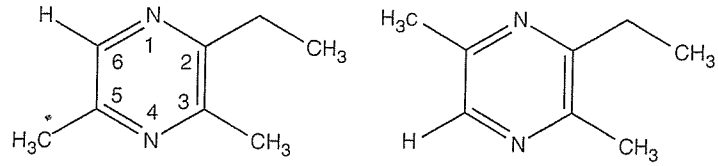

2-ethyl-3,5-dimethylpyrazine (1) 2-ethyl-3,6-dimethylpyrazine (2)

Figure 1. Structures of 2-ethyl-3,5-dimethylpyrazine (1) and 2-ethyl-3,6-dimethylpyrazine (2).

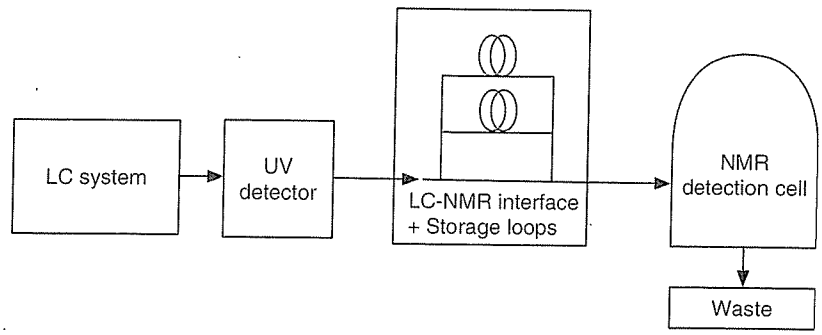

Figure 2. Schematic of LC–NMR system. The LC system is connected to the NMR detection cell through a UV detector. On on-flow mode, real-time sequence of ^{1}H-NMR is acquired after the constituents are separated by the LC system. On fraction loop mode, the separated constituents are collected into fraction storages with trigger of UV detection and then each constituent is moved to NMR detection cell.

not easily determined because standards for the two regio-isomers are not commercially available. Therefore, to establish the quality control of this flavouring agent, it is important to develop a detection method without standards. In this paper, we report the application of LC–NMR to the direct identification of the two regio-isomers of ethyl-dimethylpyrazine as part of studies to evaluate its quality and safety as a food flavouring agent in Japan.

Materials and methods

Materials

A sample of commercial ethyldimethylpyrazine product was obtained through the Japan Food Additives Association. Acetonitrile (CH_3CN) was of HPLC grade and was used without further purification. NMR solvents, deuterium oxide (D_2O) and acetonitorile-d_3 (CD_3CN) were purchased from Isotec Inc. (Miamisburg, OH, USA).

Instrumentation

The LC–MS system (Waters, Milford, MA, USA) consisted of an Alliance 2695 HPLC, a 2996 photodiode array detector (PDA) and a ZQ single-quadropole mass spectrometer (MS) equipped with a Z-spray electrospray interface. The GC–MS system (Shimadzu, Kyoto, Japan) consisted of a GC-17A GC and QP-5050A MS. The LC–NMR system consisted of a NANOSPACE SI-2 series HPLC (Shiseido, Tokyo, Japan) equipped with storage loops for fraction mode and a JNM-ECA (500 MHz; JEOL, Tokyo, Japan) installed HX/FG LC probe (JEOL). The diagrammatic illustration of LC–NMR system is shown in Figure 2.

LC–MS and GC–MS analyses

Commercial ethyldimethylpyrazine product (10 µl, liquid) was dissolved in CH_3CN (1.0 ml) and 1 µl of the solution was then injected into the LC–MS and GC–MS systems under the following conditions. LC–MS conditions: column: TSK-gel ODS-80TsQA (2.0 mm I.D. × 250 mm) (Tosoh, Tokyo, Japan); mobile phase: water/CH_3CN (20:80, v/v); flow-rate: 0.1 ml min^{-1}; The on-line PDA detector was monitored between 192 and 400 nm, and peak detections were at UV 278 nm. The electrospray source ran at 3.0 kV capillary voltage, 120 and 350°C source and desolvation temperatures, respectively, and 350 and 50 l h^{-1} desolvation and cone gas flow-rates, respectively. The cone voltage was 30 V for positive-ion detection. Full-scan acquisition between *m/z* 50 and 300 was performed at a scan speed of 0.1 s scan^{-1} with a 0.1-s inter-scan delay.

GC–MS conditions: column: Inert Cap WAX (0.25 mm I.D. × 30 m, 0.25 μm thickness (GL Sciences, Tokyo, Japan)); column temperature: 50°C → 5°C min^{-1} → 230°C (4 min); inject temperature: 250°C; carrier gas: He, 1.5 ml min^{-1}; split ratio: 1:200; ionization voltage: 70 eV; accelerator voltage: 1.0 kV; scan range: *m/z* 50–300. Mass spectra were referred to the NIST 147 database.

