Effect of wood vinegar on lipid peroxidation of fish

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SUMMARY. The lipid peroxidation in smoked salmon and two wood vinegars (commercial wood vinegar (WV) and cherry wood vinegar (CWV)) treated fish meat was evaluated using malonaldehyde (MA), 4-hydroxy-2E-hexenal (HHE) and carbonyl protein (CP) as indices for a 7-day storage period at 4°C. Smoked salmon samples showed a high variation of MA, and extremely high levels of MA and HHE indicate uneven distribution of smoke constituents and high pro-oxidative activity respectively, making smoking unsuitable. Of the two wood vinegars, CWV treated fish meat gave high levels of HHE, MA and CP compared with that of the control, demonstrating CWV as a pro-oxidant. However, in WV treated samples, the control showed a higher level of MA compared with that of treated samples, whereas the treated samples showed higher levels of HHE and CP compared with that of the control, making it difficult to decide whether WV suppresses or stimulates the peroxidation. In both wood vinegar treated samples, the variation of indices during storage period does not show a clear pattern in relation to type of wood vinegar used or its concentration. This indicates a complexity of the peroxidative mechanism due to its high dependency on numerous factors.

KEY WORDS: lipid peroxidation, malonaldehyde, 4-hydroxy-2E-hexenal, carbonyl protein, smoked salmon, wood vinegar, cherry wood vinegar

INTRODUCTION

Smoking, one of the oldest methods of food preservation, is still employed primarily to enhance the color and flavor of muscle foods.1) However, liquid smoke flavorings (LSF) such as wood vinegars are being used instead, due to their numerous advantages.2,3) As with traditional smoking, the flavor of LSF depends too much on type of wood, method of generation, etc.4) Furthermore, researches have been done to identify the constituents of different LSF with the aim of finding other flavor constituents and harmful substances.5,6) Of the identified compounds, phenols are responsible for the flavor7) and anti-microbial activity.8) The pyrocatecols, lignin oligomers and di-tert-butylhydroxy toluene are found in some LSF and considered to have a strong anti-oxidative effect.9)

On the other hand, fish is a major muscle protein source with a high content of minerals and polyunsaturated fatty acids, particularly n-3 fatty acids. However, both these factors accelerate the lipid peroxidation, resulting in organoleptic changes and reducing shelf life.9) Furthermore, some of the peroxidative products such as malonaldehyde (MD) and 4-hydroxy-2E-hexenal (HHE), are cytotoxic, mutagenic, and carcinogenic.10) In addition, aldehydes react with some amino acids, such as lysine and arginine of proteins to form protein carbonyls (CP) resulting in further nutritional losses.10-13)

In a food hygienic point of view, it is necessary to prevent or reduce the pace of lipid peroxidation to extend the shelf life of fishery products while preventing nutritional losses.

On the other hand, consumers prefer products containing natural substances such as LSF, to chemicals as shelf life extenders. In this context, we investigated MD, HHE, and CP in smoked salmon and wood vinegar treated fish meat.

MATERIAL AND METHODS

Materials

The 1,3-diethyl-2-thiobarbituric acid (DETBA) was purchased from Aldrich Chemical Co. Inc. Butyl hydroxytoluene (BHT) was from Tokyo Kasei Kogyo Co. Ltd., Japan. All the other chemicals were of either HPLC grade or the purest grade available. The commercial wood vinegar was from the Banyu Co. The cherry wood vinegar was prepared by the method of Matsui et al.14)

Preparation of samples

Five packets of smoked salmon were bought from a commercial market. Muscles in each packet were combined, minced separately in a food processor for 1 min to prepare 5 samples and analyzed for MA, HHE and CP. In fish meat, a filet of yellowtail (Seriola...
quinqueradiata) was brought to the laboratory under refrigeration. The skin and the red muscle was removed, cut into small pieces and minced in a food processor for 1 min. The minced muscle was divided into four portions, of which three were treated with wood vinegar to prepare 0.1%, 0.5% and 1.0% samples, and the remainder was used as the control. All the samples were mixed well and stored at 4°C. Samples were analyzed for MD, HHE and CP on day 0, 3, 7.

