The Effect of Some Polyphenols on Minced Pork during Refrigeration Compared with Ascorbic Acid

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Abstract

Quality of meat and meat products by two oxidative processes is affected. The two processes are lipid peroxidation and myoglobin oxidation. To delay oxidative processes, in meat and meat products are added compounds with antioxidant activity.

The aim of this study was to evaluate the antioxidant activity of (±)-catechin, quercetin, and gallic acid, on oxidative processes in minced pork, comparatively with ascorbic acid in the same concentration. The ability of antioxidants to reduce DPPH• and to chelate Fe (II) were determined. Minced pork samples separately with the chemicals were treated. The treated samples with antioxidants were preserved for seven days at refrigeration temperature. Myoglobin, oxymyoglobin, metmyoglobin concentrations, peroxide value, conjugated dienes and trienes levels and TBARS value were determined. Polyphenols have showed important hydrogen radical donator and iron chelator activity, higher as ascorbic acid activities. All antioxidants were effective in myoglobin oxidation and lipid peroxidation inhibition on minced pork, but the three polyphenols (±)-catechin, quercetin, and gallic acid) protected better myoglobin than ascorbic acid as showed the results.

Keywords: (±)-catechin, quercetin, gallic acid, lipid peroxidation, metmyoglobin

Introduction

The current trend of modern consumers and food processors is the return to natural products. The consumer requires natural product; the processor wants to provide this type of product but with shelf life comparable to preserved products with synthetic preservatives. Lipid peroxidation and myoglobin oxidation lead to changes in meat colour, flavour and appearance of undesirable odour. These negative aspects draw the consumer’s refusal to buy the oxidized products (Gahruie et al., 2017; Papuc et al., 2017a).

Antioxidants are compounds able to inhibit oxidative processes by different mechanisms: electron donating to unpaired species stopping the chain reaction such as hydroxyl radical (HO•), peroxyl radical (ROO•), or by chelating transitional metal ions. To inhibit the oxidative processes, in met industry synthetic antioxidants such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate), and TBHQ (tert-butylhydroquinone) are used. Recent studies have shown that synthetic antioxidants used in the meat industry are supposed to be involved in the development of different diseases (Kumar et al., 2015). For this reason, food industry processors are looking for new sources of antioxidants like natural polyphenols. Epidemiological studies suggested that long term consumption of diets rich in products containing polyphenols offered
some protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Pandey and Rizvi, 2009).

The aim of the present work was to complete the knowledge on the possibility to use phenolic compounds as antioxidants in the meat industry. We have determined the inhibitory activity of (±)-catechin, quercetin and gallic acid, in equal concentrations, on lipid peroxidation and myoglobin oxidation in minced pork during refrigeration storage.

The primary objective of this study was to determine the ability of (±)-catechin, quercetin and gallic acid to function as hydrogen donator and Fe$^{2+}$ chelators, comparatively with the most antioxidant used in meat industry, ascorbic acid, in some concentration. The second objective was to determine the effects of mentioned compounds on myoglobin, oxymyoglobin, metmyoglobin, conjugated dienes, conjugated trienes, and TBARS levels in minced pork meat, subject to refrigeration, comparatively with ascorbic acid in the same concentration.

**Materials and methods**

**Antioxidant activity determination**

**DPPH radical scavenging activity.** Scavenging activity of 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH•) of the mentioned compounds was determined according to the method described by Burits and Bucar (2000). Briefly, to the alcoholic solution of DPPH• (100 μM), 100 μL of alcoholic antioxidant solutions were added. For the control preparation, 60% (v/v) ethanol was added. Absorbances of the samples and control at 517 nm were read using a UV-VIS spectrophotometer (Jasco V670). The results were expressed as % Inhibition.

**Fe$^{2+}$ chelating activity.** The ability of antioxidants to chelate Fe$^{2+}$ ions was determined by a photocolorimetric method (Minotti, 1993). In this assay, the compounds bind Fe$^{2+}$ ion, released by iron (II) sulfate. 0.85 mL of each alcoholic solution was mixed with 1.5 mL of Tris-HCl buffer (0.1M, pH 7.4), followed by the addition of 1.5 mL of 500μM iron (II) sulfate. The mixture was left at room temperature for 5 min, and then 0.15 mL of 0.25% aqueous 1,10-phenanthroline were added. The absorbance of the solution at 510 nm against blank was read, using a UV-VIS spectrophotometer (Jasco V670). The results were expressed as % Chelation.

