

亜臨界含水エタノール中でのN-アセチル-D-グルコサミンの消失速度

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Kinetics of the Disappearance of *N*-Acetyl-D-glucosamine in Subcritical Aqueous Ethanol

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N-Acetyl-D-glucosamine (GlcNAc) was treated in subcritical fluids with ethanol contents of 0%–80% (w/w) using a tubular reactor at 190°C. The disappearance of GlcNAc in all the aqueous ethanol fluids obeyed first-order kinetics, and the rate constant was roughly proportional to the water molarity of the aqueous ethanol; this indicates that water plays an important role in the disappearance of GlcNAc. The pH of the reaction mixture decreased as the reaction proceeded and then plateaued because of the formation of a buffer system by glucosamine and the acetic acid liberated from GlcNAc. The ultraviolet absorption spectra of the reactor effluents suggested the formation of both carboxylic acids and furfurals. The formation of furfurals and color development were suppressed at higher ethanol contents.

Keywords: *N*-acetyl-D-glucosamine, subcritical aqueous ethanol, kinetics

1. Introduction

Water that remains a liquid from 100 to 374°C under pressurized conditions is called subcritical water. Subcritical water has two distinct features: A high ion product and low relative dielectric constant. Due to the high ion product and temperature, subcritical water not only catalyzes hydrolysis [1–5] and thermal degradation [5,6] but also condensation [7,8] and isomerization [9]. The relative dielectric constant of subcritical water is almost equivalent to those of methanol and ethanol at room temperature. Subcritical water is used to extract useful substances from bioresources, such as rosemary plants [10], coriander seeds [11], defatted rice bran [12,13], and rice straw [14].

N-Acetyl-D-glucosamine (GlcNAc) is a constituent of chitin, which comprises the outer skin of crustaceans and insects. GlcNAc and glucosamine prepared from crab shells are used as components of health food supplements. Subcritical water treatment of the crab skin has been reported to produce chitin oligomers [15]. To elucidate the phenomena occurring during the treatment, we investigated the degradation kinetics in subcritical water [16].

Under subcritical conditions, a mixture of ethanol and

water was more effective than water alone for the extraction of phenolic and antioxidative substances from defatted rice bran and rice straw [17,18]. The isomerization of common saccharides to rare ones was promoted by the addition of ethanol to water [19,20]. Although the treatment of chitin in subcritical aqueous ethanol was not examined, similar treatment of GlcNAc and glucosamine should provide basic knowledge on the phenomena that occur during the treatment.

In this context, the disappearance of GlcNAc in subcritical aqueous ethanol was examined and kinetically analyzed.

2. Materials and Methods

2.1 Materials

GlcNAc (>98%) was supplied by Koyo Chemicals (Osaka, Japan). Ethanol was purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2 Treatment of GlcNAc in Subcritical Aqueous Ethanol

GlcNAc was dissolved in 0% (water), 20%, 40%, 60%, or 80% (w/w) aqueous ethanol at a concentration of 0.5% (w/w). The feed solution was sonically degassed before treatment under subcritical conditions and then connected to a nitrogen gas bag to prevent redissolution of

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atmospheric oxygen. The feed solution was delivered into a coiled stainless steel (SUS 316) tubular reactor (0.8 mm I.D.×2.0 m length) immersed in a bath filled with an SRX 310 silicone oil (Toray-Dow-Corning, Tokyo, Japan) with a residence time of 20–240 s using an L-7100 HPLC pump (Hitachi, Tokyo, Japan). The residence time was calculated based on the inner diameter and length of the stainless steel tube and the density of the water-ethanol mixture under subcritical conditions according to our previous study [16]. The treatment was conducted at 190°C. The reactor effluent was directly introduced into a stainless steel tube (0.8 mm I.D.×1.0 m length) immersed in an ice-water bath to terminate the reaction and then collected in a sampling vessel. The pressure inside the tube was regulated at 10 MPa using a back-pressure regulator (high-pressure adjustable BPR P-880; Upchurch, Washington, USA). The effluent aliquot (usually 0.70 mL) in the sampling vessel was evaporated under reduced pressure. The residue was dissolved in the same volume of distilled water to prepare the sample for HPLC analysis and pH measurement.