LC–NMR and NMR analyses

Commercial ethyldimethylpyrazine product (20 μl) was dissolved in D_2O/CH_3CN (20:80, v/v) (1.0 ml) and 2 μl of the solution was injected into the LC–NMR system. LC–NMR was performed under the following conditions: column: TSK-gel ODS-80TsQA (2.0 mm I.D. × 250 mm) (Tosoh); mobile phase: D_2O/CH_3CN (20:80, v/v); flow-rate: 0.1 ml min^{-1}; detection, UV 278 nm. LC–NMR spectra were recorded in the two-dimensional (2D) on-flow and fraction loop modes using a HX/FG LC probe with a flow cell of 60 μl active volume at 30°C. Water suppression enhanced through T1 effect (WET) solvent suppression (Smallcombe et al. 1995) and related sequences were used to suppress the peaks of CH_3CN, its ^{13}C satellites and the residual HOD in D_2O. In the 2D on-flow mode, FID was collected with 4K data points, and eight scans with 1 s repletion time were accumulated; other parameters were the defaults for conventional NMR depending on JEOL. In fraction loop mode, FIDs of the two regio-isomers were collected with the conventional default parameters and over 200 scans were accumulated, respectively. Assignments of proton and carbon signals were confirmed by pulse-field gradient (PFG) heteronuclear multiple quantum coherence (HMQC) and PFG heteronuclear multiple bond connectivity (HMBC) experiments with WET (Smallcombe et al. 1995).

Results and discussion

LC–MS and GC–MS analyses of commercial ethyldimethylpyrazine product

To estimate the relative amounts of the two regio-isomers, 2-ethyl-3,5-dimethylpyrazine (**1**) and 2-ethyl-3,6-dimethylpyrazine (**2**), in the commercial product, LC–MS and GC–MS were performed. The LC profile of the commercial product with detection at 278 nm is shown in Figure 3. The separation of two regio-isomers was accomplished using isocratic LC separation, and two peaks were observed at 31.9 and 33.8 min at 278 nm, which must be derived from **1** or **2**, respectively. The PDA spectra of peaks A and B, and the ESI–MS (positive mode) with the molecular-related ion peak at *m/z* 137 $[M+H]^+$ were almost identical (Figure 4). Furthermore, although in-source collision-induced decomposition (CID) experiments were performed by varying the sampling-cone voltage ($\Delta V = 30$ V), peaks A and B showed that the same fragment ions at *m/z* 59, 83, 102 and 116 with very similar intensities. Thus, the difference between peaks A and B was not observed by PDA and ESI–MS spectra.

The GC–MS profile is shown in Figure 3. Two peaks X and Y were observed at 10.6 and 11.0 min from the total ion chromatogram. The area magnitudes of peaks X and Y were 43.4 and 56.6, respectively, and was very close to the area ratio of peaks B and A (B/A = 45.0:55.0), which was observed at 278 nm on LC. Therefore, we presumed that peaks X and Y on GC correspond to peak B and A on LC, respectively. The EI–MS spectra of peaks X and Y are shown in Figure 5. The EI–MS spectra of peaks X and Y had no significant differences, and the relative intensities of fragment ions at *m/z* 56, 107 of peak Y differed only slightly from the corresponding fragment ions for peak X. Although the spectra of peaks X and Y were referred to the NIST 147 database, unambiguous identification could not be provided. Therefore, it was concluded that the structural determinations of the two regio-isomers in the commercial ethyldimethylpyrazine product by LC–MS and GC–MS was impossible.

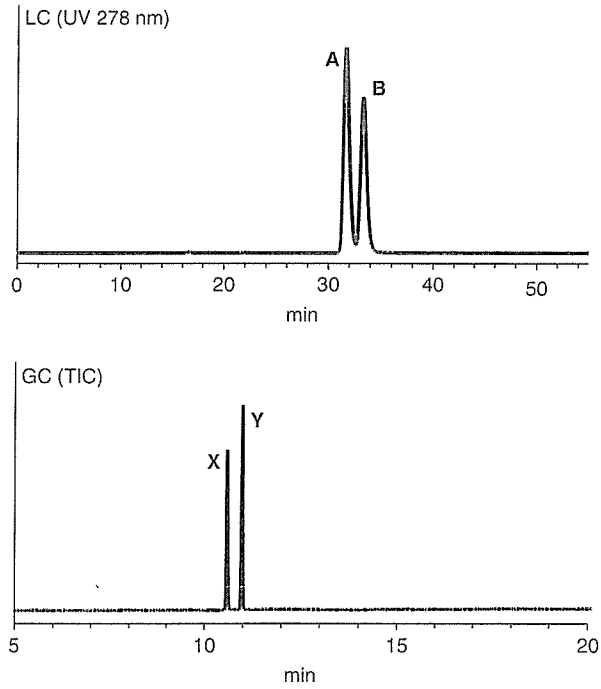

Figure 3. LC and GC profiles of commercial ethyldimethylpyrazine product. GC and LC conditions are described in the experimental section.