**Refrigerated minced pork treatments.**

Pig pulp was bought from a local butcher. First, the pork was chopped manually and then was minced in a laboratory grinder. The minced pork was divided into 5 portions of 200 grams. Four portions were treated with commercially (±)-catechin (T1), quercetin (T), gallic acid (T) and ascorbic acid (AA) (T), obtaining 50 ppm antioxidant levels in the pork mince. The samples were packed in polyethylene bags and stored by refrigeration at 4 ºC. The fifth portion was packed and refrigerated in the same conditions for obtaining the control sample (C). Lipid peroxidation and myoglobin oxidation were investigated at 2-day intervals for 7 days.

**Effect of antioxidants on myoglobin stability in refrigerated minced pork**

**Determination of myoglobin, oxymyoglobin and metmyoglobin concentrations.** Myoglobin (Mb), Oxymyoglobin (OxyMb) and metmyoglobin (MetMb) concentrations in pork samples were determined using a spectrophotometric method (Krzywicki, 1982). Briefly, 2 g of minced pork and 20 mL of cold 40 mM phosphate buffer (pH 6.8) into a centrifuge tube were transferred. The mixture was homogenized and centrifuged at 3000 g at 4°C for 30 min. For the clear supernatant of each sample and control, the profile of the spectra between 400 and 700 nm were performed, using a UV-VIS spectrophotometer (Jasco V670). Mb, OxymMb, and MetMb concentrations using formulas were calculated:

\[
mM\text{ Mb} = \frac{A_{525}/(7.6 \text{ mM} \cdot \text{cm}^{-1} \cdot \text{x} \text{ cm})}{7.6 \text{ mM} \cdot \text{cm}^{-1}} \times 100
\]

\[
% \text{ OxyMb} = (0.882R_1 - 1.267R_2 + 0.809R_3 - 0.361) \times 100
\]

\[
% \text{ MetMb} = (- 2.514R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100
\]

Where \( R_1 \) is A572/A525, \( R_2 \) is A565/A525, and \( R_3 \) is A545/A525.

**Effect of antioxidants on lipids stability in refrigerated minced pork**

**Determination of the peroxide value (PV).** Peroxide value was determined as described by Romero et al. (2008). Briefly, 0.2 mL of total lipids extract was dissolved in 9.8 mL methanol: chloroform mixture (70:30, v/v) and then 0.1 mL of 30% ammonium thiocyanate was added.
and mixed. After 5 min, 0.1 mL ferrous chloride prepared in 3.5% HCl to the previous mixture was added. The absorbance at 501 nm was measured using a Jasco V670 spectrophotometer. A standard curve was prepared using cumene hydroperoxide at a concentration range of 0.5 – 2 ppm. Peroxide value as μmole peroxide/kg meat was expressed.

**Determination of the conjugated diene (CD) and conjugated triene (CT) values.** After total lipid extract was obtained, it was used for evaluation of conjugated dienes and conjugated trienes, as follow: 20 μL lipid extract was mixed with 2 mL isoctane and absorbance was measured at 233 nm for CD and at 268 nm for CT, using a Jasco V670 spectrophotometer (Pegg, 2005). The results were expressed as absorbance units. CD and CT levels are expressed as absorbance units at 233 nm and 268 nm, respectively. However, the calculation of CD and CT concentrations requires knowing the extinction coefficient of the sample, which itself requires knowledge of the specific fatty acid composition of each sample (Wrolstad et al., 2005). Because this information was not available for this specific research, CD and CT levels were expressed in units of absorbance.

**Determination of thiobarbituric acid reactive substances (TBARS).** TBARS value was determined using a spectrophotometric method described by Buege and Aust (1978). In the presence of thiobarbituric acid (TBA), malonaldehyde form pink chromogen with maximum absorbance at 532 nm. 0.5 g of meat was homogenized with 2.5 mL of a solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl, using the homogenizer. The mixture was heated in a boiling water bath (95-100 ºC) for 10 min to develop a pink colour. The tubes were cooled with running tap water. The absorbance at 532 nm was measured using a TBARS value was calculated using $1.56 \times 10^5/M/cm$ as the extinction coefficient of the pink TBA chromogen. The TBARS value was expressed as ppm malonaldehyde (mg MDA/kg).

**Statistical analysis.** The SPSS v.20 (SPSS-IBM) software was used for statistical analysis. Significant differences as were considered at p ≤ 0.05. All determinations were carried out in triplicate, and the results were expressed as mean values ± standard deviation (SD).

**Results and Discussion**

**Antioxidant activity determination**

The ability of antioxidants to function as hydrogen atom donor and Fe (II) chelate are very important tests for antioxidant activity evaluation in food science. By donating hydrogen radicals, antioxidants can slow down lipid oxidation and consequently myoglobin oxidation. The evaluation of DPPH• scavenging activity reflects the ability of an antioxidant to function as atom hydrogen donator, and results from table 1 show that all compounds are good DPPH• scavengers, but polyphenols have higher scavenging activity.

