatic and chemical mechanisms compete, efficient inhibitors were rhubarb roots combined with black currant or chokeberry berries and rutin, but the most efficient were sodium nitrite and gallic acid. In the thermally processed samples, where only chemical oxidation is possible, efficient radical scavengers were mixtures containing dark berries that contain anthocyanins, but most outstanding radical scavengers were again sodium nitrite and rhubarb petiols that may also contain nitrite, reduced from nitrate by nitrate reductase. Polyphenols rutin and gallic acid were not as good radical scavengers in cooked meat as inhibitors of oxidation enzymes in the raw meat. TOC 171 is correlated with content of products of the second phase of oxidation, represented by malondialdehyde (MDA) (Figure 1). Concentration of the most toxic oxylipins (leukotoxin diols) remained relatively low during storage (not illustrated).

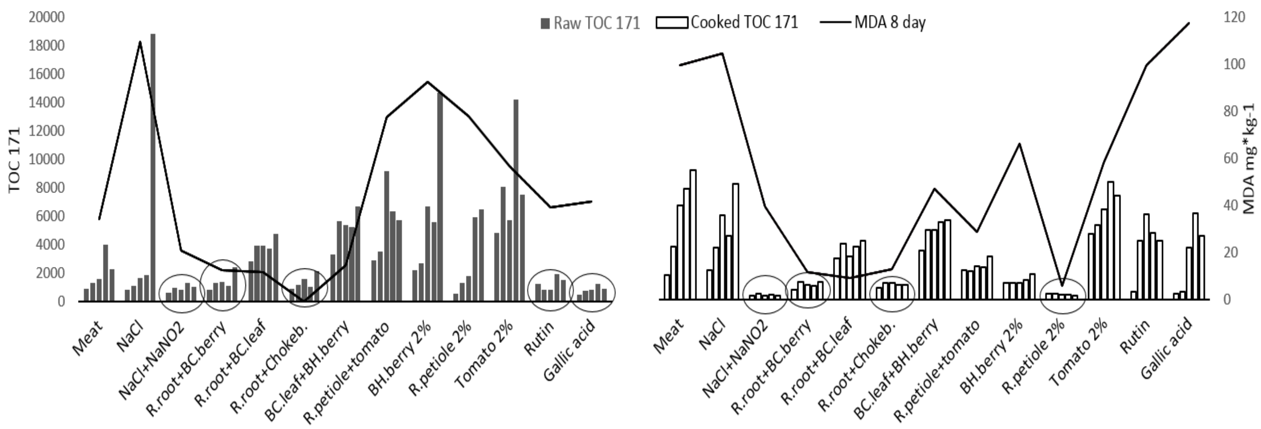

Figure 1. Dynamics of total oxylipin content (TOC 171) and malondialdehyde (MDA) content of raw and cooked samples at different days of storage. R = rhubarb, BC = black currant; BH = blue honeysuckle.

IV CONCLUSION

The best and most versatile antioxidant was nitrite ion that slowed down both enzymatic and chemical routes of LA oxidation as a single antioxidant additive as well as a possible component of rhubarb petiols. Obviously, nitrite ions are able to inactivate heme-containing oxidation enzymes such as cytochrome P450 by formation of complexes with heme and iron ions [7]. Nitric oxide radicals, formed from nitrite ions, may participate in scavenging of various oxylipin radicals during chemical oxidation of LA.

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