Decolorization and Degradation Products of Melanoidin by Active Oxygens

II. Decolorization and Degradation Products of Melanoidin on Ozonolysis

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Nondialyzable melanoids prepared from a glucose-glycine system were investigated as to their decolorization and degradation products on ozone treatment. Melanoids were decolorized to degree of 84% and 97% after ozonolysis at −1°C for 10 min and 90 min, respectively, and the mean molecular weight of melanoids decreased from 7,000 to 3,000 after ozonolysis for 40 min.

IR measurement showed that the absorption at 1,290 cm⁻¹ disappeared and that at 1,720 cm⁻¹ newly appeared on ozonolysis, and the absorption at 1,620 cm⁻¹ disappeared on acid hydrolysis after ozonolysis.

Furthermore, the major degradation products in the ether-soluble fractions obtained from ozone-treated melanoids were identified as butanedioic acid, glycolic acid, 2-hydroxybutanoic acid and so on.

In the aqueous fraction, one of the major products was glycine, which was produced to the level of 1.05% on ozonolysis which increased to 5.75% per melanoidin on acid hydrolysis after ozonolysis.

From these findings and the IR results, it is postulated that glycine was considerably incorporated into melanoidin molecules as the amide form.

Introduction

Melanoids are nitrogenous brown polymers, which are formed on the interaction between amino acids and carbohydrates. It is considered that melanoids extensively exist in foods as well as sea-food products, and environmental systems containing amino and carbonyl compounds (Mauron, 1981), and that they are very important from nutritional, physiological and environmental aspects.

In order to characterize the chemical structure of melanoids, chemical (Kato and Tsuchida, 1981; Hayase and Kato, 1981; Hayase et al., 1982) and biological degradations (Watanabe et al., 1982) have been examined. Kato and Tsuchida (1981) identified oxamic acid, pyrazines on pyrolysis and permanganate oxidation of ammonia-glucose system melanoids.

Hayase and Kato (1981) and Hayase et al. (1982) also reported volatiles formed on pyrolysis of glucose-glycine system and glucos-butylamine system melanoids. A recent study using 13C CPMAS NMR (Benzing-Purdie and Ripmeester, 1983) and 15N NMR (Benzing-Purdie et al., 1983) showed that the behavior of carbon and nitrogen in melanoids was similar to in humic substances in solis.

In the previous paper (Hayase et al., 1984), we reported the examination of the decolorization and degradation products of melanoids with the use of hydrogen peroxide in order to characterize their...
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chemical structure, considered the environmental chemical aspects of waste treatment, and discussed their partial chemical structure.

In the present work, we investigated the decolorization and degradation products of melanoidins prepared from a glucose-glycine system with the use of ozone, which is expected to be a good germicidal agent for foods, air conditioning and bleaching agents.

Materials and Methods

(1) Preparation of nondialyzable melanoidins.

Nondialyzable melanoidins were prepared on heating an aqueous solution (500 ml) containing 1 mol D-glucose, 1 mol glycine and 0.2 mol sodium bicarbonate by the methods described in the previous paper (Hayase et al., 1984).

(2) Ozonolysis of nondialyzable melanoidins.

Ozone was generated by passing extra dry oxygen through a NIHON OZONE MODEL 0-3-2 laboratory ozonator as shown in Figure 1. Ten milliliters of 2% nondialyzable melanoidins (pH 4.4) was maintained at -1°C in a constant temperature bath. A 3% ozone-oxygen stream was fed into the aqueous solutions of melanoidins at a rate of 0.5 liter/min for 90 min. The resulting reaction mixtures were purged with nitrogen to remove any residual ozone. Each ozonated solution was reduced with sodium borohydride overnight, and then acidified with hydrochloric acid and evaporated under reduced pressure. The residue was coevaporated seven times with methanol to remove borate as trimethyl borate and then lyophilized.

(3) Fractionation of degradation products of nondialyzable melanoidins on ozonolysis.