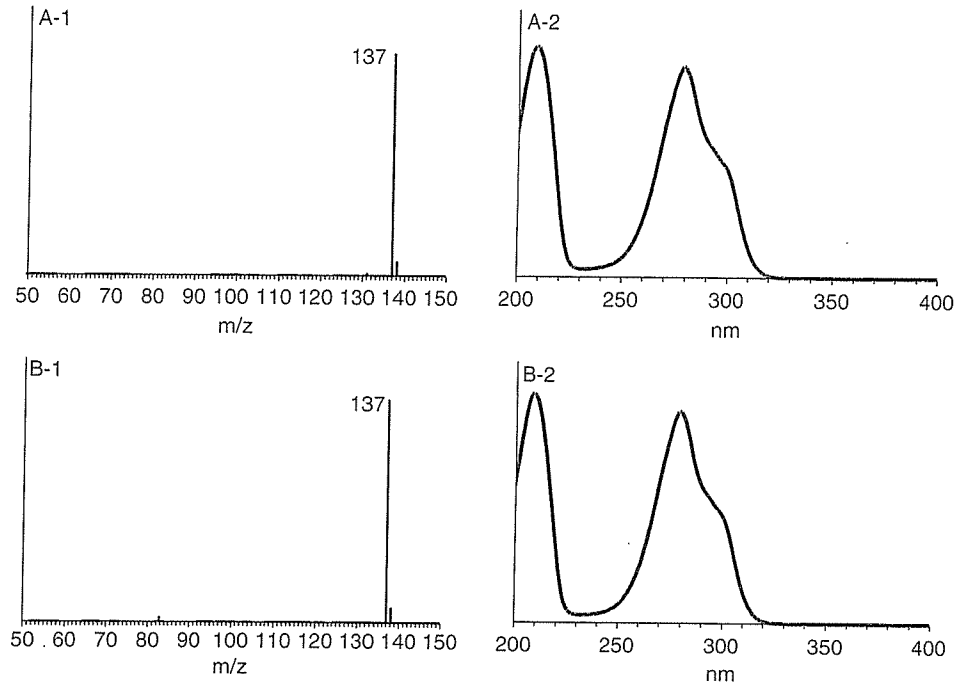

Figure 4. ESI–MS and PDA spectra of peaks A and B. (A-1) ESI–MS of peak A. (A-2) PDA of peak A. (B-1) ESI–MS of peak B. (B-2) PDA of peak B.

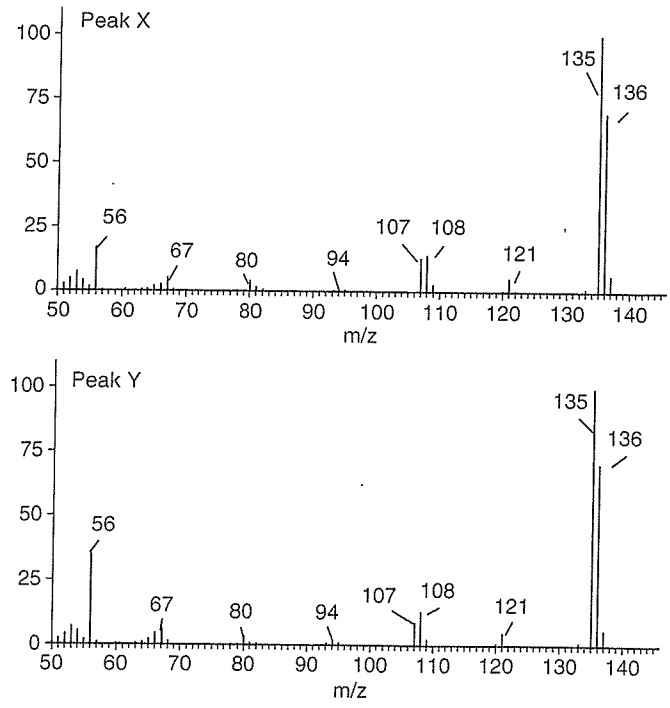

Figure 5. EI–MS spectra of peaks X and Y.

Determination of regio-isomers of ethyldimethylpyrazine by LC–NMR

As a preliminary step, we obtained the ^{1}H-NMR spectrum of the commercial product of ethyldimethylpyrazine using conventional NMR spectroscopy without separation, but it was difficult to distinguish signals of two regio-isomers because most signals overlapped except δ 8.36 and 8.38 ppm signals on the pyrazine ring of each regio-isomer. Also, the coupling constants (J) of most of signals could not be read exactly. Thus, it was necessary for