2.3 Determination of *N*-Acetyl-D-glucosamine

The concentration of GlcNAc in the effluent was determined using an HPLC consisting of an L-7100 pump (Hitachi, Tokyo, Japan), a COSMOSIL Hilic column (3.0 mm I.D.×150 mm, Nacalai Tesque, Kyoto, Japan), and an L-3350 refractometer (Hitachi, Tokyo, Japan). A mixture of 10 mmol/L ammonium acetate and acetonitrile (10/90, v/v) was used as the eluent at a flow rate of 0.4 mL/min. The column temperature was maintained at 30°C in an L-7300 column oven (Hitachi, Tokyo, Japan).

2.4 pH and Absorption Spectra Measurements

The pH of the effluent was measured at room temperature using an F-14 pH meter (Horiba, Kyoto). The effluents were diluted 200 and 10 times using distilled water for measuring the ultraviolet (200–350 nm) and visible light (350–650 nm) absorption spectra, respectively, using a Multiskan GO Microplate spectrophotometer (Thermo Scientific, Vantaa, Finland).

3. Results and Discussion

3.1 Disappearance of *N*-Acetyl-D-glucosamine in Subcritical Aqueous Ethanol

First, 0.5% (w/w) GlcNAc samples dissolved in 0% to

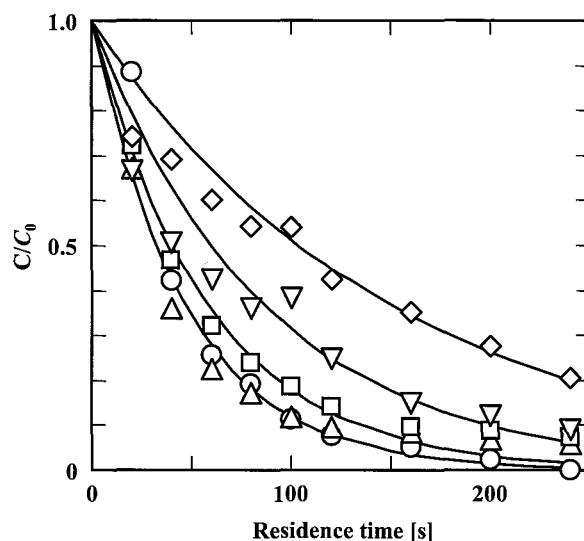


Fig. 1 Disappearance of *N*-acetyl-D-glucosamine (GlcNAc) in subcritical aqueous ethanol at 190°C. The feed concentration of GlcNAc, C_0 , was fixed at 0.5% (w/w) in all aqueous ethanol solutions, and C represents the GlcNAc concentration in the reactor effluent. The ethanol content was (○) 0% (water), (△) 20%, (□) 40%, (▽) 60%, and (◇) 80% (w/w).

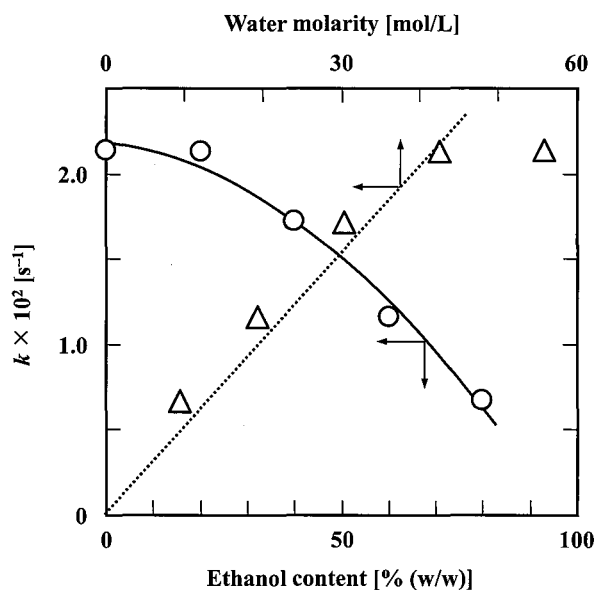


Fig. 2 Dependence of the rate constant, k , for the disappearance of *N*-acetyl-D-glucosamine at 190°C on the (○) ethanol content and (△) water molarity.