One part of degradation products obtained from melanoidins was subjected to gel permeation chromatography (GPC), elementary analysis, IR measurement and thin layer chromatography (TLC, Avicell, Funacoshi Co.).

The ninhydrin positive compounds were analyzed by TLC and determined with an amino acid analyzer (HITACHI 835 Type Amino Acid Analyzer). The TLC plates were developed with butanol/acetic acid /H2O (4/1/2). The other parts were fractionated into ether-soluble and aqueous fractions with ether. The ether-soluble fractions were fractionated into neutral, basic and acidic fractions according to the method of Hayase and Kato (1981). Acids in the acidic fraction were methylated with diazomethane. One hundred and eighty micrograms of heptadecane was added to the acidic fraction as an internal standard. Then, low molecular substances in the acidic fraction were identified and determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). On the other hand, the aqueous fraction was further fractionated into fractions (F-1: above MW 10,000, F-2: MW 5,000 to 10,000, F-3: MW 1,000 to 5,000, F-4: MW 500 to 1,000 and F-5: below MW 500) with a membrane filter, and the mean molecular weight of each fraction was determined by GP-HPLC and the elemental compositions were also analyzed.

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![Diagram](image-url)  
**Fig. 1.** Apparatus for ozonolysis of melanoidins.
(3) Acid hydrolysis of melanoids and ozone-treated melanoids.

Melanoids (5 mg) and ozone-treated melanoids (5 mg) were dissolved in 6 N HCl (5 ml) with and without 5% (v/v) thioglycolic acid in order to prevent the formation of humic substances, and then hydrolyzed at 119°C for 48 hr under a nitrogen stream. After cooling, the solutions were evaporated under reduced pressure. Each residue was dissolved in 0.02 N HCl (10 ml) and analyzed with an amino acid analyzer.

(5) Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

The experimental conditions were similar to those described in the previous paper (Hayase et al., 1984).

(6) Gel permeation-high performance liquid chromatography (GP-HPLC).

The mean molecular weight and the distribution of melanoids and ozone-treated melanoids were estimated by GP-HPLC. High performance liquid chromatography (HPLC) was performed with a HITACHI 638-30 Liquid Chromatograph as follows; column: a stainless steel column (50 cm×4 mm) pre-packed with Shodex OH PAK B-803 connected to a precolumn of Shodex OH PAK B-800P. detector; a Shodex RI SE-31 and a spectrophotometer (HITACHI Model 100-50 spectrophotometer). The column was eluted with 0.1 N NaCl containing 1mM sodium azide at a flow rate of 1.0 ml/min and the operating pressure was 6 kg/cm². Pullulan (Shodex, Mw 12,000 and Mw 5,300) and stachyose (Kanto Chemicals Co.) were used as standards for the estimation of the mean molecular weight.

(7) DEAE-Cellulose column chromatography.

Melanoids (10 mg) and ozone-treated melanoids (30 mg) were applied to a DEAE-Cellulose column (2.0×20.5 cm) equilibrated with 0.05 M Tris-HCl buffer (pH 6.8). The column was washed with the same buffer and developed with a linear gradient of 0 M to 0.5 M NaCl. The flow rate was 36 ml per hr and fractions of 5 ml were collected.

(8) Infrared (IR) analysis.

Well-dried KBr (IR grade, Nakarai Chem. Ltd.) and samples (melanoids, ozone-treated and acid hydrolyzed melanoids) were mixed at 20:1 (w/w), and then pressed into a clear micro-pellet. Absorbance spectra were measured with a JASCO A202 infrared spectrometer.

Results

1. Changes of melanoids on ozonolysis.

(1) Decolorization

![Fig. 2. Changes in decolorization degree and pH during ozonolysis of melanoids. 200 mg of melanoids was made up to 10 ml and the mixtures were treated with 3% ozone/oxygen at a rate of 0.5 liter/min at -1°C. Decolorization degree was determined by measurement of the absorbance at 470 nm and 275 nm.]

