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Pyrolysis–evolved gas-Fourier transform-infrared spectroscopy analysis of aminobutanoic acid isomers

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Abstract

Linear temperature programmed pyrolysis (100–600°C)–evolved gas-Fourier transform-infrared (EG-FT-IR) analyses have been applied to the identification of the thermal reaction products of 2-, 3- and 4-aminobutanoic acids.

The results of analyses indicate that many compounds in different quantities can be identified from the composition of the evolved gases, and the evaluation profiles of selected compounds depend on the amino acid structures. The pyrolysis of aminobutanoic acids involves two different reaction steps: the first is the induction period and free radical formation, determined by ESR spectroscopy, and the second is the steady-state reaction step and the formation of different organic compounds, determined by the FT-IR analysis.

Keywords: Aminobutanoic acids; EG-FT-IR spectroscopy; ESR; Pyrolysis

1. Introduction

Adequate characterization of amino acid and protein pyrolysates now widely used in food chemistry and food processing is necessary because of the composition-dependent variation in properties. Pyrolysis–evolved gas-Fourier transform-infrared (EG-FT-IR) analysis could be used in food industry scale production to measure the effects of applied temperatures on the changes of food composition in processing and preservation, as well as in accidental cases.

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The mutagenic properties of certain substances, formed by pyrolysis of amino acids, proteins and foods, have recently been reported by Kosuge et al. [1], Uyeta et al. [2] and Uchiyama and Uchiyama [3]. Kosuge et al. [1] reported that the basic fractions of tryptophan, phenylalanine, glutamic acid, serine and lysine pyrolysates showed more potent mutagenicities than those of other organic substances, i.e. polycyclic aromatic hydrocarbons or pyrolysates of non-nitrogen compounds.

The application of FT-IR spectroscopy for continuous analysis was pioneered by Liebman et al. [4], and then further developed by Lephardt and Fenner [5] and Fenner and Lephardt [6]. Both inert and oxidative atmospheres were used to study pyrolysis and combustion behaviour. The subject was more thoroughly reviewed by Lephardt [7] who pointed out that EG-FT-IR analysis could be used for both qualitative and quantitative analyses, in thermal stability and degradation studies. Compounds could be identified by spectral subtraction of recognizable species and evolution profiles could be plotted in terms of temperature or time. Coincident evolution profiles for two or more components could be strongly suggestive of a common reaction origin, so that more information on the underlying chemistry could become available.

Many reported papers [2,3,8,9] proved that there was a correlation between mutagenic activities and the level of nitrogen containing compounds in foods exposed to temperatures in normal processing and in accidental cases. The same authors studied an energy-requiring reaction for foods by ESR spectroscopy, and reported that the concentration of free radicals formed increased by increasing the energy level.

There are no detailed studies on the properties of free radicals and end-reaction products formed by the pyrolysis of foods, proteins and amino acids. The aim of this investigation is to establish the reaction pathway of the pyrolysis of amino acids, with amino groups at different positions in the hydrocarbon chain, and the end-reaction products.

2. Experimental

The aminobutanoic acids used for pyrolysis were of commercial CHR grade (Koch-Light, UK). To establish the appearance of free radicals in the induction step of pyrolysis, 5×10^{-4} mol of 2-, 3- and 4-aminobutanoic acid, respectively, were heated in an evacuated (13.3 kPa) Bruker double cavity TE 104 at 100, 200, and 600°C for 5 min. The free radicals were detected on a Bruker 200D ESR spectrometer, under the following instrumental conditions: scan range, 10 mT; time constant, 1.0 s; modulation amplitude, 0.1 mT; microwave power, 16 mW; field set, 346 mT; scan time, 500 s; modulation frequency, 100 kHz; microwave frequency, 9.515 GHz. The g-values were determined using the Bruker double cavity TE 104 with 1,1-diphenyl-2-picrilhydrazyl (DPPH) ($g = 2.0036 \pm 0.0002$) as a standard. To determine the pyrolysis end-reaction compounds, pyrolyses were carried out by heating 5×10^{-4} mol of 2-, 3- and 4-aminobutanoic acid respectively, in an atmosphere of helium (99.996% purity) in a quartz tube at 100, 200, 300, 400, 500 and

