

Note

Degradation Kinetics of Branched-Chain Amino Acids in Subcritical Water

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The degradation of branched-chain amino acids (valine, leucine, and isoleucine) in subcritical water was measured from 290 °C to 330 °C. The degradation process at any temperature obeyed first-order kinetics. The energy of activation for the degradation of each amino acid could be estimated according to the Arrhenius equation. The pH value of the reaction mixture increased during the degradation process.

Key words: branched-chain amino acid; degradation; subcritical water

Water, maintaining its liquid state at a high temperature but below 374 °C, under pressurized conditions, is called subcritical water or compressed hot water. Subcritical water possesses two distinct properties compared to ambient water; one is a lower dielectric constant and the other is a higher ion product at high temperatures. The latter indicates the possibility that subcritical water can act as an acidic or basic catalyst because of the high concentrations of both hydrogen and hydroxide ions.

The degradation of bio-based waste by subcritical water as a renewable bioresource has been the focus of research to obtain the constituent sugars and amino acids.^{1–3)} Since sugars and amino acids are exposed to subcritical water during their treatment, knowledge about the degradation of sugars and amino acids is also required to profoundly understand the phenomena occurring during the treatment. Although there are some reports dealing with the degradation kinetics of sugars,^{4–7)} only a few have dealt with the degradation kinetics of such amino acids as glycine, alanine, aspartic acid, and serine.^{8–10)} However, the degradation kinetics of branched-chain amino acids (BCAAs), which are a group of amino acids having particular structural characteristic and physiological functions, have not been fully examined. We therefore investigated in this study the degradation kinetics of BCAAs, *i.e.*, valine, leucine, and isoleucine, in subcritical water to identify the conditions for their effective recovery from proteinaceous waste by a subcritical water treatment.

BCAAs (valine, leucine, and isoleucine), which were purchased from Wako Pure Chemical Industries (Osaka, Japan), were degraded in subcritical water by using a flow reactor system similar to that used in previous studies.^{6,7)} Each amino acid was dissolved in distilled water at a concentration from 5 to 15 mmol/l. The solution in a glass bottle was sonicated to degas it under

reduced pressure, and then the bottle was connected to a helium gasbag to prevent the re-dissolution of oxygen. The solution was delivered to the flow reactor at a constant flow rate to give a residence time from 10 to 180 s. The flow reactor system consisted of an HPLC pump (LC-10ATVP, Shimadzu, Kyoto, Japan) connected to an SUS 316 reaction coil (0.8 mm I.D. × 1 m), which was immersed in a salt bath (Celsius 600H thermometer inspecting bath, Thomas Kagaku Kikai, Tokyo, Japan) at a temperature from 290 °C to 330 °C, and to the SUS coil immersed in an ice bath to immediately terminate the reaction. The pressure was maintained at *ca.* 20 MPa by a high-pressure adjustable back-pressure valve (Upchurch, Oak, Harbor, WA, USA). The effluent was collected in a test tube for analysis after *ca.* 10 residence times had elapsed.

The concentration of the residual amino acid in the effluent was determined by HPLC equipped with an LC-10ATVP HPLC pump (Shimadzu) and an SPD-10AVVP UV detector (Shimadzu) at a wavelength of 220 nm. The column was a YMC-Pack ODS AQ (4.6 mm I.D. × 150 mm, YMC, Kyoto), and the eluent was distilled water adjusted to pH 2.5 with phosphoric acid (Wako) at a flow rate of 1.0 ml/min. The pH value of each sample was measured with an F-13 pH meter (Horiba, Kyoto) at 25 °C.

Figure 1 shows the degradation processes for the three amino acids at different temperatures. The amino acids degraded faster at the higher tested temperatures. The concentration of a residual amino acid, *C*, exponentially decreased with increasing residence time. Irrespective of the kind and concentration of the amino acid, each plot lay on straight line for a semi-logarithmic scale, indicating that the degradation kinetics for these amino acids could be regarded as a first-order reaction. The degradation rate for each amino acid decreased in the order of leucine > isoleucine > valine at a constant temperature, indicating that the amino acid with a short side-chain was more thermostable.

