Volatile Compounds Generated from Thermal Degradation of *N*-Acetylglucosamine

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N-Acetylglucosamine was pyrolyzed at 200 °C for 30 min. Thermal degradation products were isolated and analyzed by GC and GC/MS. The volatile compounds that were identified included pyrazines, pyridines, pyrroles, and furans. The compound 3-acetamido-5-acetylfuran was found to be the major degradation product, with 2-acetylfuran, 3-acetamidofuran, pyrazine, pyridine, ethylpyrazine, methylpyrazine, 2-ethyl-6-methylpyrazine, 2,3-dimethylpyrazine, and acetamide in decreasing order. The compound 3-acetamido-5-acetylfuran was isolated by preparative column chromatography, and its NMR spectrum was obtained to confirm its structure.

Keywords: *N-Acetylglucosamine; glucosamine; chitin; chitosan; acetamido; acetylfuran*

INTRODUCTION

N-Acetylglucosamine is the monomer of chitin, while glucosamine is the monomer form of chitosan. Next to cellulose, chitin is the second most abundant biopolymer in nature. Chitin exists prominently in lower animals and plants, especially in marine invertebrates, insects, fungi, and yeast. Chitin represents 14−27% and 13−15% of the dry weight of shrimp- and crab-processing wastes, respectively (Dreher, 1987). Chitin is usually conjugated with protein in nature, which is called mycoprotein. Chitin is the major skeletal and support matrix for crustaceans and fungi and serves as a structural defensive material. Chitin can be obtained from crustacean shell, such as crab- or shrimp-processing waste. Protein separation and calcium carbonate separation are the two main steps during the chitin preparation process (Knorr, 1984). Chitosan is a deacetylated product of chitin. There is an increasing interest in the applications of chitin and chitosan because of their functional properties and availability (Knorr, 1984).

Chitin can be used as a tobacco extender and cigarette filter. One study compared volatiles generation between tobacco leaves and chitin when both materials were pyrolyzed at 900 °C (Schlotzhauer et al., 1976). Other researchers identified acetamide and a series of methyl-substituted pyrazines when chitin was heated to over 300 °C (Koell et al., 1979; Knorr, 1985). Glucosamine possesses the structure of an α-amino carbonyl, which is one of the important precursors of pyrazines. One paper studied the formation of pyrazines from the thermal treatment of some aminohydroxy compounds, which included glucosamine. When glucosamine was heated to 200 °C for 4 h and the volatiles were purged and trapped, 2-methylpyrazine and 2,5-dimethylpyrazine were found to be the major degradation products with pyrazine, 2,3-dimethylpyrazine, trimethylpyrazine, 2-ethyl-5-methylpyrazine, and pyridine (Wang and Odell, 1973). Thermal degradation of glucosamine in an aqueous solution at 150 °C was recently reported (Shu, 1997). Furfurals were the major products when the pH of the solutions were 4 or 7, while pyrazines, 3-hydroxypyridine, 1*H*-pyrrole-2-carboxaldehyde, furanones, and hydroxyketones were generated when the pH of the solution was 8.5. One of our studies reported the thermal degradation products of glucosamine (Chen and Ho, 1998). A series of furyl-substituted pyrazines was identified, and their formation mechanism was proposed. Some of those furyl-substituted pyrazines exist in volatiles of coffee (Friedel et al., 1971).

Pyrolysis of *N*-acetylglucosamine in vacuo has been reported (Franich et al., 1984). 3-Acetamidofuran, 3-acetamido-5-acetylfuran, and acetamidoacetaldehyde were isolated from the tar in 5, 2, and 3% yield, respectively. However, those compounds isolated have been tentatively assigned structures only from their mass spectra (Franich et al., 1984). The present work studied the thermal degradation of *N*-acetylglucosamine at 200 °C. Several groups of volatile compounds were identified, and 3-acetylamido-5-acetylfuran was found to be the major degradation product. The structure of this major degradation product was verified by NMR spectrum after purification by preparative LC. These studies should be helpful in understanding the aroma contribution of *N*-acetylglucosamine and glucosamine and also in elucidating the flavor roles of chitin and chitosan in their existing systems.

