LIPID OXIDATION WHEN ADDING VEGETAL OILS AND WALNUT IN MEAT PRODUCTS

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Abstract

Lipid oxidation of pasteurized meat products with high content of unsaturated fatty acids has been assessed. The increase in unsaturated fatty acids level has been achieved by adding vegetal oils and walnuts (Juglans Regia L.) to a salami type meat product.

Peroxide content, α, β-unsaturated aldehydes and the superoxide ions have been assessed from the crude fat and results have been expressed in relation to the lipid profile and the antioxidant mixture utilized in each compositional formula. Lipid oxidation has been assessed by measuring the peroxide value, the anisidine value and the antioxidant capacity of the lipid phase by photochemiluminescence, in meat compositions with different lipid sources.

Lipid oxidation has higher values, and p-anisidine value was: 28 to 36.8, in the compositions were vegetal oils have been added, compared to the compositions with sea buckthorn oil and walnut, where p-anisidine values were: 9.25 to 12.37.

The way lipids are added to salami has a great impact over the extent of oxidation processes. Adding lipids in their source matrix (walnuts) rather than extracted (vegetal oils), to salami, will increase stability of the product. Antioxidants are more efficient when used as a mixture compared to only one type of antioxidant.

Key words: Lipid oxidation, Meat products, Vegetal oils, Walnut, Antioxidants.

1. Introduction

In order to modify the lipid profile of meat products by increasing the unsaturated fatty acids level different published technological methods, and polyunsaturated fatty acids (PUFA) sources ingredients such as: vegetal oils, oilseeds, algae with high polyunsaturated fatty acids content, have been studied.

The technological methods studied the partial substitution of fat, and have been adapted to the texture and shape of the ingredients, thus the vegetal oils have been added directly, emulsified or microencapsulated [1], to finely comminuted meat products like frankfurters [2] and fermented salami [3], [4]. Hardon et al., [5] have shown that by replacing fat, in heterogeneous meat products, with variable quantities of olive oil most of the physical and sensory characteristics have not been affected, except hardness and the a* values. However, above 15% oil addition will influence product taste, providing the product with a pronounced oily taste.

By adding vegetal oil it was found that the conservability of the product would proportional decrease accordingly the percentage of added oil. López-López et al., [6] had partially substituted fat with 5.5 % algae from the Himanthalia elongata (aiming to obtain a content of 400 mg DHA/100 g product) and 50% olive oil, source of monounsaturated fatty acids (MUFA), as well as a combination of these two ingredients in different percentages. They have obtained a PUFAs/SFAs ratio of 0.4 a known indicator of nutritional quality of fats recommended by nutritionists [7]. Zapata [8] added coarsely ground walnuts (Juglans regia L.) at levels of 5, 10 and 15%, in meat burgers and finely comminuted beef. The authors have reported issues regarding color and texture for both products and, additional, the finely comminuted product had greatly diminished its water retention capabilities.
Lipid profile modification experiments, using several nut species as main ingredient, have studied the maximum percentage of nuts that can be added to meat products and reported problems regarding texture, taste and shelf life [9, 10, 11, 12]. By studying product development in this field, Jimenez-Colmenero [1] found out that a fundamental requirement in designing and reformulating the lipid profile of meat products is the crude lipid content and the optimum lipid profile of the fatty acids from the crude lipid content. Expert groups of the World Health Organization and of Food and Agriculture Organization are recommending a fatty acid ratio of SAT/MUFA/PUFA = 1/1/1 [13]. The present study has evaluated and compared two technological methods for lipid profile optimization, for cooked salami, by partially replacing fat by a vegetal oil mixture and with sea buckthorn oil and walnut (Juglans regia L.). The crude fat of each composition consists of a different mixture of triacylglycerol given by each lipid source: meat, vegetal oil and walnut. The lipid oxidation level of each compositional formulation was assessed, by measuring the peroxide value (PV) and anisidine value (AV). The efficiency of the antioxidant formulations from each composition was evaluated by photochemiluminescence in regard to the lipid profile.