600°C, during the reaction period which lasted from 2 to 15 min. The flow rate of helium, as the carrier gas, was 5.0×10^{-5} m³/min. The products of pyrolysis, for each examined system at different temperatures and times, carried out with helium as aerosol, were subjected to FT-IR analyses.

FT-IR spectra were recorded on a Bruker IFS-88 FT-IR spectrometer at 4 cm^{-1} resolution, 2 scan/s, MCT detector, using the Bruker GC/IR software and 25 scans per point. Trace files were adjusted to the same temperature range, number of points, and point interval, and normalized for sample mass to give evolution profiles using modified Bruker software. This allowed direct comparison and subtraction of profiles from different samples. The evolved gases were passed through a 4×10.25 cm gas cell made from stainless steel with removable KBr windows. Cell and transfer lines were operated at room temperature. Condensation losses were not a problem, owing to the smallness of the samples and the geometry of the system.

3. Results and discussion

ESR studies of pyrolyzation series consisting of different amino acids were carried out as part of a continuing investigation [10–13] of the chemistry of the Maillard reaction.

The aminobutanoic acid isomer samples pyrolyzed at temperatures below 310, 150 and 230°C, for 2-, 3- and 4-aminobutanoic acid, respectively, revealed no observable ESR signals, while all other pyrolyzed samples above these temperatures showed almost identical symmetrical spectra with $g = 2.0061$, 2.0054 and 2.0048 (Fig. 1).

The lineshapes of the spectra were of lorentzian type in the middle, while the wings were of gaussian shape. The behaviour of the obtained lineshape spectra could be explained by the exchange interaction between the electron spins in identical organic radicals, which caused the transition to have a nearly lorentzian shape, or by the interaction of organic radicals of different origin. The estimation from the linewidth, $H_{\text{p–p}}$, indicated that the frequency of this interaction was about 10^7 s^{-1}, compared with a DPPH single crystal value of 10^{11} s^{-1}, as a well known stable organic free radical.

All the pyrolyzed samples examined, at temperatures of 310, 150 and 230°C, for the 2-, 3- and 4-aminobutanoic acids, respectively, showed saturation phenomena for the maximum microwave power at the resonator 10 mW. Evaluation of the spin–lattice relaxation time, T_1, from the saturation behaviour of the samples pyrolyzed at 400°C, gave $T_1 = 10^{-6}$ s. The saturation broadening of the samples pyrolyzed between 310, 150 and 230°C for 2-, 3- and 4-aminobutanoic acids, respectively, and 400°C was inhomogeneous, indicating that the linewidth, $H_{\text{p–p}}$, in this temperature range was mainly determined by the unresolved hyperfine structure of the protons. The spin concentrations of the examined samples changed dramatically within the given temperature range. From the temperatures of 310, 150 and 230°C to 360, 200 and 280°C for 2-, 3- and 4-aminobutanoic acids, respectively, the
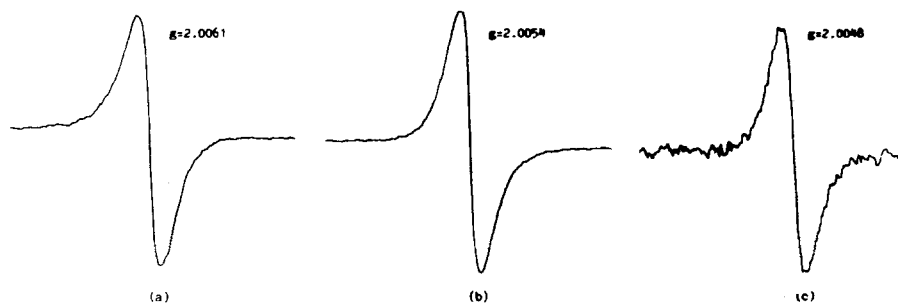
Fig. 1. ESR spectra of free radicals obtained from pyrolysis of (a) 2-, (b) 3- and (c) 4-aminobutanoic acid at 400°C.

