The Rate and Mechanism of Long Chain 2, 3-Dialkyl Acroleins Formation in Meat by Heat Treatment

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The long chain (C₂₄–C₃₆) 2,3-dialkyl acroleins in heated meat were produced by aldol condensation and dehydration reaction of fatty aldehyde via hydrolysis of plasmalogen. This work was undertaken to determine the rate of aldol condensation of fatty aldehyde under various temperatures. Two-dimentional thin-layer chromatography for determining the plasmalogen-aldehyde, free fatty aldehyde and 2,3-dialkyl acrolein in heated meat was also described. The reaction-rate data indicated that it appeared to be reasonable to assume the following complex reaction including consecutive and competitive reaction mechanism as an over-all picture of the reaction. However at higher temperatures the reaction was found to be more complex than at lower temperature.

\[
\begin{align*}
&\text{Plasmalogen-aldehyde} \xrightarrow{k_1} \text{Free fatty aldehyde} \\
&\text{Free fatty aldehyde} \xrightarrow{k_2} \text{2,3-Dialkyl acrolein} \\
&\text{Free fatty aldehyde} \xrightarrow{k_3} \text{Unidentified compounds}
\end{align*}
\]

The apparent energy and entropy of activation for long chain 2,3-dialkyl acrolein formation were calculated to be 10.1 kcal/mole and -49.8 cal/deg-mole, respectively. The large negative entropy of activation could be explained that the number of degrees of freedom were decreased by this reaction. From a view point of energy of activation, aldol condensation in heated meat was relatively easy to occur.

The amino groups acts as a basic catalyst in the aldol condensation of the carbonyl compounds, followed by dehydration of the condensation products to yield the alk-2-enals. It has previously been reported that the condensation of aldehydes to alk-2-enals was catalyzed by phospholipids which contained amino group. Montgomery and Day suggested that aldol condensation reaction led to the formation of flavor compounds and polymeric pigments in foods. Burton et al. also showed that the development of alk-2-enals in many foods were probably the first step in chromophores development.

We have shown recently that the long chain (C₂₄–C₃₆) 2,3-dialkyl acroleins (abbreviated as DAA) were formed in chicken, pork and beef by heat treatment, and DAA were produced by aldol condensation reaction of fatty aldehyde (abbreviated as F-Al) via hydrolysis of plasmalogen. This result suggested that aldol condensation reaction occurred in meat or meat products during storage or heat processing.

The purpose of this paper is to obtain information of the kinetic behavior of these reactions in meat. For this purpose, we describe in this paper the determination of the rate of aldol condensation of F-Al under various temperatures and the elucidation of the rate laws for disappearance of aldehyde in plasmalogen (abbreviated as Pl-Al), the production and disappearance of F-Al, and the production of DAA in meat.

Two-dimentional thin-layer chromatographic procedure for determining the Pl-Al,
F-Al and DAA contents in heated meat is also described.

EXPERIMENTAL

1. Solvents and reagents. All the solvents used were of reagent grade quality. By dissolving HCl gas, an approximately 3% HCl solution in methanol was prepared, in which commercial pure 2,4-dinitrophenyl hydrazine was dissolved to concentration of 2% (DNP-reagent).

2. Materials. Sample of muscle was taken from M. longissimus dorsi section of pig after about 1 day of slaughter.

3. Preparation of heated meat. Twenty grams of M. longissimus dorsi of pig was placed in the test tube (hard glass, thick-wall, 1.4 × 20 cm). After the atmosphere was replaced by nitrogen gas, the tube was flame-sealed to prevent evaporation and oxidation during reaction at elevated temperature. The sealed tube was placed in a temperature-controlled oil bath at the desired temperatures (±1°C). After heating, the tube was placed in dry ice-ethanol and stored in this solution until required.