### 2. Materials and Methods

#### 2.1 Materials salami samples preparation

Two compositional structures have been manufactured: one by adding emulsified oils (sea buckthorn, soy and rapeseed) to the finely comminuted phase, and the other by adding emulsified sea buckthorn oil to the finely comminuted phase and walnut in the meat grind. The meat batter has been manufactured from finely comminuted meat and meat grind, in different compositional variations (Table 1).

The meat has been bought from the same supplier: Prahova, Romania. The finely comminuted batter has been manufactured from refrigerated pork leg (Semi-membranosus muscle), with a fat content of 8 - 12% total fat, measured by Soxhlet extraction with petroleum ether.

The meat has been finely comminuted in the mix bowl cutter with ice, milk protein, salt, usual technological additives and antioxidants: rosemary extract, green tea extract, sodium nitrate, Na erythorbate, in different amount (Table 1), the vegetal oil: mixture of sea buckthorn oil; sunflower oil and rapeseed oil, that has been added directly to this mixture.

### Table 1 Salami sample composition, %

<table>
<thead>
<tr>
<th>Samples</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>PN1</th>
<th>PN2</th>
<th>PN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finely comminuted phase</td>
<td>26.13</td>
<td>26.13</td>
<td>26.13</td>
<td>17.0</td>
<td>17.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Meat grind</td>
<td>73.87</td>
<td>73.87</td>
<td>73.87</td>
<td>82.98</td>
<td>82.99</td>
<td>83.0</td>
</tr>
<tr>
<td>Pork leg</td>
<td>51.78</td>
<td>51.78</td>
<td>51.78</td>
<td>55.13</td>
<td>50.37</td>
<td>36.72</td>
</tr>
<tr>
<td>Beef leg</td>
<td>34.52</td>
<td>34.52</td>
<td>34.52</td>
<td>18.07</td>
<td>22.49</td>
<td>35.82</td>
</tr>
<tr>
<td>Water</td>
<td>6.90</td>
<td>6.90</td>
<td>6.90</td>
<td>18.78</td>
<td>18.69</td>
<td>18.60</td>
</tr>
<tr>
<td>Oil mixture</td>
<td>4.31</td>
<td>4.31</td>
<td>4.31</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sea buckthorn oil</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.81</td>
<td>1.8</td>
<td>1.79</td>
</tr>
<tr>
<td>Walnut</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.9</td>
<td>2.35</td>
<td>2.79</td>
</tr>
<tr>
<td>Salt</td>
<td>1.28</td>
<td>1.28</td>
<td>1.28</td>
<td>1.5</td>
<td>1.5</td>
<td>1.49</td>
</tr>
<tr>
<td>Technological additives, spices</td>
<td>1.19</td>
<td>1.19</td>
<td>1.19</td>
<td>1.79</td>
<td>1.79</td>
<td>1.79</td>
</tr>
<tr>
<td>Total batter</td>
<td>99.98</td>
<td>99.98</td>
<td>99.98</td>
<td>99.98</td>
<td>99.99</td>
<td>100</td>
</tr>
</tbody>
</table>

#### Antioxidants

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>PN1</th>
<th>PN2</th>
<th>PN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₂, mg/kg</td>
<td>150</td>
<td>45</td>
<td>45</td>
<td>79</td>
<td>79</td>
<td>79</td>
</tr>
<tr>
<td>Na Erythorbate, mg/kg</td>
<td>300</td>
<td>220</td>
<td>200</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ascorbyl Palmitate, mg/kg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>Rosemary extract, mg/kg</td>
<td>-</td>
<td>98 (3.9)^1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tea extract, mg/kg</td>
<td>-</td>
<td>-</td>
<td>200(40)^1</td>
<td>150 (30)^1</td>
<td>100 (20)^1</td>
<td>80 (16)^1</td>
</tr>
</tbody>
</table>

^1 active ingredient content.
Pork leg, beef leg was chopped, and cured with: 18 % brine salt, milk protein, usual technological additives and antioxidants. The curing has been made by vacuum tumbling for 18 hours. The cured meat has been ground through the 13 mm sieve (pork and walnut) and the 3 mm sieve (beef), walnut has been added without any prior treatment.