80% (w/w) aqueous ethanol solutions were heated at 190°C, and the disappearance of GlcNAc in the reactor effluent was observed (Fig. 1). The rate of the disappearance of GlcNAc decelerated at higher ethanol contents. Because the disappearance of GlcNAc in subcritical water can be expressed using first-order kinetics [16], the following kinetics were adopted to describe the

changes in the concentration of GlcNAc in aqueous ethanol:

$$C/C_0 = \exp(-k\tau) \quad (1)$$

where k is the rate constant of the disappearance, C denotes the concentration of the remaining substrate (GlcNAc) in the reactor effluent, C_0 is the substrate concentration of the feed, and τ is the mean residence time of the substrate solution in the reactor. Plots of the fraction of the remaining substrate, *i.e.*, C/C_0 , versus t on the semi-logarithmic scale were linear, and k was estimated from the slope of the line at each ethanol content. The solid curves in Fig. 1 were drawn by substituting the estimated rate constants in Eq. (1). Figure 2 shows the dependence of the rate constant on the ethanol content. The rate constant was lower at higher ethanol contents. The water molarity was also lower at higher ethanol contents. The water molarity at room temperature was roughly evaluated assuming additivity of the volumes of water and ethanol. The rate constants were also plotted against water molarities (the upper abscissa) in Fig. 2. It was roughly proportional to water molarity, which indicates that water plays a major role in the disappearance of GlcNAc under subcritical conditions, while ethanol acts merely as a diluent. A reason for the deviation from linearity at the highest water molarity, which corresponds to water alone, remains unclear.

3.2 pH Changes during Treatment

The pH values of the reactor effluents are shown in Fig. 3. The pH of the reactor effluent decreased as

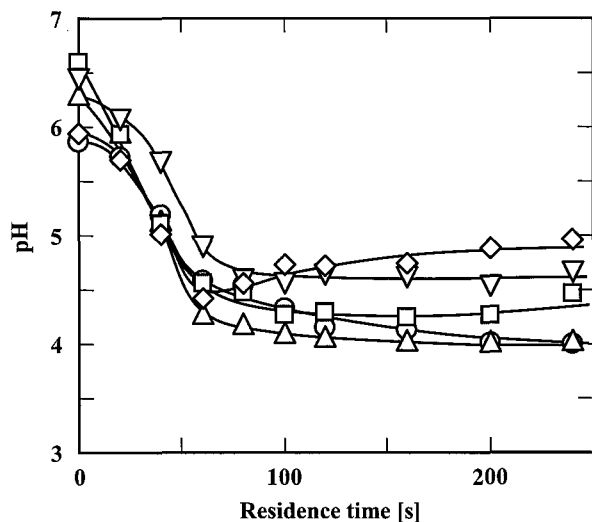


Fig. 3 Changes in the pH of the reactor effluents for the treatment of *N*-acetyl-D-glucosamine at 190°C in subcritical fluids with different ethanol contents. The symbols are the same as those in Fig. 1.

GlcNAc degraded. The rate of the change in pH with residence time decelerated at higher ethanol contents; this indicates that acidic compounds formed at lower ethanol contents and the concentration of hydrogen ion increased during treatment [21–23]. However, the decrease in pH leveled off at a residence time of 60 s or longer. As shown in section 3.3, the ultraviolet absorption spectra of the reactor effluent suggest the formation of