Figure 2 shows the time course of the decolorizations and the pH of the reaction mixture on ozonolysis. The degree of decolorization of melanoids determined from the changes in absorbance at 470 nm increased markedly in the initial stage of ozonolysis and reached 84% and 95% after ozonolysis for 10 min and 40 min, respectively, and then reached 97% after 90 min. In addition, the decrease in the absorbance at 275 nm of melanoids was lower in comparison with that at 470 nm, and reached 45% and 72% after ozonolysis for 10 min and 40 min, respectively, and then reached 74%
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after 90 min. The pH of the reaction mixture decreased gradually with the ozonolysis time from the initial pH 4.4 to pH 3.4 after 90 min. The melanoidins before and after ozonolysis showed no absorption maximum in the visible and ultraviolet regions (Fig. 3 and Fig. 4).

Figure 5 and 6 show the changes in the mean molecular weight. Ozone-treated melanoidins showed the tendency of a decrease in the overall mean molecular weight, and showed a weak shoulder on GP-HPLC chromatograms. The GP-HPLC profile obtained with the RI detector, as shown in Figure 5, coincided with that of GP-HPLC with the UV detector (280 nm). The mean molecular weight of melanoidins decreased with ozonolysis time from 7,000 to 3,000.

Fig. 3. Visible light absorption spectra of ozone-treated melanoidins.

Fig. 4. Ultraviolet absorption spectra of ozone-treated melanoidins.

Fig. 5. Gel permeation-HPLC of melanoidins before and after ozonolysis. A: control, B: ozonolysis for 90 min, C: MW 1,000 to 5,000 fraction after membrane filtration of B, D: acid hydrolysis of C with 6 N HCl for 48 hr in the presence of thioglycolic and (4%, v/v) and under N₂ stream.

Table 1. Changes in molecular weight, bromine consumption and weight of reaction products during ozonolysis of melanoidins

<table>
<thead>
<tr>
<th>Ozonolysis time(min)</th>
<th>Average MW(b)</th>
<th>Br₂ consumed/ Melanoidins(c)</th>
<th>Weight Gain, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7,000</td>
<td>89.3</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>5,000</td>
<td>5.2</td>
<td>3.8</td>
</tr>
<tr>
<td>25</td>
<td>3,700</td>
<td>4.8</td>
<td>9.0</td>
</tr>
<tr>
<td>40</td>
<td>3,000</td>
<td>2.7</td>
<td>10.4</td>
</tr>
<tr>
<td>60</td>
<td>3,000</td>
<td>1.8</td>
<td>12.2</td>
</tr>
<tr>
<td>90</td>
<td>3,000</td>
<td>1.8</td>
<td>11.8</td>
</tr>
</tbody>
</table>

a) Determined by the iodometric titration method.
b) Measured by HPLC with a GPC column.
c) Calculated as mole of Br₂ consumed per mole of each ozone-treated melanoidin.
Fig. 6. Determination of average molecular weight of melanoids before and after ozonolysis by gel permeation-HPLC. M: melanoids, S-10, S-25 and S-40 to S-90 mean ozonolysis products of M at -1°C for 10 min, 25 min and 40 to 90 min, respectively. After 40 min, and thereafter the molecular weight did not change (Table 1). On UV and RI detection after GP-HPLC, melanoids and ozone-treated melanoids were found to show similar patterns.

The ozone-treated melanoids were fractionated into F-1 (above Mw 10,000), F-2 (Mw 5,000 to 10,000), F-3 (Mw 1,000 to 5,000), F-4 (Mw 500 to 1,000) and F-5 (below Mw 500) on a membrane filter. Table 2 shows the mean molecular weight, yields, C/N values and empirical formula calculated from elementary analysis of each fraction (F-1 to F-5) obtained from the degradation products of melanoids on ozonolysis. F-3 was the most abundant fraction and occupied about 51% of the total fractions. F-4 and F-5 comprised 27% and 13%, respectively, and fractions above Mw 5,000 (F-1 and F-2) occupied merely 10% of the total.