Malonaldehyde (MA) analysis

1,3-diethyl-2-thiobarbituric acid (DETBA) assay was based on the method of Sakai et al. Briefly, 1 g of sample was homogenized with 9 vol of ice-cooled 10 mM sodium phosphate buffer (pH 7.0) at 0°C. An aliquot (0.4 ml) of the homogenate was treated with 0.2 ml of 8% SDS, 0.2 ml of 20 mM butyl hydroxytoluene in ethanol, and 3.2 ml of 12.5 mM DETBA in sodium phosphate buffer (0.125 M, pH 3.0). The solution was mixed and heated in a water bath at 95°C for 15 min. Then it was cooled quickly and shaken vigorously with 4 ml of ethyl acetate to extract the DETBA-MA adduct. An ethyl acetate extract (2.4 ml) containing the DETBA-MA adducts was transferred to another tube and evaporated in vacuo. The residue was dissolved in 150 µl of ethanol, and 10 µl of the sample was applied to HPLC under the following conditions: column, Inertsil ODS (5 µm particle size, 250 x 4.6 mm i.d.; GL Sciences, Japan); mobile phase, acetonitrile-0.1 M sodium chloride (75:25, v/v); flow rate, 1.0 ml/min; detection, excitation 515 nm and emission 555 nm.

4-Hydroxy-2E-hexenal (HHE) analysis

2,4-Dinitrophenylhydrazine (DNPH) conversion of HHE essentially followed the procedure of Sakai et al. A 5 g of sample was mixed with 25 mg of butylhydroxytoluene (BHT), and 50 ml of 1 N HCl containing 2.5 mmol DNPH was added to the BHT mixture. Then the reaction was carried out in dark for 2 h at room temperature. DNPH derivatives were extracted three times with 3 vol. of dichloromethane. The dichloromethane thus obtained was evaporated in vacuo to dryness and re-dissolved in 0.2 ml of chloroform. Then applied to a disposable silica gel extraction column (Baker, USA) that had been pre-equilibrated with n-hexane/chloroform (2:1 vol/vol). The same solvent mixture was used to wash off the highly lipophilic DNPH derivatives in a discrete band and remaining material was eluted with chloroform. The chloroform elute was evaporated in vacuo to dryness and re-dissolved in 0.5 ml of methanol. The HHE-DNPH derivatives were analyzed by the HPLC method under the following analytical conditions: column, Ultrasphere C18 (25 cm x 4.6 mm i.d., Backman); mobile phase, 30 mM sodium citrate/27.2 mM acetate buffer (pH 4.75); methanol = 35:65; flow rate, 1 ml/min; column temperature, 40°C; detection wavelength, 365 nm.

Analysis of carbonyl protein

Carbonyl protein (CP) contents were determined by 2,4-dinitrophenylhydrazine (DNPH) method of Nakamura and Goto. Protein extracted from the sample with 10 to 20 vol of 50 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA was precipitated with 10% trichloroacetic acid. The precipitates were treated with 2 N HCl solution containing 10 mM DNPH (PC) or 2 N HCl alone (control) at 15°C for 1 hr. The excessive DNPH was washed off with ethanol: ethyl acetate (1:1 vol/vol) solution and final precipitates were dissolved in 8 M urea solution. The absorbance was measured at 360 nm, and the carbonyl content was obtained as nmol per mg of protein using a molar extinction coefficient of 22,000. Concentration of protein was measured with a BioRad assay kit using bovine serum albumin as standard.

Statistical analysis

Results were analyzed by Duncan’s multiple range test.

RESULTS

Smoked salmon

Of the five samples tested, MA and CP were detected in all the samples while HHE was limited to only two samples that showed higher MA contents (Table 1).

<table>
<thead>
<tr>
<th>Sample No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA (µmol/kg)</td>
<td>13.2</td>
<td>9.3</td>
<td>25.1</td>
<td>42.1</td>
<td>42.1</td>
</tr>
<tr>
<td>HHE (µmol/kg)</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>27.2</td>
<td>27.2</td>
</tr>
<tr>
<td>CP (nmol/mg protein)</td>
<td>27.8</td>
<td>27.1</td>
<td>16.3</td>
<td>25.1</td>
<td>24.9</td>
</tr>
</tbody>
</table>

nd = Not detected.

Commercial wood vinegar (WV)

The MA of WV treated samples showed a decrease on day 3 followed by an increase towards day 7. On day 3, MA of the control was not detected due to a technical error. The highest MA was found for the 1.0% sample followed by the 0.5% and the 0.1% samples. On day 7, the control had the highest MA level followed by the 1.0%, the 0.1 % and the 0.5%. However, the significance of these changes were not significant (Table 2).