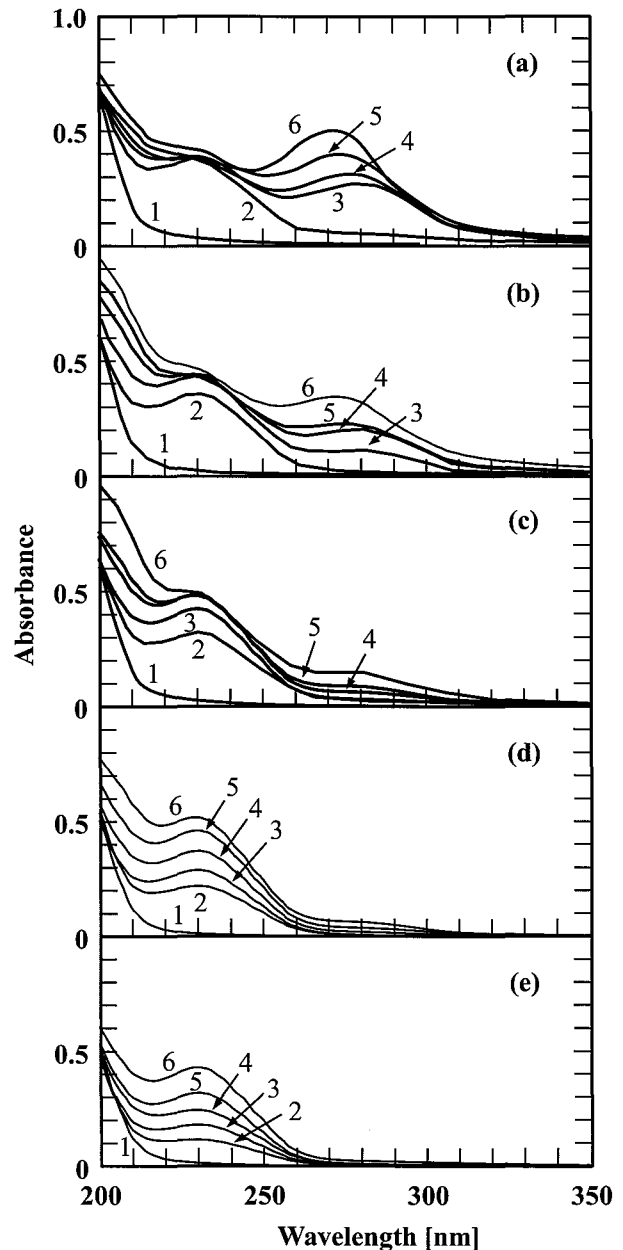


Fig. 4 Ultraviolet absorption spectra of the reactor effluents after the treatment of *N*-acetyl-D-glucosamine at 190°C in subcritical fluids with different ethanol contents. The spectra were measured after 200 times dilution of the reactor effluents. The ethanol contents were (a) 0% (water), (b) 20%, (c) 40%, (d) 60%, and (e) 80% (w/w). The number symbols, 1–6, indicate residence times of 0 (feed solution), 40, 80, 120, 160, and 240 s, respectively.

carboxylic acids. Liberation of the acetyl group from GlcNAc produces acetic acid and glucosamine, which generate a buffer system. This is likely the reason that the pH reaches a minimum value during the degradation of GlcNAc.

3.3 Absorption Spectra

Figure 4 shows the ultraviolet absorption spectra from 200 to 350 nm for the reactor effluents with different residence times, which were diluted 200 times with water. In subcritical water (Fig. 4(a)), the absorbance near 230 nm increased rapidly at short residence times and maintained almost a constant value at longer residence times. The absorbance near 230 nm suggests the formation of carboxylic acids, and the increase in the absorbance observed near 270 nm suggests the formation of furfu-

rals [24]. The increase in the absorbance near 230 nm was also observed in subcritical aqueous ethanol (Figs. 4(b)–(e)), although the rate of the increase was slower at higher ethanol contents. The increase in the absorbance near 270 nm was suppressed with increasing ethanol content. No increase in the absorbance was observed in 80% (w/w) ethanol.

Figures 5(a) and (b) show the changes in the absorbances at 230 and 269 nm, respectively, with residence time. The rate of the increase of the intensity of the absorbance at 230 nm was rapid at short residence times and slower with lower ethanol content, i.e., higher water molarity. This indicates that water plays an important role in the degradation of GlcNAc. The absorbance reached a constant value of *ca.* 0.4 and then plateaued. The absorbance at 269 nm also increased faster at lower ethanol contents. A lag between the increase in the absorbance peaks at 230 and 269 nm, respectively, was observed; this is likely because GlcNAc degrades to acetic acid and glucosamine, which is then further degraded to furfurals. The consecutive generation of furfurals results in the time lag in the increase of the absorbance at 269 nm.