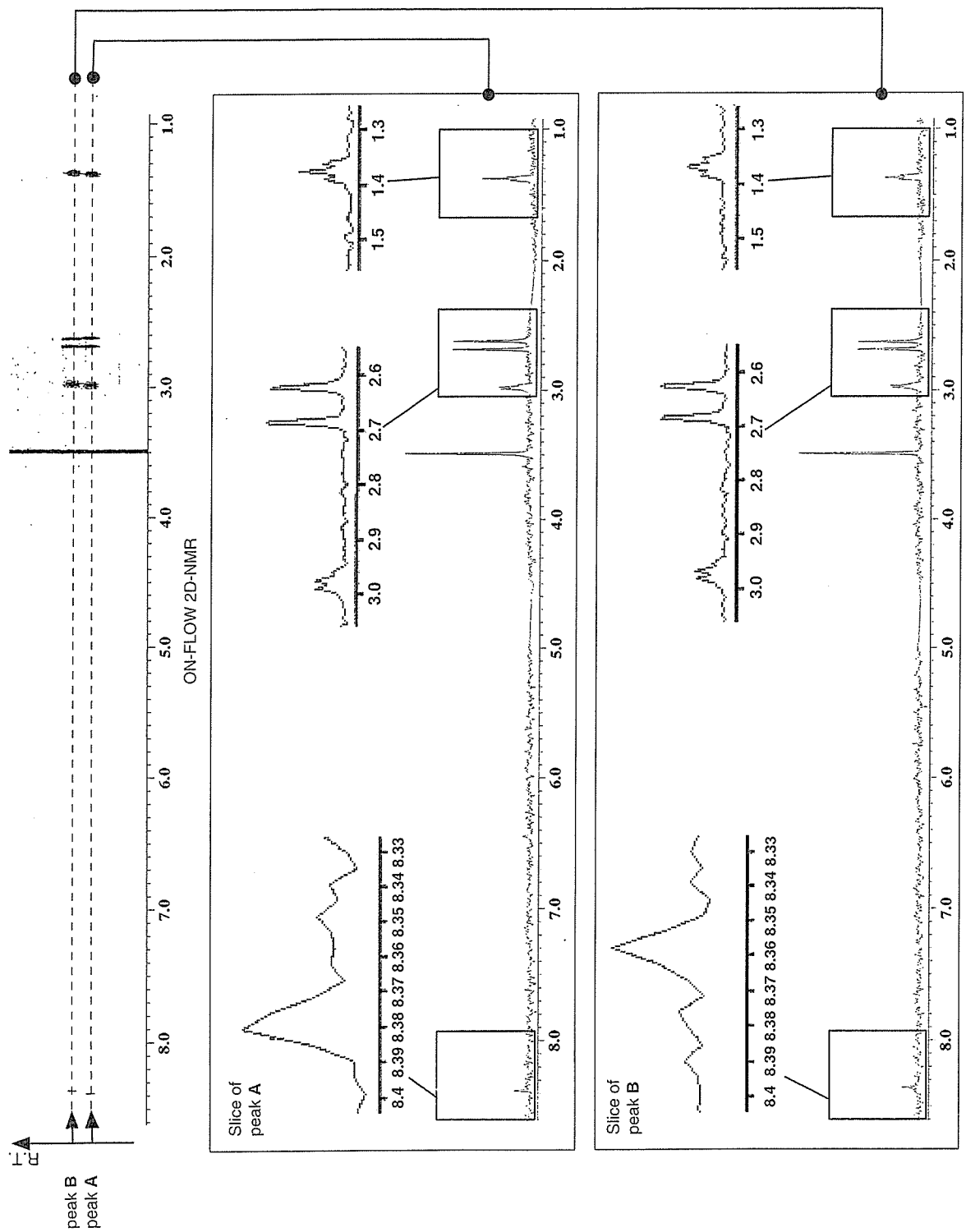

Figure 6. ^{1}H-NMR data of peaks A and B using 2D on-flow mode LC–NMR.

Table I. NMR data (δ, ppm, D_2O/CH_3CN (20:80, v/v)) for peaks A (1) and B (2).

No.	Peak A			Peak B		
	2-Ethyl-3,5-dimethylpyrazine (1)			2-Ethyl-3,6-dimethylpyrazine (2)		
	^1H	^{13}C	HMBC	^1H	^{13}C	HMBC
2	–	161.7	–	–	162.0	–
3	–	155.0	–	–	155.0	–
5	–	155.0	–	8.36 (1H, s)	141.9	C-3, C-6
6	8.38 (1H, s)	141.3	C-2	–	154.5	–
3-CH_3-	2.68 (3H, s)	20.9	C-2, C-3	2.69 (3H, s)	20.9	C-2, C-3
6-CH_3-	–	–	–	2.63 (3h, s)	20.9	C-5, C-6
5-CH_3-	2.62 (3H, s)	20.7	C-5, C-6	–	–	–
2-CH_3-CH_2-	1.38 (3H, t, J=7.5 Hz)	12.7	C-2	1.37 (3H, t, J=7.5 Hz)	13.1	C-2
2-CH_3-CH_2-	2.98 (2H, q, J=7.5 Hz)	27.8	C-2	2.97 (2H, q, J=7.5 Hz)	27.9	C-2

^{13}C chemical shifts were assigned indirectly by HMQC and HMBC.

identification and structural determination of two regio-isomers to carry out a separation. However, general preparative separations, which consist of isolation and purification steps, are time-consuming and tedious. We concluded that LC–NMR would be an ideal method for obtaining NMR spectral information for individual isomers.