spin concentration increased sharply and then dropped substantially to 600°C. In order to characterize organic free radicals in the samples pyrolyzed at 400°C the signal intensities of the ESR spectra were measured as a function of temperature in the range from −80 to 0°C, and the results were compared with those from DPPH and the Bruker pitch. For the temperature range employed, the signal intensity of spin concentration increased with decreasing temperature at the rate predicted by the Curie–Weiss law. The system following this law often have a paramagnetic doublet ground state independent of their surroundings. The absence of hyperfine structure, a relatively narrow linewidth independent of temperature, as well as quazi-lorentzian lineshape at the *g*-values, could support the hypothesis that there is a strong exchange interaction between the identical organic free radicals. In order to gain further information about the species of organic free radicals in the pyrolyzed samples of different isomers of aminobutanoic acid, the electron nuclear double resonance spectroscopy technique must be employed.

Fifteen experiments carried out by pyrolysis of 2-, 3- and 4-aminobutanoic acids under the above-mentioned conditions, which enabled identification of the stable reaction products, are listed in Table 1.

Some of the reaction products formed from pyrolysis of examined amino acids were found only in trace amounts (non-listed substances in Table 1), producing ill-defined EG-FT-IR analysis signals which represented $<10^{-10}$ mol in the reactions.

Organic substances listed in Table 1 were obtained from evolution profiles in the temperature ranges from 180 to 370°C, from 30 to 230°C, and from 130 to 280°C, for 2-, 3- and 4-aminobutanoic acids, respectively. These temperature ranges were chosen according to differential scanning calorimetry analyses of aminobutanoic acid isomers [14], in which the examined amino acids showed maximum loss of mass or maximum reactivity.

As expected from our previous studies [14], the pyrolysis of 2-aminobutanoic acid at temperatures from 180 to 280°C sustained the decarboxylation to produce *n*-propylamine as the major organic product. The second process, considered also as the primary decomposition mode, involved double dehydration, yielding dipeptide first and subsequently 2,5-diketopiperazine (2,5-piperazinedione). The above

reactions provide strong evidence for its involvement at 340°C. The reaction of deamination has been suggested to be the primary, although minor, pathway. The absence of the corresponding carboxylic acids indicated that ammonia, as 14.2% of the total mass, was formed as a product from the secondary reaction which was involved in the pyrolysis reaction. The possibility of a minor pathway in which the ammonia could be formed as a primary product, however, cannot be eliminated. An aldehyde containing one carbon less than the parent amino acid was detected in the evolution profile in the temperature range 300–320°C during pyrolysis. The next mode, from 320 to 330°C, possibly representing the fragmentation of primary products, involved chain homolysis and led to a series of both saturated and unsaturated hydrocarbons and their amino, nitrile and amido derivatives, but in small amounts, never exceeding 3%.

Evolution profiles of the pyrolysis of 3-aminobutanoic acid, the compounds listed in the second column of Table 1, showed the most striking difference from the thermal decomposition of 2-aminobutanoic acid. Whereas 2-aminobutanoic acid underwent pyrolysis at 180°C, leading to decarboxylation at most, which was minor compared with deamination, the pyrolysis of 3-aminobutanoic acid started from temperatures of 35°C, leading to a mass reduction of 27% at 97°C and about 82% at 138°C, yielding, in primary mode, unsaturated acids and ammonia as the major products. The pyrolysis of 3-aminobutanoic acid gave both *cis*- and *trans*-crotonic acids, as well as 3-butenoic acids, in the amounts 20.9%, 29.4% and 19.2%. The detected ammonia yield was 19.6%. While 2,3-unsaturated acids were the more abundant products, the ratio of these to 3,4-unsaturated acids was not necessarily relevant because their formation depended on their relative rates of formation.