MATERIALS AND METHODS

Materials. D-*N*-Acetylglucosamine, anhydrous sodium phosphate, silica gel (130−230 mesh), and thin layer plate (250 μm thickness, 2−50 μm particle size) were purchased from Aldrich Chemical Company (Milwaukee, WI). White quartz sand (50−70 mesh) was purchased from Sigma Chemical Company (St. Louis, MO). All organic solvents were obtained from Fisher Scientific (Springfield, NJ).

Pyrolysis of *N*-Acetylglucosamine. Similar to the procedures described in the previous paper (Chen and Ho, 1998), an equalmolar (20 mmol) amount of *N*-acetylglucosamine and anhydrous sodium phosphate (Na_2HPO_4) were ground and mixed with 50 g of quartz sand. The mixture was sealed in a 100-mL stainless steel vessel (Taiatsu, Japan) and then put

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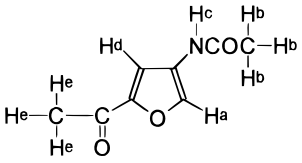

1. MS data obtained from GC/MS:

m/z:	168	167	125	124	110	96	83	54	43
Relative intensity:	2	23	44	16	51	10	11	12	100

2. Proton NMR data:

Proton:	a	b	c	d	e
Peak area:	1	3*	1	1	3
Peak form:	singlet	singlet	broad	singlet	singlet
Chemical shift:	8.18	2.06	9.38	7.15	2.40

*: peak overlap with that of solvent acetone-d 6.

Figure 1. MS and NMR data of 3-acetamido-5-acetylfuran, the major degradation product of *N*-acetylglucosamine.

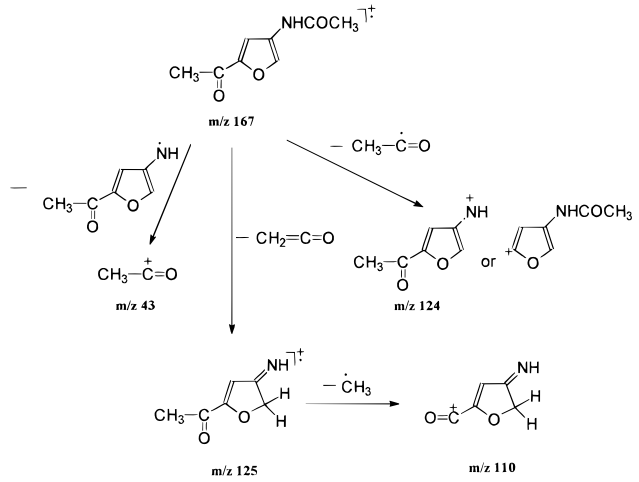

Figure 2. Proposed mass fragmentation of 3-acetamide-5-acetylfuran.

in a 200 °C oil bath and heated for 30 min. After cooling to room temperature under tap water, the heated mixture was ground and then extracted with 3 × 50 mL of 0.1 M HCl. The pH value of the aqueous solution was adjusted to 11.0 with 1 M NaOH solution. The solution was then extracted with 3 × 30 mL of CH_2Cl_2 after spiking with tridecane as the internal standard. The organic phase was dried over anhydrous sodium sulfate and concentrated to approximately 0.5 mL under a gentle stream of nitrogen gas. The concentrated sample was ready for GC and GC/MS analysis.

Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS) Analysis. The gas chromatography was performed on a Varian model 3400 equipped with a flame ionization detector (FID) and a nonpolar fused silica capillary column (DB-1, 60 m × 0.32 mm (i.d.), 1.0 μm film thickness, J&W Scientific). The column temperature was programmed from 40 to 260 °C at a rate of 3 °C/min. The injector and detector temperatures were maintained at 270 and 300 °C, respectively. The flow rate of the helium carrier gas was 1 mL/min. The volume of the injected sample was 1