The mixture of two regio-isomers was first analyzed by on-flow mode LC–NMR using LC conditions identical to LC–MS. By on-flow mode, NMR spectra of a mixture of two regio-isomers of ethyldimethylpyrazine were displayed as a 2D matrix showing NMR spectrum against retention time, similar to an LC–PDA plot. Figure 6 shows the 2D data for the NMR region from δ 1 to 9 ppm. So the individual spectra of regio-isomers could be extracted from 1D slices along the x-axis, the stacked plots were sliced from each retention time of peaks A and B, and the coupling constants and chemical shifts could be read. However, it was not possible to determine each isomer structure from 1D slice data because the resolution in the individual spectra was lower than that of conventional NMR and the signals were very similar, except the δ 8.36 and 8.38 ppm signals on the pyrazine ring of each regio-isomer. On on-flow mode, it is impossible to carry out 2D-NMR data, such as HMQC and HMBC, which provide more structural information, because these 2D-NMR experiments need more accumulation time than ^1H-NMR.

To determine the individual isomers exactly, the mixture was analyzed by fraction loop mode LC–NMR (Figure 2). Using fraction loop mode, the fractions of the eluent flowing from LC system were separated by a storage device that consisted of fraction loops. Without interrupting the separation, peaks could be trapped in the fraction loops. The fractionated peaks were transferred into a LC–NMR probe, respectively, and NMR spectra of each peak could be carried out as with conventional NMR. The resolution is also better than that of on-flow mode.

After peaks A and B were fractionated into the loops using the same conditions for LC–MS, the contents in the loops were transferred into a LC–NMR probe. Accumulated times of 200 were used to get unambiguous spectral data of ^1H-NMR and 2D HMQC and HMBC. The results were two sets of spectra for peaks A and B (Table I). ^{13}C-NMR data could not be observed directly on LC–NMR because the amount of samples was insufficient. This was a disadvantage against conventional NMR but the ^{13}C-NMR data could be indirectly attributed from correlations of 2D HMQC and HMBC experiments. The observed HMBC correlations are listed in Table I. The ^1H-NMR spectrum of the content of peak A exhibited five proton signals at δ 8.38 (1H, s), 2.68 (3H, s), 2.62 (3H, s), 1.38 (3H, t, J=7.5 Hz) and 2.98 (2H, q, J=7.5 Hz), which were attributed to a proton on the pyrazine ring, two methyl protons, and methyl and ethylene protons on the ethyl group, respectively. ^{13}C-NMR data was also attributed indirectly by HMQC. A HMBC correlation was observed between methyl protons on the ethyl group at δ 1.38 and quaternary carbon signal at δ 161.7, suggesting the carbon signal was at C-2. Furthermore, a proton on the pyrazine ring at δ 8.38 was correlated to C-2 carbon. Based on these result, the structure of the content of peak A was determined as 2-ethyl-3,5-dimethylpyrazine (1). On the other hand, as no correlation was observed between a proton on the pyrazine ring at δ 8.36 (H-5) and quaternary carbon signal at δ 162.0, the structure of the content of peaks B was determined as 2-ethyl-3,6-dimethylpyrazine (2). It took only 2 days to determine the structures of two regio-isomers using LC–NMR, but it would take over 1 week using general isolation and purification.

Conclusions

In general, if standard compounds cannot not be obtained, to identify a structure it is necessary to fractionate the mixture and obtain rather pure compounds for characterization by NMR, MS, IR, etc. Preparative separations of mixtures are difficult and time-consuming, so identification of structures is troublesome. It was not necessary to obtain pure compounds for LC–NMR. NMR spectra were obtained easily by LC–NMR and structural information derived by analysis of the data. In this study, we demonstrated that the two regio-isomers of commercial ethyldimethylpyrazine were directly identified using LC–NMR. 2D on-flow mode analysis provided ^{1}H-NMR data for each isomer clearly without the need for a general preparation method. Fraction loop mode analysis gave not only detailed ^{1}H-NMR data but also ^{13}C-NMR data indirectly by 2D HMQC and HMBC experiments. These data were useful for structure determinations. We believe that the use of LC–NMR for the rapid identification of constituents may be a useful tool for quality control of food additives.

Acknowledgements

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Note

Standard Infrared Absorption Spectrum of Betaine and Optimal Conditions for its Measurement

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The infrared absorption (IR) spectrum is often used as a standard reference in identification tests of food additives in Japan. In the case of betaine, many different IR spectra have been reported and, therefore, it is necessary to establish an IR spectrum that is reproducible and reliable enough to be used as a standard for identification. In the present study, suitable conditions to obtain a standard IR spectrum were examined from various viewpoints, including pretreatment, selection of method, and measuring technique. The KBr disk method, which has generally been used to identify betaine, was found to be humidity-dependent, and there was also an interaction between betaine and KBr. A reproducible IR spectrum suitable as a standard could be obtained by drying betaine at 105℃ for 3 hours over phosphorus pentoxide, and then measuring the IR spectrum by the liquid paraffin (Nujol) paste method.

