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# Correlations between microbial growth and lipid oxidation markers and polyphenol contents in minced pork supplemented with plant powders (#309)

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#### Introduction

Microbial growth and oxidation of polyunsaturated fatty acids are the two main routes of meat deterioration during storage. Lipid oxidation occurs by both autoxidative (radical) and enzymatic mechanisms. Both harmful processes are inhibitable by various phytochemicals, particularly polyphenols. Dynamics of microbial growth and markers of two main consecutive phases of linoleic acid oxidation (oxylipins and malondialdehyde) was studied in fresh and cooked minced pork, enriched with plant powders and also with NaCl, NaNO<sub>2</sub> and polyphenols rutin and gallic acid (GA).

#### Methods

Meat: Minced pork with fat content 27.2%, PUFAs content 3.5%.

**Plant supplements:** Freeze-dried non-sterile powders of roots (Rroots) and petioles (Rpetioles) of garden rhubarb, tomatoes, berries and leaves of black currant (BC), berries of chokeberry (CB) and edible honeysuckle (BHS) were mixed with minced pork either by two (1%+1%) or singly (2%) in two replicates. Amount of added nitrite was 150 mg/kg and of rutin and GA 80 mg/kg of meat. Half of every mixture was cooked. Samples were stored at +4°C for 8 days.

**Analytical sample preparation:** 2 g of minced pork was extracted with 4 ml of methanol, extract was shaken, 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 with Zorbax 300SB-C18 column for identification and quantitation of oxylipins and polyphenols.

**Quantitation of microorganisms:** Total microbial counts (log cfu/g) were determined by surface plating technique.

**Quantitation of meat oxidation markers:** Total oxylipin contents (TOC) were expressed in arbitrary units as areas under the extracted ion chromatograms of the  $MS^2$  fragment with m/z =171 (AUC<sub>171</sub>), as described in [1]. Malondialdehyde (MDA) was quantitated by TBARS method. Total phenolics contents (TPC) were expressed as areas under chromatographic curves at 280 nm.

#### Results

Most important results are the following.

There is a weak promotion of microbial growth by NaCl and most plant mixtures, except of rhubarb petioles and tomato (Fig. 1). It could be explained by pH raising effect of most polyphenols, expressed by positive linear correlation between TPC and pH of enriched meats (r=0.80). However, the fact that pH of all enriched samples is lower than of pure meat ( $5.66\pm0.12$ ), vice versa refers to the possible inhibition of bacterial growth. Another explanation is nutritive effect of phytochemicals, accelerating bacterial growth. Rhubarb petioles decrease meat pH to  $4.38\pm0.08$  that is already unsuitable for growing of most bacteria. Nitrites are expectedly potent inhibitors of bacterial growth and inhibitory effect have also single polyphenols GA and rutin. **Figure 1.** Positive logarithmic correlation between TPC and total microbial counts at  $8^{\text{th}}$  day of storage of raw meat samples.

**Figure 2.** Strong negative logarithmic correlation between TPC and MDA at 8<sup>th</sup> day of storage of raw meat samples.

Plant polyphenol mixtures inhibit PUFA oxidation in the concentration dependent manner (Fig. 2). However, single polyphenols GA and particularly rutin have even remarkably higher inhibitory effect than expected by their content in mixture. NaNO<sub>2</sub> with NaCl is also a powerful antioxidant, while NaCl alone accelerates oxidation by opening meat structure for free radicals. BCleaves have intrinsically high content of oxidation markers.

Figure 3. Strong correlation between parameters of two consecutive phases of linoleic acid oxidation in raw and intermediate correlation in cooked samples.

NaCl promotes both phases of oxidation (Fig. 3). Mixtures, containing Rroots and dark berries are in turn efficient inhibitors of both phases, but both single polyphenols and nitrite (radical scavenging) and petioles (Fe<sup>2+</sup>-chelating by oxalic and other dicarboxylic acids) perform expectedly more efficiently during the 1<sup>st</sup> phase. Tomatoes as well as BCleaves and BHSberries have higher own content of oxylipins, expressed by their TOC values.

Somewhat weaker correlation for cooked meat is explainable by remarkable changes in the total antioxidant capacity of meat during cooking, caused by denaturation and exposure of reactive protein sites, releasing of heme iron, impairment of meat own pro-oxidant-antioxidant system and formation of new antioxidants like Maillard reaction products [2]. Surprisingly, two individual polyphenols (excluded from correlation) don't work in the case of cooked meat. Very efficient are again Rroots with both BCberries and CB

## **Notes**

berries, NaNO<sub>2</sub> and petioles during the 1<sup>st</sup> phase of oxidation. Tomato fruits and BCleaves differentiate again by their higher TOC values.

There is no correlation between log cfu/g and TOC or MDA, r values are +0.17 and -0.12, respectively.

## Conclusion

Plant powders containing powerful antioxidant polyphenols are not necessarily potent antimicrobials in minced meat. Sodium nitrite has one of the strongest antibacterial as well as antioxidant effects at its maximum permitted level.

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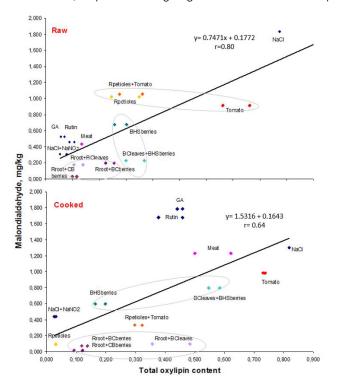


Figure 3.

Strong positive correlations between parameters of two phases of linoleic acid oxidation

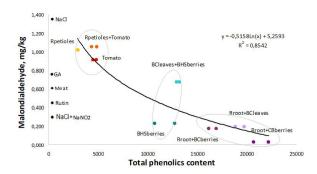
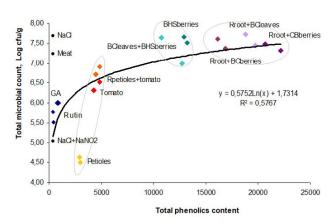


Figure 2.

Strong negative logarithmic correlation between TPC and MDA in raw at 8th day.



**Figure 1.**Positive logarithmic correlation between TPC and total microbial counts at 8th day

**Notes**