Quercetin and (±)-catechin showed the highest DPPH• scavenging activity (Table 1), indicating that the flavonoids are most potent hydrogen atom donor as the studied phenolic acid. These results were consistent with the results reported by Majewska et al (2011) who found highest DPPH• scavenging activity for quercetin in low concentration, comparatively with ascorbic acid. It is well known that polyphenols are effective metal chelators (Papuc et al, 2017b). This property of polyphenols is very important because in the most cases, lipid and myoglobin oxidation in meat can be initiated by the most potent free radical [hydroxyl radical (HO•)], generated in the reaction

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>DPPH• scavenging activity (% Inhibition)</th>
<th>Fe(II) chelating ability (% Chelation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>78.12±5.61</td>
<td>79.54 ± 6.87</td>
</tr>
<tr>
<td>Quercetin</td>
<td>89.11 ± 7.54</td>
<td>74.52 ± 6.35</td>
</tr>
<tr>
<td>(±)-Catechin</td>
<td>79.04 ± 6.74</td>
<td>82.98 ± 7.81</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>76.24 ± 7.14</td>
<td>69.31 ± 6.17</td>
</tr>
</tbody>
</table>

Note: Each value represents mean ± SD (n=3).

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of Fe (II) with hydrogen peroxide, known as Fenton reactions (Papuc et al., 2017a). Therefore, polyphenols as iron chelators can inhibit lipid oxidation, and indirectly myoglobin oxidation. Fe$^{2+}$ chelating ability of tested antioxidants increased as follow: ascorbic acid, quercetin, gallic acid, and catechin (Table 1). Results suggested that tested polyphenols are important iron chelators just like ascorbic acid, due to phenolic – OH groups.

**Effect of antioxidants on myoglobin concentration in refrigerated minced pork**

The results regarding antioxidants effect on myoglobin stability in refrigerated minced pork are presented in table 2. During refrigeration, for all treatments myoglobin concentration decreased and metmyoglobin concentration increased, but the highest increases of metmyoglobin concentration were found in control samples (untreated meat) (p < 0.05). The lowest increase of metmyoglobin concentration was found in samples treated with gallic acid (p < 0.05). The concentration of metmyoglobin was higher in the samples treated with the flavonoids (±)-catechin and quercetin than its values found in the samples treated with ascorbic acid (p < 0.05). During the experiment, oxymyoglobin concentration increased for meat samples treated with antioxidants, while in untreated meat samples oxymyoglobin concentration decreased (p < 0.05). Increasing of oxymyoglobin concentration was due probably to the reperfusion produced during T1 – T4 samples homogenization. Obtained results show that gallic acid protects better myoglobin from refrigerated minced pork, compared to the other antioxidants in the last two days of investigation (p < 0.05).

Values in the same row and subtable not sharing the same subscript are significantly different at p< 0.05 in the two-sided test of equality for column means. Each value represents mean ± SD (n=3).

**Table 2. Effect of antioxidants on myoglobin stability in refrigerated minced pork**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Myoglobin (mM)</th>
<th>% Methmyoglobin</th>
<th>% Oxymyoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
<td>Day 5</td>
</tr>
<tr>
<td>T1</td>
<td>0.77±0.04</td>
<td>0.74±0.03</td>
<td>0.68±0.05</td>
</tr>
<tr>
<td>T2</td>
<td>0.76±0.03</td>
<td>0.71±0.03</td>
<td>0.65±0.05</td>
</tr>
<tr>
<td>T3</td>
<td>0.79±0.05</td>
<td>0.72±0.03</td>
<td>0.69±0.04</td>
</tr>
<tr>
<td>T4</td>
<td>0.79±0.05</td>
<td>0.73±0.03</td>
<td>0.62±0.04</td>
</tr>
<tr>
<td>C</td>
<td>0.77±0.03</td>
<td>0.59±0.04</td>
<td>0.47±0.04</td>
</tr>
<tr>
<td></td>
<td>18.71±0.20</td>
<td>20.68±1.14</td>
<td>25.32±2.70</td>
</tr>
<tr>
<td>T2</td>
<td>18.38±2.10</td>
<td>20.87±3.37</td>
<td>25.99±3.00</td>
</tr>
<tr>
<td>T3</td>
<td>16.48±2.50</td>
<td>18.20±2.75</td>
<td>24.05±3.00</td>
</tr>
<tr>
<td>T4</td>
<td>18.11±2.00</td>
<td>24.00±2.00</td>
<td>26.04±2.00</td>
</tr>
<tr>
<td>C</td>
<td>19.63±2.51</td>
<td>25.20±2.25</td>
<td>27.37±2.15</td>
</tr>
<tr>
<td></td>
<td>10.09±1.01</td>
<td>10.15±2.01</td>
<td>10.29±2.06</td>
</tr>
<tr>
<td>T2</td>
<td>10.11±2.00</td>
<td>10.30±2.06</td>
<td>11.15±1.90</td>
</tr>
<tr>
<td>T3</td>
<td>10.50±2.50</td>
<td>10.57±2.50</td>
<td>11.48±2.50</td>
</tr>
<tr>
<td>T4</td>
<td>10.55±1.50</td>
<td>10.95±2.00</td>
<td>11.55±2.31</td>
</tr>
<tr>
<td>C</td>
<td>10.01±2.00</td>
<td>9.03±3.00</td>
<td>9.07±2.00</td>
</tr>
</tbody>
</table>