Contrary to that of MA, HHE levels showed an increase on day 3. The 1.0% and 0.5% samples had higher levels while the 0.1% had a lower level compared with that of the control. On day 7, a reduction was observed for the 1.0% and the 0.5% samples while an increase was observed for the 0.1% and the control. However, the significance of these
**Table 2** Variation of MA, HHE and CP in commercial wood vinegar treated fish meat during 7 day storage period at 4°C

<table>
<thead>
<tr>
<th>Index</th>
<th>Days</th>
<th>Contol</th>
<th>0.1%</th>
<th>0.5%</th>
<th>1.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA</td>
<td>0</td>
<td>9.5±5.4</td>
<td>9.5±5.4</td>
<td>9.5±5.4</td>
<td>9.5±5.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>nd</td>
<td>4.1</td>
<td>4.2±1.2</td>
<td>4.6±0.6</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>14.3±1.9</td>
<td>9.8±3.8</td>
<td>8.3±5.4</td>
<td>13.8±3.7</td>
</tr>
<tr>
<td>HHE</td>
<td>0</td>
<td>0</td>
<td>0.079±0.061</td>
<td>0.05±0.027</td>
<td>0.132±0.072</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.133</td>
<td>0.128±0.099</td>
<td>0.10±0.092</td>
<td>0.186</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.88±1.42</td>
<td>1.88±1.42</td>
<td>1.88±1.42</td>
<td>1.88±1.42</td>
</tr>
<tr>
<td>CP</td>
<td>0</td>
<td>2.20±0.51</td>
<td>3.56±0.74</td>
<td>4.02±0.28</td>
<td>1.40±1.63</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.04±0.74</td>
<td>1.93±0.17</td>
<td>2.60±0.28</td>
<td>1.93±0.17</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.09±1.23</td>
<td>1.09±1.23</td>
<td>1.09±1.23</td>
<td>1.09±1.23</td>
</tr>
</tbody>
</table>

a-d Means ± standard deviation within same raw with no common superscript differ significantly (P < 0.05)

x-z Means ± standard deviation within same column with no common superscript differ significantly (P < 0.05).

The 0.5%, 0.1% and control samples showed an increase of CP on day 3 while the 1.0% sample showed a decrease. The CP decreased in all samples on day 7. Moreover, the highest CP level was found for the 0.5% sample and was significantly higher than that of the 1.0%, and than those of the 1.0% and the control, on day 3 and day 7, respectively.

**Cherry wood vinegar**

An increase of MA was observed for all samples throughout. On day 3, the control had the highest MA level followed by the 0.5% and the 0.1% samples. The 1.0% sample had a significantly lower level than that of the rest. On day 7, MA content of the 0.5% sample increased rapidly and was significantly higher than that of the rest (Table 3).

The treated samples had a concentration dependent increase of HHE throughout the storage period, whereas the control had a decrease on day 7. Furthermore, HHE content of the 1.0% sample was significantly higher than that of the rest on day 3 while the 1.0% and the 0.5% samples were found significantly higher than that of the 0.1% and the control on day 7.

The CP contents of the treated samples showed an increase on day 3 followed by a decrease. Whereas, an initial decrease was observed for the control followed by an increase. On day 7, the control had a significantly higher level than that of the 0.1% and the 1.0% samples.

**DISCUSSION**

In lipid peroxidation, MA and CP are used as common indices irrespective of the source, while HHE is specific for n-3 fatty acids. In smoked salmon, the limitation of HHE to samples that had high MA, indicates either resistance of n-3 fatty acids or usage of them as secondary substrate by pro-oxidants under these conditions. Furthermore, a great variation of MA may be due to uneven distribution of smoke constituents.

In WV treated samples, the control had the highest MA on day 7, demonstrating suppression of peroxidation by WV. In contrast, high HHE contents in treated samples indicate an induction of n-3 fatty acid breakdown by WV. Furthermore, high CP for treated samples also indicates WV as a pro-oxidant. However, the lowest CP content was found for the 1.0% sample. This may be due to the changes, such as pH, that hinder CP formation.

The significantly high levels of HHE and MA on day 7 for higher concentrations of CWV imply it as a...
pro-oxidant. The variation of CP also points out pro-oxidative activity of CWV on day 3. The reduction of CP on day 7 may be due to the conformational changes of formed CP so that they cannot be detected by DNP derivative methods.21)

As a whole, the uneven distribution of smoke constituents and extremely high levels of MA and HHE in smoked salmon indicates that LSF is superior to that of smoking. Furthermore, CWV can be considered as pro-oxidative. However, the role of WV on lipid peroxidation is not clear, as different indices have given different implications. In both experiments, the indices did not demonstrate a clear relationship with the wood vinegar type or the concentration used. This indicates a complexity of the peroxidative mechanism due to its high dependency on numerous factors.

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REFERENCES