The two semi-products: finely comminuted batter and the meat grind, have been mixed in different combinations (Table 1), stuffed into 60 - 90 mm synthetic casings, using the vacuum filling machine, and cooked according to the cooked and smoked salami technology: drying, hot smoking, cooking, drying, smoking, for 120 - 280 minutes. The salami samples have been stored at 3 - 4 °C. The analytic measurements were conducted after 4 days of storage. The test samples have been labelled P1, P2 and P3 for the salami where the vegetal oils were added to the finely comminuted phase, and test samples with seabuckthorn oil added to the finely comminuted phase and walnut in the meat grind with PN1, PN2 and PN3 (Table 1).

2.2 Methods

2.2.1 Crude fat (CF)

CF extraction, for peroxide value measurement, was performed in accordance with AOAC 965.33/2006. Approximately 250 - 300 g from the salami samples have been ground (1 - 1.5 mm) and homogenized by using an 800 W Gorenje mixer. 70 g of ground salami has been weighted into a 250 mL Erlenmeyer where 40 g anhydrous NaSO₄ and 100 mL chloroform were added. Chloroform was used as the extraction agent, as it has a great solubilizing power for saturated fatty acids and an average extraction yield for the polyunsaturated fatty acids with high number of double bonds and methyl groups. The samples were thoroughly mixed for 10 minutes. Non-polar lipid extraction, composed mainly of triacylglycerol and liposoluble vitamins was performed through diffusion for 15 - 20 minutes, in a dark room followed by sample filtration. From the total chloroform filtrate, aliquots have been transferred for analytic parameter measurement: 20 mL filtrate for primary oxidation compounds measurement (peroxide value), 20 mL filtrate for salami crude fat content determination and 5 mL filtrate for antioxidant capacity measurement. After solvent evaporation, from the crude fat samples, aliquots have been transferred: 0.01 g for secondary lipid oxidation compounds measurement (p-anisidine value) and 1g for crude fat content chemical analysis by ¹H-NMR spectroscopy.

2.2.2 Fatty acid composition of crude fat by ¹H-NMR

The fatty acid composition of the crude fat samples was measured by ¹H-RMN (Bruker Biospin GMBH, Sillerstreifen, Germany) by diluting the sample with deuterated solvent 2:8 (fat:CDCl₃, v/v) and transferring approximately 0.7 mL of the mixture to MRI. The ¹H-NMR spectrums were recorded on a Bruker Ascend 400 spectrometer, with pulses and Fourier transform, with field gradients on z axis operating at 9.4 Tesla, corresponding to the resonance frequency of 400.13 MHz for the ¹H nucleus. The chemical signal attribution to spectrum was in accordance with the method described by Taha [14] and Balaban [15]. Fatty acid content and the number of double bonds have been assessed based on the values of the signals integrals. The spectrums have been processed using the Topspin software, and the fatty acid content has been evaluated according to the chemometric equations ([16, and 17]).

2.2.3 Peroxide value

This value was measured in accordance with AOAC 965.33/2006, by oxidizing the iodine ions by peroxides, as an indicator of auto-oxidation process. Results have been expressed as milliequivalent active oxygen on kg crude fat (mEq O₂/kg crude fat).

2.2.4 Anisidine value

Anisidine value was measured from the crude fat in accordance with ISO 6885:2006. Spectrophotometric method that implies the reaction between p-methoxy-aniline and α, β-unsaturated aldehydes, as an indicator of secondary lipid oxidation compounds presence. The reaction occurs in acid medium with the formation of yellow compounds that absorb at 350 nm. The UV/VIS, Jasco V-550 spectrophotometer with quartz cuvettes (l = 10 mm) was used.