Note: T1 – gallic acid 50 ppm, T2 – quercetin 50 ppm, T3 – (±)-catechin 50 ppm, T4 – ascorbic acid, C – control.
Many researches showed that polyphenols exhibit antioxidant activity on meat lipids (Ahn et al., 2007; Papuc et al., 2010; Vamanu and Nita, 2014). The antioxidant is a compound that delays or even prevents lipids oxidation by inhibiting initial free radical formation (Fennema, 1996). Antioxidants can bind metals, scavenge reactive species that initiate or perpetuate oxidation, quench high-energy oxygen species-preventing formation of peroxides, or decompose lipid peroxides, and the result is represented by the improvement both color and flavor in meats (Xiong et al., 1993).

Values in the same row and subtable not sharing the same subscript are significantly different at p<0.05 in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances. Each value represents mean ± SD (n=3).

The effect of antioxidants on lipid peroxidation in minced pork, subject to refrigeration is shown in Table 3. Comparatively with untreated meat, for all samples treated with antioxidants the level of peroxide value (PV), conjugated dienes (CD), conjugated trienes (CT) and TBARS were lower. The indicators that reflect the levels of primary products of lipid peroxidation (PV, CD, and CT) had the lowest values in the samples treated with the flavonoids (±)-catechin and quercetin. The level of the primary products of lipid peroxidation were lower in the meat sample treated with gallic acid than those found in the meat treated with ascorbic acid. The levels of secondary products of lipid peroxidation (TBARS value) increased and decreased during refrigeration (p < 0.05). This aspect was observed also by other researchers (Addeen et al., 2016, Maqsood and Benjakul, 2011), and was due to the decomposition of these compounds during refrigeration storage. The level of the TBARS value was highest in control sample, and the lowest in the samples treated with ascorbic acid, in the refrigeration time. The results obtained in this experiment show that antioxidants are effective in myoglobin oxidation and lipid peroxidation inhibition. The used polyphenols, (±)-catechin, quercetin and gallic acid, protected myoglobin in the oxidative processes better than ascorbic acid (p < 0.05), but ascorbic acid protected better the fatty acids than polyphenols in the lipid peroxidation reactions.

Table 3. Effect of antioxidants on primary and secondary lipid peroxidation products level in refrigerated minced pork.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PV (µmol/kg meat)</th>
<th>CD (µmol/kg meat)</th>
<th>CT (µmol/kg meat)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
<td>Day 5</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>0.19±0.03</td>
<td>0.14±0.03</td>
<td>0.08±0.02</td>
</tr>
<tr>
<td>T3</td>
<td>0.20±0.03</td>
<td>0.16±0.02</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>T4</td>
<td>0.22±0.02</td>
<td>0.15±0.02</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>C</td>
<td>0.24±0.02</td>
<td>0.20±0.03</td>
<td>0.12±0.01</td>
</tr>
</tbody>
</table>

Note: T1 – gallic acid 50 ppm, T2 – quercetin 50 ppm, T3 – (±)-catechin 50 ppm, T4 – ascorbic acid, C – control.
Pearson correlation revealed that CD concentration were positively correlated with the concentration of CT, PV and TBARS (r=0.218, p<0.01; r=0.245, p<0.01 and r=0.053, p<0.05). On the other hand, significant negative correlations were found between myoglobin concentration and methmyoglobin concentration (r=-0.635, p<0.01).

**Conclusion**

Quercetin, (±)-catechin and gallic acid are stronger hydrogen radical donators and Fe (II) chelators as ascorbic acid. Quercetin, (±)-catechin and gallic acid, in concentration 50 ppm are more effective than ascorbic acid, in the same concentration, in the inhibition of myoglobin oxidation reaction in the refrigerated minced pork. Ascorbic acid is more effective than investigated polyphenols in the inhibition of lipid peroxidation in refrigerated minced pork. Quercetin, (±)-catechin and gallic acid added in minced pork in concentration 50 ppm, are able to protect fatty acids and myoglobin as effective as ascorbic acid in concentration 50 ppm.

The obtained results recommend quercetin, (±)-catechin and gallic acid to be use for preserving meat and meat products.

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**References**


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