Propene was also determined as an important product in the first reaction mode, but its formation probably depended on the decarboxylation of 3-butenoic acid.

From the results of evolution profiles of pyrolysis of 3-aminobutanoic acid, the second mode, from 105 to 140°C, involved the formation of unsaturated nitriles with the same carbon numbers as the parent amino acids. The formation of nitriles are most reasonably accounted by the dehydration of unsaturated amides, similar to the case of pyrolysis of 2-aminobutanoic acid.

The third column in Table 1 represents compounds obtained by pyrolysis of 4-aminobutanoic acid in the temperature range 130–300°C, and detected by GC/MS and EG-FT-IR methods. Relative amounts of obtained compounds were derivatized from evolution profiles of pyrolysis, in which 4-aminobutanoic acid at a temperature of about 275°C, gave five-membered cyclic lactam as the major product. The minor and secondary products obtained by pyrolysis of this amino acid was different from both the 2- and 3-aminobutanoic acids and produced an interesting trend. While 3-butenoic acid, obtained by pyrolysis of 3-aminobutanoic acid, and the corresponding unsaturated amide were formed presumably via processes similar to those occurring on pyrolysis of 2-aminobutanoic acid, 4-aminobutanoic acid, on pyrolysis in the secondary mode, at temperatures from 280°C gave small yields of 1,4-butanolide (γ-butyrolactone), which was formed by an intramolecular nucleophilic attack of oxygen.

Table 1
Organic compounds obtained from the pyrolysis of 2-, 3- and 4-aminobutanoic acids