(3) Changes in bromine consumption and weight.

As shown in Table 1, bromine consumption of melanoids decreased predominantly in the initial stage of ozonolysis and changes after 60 min were not seen. On the other hand, the weight of melanoids gradually increased with the ozonolysis time and the increase after 90 min was 11.8%.

(4) Changes in DEAE-cellulose chromatograms.

Table 2. Average molecular weight distributions and elemental compositions of melanoids after ozonolysis

<table>
<thead>
<tr>
<th>Ozonolysis time (min)</th>
<th>Fractiona)</th>
<th>Mw distribution (weight %)</th>
<th>N/C</th>
<th>Empirical formula as N=1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>whole</td>
<td>0.13</td>
<td>(C9.3H12.8N1.7O5.17)n, n=30.6</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>whole</td>
<td>0.14</td>
<td>(C8.8H12.8N1.7O6.17)n, n=11.9</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>F-1</td>
<td>6.0</td>
<td>0.14</td>
<td>(C8.8H12.8N1.7O6.17)n, n=86.1</td>
</tr>
<tr>
<td>90</td>
<td>F-1</td>
<td>4.0</td>
<td>0.13</td>
<td>(C9.2H12.8N1.7O5.17)n, n=37.5</td>
</tr>
<tr>
<td>90</td>
<td>F-3</td>
<td>50.5</td>
<td>0.14</td>
<td>(C8.8H12.8N1.7O6.17)n, n=4.6</td>
</tr>
<tr>
<td>90</td>
<td>F-4</td>
<td>27.0</td>
<td>0.14</td>
<td>(C8.8H12.8N1.7O6.17)n, n=3.6</td>
</tr>
<tr>
<td>90</td>
<td>F-5</td>
<td>12.5</td>
<td>0.06</td>
<td>—</td>
</tr>
</tbody>
</table>

a) Fractionated into F-1 to F-5 with a membrane filter.
F-1, above Mw 10,000; F-2, 5,000 to 10,000; F-3, Mw 1,000 to 5,000; F-4, Mw 500 to 10,000; F-5, below Mw 500.
b) Calculated from elementary analysis results.
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The chromatogram showed maximum absorption at 288 nm on UV scanning. In the melanoids before ozonolysis, the absorption at 470 nm of the second peak was greater than that of the third peak, whereas, after ozonolysis, the second peak was smaller than the third peak.

However, UV absorption of the second peak in the case of ozonized melanoids increased more than that of the third peak.

Fig. 8. Infrared (IR) spectra of melanoids (A), ozone-treated melanoids (B) and acid hydrolyzed melanoids of ozonolysis (C). A, before ozonolysis; B, treated with ozone at -1°C for 93 min; C, permethylated with methylsulfonyl carbonon after acid hydrolysis of the MW 1,000 to 5,000 fraction obtained on membrane filtration of B.

Table 3. Infrared absorption bands of melanoids and ozone-treated melanoids

<table>
<thead>
<tr>
<th>Absorption bands (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,353(s)</td>
<td>OH stretching</td>
</tr>
<tr>
<td>2,850(s)</td>
<td>CH symmetric stretching methylene</td>
</tr>
<tr>
<td>1,720(s)</td>
<td>C=O stretching</td>
</tr>
<tr>
<td>1,620(m)</td>
<td>NH deformation, amide-like</td>
</tr>
<tr>
<td>1,370(m)</td>
<td>CH symmetrical band</td>
</tr>
<tr>
<td>1,290(w)</td>
<td>C=C double bond</td>
</tr>
<tr>
<td>1,210(m)</td>
<td>C-N stretching</td>
</tr>
<tr>
<td>1,040(s)</td>
<td>-C=O- stretching</td>
</tr>
</tbody>
</table>

a) Newly appeared band after permethylation of acid hydrolyses.
b) Newly appeared band after ozonolysis.
c) Disappeared band after permethylation of acid hydrolyses.
d) Disappeared band after ozonolysis.