2.2.5 Antioxidant capacity (AC)

AC was measured by photochemiluminescence with Photochem (Analytik Jena AG, Konrad-Zuse-Straße 1, Germany). The method implies the optical excitation of a photosensitive substance that will generate free superoxide O₂⁺, without generating ¹O₂. The measurement of the remaining free radicals, after the interaction with the antioxidants, is performed with luminol. The antioxidant capacity is calculated in relation to the calibration curve, established with the ACL kit TROLOX: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, (Analytik Jena AG, Konrad-Zuse-Straße 1, Germany), and the results were expressed as Trolox units (μmol TE / 1 g fat). Depending on the sample relation to the calibration curve dilution were performed, and the antioxidant capacity was recalculated with the following formula (1) and (2):

\[
\text{CF [µg]} = \left[ V \times D \times \text{CF} \right] / V
\]

\[
\text{AC [µmole TE/g fat]} = \left[ \text{AC} / \text{CF} \right] \times 10^3
\]

Where: AC - antioxidant capacity; CF - crude fat in V chloroform extract analyzed for AC, expressed as µg;
V - volume of chloroform extract analyzed for fat content measurement CF, in ml; Vₐ - volume of chloroform extract analyzed for AC measurement, in µl; CF - fat measured from Vₐ, in g; D - the dilution of the chloroform extract analyzed.

3. Results and Discussion

3.1 Crude fat

For the samples formulated with oil added to the finely comminuted meat phase an average quantity of crude fat has been obtained 6.59 ± 0.29 g / 100 mL filtrate: P1 6.4 g / 100 mL, P2 6.44 g / 100 mL and P3 6.93 g / 100 mL. By calculating the partial lipid balance the estimated quantity of CF has a value of 11.24% in the final product (including 20% losses during cooking), with a total fat content in meat of 1.8 ± 0.1% for beef and 8.5 ± 0.5% for pork. An average cold diffusion extraction yield for CF, of 58.63 ± 0.29% was registered. For the samples where seabuckthorn oil and walnuts were added to the meat grind a different quantity of CF was obtained for each sample: PN1 11.51 g / 100 mL, PN2 8.62 g / 100 mL, PN3 6.67 g / 100 mL, result of different meat content, species, and lipid source. According to lipid balance for each sample (total fat content of beef is 2.3 ± 0.2%, for pork 12.0 ± 0.4%) the quantity of total fat has been estimated at 13.4% for PN1, 11.44% for PN2 and 10.09% for PN3. We notice a decrease of CF content as pork meat grind quantity decreases, while the quantity of seabuckthorn oil added to the finely comminuted phase and the quantity of walnut from the meat grind are approximately equal. For a pork/beef ration of PN1: 3.05, PN2: 2.24 and PN3: 1.02, CF quantity decreases in relation to PN1 by 2.89 g / 100g CF and 2.25 g / 100g CF reduction in saturated fatty acid content, while trienoic PUFA increases by 2.49 g / 100g CF when compared to control. Sample PN3: 3.5 / 4.3 / 2.2, having the highest quantity of beef (pork/beef ration of 1.02) has registered 2.5 g / 100g CF more saturated fatty acids and 2.5 g / 100g CF more PUFA’s when compared to control.

3.2 Lipids profile to crude fat

The fatty acid content of the crude fat has been assessed, similar values of the lipid profile have been found for P1, P2 and P3 (Table 2). Adding vegetal oils to salami batter will reduce the saturated fatty acid (SFA) content by 18.12 - 22.86%, from 32.89 g / 100 g CF in the salami formulated only with fat in control (C), with a content of 13.0 - 13.5 g/100g, to 25.37 - 26.93 g / 100g CF in samples. Monounsaturated fatty acids (MUFA) content is similar to control, a 4% smaller quantity was found in P1. The content of polyunsaturated fatty acids (PUFA) increases when compared to control: dienoic PUFA registered a increase of 28.3 - 30.4%, and the trienoic PUFA have increased by 61.58%. The closest ratio of (SFA / MUFA / PUFA) to the lipid nutritional quality recommendations was found in P1 (2.7 / 4.5 / 2.8), samples P2 and P3 have similar lipid profiles, differences were registered for saturated fatty acids: P2 > P3 by 1.4 g / 100g CF and dienoic PUFA: P2 < P3 by 1.6 g / 100g CF. Sample P2 (2.7 / 4.6 / 2.7) and P3 (2.5 / 4.6 / 2.8) have a better nutritional lipid profile when compared to control: 3.3/4.7/1.9.