4. Extraction of lipids. The procedure of Folch et al. was used for extraction of lipids. The sample was homogenized in 300 ml of chloroform-methanol (2: 1 v/v) in a high speed blender (Nippon seiki). After standing for 15 min with stirring, the suspension was filtered through filter paper on a Büchner funnel. The filtrate was shaken for 2 ~ 3 min with 0.2 volume of 0.9% NaCl solution. After standing it for about 1 hr at 0°C, the separated lower layer was evaporated to dryness in vacuo, and dissolved in 10 ml of chloroform.

5. Preparation of thin-layer plates. Glass plates (20 × 20 cm) were coated with Silica Gel H (E. Merck) to a thickness of about 300 μ using a thin-layer applicator (Toyo Kagaku). The plates, after air-drying at room temperature, were activated in an air-oven at 120°C for 2 hr.

6. Two-dimensional thin-layer chromatography. One hundred and fifty μl of the solution of extracted lipid in chloroform was spotted, at the lower left hand corner, 2 cm from each side of a thin-layer plate by 50 μl volumetric micro syringe (Jintan Co., Japan, for gas chromatography) (Fig. 1). The plate was developed to height of 12 ~ 15 cm in TLC chamber containing n-hexan-ethyl ether (90: 10 v/v) as a developing solvent. The plate was removed from the TLC chamber and dried with a stream of dry nitrogen. A strip of 4 cm wide at the left side of the plate was then sprayed in excess with a DNP-reagent (Fig. 1) for quantitative formation of DNP-hydrazone of Pl-Al, F-Al and DAA, and then dried for 10 min under a stream of dry nitrogen in TLC chamber which contained calcium chloride. In our earlier work, formation of DNP-hydrazones of these compounds on TLC plate was shown to be quantitative.

As reference standards, DNP-hydrazones of palmitaldehyde and 2-tetradecyl-2-octadecenal were spotted at the upper left hand corner of the plate, and then developed in second dimension with developed components in the first dimension from a left edge with the same n-hexan-ethyl ether (90: 10 v/v) as a developing solvent. When the solvent rose to a height of about 15 cm, the plate was removed from the chamber and dried in atmosphere. Thus, the position of yellow spots of DNP-hydrazones of Pl-Al and F-Al, and orange colour spot of DNP-hydrazone of DAA were detected.

7. Quantification of DNP-hydrazones. Each outlined spot of each DNP-hydrazones was scraped directly into the glass stoppered centrifuge tube of 10 ml volume, to which 10 ml or 5 ml of chloroform was added, and then centrifuged at 1,000 rpm for 5 min after shaking. The absorbances of the upper chloroform solution of DNP-hydrazone of Pl-Al and F-Al
Kinetics of 2,3-Dialkyl Acrolein Formation in Meat

were determined at 358 m
and that of DAA was determined at 382 m
in 1 cm cells against a blank prepared in the absence of DNP-hydrazine, with Hitachi-Perkin Elmer 139 spectrophotometer. The concentrations of Pl-Al and F-Al, and DAA were calculated from a molar absorptivity of 21,600, and 29,000 described in our previous reports, re
pectively.

RESULTS AND DISCUSSION

1. Reproducibility of the method

The results of the analysis of Pl-Al, F-Al and DAA contents in five samples heated under the same condition (at 100°C for 4 hr) were presented in Table I. The values of mean, variance, standard deviation and coefficient of variation of five measurements were presented in Table I. The statistical data showed satisfactory reproducibilities among five measurements.

TABLE I. REPRODUCIBILITY OF THE METHOD

The values of Pl-Al and F-Al, and DAA were absorbancy at 358, and 382 m
of DNP-hydrazine derivatives, respectively. The samples were heated at 100°C for 4 hr. Experimental details were given in the text.