The pH values of the effluent were measured at different residence times. Figure 2 shows the relationship between the pH value and the concentration of the reacted amino acid, ΔC . The pH value increased with increasing ΔC value. This fact indicates that the formation of basic compounds would occur. Sato *et al.* have reported that ammonia and methyl or ethyl amine were formed from glycine or alanine by decarboxylation.^{8,9)} The increase in pH value observed in this study and previous reports^{8,9)} would indicate decarboxylation

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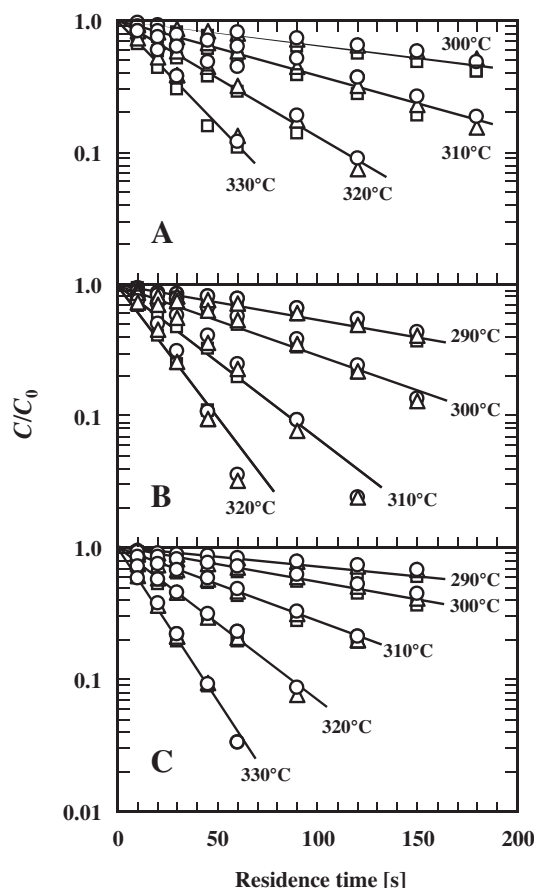


Fig. 1. Time-Course Plots for the Degradation of Branched-Chain Amino Acids in Subcritical Water.

A, valine from 300°C to 330°C; B, leucine from 290°C to 320°C; C, isoleucine from 290°C to 330°C. The initial concentrations, C_0 , of the amino acids were ($\square$) 5 mmol/L, ($\triangle$) 10 mmol/L, and ($\circ$) 15 mmol/L.

to be one of the predominant reactions during the degradation of amino acids to finally form ammonia and amines. The change in pH value only slightly depended on the kind and concentration of the amino acids used in this study.

The relationship between the rate constant for degradation and the reciprocal of the temperature could be linearly expressed on a semi-logarithmic scale (Fig. 3). The energy of activation and frequency factor for the degradation were respectively estimated to be 186 kJ/mol and $1.0 \times 10^{15} \text{ s}^{-1}$ for leucine, 207 kJ/mol and $4.2 \times 10^{16} \text{ s}^{-1}$ for isoleucine, and 229 kJ/mol and $3.8 \times 10^{18} \text{ s}^{-1}$ for valine according to the Arrhenius equation. Sato *et al.*⁸⁾ have reported the energy of activation for the degradation of alanine (154 kJ/mol) and glycine (166 kJ/mol). These facts indicate that the degradation of branched-chain amino acids was more thermosensitive than that of the short-chain amino acids. Furthermore, a plot of the energy of activation vs. the frequency factor gave a straight line (inset to Fig. 3), indicating that entropy-enthalpy compensation held for these hydrophobic amino acids (valine, leucine, isoleucine, glycine, and alanine), and that the mechanism for degradation of these amino acids was similar. However, the points for serine and aspartic acid did not lie on the plotted line. These hydrophobic amino acids would therefore be degraded in subcritical water through a