Each sample, where seabuckthorn oil and walnuts were added, had their own individual lipid profiles, the variance was caused by the quantity and the lipid profile of meat. PN2 formulated with 50.37% pork leg, and 22.5% beef leg, registered the best lipid profile, with a fatty acid ratio of 2.8 / 4.0 / 3.2, when compared to control C: 3.3 / 4.7 / 1.9. PN1: 3.1 / 4.5 / 2.4 registers a 2.25 g / 100g CF reduction in saturated fatty acid content, while trienoic PUFA increases by 2.49 g / 100g CF when compared to control. Sample PN3: 3.5 / 4.3 / 2.2, having the highest quantity of beef (pork/beef ration of 1.02) has registered 2.5 g / 100g CF more saturated fatty acids and 2.5 g / 100g CF more PUFA’s when compared to control.

3.3 Lipid oxidation indicators, Peroxide value, Anisidine value and Antioxidant capacity

The salami matrix is a complex system with multiple pro-oxidant and free radical sources. The free radicals have different physicochemical characteristics. These will interact through specific mechanisms according to the environmental parameters of each system. The antioxidant response is specific to the source of oxidation. For example, carotenoids are not good reducing agents against peroxyl (**O₂·**), preferentially react with singlet oxygen (**O₁**), converting it to the triplet state, where most antioxidants and polyphenols are inefficient. Vegetal oils, especially seabuckthorn oil, have a variable content of tocopherols and β-carotene, which can help prevent against ROS action on the double bonds of PUFA’s, thus increasing the overall antioxidant

Table 2. Contents of fatty acids (g / 100 g CF) of CF extracted from the salami samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cn : 0</th>
<th>Cn : 1</th>
<th>Cn : 2</th>
<th>Cn : 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>32.89 ± 0.31³</td>
<td>46.66 ± 0.28³</td>
<td>17.8 ± 0.19³</td>
<td>1.36 ± 0.39³</td>
</tr>
<tr>
<td>P1</td>
<td>26.93 ± 0.55¹</td>
<td>44.77 ± 0.82³</td>
<td>24.77 ± 0.29³</td>
<td>3.54 ± 0.0³</td>
</tr>
<tr>
<td>P2</td>
<td>26.78 ± 0.51¹</td>
<td>46.46 ± 0.35³</td>
<td>23.21 ± 0.11³</td>
<td>3.54 ± 0.0³</td>
</tr>
<tr>
<td>P3</td>
<td>25.37 ± 0.25¹</td>
<td>46.26 ± 0.64³</td>
<td>24.82 ± 0.69³</td>
<td>3.85 ± 0.22³</td>
</tr>
<tr>
<td>PN1</td>
<td>30.64 ± 0.39³</td>
<td>45.55 ± 0.23³</td>
<td>19.95 ± 0.56³</td>
<td>3.85 ± 0.22³</td>
</tr>
<tr>
<td>PN2</td>
<td>28.36 ± 0.53³</td>
<td>40.12 ± 0.33³</td>
<td>30.2 ± 0.12³</td>
<td>1.32 ± 0.11³</td>
</tr>
<tr>
<td>PN3</td>
<td>35.4 ± 0.38³</td>
<td>42.94 ± 0.14³</td>
<td>19.06 ± 0.34³</td>
<td>2.6 ± 0.33³</td>
</tr>
</tbody>
</table>

³Cn:0: saturated fatty acid (SFA); ²Cn:1: monounsaturated fatty acids (MUFA); ³Cn:2: dienoic polyunsaturated fatty acids (PUFA); ⁴Cn:3: trienoic polyunsaturated fatty acids (PUFA); ¹The values represent means of duplicates ± standard deviation.