Figure 8 shows IR spectra of melanoids before and after ozonolysis. The absorption at 1,723 cm⁻¹, which is assigned to carbonyl and carboxyl groups, newly appeared and the absorption at 1,290 cm⁻¹, which is assigned to carbon-carbon double bonds, completely disappeared after ozone treatment. Furthermore, the absorption at 1,620 cm⁻¹, which is regarded as the amide type, disappeared on acid hydrolysis with 6 N HCl of melanoids after ozonolysis (Table 3).

2. Fractionation and identification of degradation products of melanoids on ozonolysis.

(1) Ether soluble fraction.

Melanoids after ozonolysis were fractionated into acidic, neutral and basic fractions with ether. The acidic fraction was methylated with diazomethane, and identified and determined by GC-MS and GC. In the ether-soluble fraction obtained from ozone-treated melanoids, only acidic compounds were found, i.e. neutral and basic compounds were not detected.

Fig. 9. Gas chromatogram of the acidic fraction obtained from the ether extract of melanoids after ozonolysis at -1°C for 90 min. The acidic fraction was methylated with diazomethane. n-Heptadecane was used as an internal standard (IS). BHT denotes butylated hydroxytoluene originating from ether.

Figure 9 shows the gas chromatogram of the acidic fraction methylated with diazomethane, and the major compounds identified and their relative amounts are shown in Table 4.

The major degradation compounds in the acidic fraction were identified as methoxyacetic acid, 2-
Table 4. The main compounds of the acidic fraction obtained from the ether extract of the ozonization products of melanoidins

<table>
<thead>
<tr>
<th>Compound</th>
<th>Peak No.</th>
<th>Ratio to IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Methoxyacetic acid</td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td>2-Hydroxybutanoic acid</td>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td>Glycolic acid</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>Methylpropanedioic acid</td>
<td>6</td>
<td>0.3</td>
</tr>
<tr>
<td>Propanedioic acid</td>
<td>7</td>
<td>0.9</td>
</tr>
<tr>
<td>3-Hydroxy-3-methylbutanoic acid</td>
<td>8</td>
<td>0.4</td>
</tr>
<tr>
<td>2-Hydroxy-2-methylpropanoic acid</td>
<td>9</td>
<td>0.4</td>
</tr>
<tr>
<td>Butanedioic acid</td>
<td>10</td>
<td>2.1</td>
</tr>
</tbody>
</table>

a) The acidic fraction was methylated with diazomethane.
b) Ozonized at −10°C for 90 min.
c) IS denotes Internal Standard (n-heptadecane).

hydroxybutanoic acid, glycolic acid, propanedioic acid and butanedioic acid. Among the minor products, compounds having methyl side chains, such as 3-hydroxy-3-methylbutanoic acid and 2-hydroxy-2-methylpropanoic acid were also identified.

(2) Aqueous fraction.
The aqueous fraction was concentrated under reduced pressure, analyzed by TLC and determined with an amino acid analyzer. In this fraction, the main ninhydrin positive component obtained from ozonated melanoidins was identified as glycine.

Table 5. Amounts of glycine produced from ozone-treated melanoidins on acid hydrolysis

<table>
<thead>
<tr>
<th>Ozonolysis time (min)</th>
<th>Hydrolysis, % before</th>
<th>Hydrolysis, % after</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.03</td>
<td>1.95</td>
</tr>
<tr>
<td>25</td>
<td>1.00</td>
<td>5.21</td>
</tr>
<tr>
<td>40</td>
<td>1.07</td>
<td>5.92</td>
</tr>
<tr>
<td>60</td>
<td>1.05</td>
<td>5.72</td>
</tr>
<tr>
<td>+ TGb)</td>
<td>1.05</td>
<td>5.73</td>
</tr>
<tr>
<td>90</td>
<td>1.05</td>
<td>5.75</td>
</tr>
<tr>
<td>+ TG</td>
<td>1.05</td>
<td>5.72</td>
</tr>
</tbody>
</table>

a) Hydrolysed with 6N HCl for 48 hr under a N₂ stream.
b) Hydrolysed in the presence of thioglycolic acid (4%, v/v).