<table>
<thead>
<tr>
<th>Run No.</th>
<th>Pl-Al</th>
<th>F-Al</th>
<th>DAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.302</td>
<td>0.090</td>
<td>0.155</td>
</tr>
<tr>
<td>2</td>
<td>0.324</td>
<td>0.083</td>
<td>0.169</td>
</tr>
<tr>
<td>3</td>
<td>0.315</td>
<td>0.091</td>
<td>0.150</td>
</tr>
<tr>
<td>4</td>
<td>0.298</td>
<td>0.096</td>
<td>0.149</td>
</tr>
<tr>
<td>5</td>
<td>0.334</td>
<td>0.085</td>
<td>0.178</td>
</tr>
<tr>
<td>Mean</td>
<td>0.315</td>
<td>0.089</td>
<td>0.160</td>
</tr>
<tr>
<td>Variance</td>
<td>0.00018</td>
<td>0.000021</td>
<td>0.00013</td>
</tr>
<tr>
<td>SD</td>
<td>0.0134</td>
<td>0.0046</td>
<td>0.0114</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>0.043</td>
<td>0.052</td>
<td>0.071</td>
</tr>
</tbody>
</table>

2. Relationships between Pl-Al, F-Al and DAA concentrations and heating times at various temperatures

At heating temperatures of 100°C, 110°C, 120°C and 130°C, the relations of the concentrations calculated as molar fractions of Pl-Al, F-Al, DAA and UIC to the heating times were shown in Fig. 2, 3, 4 and 5, respectively. UIC should be the sum of all possible reaction products and calculated as follows;

$$[\text{UIC}] = A_0 - [\text{Pl-Al}] - [\text{F-Al}] - [\text{DAA}]$$

Where $A_0$ represents initial concentration of Pl-Al.

From experimental finding (Figs. 2, 3, 4,
and 5), following assumptions may be made to define the kinetic behaviour of the reactions.

1) Rate of disappearance of Pl-Al. Figure 6 gives curves showing the logarithm of the concentrations of Pl-Al as a function of the time at 100°C, 110°C, 120°C and 130°C (first-order plots of disappearance of Pl-Al). The linear relationships of every heat treatments were obtained. This result meant that Pl-Al disappeared according to a first order reactions, that is, F-Al formation was regarded as simple first-order term with respect to Pl-Al.

2) Change of F-Al concentration. From F-Al concentration-heating time curves of Figs. 2, 3, 4 and 5, maximum concentrations of F-Al were observed in every cases. The result obtained meant that F-Al was considered to be an intermediate in a consecutive reaction.

3) Change of DAA concentration. From the curves in Figs. 2, 3, 4 and 5, DAA concentrations increased in the time course, but the slope of curves became sluggish and finally equilibrated. Discussion of this point would be described later. However, in the case of 130°C treatment (Fig. 5), DAA concentration slightly decreased at the final stage. This observation suggested that DAA → UIC reaction occurred in meat by heat treatment at higher temperature but the rate of its reaction was very slow. Burton et al. showed that α,β-unsaturated straight chain aldehydes greatly accelerated by initial development of chromophores in foods, but this effect was not shown by the unsaturated aldehydes with alkyl substituent on the α-carbon atom (2,3-dialkyl acroleins). On the basis of these data, DAA seemed to be stable in meat.

The aldol condensation reaction in the presence of basic catalyst in general was
first order term with respect to aldehyde, and that in the presence of amine or phosphatidyl ethanolamine was also first order term with respect to aldehyde. On the basis of these data, it seemed to be reasonable to assume that DAA formation followed first order term with respect to F-Al.

4) Change of UIC concentration. The reaction of UIC formation from DAA takes place, but the rate of this reaction was small enough to be neglected as previously described. This illustrated that the reaction of F-Al with meat components (such as amino group) produced UIC. On the other hand, the rate curves of formation reaction of UIC shown in Figs. 2, 3, 4 and 5 were qualitatively the same as the rate curves of DAA formation reaction. We assumed that UIC formation followed first order term with respect to F-Al.

3. Mechanism of the reaction and kinetic analysis

In the establishing an approximate kinetic scheme fitting the data given in Figs. 2, 3, 4 and 5, certain approximates were unavoidable because of the complexity of the reaction and the unavailability of information on the structure of UIC as described in above section.