Key words: betaine; seasoning; infrared spectrum; reference spectrum; anhydride; hydrate; identification

Introduction

Betaine (Fig. 1) occurs widely in nature, especially in beets, and is used as a food additive for seasoning or as a flavor-improving agent in Japan. An identification-test for betaine is described in "Voluntary Specifications of Existing Food Additives: 3rd Ed"[1], which suggests identification of betaine on the basis of the wave numbers of the absorption bands in an infrared absorption (IR) spectrum measured by the KBr method, as well as retention time in HPLC, and the like. However, the criteria for identification employed in the test are not comprehensive; for example, the defined wave numbers do not include that of the absorption band of the COO^- group, which is one of the characteristic bands of betaine.

Apart from their use as food additives, betaine and related compounds have attracted much interest in terms of the relationship between IR spectra and structure, specifically, the influence of hydrogen bonding[2)-5)]. IR spectra of betaine are available from databases, for example, "The Sigma Library of FT-IR Spectra"[6], "Spectral Database for Organic Compounds, SDBS"*1 of the National Institute of Advanced Industrial Science and Technology, Japan (AIST), and "WebBook"*2 of the National Institute of Standards and Technology, USA (NIST). However, these IR spectra are very different from each other and hardly appear to represent the same compound.

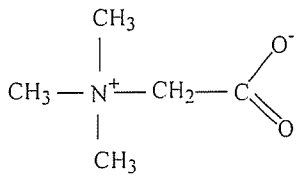

Fig. 1. Structure of betaine

In this study, we examined the influence of pretreatment, measuring method, and measurement conditions on the IR spectrum of betaine in order to establish a method to obtain a reliable and reproducible IR spectrum of betaine, to serve as a standard for an identification test.

Materials and Methods

1. *Samples and reagents*

Betaines (products of Nippon Beet Sugar Manufacturing Co., Ltd., and Danisco Japan Co., Ltd. Tokyo, Japan)

*1 http://www.aist.go.jp/RIODB/SDBS/cgi-bin/direct_frame_top.cgi

*2 http://webbook.nist.gov/cgi/cbook.cgi?ID=C590476&Units=SI&Mask=80

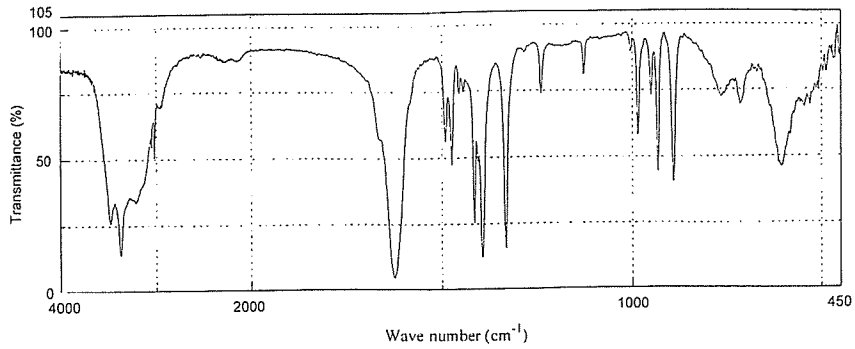

Fig. 2. IR spectrum of betaine dried over phosphorus pentoxide at 105℃ for 3 hours (the KBr method)

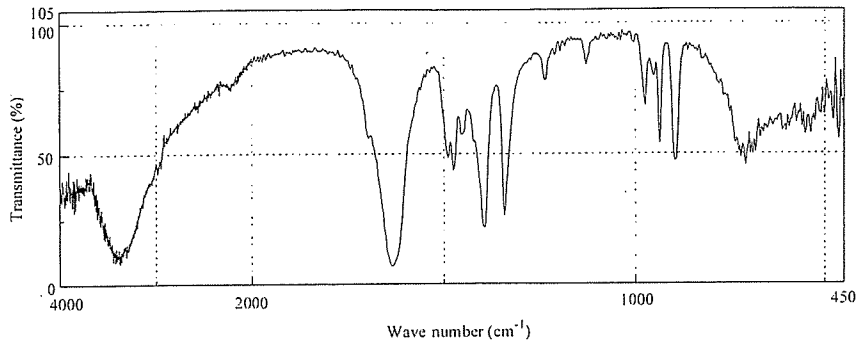

Fig. 3. IR spectrum of betaine dried over phosphorus pentoxide at 105℃ for 3 hours (the KCl method)

were provided by Japan Food Additives Association (Tokyo, Japan) and used as received. Both betaines were in the form of white crystalline powders.

Liquid paraffin (Nujol®) and potassium bromide (KBr) for IR spectroscopy were purchased from Merck AG. (Germany) and JASCO Co., Ltd. (Hachiouji, Japan), respectively. Potassium chloride (KCl) for IR spectroscopy was from JASCO Co., Ltd. (Japan).