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efficiency. Polyphenols (catechines) are efficient blockers against protein carbonyl group formation [18]. The experiments described in this paper studied the triacylglycerol oxidation of the crude fat from different salami matrices as a function of the physical state of the added lipids: liquid (vegetal oils), in solid matrix (walnut). Although the system is very complex and the specific action of each antioxidant cannot be tracked, we can study the synergistic effect of different antioxidant mixtures in salami batters with different lipid profiles. The obtained results (Table 3) have been interpreted based on the premise that a low content of unsaturated aldehydes and peroxides is indicator of a good antioxidant activity of the antioxidant mixture. PV can be an indicator of the early onset of the lipid oxidation, history in regard to the unsaturated aldehyde quantity.

For samples P1, P2 and P3, formulated form the same meat batter with similar lipid profiles we notice a different evolution of the oxidation processes as a result of the different antioxidant content. P1 registered the highest quantity of unsaturated aldehydes and superoxide ions, indicator that the mixture of NaNO2 / Na erythorbate had the smallest antioxidant activity. Sample P2 registered a small quantity of hydroperoxide: 12.54 meq O2/g CF smaller compared to P1, and aldehyde content smaller by 3.76 units in regard to P1, an indicator of the early onset of oxidation. The antioxidant capacity has the smallest value of the three samples, indicator of the rosemary extract efficiency even at low concentrations: 3.9 mg/kg fats, while the minimum accepted quantity is set at 15 mg/kg fats.

The carbonyl compounds generated by the protein oxidation may have contributed to the high AV. The substitution of rosemary extract with tea extract, in sample P3, may lead to very different lipid oxidation reactions. Thus the aldehyde content has the smallest value, indicator of antioxidant activity of the catechines on the protein oxidation. The high value of the peroxides shows a delayed onset of the oxidation reactions while the high values of O2⋅, indicate a reduced antioxidant activity in the lipid phase. It was found that for a lipid profile with 8.6% more PUFA's compared to control, achieved by adding vegetal oil to the finely comminuted phase, the most efficient antioxidant activity was achieved by the rosemary extract / green tea extract mixture as opposed to using only one antioxidant. The samples with added vegetal oil and walnut, registered significant reduction in the lipid oxidation reactions compared to the samples with vegetal oils because of the difference in the lipid profile and by exposing the triacylglycerols to the matrix environmental factors. The samples with added walnut had 2 - 9% more saturated fatty acids and 3.7 - 5.8% less PUFA's when compared to the samples formulated only with vegetal oils. The smallest quantity of aldehydes was found in PN1, which also contained the highest concentration of tea extract, 150 mg / kg meat as well as the highest values for O2⋅, with reduced antioxidant activity in the lipid phase for the smallest content of ascorbyl palmitate and the highest content of trienoic PUFA. Aldehyde content rises in PN2 and PN3 as green tea extract is decreased, while O2⋅ decreases with the addition of ascorbyl palmitate in correlation to the lipid profile of the CF: higher PUFA content PN2: 31.52 g/100 g CF compared to PN3: 21.66 g/100g CF. This behavior demonstrates that the ascorbyl palmitate has an efficient antioxidant activity for the lipid phase. The tests prove that for each of the studied compositions the antioxidant mixture needs to be optimized in relation to the lipid profile of meat and the manufacturing technology.

4. Conclusions

- Measuring the extent to which each pro-oxidant molecule and ROS contribute to lipid oxidation in a complex salami matrix is not feasible. The way lipids are added to salami has a great impact over the extent of oxidation processes. It would be better, from a stability point of view, to add lipids in their source matrix (walnuts) rather than extracted (vegetal oils).

- Adding a mixture of antioxidants against ROS (antioxidant lipids, rosemary extract) and protein carbonyl compound (polyphenols) quenching has greater efficiency rather than using only one antioxidant.

Acknowledgement
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5. References