Table 5 shows the yields of glycine formed from the ozonated melanoidins and melanoidins hydrolyzed before and after ozone treatment with 6 N HCl. Melanoidins as a control were dissolved in 0.02 N HCl and glycine was determined. The glycine content of the control melanoidins was 0.03% and glycine was formed at the level of 1.95% per melanoidin on acid hydrolysis alone. On the other hand, glycine was formed at the level of 1.05% after ozonolysis for 90 min and glycine was then liberated at the level of 5.75% per melanoidin on acid hydrolysis after ozonolysis. Accordingly, by treatment with ozone prior to acid hydrolysis, about three-fold more glycine was released than on acid hydrolysis alone.

Discussion

When melanoidins were treated with ozone, there were a remarkable increase in the decolorization degree and a remarkable decrease in carbon-carbon double bonds in the initial stage, and decreases in molecular weight and weight gain were observed.

Ozone, compared with hydrogen peroxide as a nucleophile, attacks organic compounds electrophilically (Bailey et al., 1959). The ozone molecule is known to exist as a resonance hybrid of four canonical forms, and ozone reacts with H₂O in aqueous solution to form hydrogen peroxide in part (Creegee, 1975). However, the nucleophilic activity of hydrogen peroxide mainly occurs on the alkaline side (Pearson and Edgington, 1962). As the present work was performed on the acidic side in the pH range of 3–4, the nucleophilic attack of hydrogen peroxide secondarily formed on the reaction of ozone with H₂O was considered to be suppressed much more than on the alkaline side. Consequently, the electrophilic reaction on ozone is proposed to mainly occur in the present experimental system. Especially, ozone as an amphoteric ion makes an electrophilic attack in an electron rich \( \pi \) system such as a carbon-carbon double bond, and causes cleavage of that bond. Melanoidins have carbon-carbon double bonds in their molecules (Table 1.
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and Fig. 8).

It is supposed that the decolorization and the decrease in the molecular weight of melanoidins are due to cleavage of carbon–carbon double bonds in their molecules by ozonolysis.

Homma et al. (1982) reported that when nondialyzable melanoidins were applied on a preparative flat bed of Sephadex G-100, 14 bands were clearly electrofocused in the pH range of 2.7–3.3. The authors also reported the electrofocusing of melanoidins and ozone-treated melanoidins and ozone-treated melanoidins and the pI 3.00 and pI 3.02 bands, which are main ones of melanoidins, were revealed to highly contribute in the decolorization and degradation on ozonolysis (Kim et al., 1985).

In the ether-soluble fraction, the major degradation products in the acidic fraction were identified as glycolic acid, butanedioic acid, 2-hydroxybutanoic acid and so on.

Hayase et al. (1984) identified butanedioic acid formed from glucose–glycine system melanoidins on hydrogen peroxide treatment, but glycolic acid and 2-hydroxybutanoic acid were not identified from hydrogen peroxide-treated melanoidins. It is considered that butanedioic acid is partly formed from the melanoidins peroxide secondarily produced during ozonolysis.