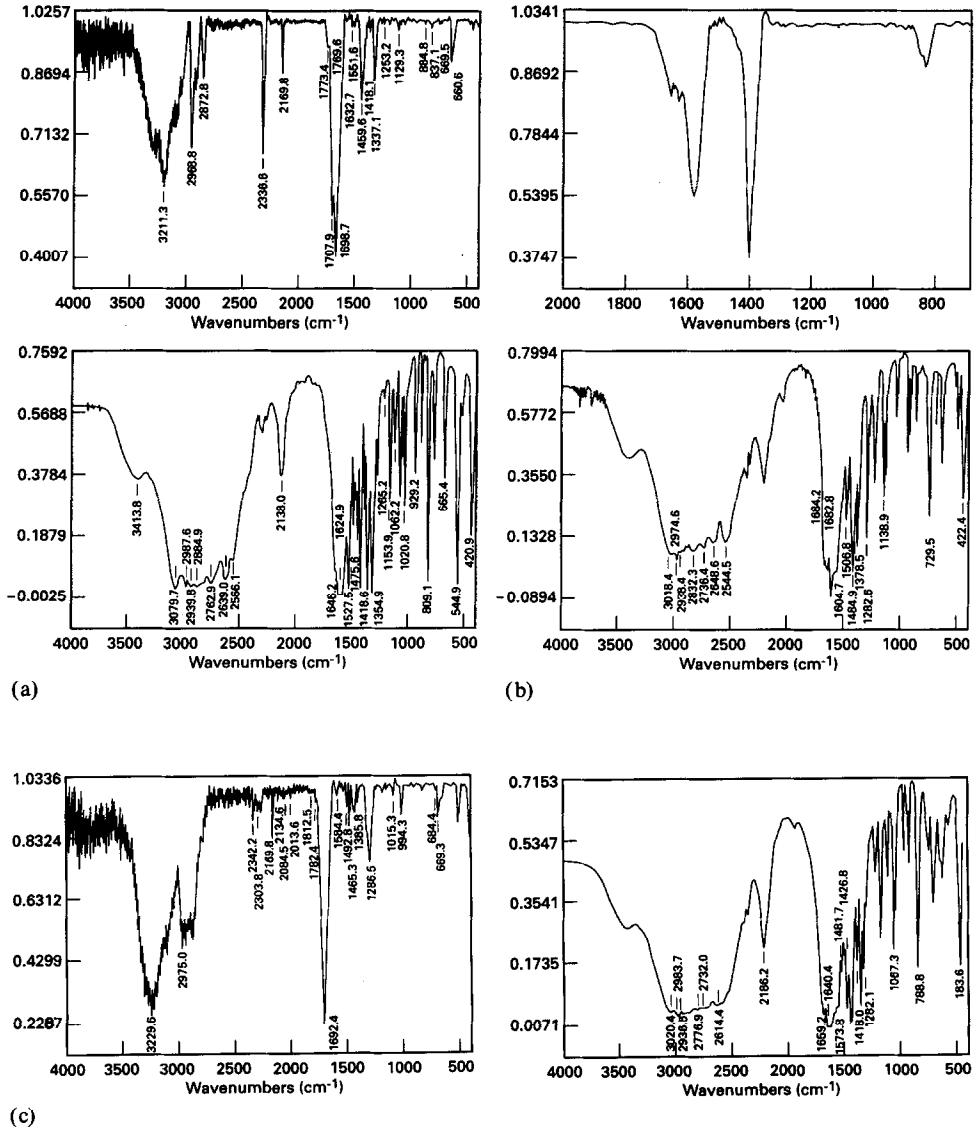| Yield (% of total pyrolysate)	2-Aminobutanoic acid	3-Aminobutanoic acid	4-Aminobutanoic acid
≥ 20%	CO_2 H_2O (propylamine, NH_2) (propanenitrile, CN)	NH_3 (but-2-enoic acid, COOH) (but-3-enoic acid, COOH) (but-2-enoic acid, COOH)	(2-pyrrolidinone) NH > 90% H_2O
5–20%	(N-propylidenepropylamine) DKP > 19.5% NH_3	CO_2 H_2O (but-1-ene) (but-3-enenitrile, CN) (but-2-enenitrile, CN)	
0.5–5%	C_2H_4 CH_3CN (propene) (propylamine, NH_2) (butanenitrile, CN) (butanamide, NH_2, O) (N-ethylidenepropylamine) (butanal, CHO)	(but-2-enamide, O, NH_2) (but-3-enamide, NH_2, O) (butanamide, NH_2, O)	CO CO_2 NH_3 (γ-butyrolactone) (but-3-enoic acid, COOH) (but-3-enamide, O, NH_2)

DKP = diketopyperazine

Fig. 2. FT-IR spectra of pyrolyzed (a) 2-, (b) 3- and (c) 4-aminobutanoic acid at 307, 138 and 221°C, respectively, compared with the IR spectra of the same commercial samples (lower) of amino acids.

Figure 2 presents the FT-IR analyses of the pyrolyzed aminobutanoic acids at 307, 138 and 221°C, for 2-, 3- and 4-aminobutanoic acids, respectively, compared with IR analyses of the same unpyrolyzed amino acids.

In conclusion, the careful identification of both major and minor reaction products suggested that examined amino acids decomposed under pyrolysis conditions along a common reaction pathway, but the evolution profiles of the pyrolysis–EG-FT-IR

analyses gave more information about critical temperatures, possible intra- and intertransformation of the compounds obtained by pyrolysis, and information about quantitative concentrations of detected compounds. On the basis that the area under the evolution profile was proportional to the total concentration of components in the gas stream, the reproducibility of the method was good enough to consider its use, not only for quantitative analysis, but also for analysis of very complex organic material, such as food, feeds, rubber, plastics, woods, etc. However, in order to establish more information about the qualitative and quantitative reproducibility of the EG-FT-IR results, the GC/MS technique must be employed.

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