2. Measurements of IR spectra

Measurements were carried out by the KBr method and the paste method using liquid paraffin as described in the 7th Ed. of Japan's Specifications and Standards for Food Additives[7] for measuring a solid sample. A KBr disk without any sample and a KBr optical plate were used as references in the KBr method and the paste method, respectively. The resolution was about 4 cm^{-1} (32 or 64 scans). The Fourier transform (FT)-IR device used was an Impact 400 FT-infrared spectrophotometer (Nicolet Co., Madison, Wis., USA) which could nominally measure up to 400 cm^{-1}, though the practical limit was about 450 cm^{-1}. The measurement was conducted in a room used exclusively for IR spectral measurement, where the humidity and temperature were controlled to 30–40% and 23℃, respectively. KBr disks and pastes were usually prepared in the controlled room, but sometimes in an ordinary laboratory. Measurements using the KCl disk or paste method were carried out under the same conditions.

3. Recommended procedure for measurement of a standard IR spectrum of betaine

Betaine as a sample was dried over phosphorus pentoxide at 105℃ for 3 hours. The IR spectrum was measured by using the paste method as described in the General Methods of the 7th Ed. of Japan's Specifications and Standards for Food Additives[7]. As a reference, a KBr optical plate was used.

Results and Discussion

1. Examination by the disk method

In the measurement by the KBr disk method, commercial betaine was used after having been dried at 105℃ for 3 hours according to the "Loss on Drying Test" in the Voluntary Specifications of Existing Food Additives: 3rd Ed.[1] The dried betaine was first subjected to measurement by the KBr method according to the General Methods of the 7th Ed. of Japan's Specifications and Standards for Food Additives[7]. The IR spectrum obtained is shown in Fig. 2. The IR spectrum agreed well with that described in SDBS, but differed from those described in The Sigma Library (the paste method), NIST (the paste method) and reference 2 cited above (the KBr method), suggesting the importance of moisture absorption during disk preparation or an interaction between betaine and KBr. To examine whether or not an interaction between betaine and KBr exists, a betaine disk was prepared using KCl, and the IR spectrum was measured. The result is shown in Fig. 3. It is clear from Figs. 2 and 3 that the spectrum of betaine in the KBr disk is quite different from that in the KCl disk throughout the entire region measured.

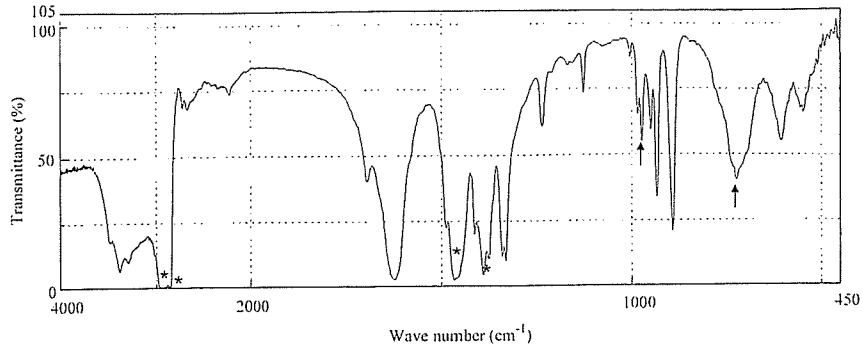

Fig. 4. IR spectrum of a 1 : 10 mixture of dried betaine and KBr (the paste method)
*Bands due to liquid paraffin.
↑ extra bands observed by the paste method.

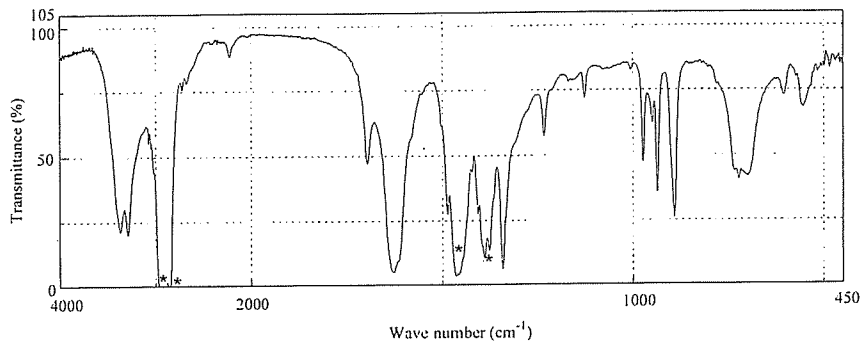

Fig. 5. IR spectrum of a 1 : 10 mixture of dried betaine and KCl (the paste method)
*Bands due to liquid paraffin.