From these results, the chemical structures adjacent to the carbon–carbon double bond originating from glucose existing in melanoidins are suggested to be as follows; \( \text{CH}_2(\text{OH})-(\text{H or OH})=\text{C}–\text{R} \) (1) and \( \text{CH}_2\text{CH}_2–\text{CH}(\text{OH})\text{C}(\text{H or OH})=\text{C}–\text{R} \) (2). In addition, 3-hydroxy-3-methylbutanoic acid and 2-hydroxy-2-methylpropanoic acid were identified as compounds containing branched chains. Accordingly, \( (\text{CH}_3)_2\text{C}(\text{OH})\text{C}(\text{H or OH})=\text{R} \) (3) and \( (\text{CH}_3)_2\text{C}(\text{OH})\text{CH}_2\text{C}(\text{H or OH})=\text{R} \) (4) are suggested as minor structures. The minor partial structure compounds having methyl side chains, such as \( \text{R–CO–CH(CH}_3)–\text{CO–R}' \), was also estimated from hydrogen peroxide-treated melanoidins (Hayase et al., 1984).

As further knowledge concerning the chemical structure of melanoidins, a recent study (Benzing-Purdie et al., 1983), with \(^{15}\text{N} \) NMR of N-15 labeled melanoidins, revealed that the nitrogen in the melanoidins was mainly of the secondary amide type, and that the sterically hindered secondary amide bond are very resistant to acid hydrolysis. The existence of the carbon bonded to the electronegative atoms, such as oxygen or nitrogen, in the melanoidins was confirmed by \(^{13}\text{C} \) CP–MAS NMR (Benzing–Purdie and Ripmeester, 1983).

The present study also indicated the presence of a little glycine (1.95%) produced on acid hydrolysis of melanoidin before ozonolysis, whereas three-fold glycine was released on acid hydrolysis of ozonated melanoidins. It is suggested that the carbon-nitrogen bonds are labile to the acid hydrolysis after the oxidation reaction with ozone. Moreover, the absorption at 1620 cm\(^{-1} \), which is regarded as the amide type, completely disappeared on acid hydrolysis of melanoidins after ozonolysis (Fig. 8). From these results, glycine is considered to be produced by the cleavage of amide bonding by acid hydrolysis after ozonolysis. It is also postulated that glycine is considerably incorporated into melanoidin molecules as the amide form.

References


Seon-Bong Kim and Yeung-Ho Park


活性酸素種에 의한 Melanoid인의 脱色 및 分解生成物

II. Ozone에 의한 Melanoid인의 脱色 및 分解生成物

金 善 奉・朴 憲 浩
釜山水産大学 食品工学科
(1985년 11월 15일 수리)

glucose-glycine 층으로부터 調製한 非濃縮性 melanoid인에 ozone 을 作用시켜서, melanoid인의 脱色 및 分解生成물을 検討하였다.  그 결과, ozone에 의한 melanoid인의 脱色率은 10분 反應으로 84%, 30분 反應으로 97%에 각각 達하였고, 平均分子量 7,000의 未處理 melanoid인이 40분의 ozone 處理에 의하여 分子量 5000까지 低分子化하였다. 또한, 赤外線吸收スペクト럼의 結果, ozone 處理에 의하여 1,290cm⁻¹의 吸收가 消失한 편에 1720 cm⁻¹의 吸収가 新しく 出現하였고, 酸加水分解에 의하여 1,620 cm⁻¹의 吸収가 完全히 消失되었다.

한편, melanoid인에 ozone 處理함으로써 얻어진 에테르 可溶性部分의 主要分解生成物은 butanediolic acid, glycolic acid 및 2-hydroxybutanoic acid 등이었고, 水溶性部分의 主要分解生成物은 glycine 으로서, ozone 處理함으로서 1.05%, 酸加水分解함으로서 1.95% 生成하였다. 따라서, ozone處理後 酸加水分解함으로서 melanoid인 류 5.75% 生成되었다.

이 結果와 赤外線吸收 스펙트럼의 結果을 합쳐 비교하여 보면, 一部의 glycine이 melanoid인 중에 amide 狀態로 結合되어 있음은 明確하였다. ozone 處理에 따라 amide 結合이 새로운 形成되었다고 생각된다.