It seems to be reasonable to assume from the discussion of the above section that the following complex reactions including consecutive and competitive reaction mechanisms appears as an over-all picture of the reaction.

\[
\begin{align*}
\text{Pl-Al} & \xrightarrow{k_1} \text{F-Al} \\
\text{F-Al} & \xrightarrow{k_2} \text{DAA} \\
\text{F-Al} & \xrightarrow{k_3} \text{UIC}
\end{align*}
\]

Where \( k_1, k_2 \) and \( k_3 \) are the rate constants.

The reverse reactions are not written in above scheme, although the possibility of these reactions can not be disregarded. The rate laws which are assumed to be applied to proposed mechanism are expressed by the following differential equations:

\[
\begin{align*}
\frac{d[\text{Pl-Al}]}{dt} &= -k_1[\text{Pl-Al}] \\
\frac{d[\text{DAA}]}{dt} &= k_2[\text{F-Al}] \\
\frac{d[\text{UIC}]}{dt} &= k_3[\text{F-Al}] \\
\frac{d[\text{F-Al}]}{dt} &= k_1[\text{Pl-Al}] - (k_2 + k_3)[\text{F-Al}] \\
[\text{DAA}] + [\text{UIC}] &= A_0 - [\text{Pl-Al}] - [\text{F-Al}]
\end{align*}
\]

Integration of eq. (4) gives

\[
[\text{Pl-Al}] = A_0 \exp(-k_1t)
\]

and then eq. (7) becomes

\[
[\text{F-Al}] = \frac{A_0 k_1}{m_1} \exp(-m_2t) - \exp(-k_1t)
\]

Substituting eq. (9) and (10) into eq. (8) we get

\[
[\text{DAA}] + [\text{UIC}] = A_0 \left\{ 1 + \frac{m_5}{m_1} \exp(-k_1t) - \frac{k_1}{m_1} \exp(-m_2t) \right\}
\]

from eq. (5) and (6)

\[
[\text{DAA}]/[\text{UIC}] = k_2/k_3
\]

Substituting eq. (12) into eq. (11) we get

\[
[\text{DAA}] = A_0 \left\{ k_2 \left( \frac{k_2}{m_2} + \frac{k_3}{m_1} \right) \exp(-k_1t) - \frac{k_1 k_3}{m_1 m_2} \exp(-m_2t) \right\}
\]

and

\[
[\text{UIC}] = A_0 \left\{ \frac{k_2}{m_1} + \frac{k_3}{m_1} \exp(-k_1t) - \frac{k_1 k_3}{m_1 m_2} \exp(-m_2t) \right\}
\]

where

\[
m_1 = k_1 - (k_2 + k_3) \\
m_2 = k_2 + k_3
\]

Assuming that the reactions are not reversible, the apparent first order rate constants are calculated from the data in Figs. 2, 3, 4 and 5, and are presented in Table II. A value of \( k_1 \) is calculated from equation (9), values of \( k_2 \) and \( k_3 \) are calculated from equation (10), (13) and (14). Figure 7 is plot of data which are computed upon the illustrative
assumption that the values of the parameters are $A_0=1.0$ and rate constants described in Table II. The good agreements of the computative curve (Fig. 7) with experimental curves (Figs. 2, 3, 4 and 5) are obtained. From

Table II. Apparent First-order Rate Constants

<table>
<thead>
<tr>
<th>$t$ (°C)</th>
<th>$k_1$ (sec$^{-1}$ $\times 10^9$)</th>
<th>$k_2$ (sec$^{-1}$ $\times 10^8$)</th>
<th>$k_3$ (sec$^{-1}$ $\times 10^5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100°</td>
<td>4.16</td>
<td>11.1</td>
<td>9.72</td>
</tr>
<tr>
<td>110°</td>
<td>9.72</td>
<td>15.0</td>
<td>16.4</td>
</tr>
<tr>
<td>120°</td>
<td>22.2</td>
<td>24.4</td>
<td>42.5</td>
</tr>
<tr>
<td>130°</td>
<td>39.4</td>
<td>30.6</td>
<td>61.7</td>
</tr>
</tbody>
</table>


a) mean values of data for 1.5 and 4 hr.
b) mean values of data for 1 and 2 hr.