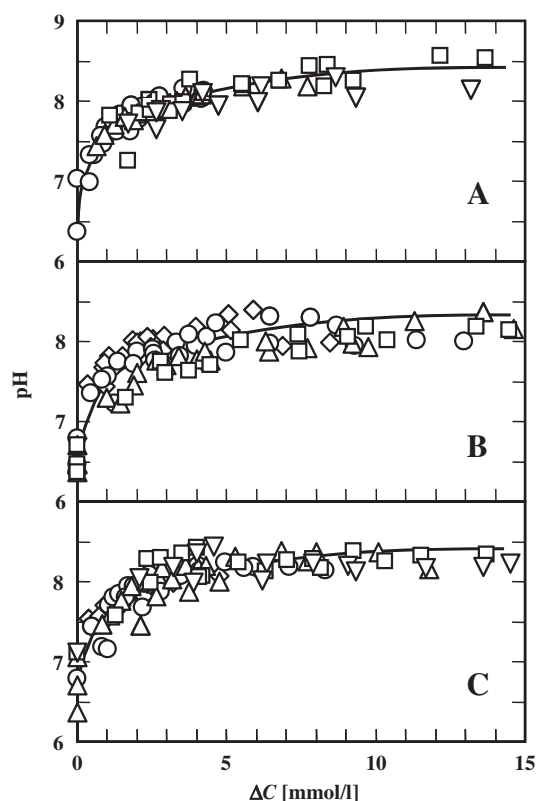


Fig. 2. Relationship between the pH Value and Concentration, ΔC , of the Reacted Amino Acids for (A) Valine, (B) Leucine, and (C) Isoleucine.

The reaction was performed at ($\diamond$) 290°C, ($\circ$) 300°C, ($\triangle$) 310°C, ($\square$) 320°C, and (∇) 330°C.

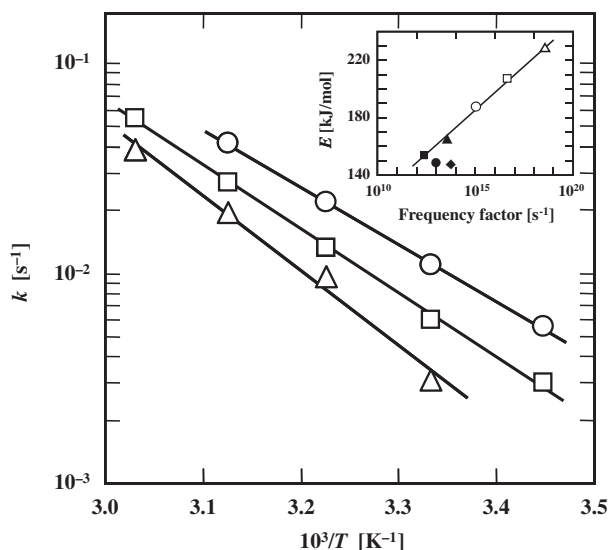


Fig. 3. Temperature Dependence of the Rate Constants, k , for the Degradation of ($\triangle$) Valine, ($\square$) Leucine, and ($\circ$) Isoleucine.

Inset: Relationship between the energy of activation, E , and frequency factor for degradation of the amino acids. Filled symbols, ($\blacktriangle$) glycine, ($\blacksquare$) alanine, ($\bullet$) serine, and ($\blacklozenge$) aspartic acid, are cited from the literature.¹⁰⁾

mechanism different from that for the amino acids with a hydrophilic side chain.

In conclusion, the degradation of branched-chain amino acids was observed from 290°C to 330°C in subcritical water. The degradation rate at any temperature obeyed first-order kinetics. The pH value increased

due to decarboxylation of the amino acids during the treatment. However, the reason for the difference in degradation mechanism among the amino acids still remains unclear. Further study is required for understanding the phenomena occurring during the subcritical water treatment of various amino acids.

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