The visible light spectra of the reactor effluents, which were diluted 10 times with water, were also measured. The spectra show a monotonic decrease in intensity with increasing wavelength. Figure 6 shows the spectra of the reactor effluents with a residence time of 240 s. The

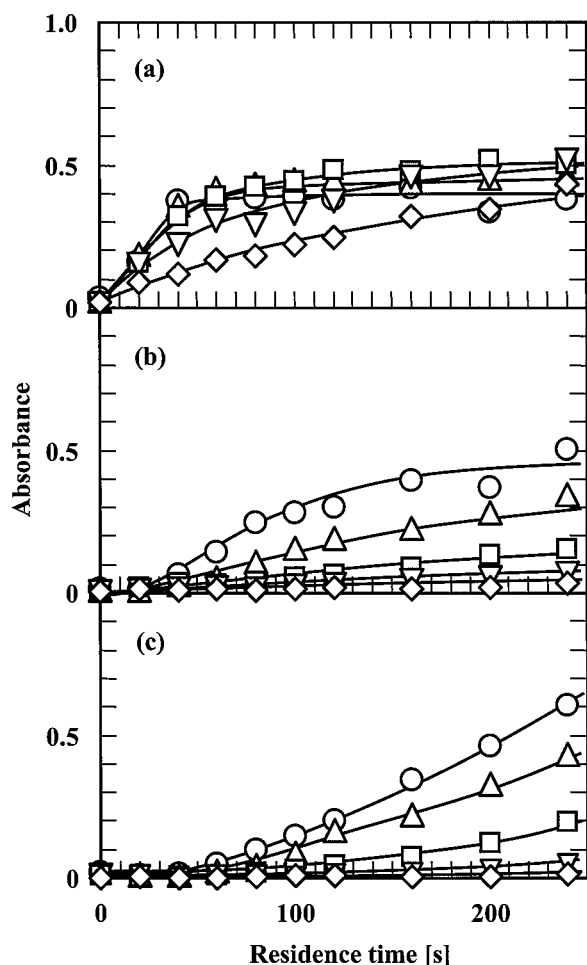


Fig. 5 Changes in the absorbance at (a) 230, (b) 269, and (c) 400 nm of the reactor effluents from the treatment of *N*-acetyl-*D*-glucosamine in subcritical fluids with different ethanol contents at 190°C. The ultraviolet and visible light absorbances of the reactor effluent were measured after 200 and 10 times dilution with water, respectively. The symbols are the same as those in Fig. 1.

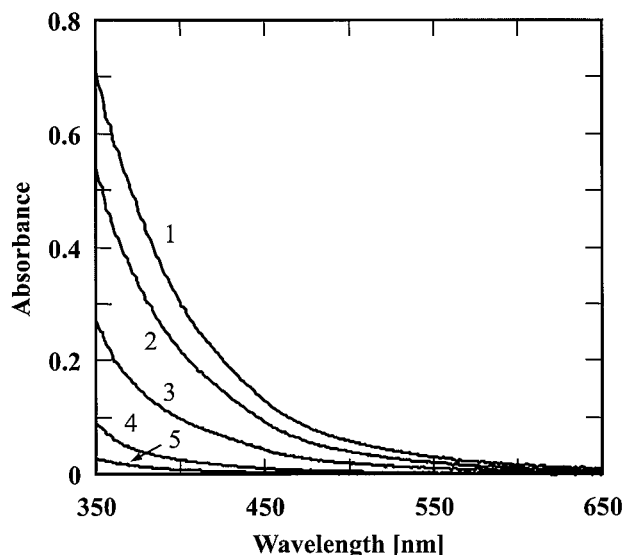


Fig. 6 Visible light absorption spectra of the reactor effluents from treatments with a residence time of 240 s at 190°C in (1) water and (2) 20%, (3) 40%, (4) 60% and (5) 80% (w/w) ethanol. The spectra were measured after 10 times dilution with water.

absorbance at any wavelength was higher for the reactor effluent in subcritical fluid with lower ethanol contents. Figure 5(c) shows the changes in the absorbance at 400 nm, which was selected as a representative wavelength for assessing the coloration of the reactor effluent in subcritical fluids with different ethanol contents. The coloration also lagged in its development, and no significant color development was observed in 80% (w/w) ethanol. Glucosamine, which was liberated from GlcNAc, undergoes the Maillard reaction to generate a yellow or brown color [25]. Therefore, the reason for the lag in color development is that it is generated in the second step or subsequent steps in the reaction. The greater color development in the fluid with lower ethanol content indicates that water also plays an important role in the coloration of the GlcNAc solution.