FIG. 7. The Computative Curve of the Changing Concentration of PI-Al, F-Al, DAA and UIC vs. Heating time.

The values of the parameters were $A_0=1.0$ and rate constants described in Table II.

a consideration of above results, it seems that experimental measurements interpreted the theoretical scope and mechanism, except that following several differences are observed between experimental curves and computative curves, especially in the case of 120°C and 130°C treatments.

a) Experimental curves show that F-Al concentration do not become zero as illustrated in computative curves, and final concentrations of F-Al, DAA and UIC approach constant values. From these reasons, following two assumptions may be made to define the kinetic behavior or reaction. First, the reverse reactions occurred. Second, degradation of functional groups such as amino groups participating the aldol condensation reaction occurred. Further aspect of mechanism will be presented in next paper.11)

b) A slight decrease of DAA concentration occurred in the case of 130°C. This observation suggested that DAA→UIC reaction actually occurred, but rate constant of this reaction is small enough to be neglected as previously described.

4. Thermodynamic quantities of the activation

The effect of temperature on the rates of reactions is revealed by Arrhenius plots of log $k_1$, $k_2$ and $k_3$ vs. the reciprocal of the absolute temperature, $T$ (Fig. 8). The straight

FIG. 8. Arrhenius Plots.

Temperature dependence of rate constants $k_1$, $k_2$ and $k_3$.

The energies of activation ($E_a$) are calculated for the slopes of the lines obtained using the formula $E_a=-2.3026R(slope)$. The frequency factor (A) and entropy of activation ($\Delta S^*$) are also calculated from the equation
log \( A = \log k - (\text{slope} / T) \) and \( A = (KT/h) \exp (\Delta S^\circ / R) \), respectively. A summary of these results are provided in Table III.

**Table III. Thermodynamic Quantities of the Activation**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>( E_a ) (kcal mole(^{-1}))</th>
<th>( A ) (sec(^{-1}))</th>
<th>( \Delta S^\circ )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pl-Al ( \xrightarrow{k_1} ) F-Al</td>
<td>22.0</td>
<td>( 3.2 \times 10^8 )</td>
<td>-20.1</td>
</tr>
<tr>
<td>F-Al ( \xrightarrow{k_2} ) DAA</td>
<td>10.1</td>
<td>( 1.0 \times 10^2 )</td>
<td>-49.8</td>
</tr>
<tr>
<td>F-Al ( \xrightarrow{k_3} ) UIC</td>
<td>20.1</td>
<td>( 5.0 \times 10^7 )</td>
<td>-23.8</td>
</tr>
</tbody>
</table>

\( E_a \) is Arrhenius activation energy; \( A \) is Arrhenius frequency factor; \( \Delta S^\circ \) is entropy (in cal/deg\( \cdot \)mole) of activation at 373 K.

It appears from Table III that the reaction has unusually large negative entropy of activation for a first-order reaction, especially for F-Al\( \xrightarrow{} \)DAA reaction (-49.8 e.u.), although value of energy of activation is relatively low. The large negative entropy of activation can be explained that the number of degrees of freedom are decreased by the reaction in meat. A decrease in the number of degrees of freedom may depend upon the reaction in meat which is heterogeneous and complex reaction system, and/or large numbers of carbon atoms of F-Al molecules, and/or change of solvation of reactants during the reaction, thereby resulting in a negative entropy of activation. From a viewpoint of energy of activation, these data indicated that the aldol condensation reaction in meat was relatively easy to occur.

**REFERENCES**