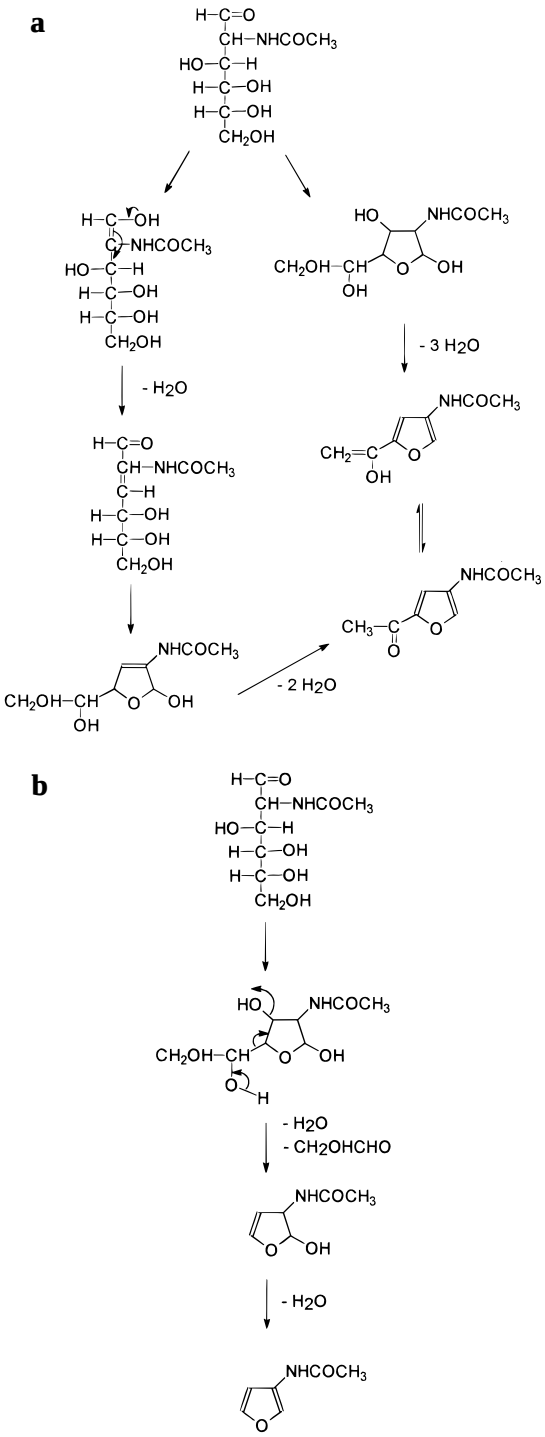

Figure 3. Proposed formation pathway of 3-acetamido-5-acetylfuran (a) and 3-acetamido-furan (b) from *N*-acetylglucosamine.

Table 1. Identified Volatile Compounds in Pyrolysis of *N*-Acetylglucosamine at 200 °C

compound[a]	RI[b]	concn (mg/kg)[c]	compound[a]	RI[b]	concn (mg/kg)[c]
pyrazine	711	18.49	vinylpyrazine	923	2.44
pyridine	728	9.22	2-ethyl-6-methylpyrazine	967	3.62
acetamide	764	2.89	2-acetylpyrazine	1003	1.67
methylpyrazine	785	4.29	2-acetylpyrrole	1047	1.22
2-furfural	808	1.85	1-methyl-2-pyrrolecarboxaldehyde	1067	0.86
2-acetylfuran	888	26.67	3-acetamidofuran	1202	18.94
ethylpyrazine	902	5.15	3-acetamido-5-acetylfuran	1619	287.78
2,3-dimethylpyrazine	908	3.07			

[a] Identification refers to the Wiley Mass Spectra Library. [b] Retention index, calculated according to the retention time of *n*-alkanes on DB-1 column. [c] Milligram of volatile per killogram of *N*-acetylglucosamine.

μL, and the split ratio was 25:1. GC/MS analysis was performed using an HP model 5790 GC coupled with an HP 5970A mass-selective detector. The capillary column and temperature program were the same as for the GC analysis. Mass spectra were obtained by electron ionization at 70 eV and mass scan from 33 to 300. Compound quantification was based on the GC/FID data, and compound identification was based on mass spectra obtained from the GC/MS.