4. Acknowledgments

N-Acetyl-D-glucosamine was supplied by Koyo Chemicals, Osaka, Japan.

References

- 1) H. Yoshida, M. Terashima, Y. Takahashi; Production of organic acids and amino acids from fish meat by subcritical water hydrolysis. *Biotechnol. Progr.*, **15**, 1090–1094 (1999).
- 2) J. W. King, R. L. Holliday, G. R. List; Hydrolysis of soybean oil in a subcritical water flow reactor. *Green Chem.*, **1**, 261–264 (1999).
- 3) T. Oomori, S. Haghighat Khajavi, Y. Kimura, S. Adachi; Hydrolysis of disaccharides containing glucose residue in subcritical water. *Biochem. Eng. J.*, **18**, 143–147 (2004).
- 4) S. Haghighat Khajavi, S. Ota, Y. Kimura, S. Adachi; Kinetics of maltooligosaccharide hydrolysis in subcritical water. *J. Agric. Food Chem.*, **54**, 3663–3667 (2006).
- 5) G. Brunner; Near critical and supercritical water. Part I. Hydrolytic and hydrothermal processes. *J. Supercrit. Fluid.*, **47**, 373–381 (2009).
- 6) S. Haghighat Khajavi, Y. Kimura, T. Oomori, R. Matsuno, S. Adachi; Degradation kinetics of monosaccharides in subcritical water. *J. Food Eng.*, **68**, 309–313 (2005).
- 7) M. Sasaki, M. Furukawa, K. Minami, T. Adschiri, K. Arai; Degradation kinetics of monosaccharides in subcritical water. *Ind. Eng. Chem. Res.*, **41**, 6642–6649 (2002).
- 8) T. Asano, A. Maeda, Y. Kimura, T. Takahashi, A. Nakamura, H. Maeda, S. Adachi; Condensation reaction between angiotensin II and dicarboxylic acid in water at high temperature without any catalytic agent additive. *Biotechnol. Progr.*, **21**, 1169–1174 (2005).
- 9) C. Usuki, Y. Kimura, S. Adachi; Isomerization of hexoses in subcritical water. *Food Sci. Technol. Res.*, **13**, 205–209 (2007).
- 10) E. Ibañez, A. Kubátová, F. J. Señoráns, S. Cavero, G. Reglero, S. B. Hawthorne; Subcritical water extraction of antioxidant compounds from rosemary plants. *J. Agric. Food Chem.*, **51**, 375–382 (2003).
- 11) M. H. Eikani, F. Golmohammad, S. Rowshanzamir; Subcritical water extraction of essential oils from coriander seeds (*Coriandrum sativum* L.). *J. Food Eng.*, **80**, 735–740 (2007).
- 12) J. Wiboonsirikul, Y. Kimura, M. Kadota, H. Morita, T. Tsuno, S. Adachi; Properties of extracts from defatted rice bran by its subcritical water treatment. *J. Agric. Food Chem.*, **55**, 8759–8765 (2007).
- 13) T.-Y. Chiou, T. L. Neoh, T. Kobayashi, S. Adachi; Antioxidative ability of defatted rice bran extract obtained by subcritical water extract in bulk oil and aqueous dispersion systems. *Jpn. J. Food Eng.*, **12**, 147–154 (2011).
- 14) B. Tangkhavanich, Y. Oishi, T. Kobayashi, S. Adachi; Properties of the rice stem extracts obtained by subcritical water/ethanol treatment. *Food Sci. Technol. Res.*, **19**, 547–552 (2013).
- 15) M. Osada, C. Miura, Y. S. Nakagawa, M. Kaihara, M. Nikaido, K. Totani; Effect of sub- and supercritical water treatments on the physicochemical properties of crab shell chitin and its enzymatic degradation. *Carbohydr. Poly.*, **134**, 718–725 (2015).
- 16) R. Wang, T. Kobayashi, S. Adachi; Degradation kinetics of *N*-acetyl-D-glucosamine and D-glucosamine in subcritical water. *Food Sci. Technol. Res.*, **17**, 273–278 (2011).
- 17) T.-Y. Chiou, T. L. Neoh, T. Kobayashi, S. Adachi; Properties of extract obtained from defatted rice bran by extraction with aqueous ethanol under subcritical conditions. *Food Sci. Technol. Res.*, **18**, 37–45 (2012).
- 18) B. Tangkhavanich, T. Kobayashi, S. Adachi; Properties of rice stem extracts obtained using subcritical fluids. *Biosci. Biotechnol. Biochem.*, **77**, 2112–2116 (2013).
- 19) D. M. Gao, T. Kobayashi, S. Adachi; Production of rare sugars from common sugars in subcritical aqueous ethanol. *Food Chem.*, **175**, 465–470 (2015).
- 20) D.-M. Gao, T. Kobayashi, S. Adachi; Production of keto-disaccharides from aldo-disaccharides in subcritical aqueous ethanol. *Biosci. Biotechnol. Biochem.*, **80**, 998–1005 (2016).
- 21) F. S. Asghari, H. Yoshida; Kinetics of the decomposition of fructose catalyzed by hydrochloric acid in subcritical water:

- formation of 5-hydroxymethylfurfural, levulinic, and formic acids. *Ind. Eng. Chem. Res.*, **46**, 7703–7710 (2007).
- 22) B.M. Kabyemela, T. Adschiri, R.M. Malaluan, K. Arai; Glucose and fructose decomposition in subcritical and supercritical water: detailed reaction pathway, mechanisms, and kinetics. *Ind. Eng. Chem. Res.*, **38**, 2888–2895 (1999).
- 23) F. Jin, Z. Zhou, H. Enomoto, T. Moriya, H. Higashijima; Conversion mechanism of cellulosic biomass to lactic acid in subcritical water and acid–base catalytic effect of subcritical water. *Chem. Lett.*, **33**, 126–127 (2004).
- 24) R. Boopathy, H. Bokang, L. Daniels; Biotransformation of furfural and 5-hydroxymethyl furfural by enteric bacteria. *J. Ind. Microbiol.*, **11**, 147–150 (1993).
- 25) M. Oyaizu; “Studies on products of browning reaction–antioxidative activities of products of browning reaction prepared from glucosamine” (in Japanese). *Eiyogaku Zasshi*, **44**, 307–315 (1986).

亜臨界含水エタノール中での N-アセチル-D-グルコサミンの消失速度

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常圧での沸点である 100°C から臨界温度の 374°C の温度域で加圧することにより液体状態を保った水を亜臨界水という。常温常圧の水に比べて亜臨界水には、イオン積が大きく、比誘電率が低いという 2 つの特徴がある。前者の特徴から、亜臨界水は水素イオンおよび水酸化物イオンの濃度が高く、酸またはアルカリ触媒として作用し、加水分解 [1-5] や熱分解 [5,6] だけでなく、縮合 [7,8] や異性化 [9] を触媒する。また、後者の性質から、亜臨界水は疎水性物質を溶解するので、各種の生物資源からの有用物質を抽出できる [10-14]。亜臨界水を抽出などの食品加工に応用するには、それらの過程で生起する諸現象に関する基礎的な知見が必要である。そのような観点から、農水産未利用資源の有効利用に関する応用的な研究に加えて、モデル系ではあるが、亜臨界水中での糖や脂質の（加水）分解や異性化などに関する速度論的な検討を行っている。

また、亜臨界状態に保った含水アルコールは、水だけのときには異なる抽出効率や反応特性を示す [17-20]。そこで本論文では、甲殻類の殻などに含まれる N-アセチル-D-グルコサミン (GlcNAc) の亜臨界含水エタノール中での反応について検討した。処理温度は 190°C で、エタノール濃度は 0~80% (w/w) とした。GlcNAc の消失過程は 1 次反応速度式で整理でき、速度定数は含水エタノール中の水の容量モル濃度に比例した。これは GlcNAc の消失に水が重要な働きを示唆する。GlcNAc の消失過程では反応液の pH が低下し、その後一定の値となった。これは GlcNAc の加水分解により生じた酢酸とグルコサミンが緩衝液系を形成することに起因すると考えられる。また、反応液の紫外吸収スペクトルはカルボン酸とフルフラールの生成を示唆した。さらに、エタノールの含有率が高くなると、フルフラールの生成や反応液の着色が抑えられた。

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