Purification of the Major Degradation Product and the Proton NMR Spectrum Analysis. Eighty millimoles of *N*-acetylglucosamine was pyrolyzed, and the degradation products were extracted and dried as in the procedures described above. The methylene chloride solution of the extract was loaded onto a preequilibrated silica gel column (130−230 mesh, 38 × 400 mm) and eluted with a mixed eluting solvent (6 parts of CH_2Cl_2 with 1 part of acetone). The fractions containing the major degradation products were pooled, and the organic solvents were dried using a rotary evaporator under vacuum. Approximately 15 mg of white crystals was obtained. The NMR analysis was performed on an NMR instrument of modified Nicolet NT-360 (Madison, WI).

RESULTS AND DISCUSSION

When *N*-acetylglucosamine was pyrolyzed at 200 °C for 30 min, a roasty, smoky, and acidic flavor was generated. The composition of isolated volatile compounds is listed in Table 1, and 15 compounds were identified. The compounds were listed according to their elution order. The identified compounds included furans, pyridines, pyrroles, pyrazines, and others. Acidic compounds were not represented in the chromatogram due to alkaline extraction conditions.

Of the generated volatile compounds, those with the largest amounts were 3-acetamido-5-acetylfuran, 2-acetylfuran, 3-acetamidofuran, pyrazine, pyridine, ethylpyrazine, methylpyrazine, 2-ethyl-6-methylpyrazine, 2,3-dimethylpyrazine, and acetamide in decreasing order. The compound 3-acetamido-5-acetylfuran was the major degradation product, and its amount exceeded the sum of the other identified volatile compounds. In terms of classification of identified volatile compounds, furans such as 2-furfural, 2-acetylfuran, 3-acetamidofuran, and 3-acetamido-5-acetylfuran; *pyridines* such as pyridine; pyrroles such as 2-acetylpyrrole and 5-methyl-2-pyrrolecarboxyaldehyde; pyrazines such as pyrazine, methylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine, vinylpyrazine, 2-ethyl-5-methylpyrazine, and 2-acetylpyrazine were identified.

Considering the certain range of polarity distribution and tridecane as a nonpolar internal standard, the quantification data listed in Table 1 should be considered semiquantitive.

Comparing the thermal volatile generation of *N*-acetylglucosamine with glucosamine (Chen and Ho, 1998), we can see glucosamine undergoes more vigorous degradation than *N*-acetylglucosamine.

Pyrazine compounds are the most important flavor compounds identified in pyrolysis of *N*-acetylglucosamine, and almost all of them were found in volatiles of pyrolysis of glucosamine (Chen and Ho, 1998). Undoubtedly, the existence of these pyrazines contributes to the roasty aroma in pyrolysate of *N*-acetylglucosamine. In the study of the thermal degradation of glucosamine, we have identified several furyl-substituted pyrazines. These furyl-substituted pyrazines were not detected when *N*-acetylglucosamine was pyrolyzed under the same conditions.

Acetamide was found to be one of the degradation products of *N*-acetylglucosamine in our experiment. This compound was reported when chitin, the polymer of *N*-acetylglucosamine, was heated to over 260 °C (Koell et al., 1979).

To confirm the structure of 3-acetamido-5-acetylfuran, which was the major degradation compound of *N*-acetylglucosamine, it was prepared and purified using a preparative column chromatography. Purified 3-acetamido-5-acetylfuran has a weak sweet and cracker odor. The NMR spectrum was obtained to confirm its structure. The MS and NMR data of this compound are listed in Figure 1. The ^1H NMR of this compound showed five singlets, δ 8.18 and 7.15 ppm for hydrogen signals on furan ring, δ 2.40 and 2.06 ppm for two methyl groups, and one broad peak for the NH group, assignable for the structure of 3-acetamido-5-acetylfuran. The mass fragmentation mechanisms of major fragments of 3-acetamido-5-acetylfuran are shown in Figure 2. The formation mechanisms of 3-acetamido-5-acetylfuran and 3-acetamidofuran from *N*-acetylglucosamine are proposed in Figure 3.

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