Thus, there was an interaction between betaine and KBr during the procedures of mixing and grinding, or compressing, which resulted in a change of the IR spectrum. In the KBr method, it is well known that a sample may form a solid complex with KBr[8], or may interact with KBr through hydrogen bonding or ion-exchange[9, 10]. To elucidate the causes of the altered spectrum, the IR spectrum of betaine was measured by the paste method using a paste prepared by mixing betaine and alkali metal halide (KBr or KCl) at the mixing ratio of about 1 : 10 (betaine : alkali metal halide), and grinding in agate mortar. The measurement was carried out using a portion of the resultant paste, with a greater thickness than usual. The results are shown in Figs. 4 and 5.

The IR spectrum measured by the paste method in the presence of KBr (Fig. 4) and that of the KBr method (Fig. 2) are in good agreement in the region between 3,500 and 3,000 cm^{-1}, and almost in agreement in the region of 1,000–800 cm^{-1} although extra bands (marked with ↑) exist. From these results, it was presumed that the spectrum of betaine is easily changed even by very mixing and grinding betaine with KBr. When the KBr disk was prepared by very brief grinding, the IR spectrum of betaine was again different from that shown in Fig. 2.

It has been reported that the spectrum of betaine measured by the KBr method is influenced by the relative humidity in the measuring room[2]. It has also been reported that the IR spectrum of betaine anhydride, which was obtained by crystallization from ethanol, coincided with that of betaine hydrate when measurement was conducted by the KBr method under the condition of 12% or higher relative humidity[2]. We measured the IR spectrum at a humidity level as low as possible (23℃, relative humidity 30%), but could not obtain the IR spectrum of betaine anhydride. The relative humidity varies greatly within Japan, and also among the cold districts (Hokkaido, North Europe, *etc.*) where betaine is mainly produced. Such regional differences in humidity may be linked to the variations observed in the IR spectra provided by the manufacturers in different districts, since test laboratories are not necessarily environmentally controlled. It was therefore considered that the KBr method does not necessarily give an IR spectrum with good reproducibility.

On the other hand, the IR spectrum measured by the paste method in the presence of KCl (Fig. 5) is in agreement with that measured by the KCl disk method (Fig. 3) in the region between 1,000 and 800 cm^{-1}. In addition, these IR spectra are almost in agreement with the IR spectrum of betaine hydrate measured by the paste method described below, and, also are similar to the IR spectrum provided by NIST. These facts indicate that the KCl method is preferable, and that betaine tends to absorb moisture to form a hydrate. Therefore, further investigation into the IR spectrum was conducted by the paste method, which was hardly affected by humidity.

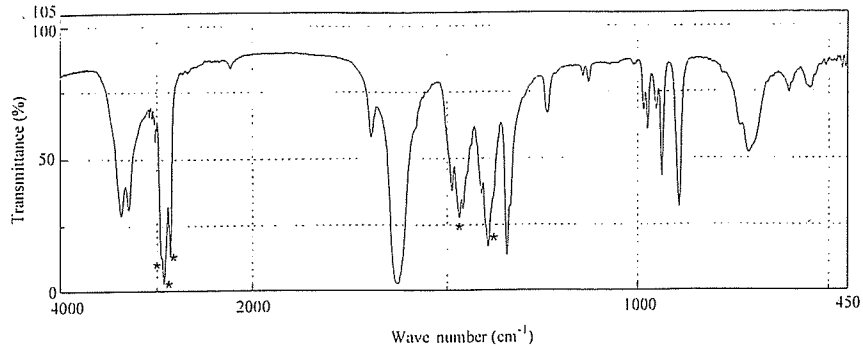

Fig. 6. IR spectrum of commercial betaine (the paste method)
*Bands due to liquid paraffin.

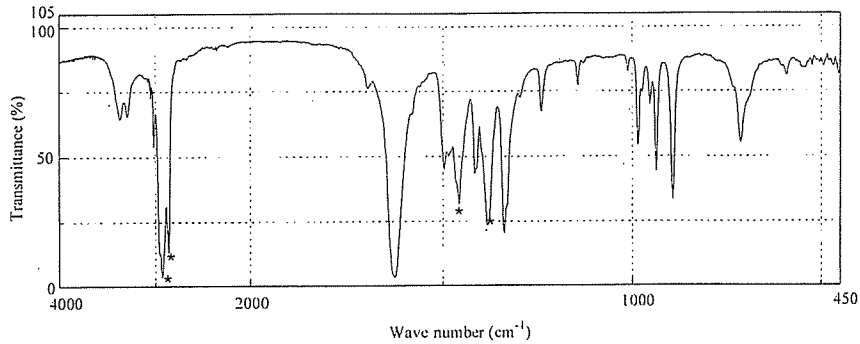

Fig. 7. IR spectrum of betaine dried over phosphorus pentoxide at 105℃ for 3 hours (the paste method)
*Bands due to liquid paraffin.

2. Examination by the paste method

In the paste method, a target substance is coated with liquid paraffin when preparing a paste for measurement. Therefore, the IR spectrum should be littel affected by humidity, even if the substance is hygroscopic. Figure 6 shows the IR spectrum of a betaine product (commercially available bulk powder) measured by the paste method without drying treatment. The high similarity of the IR spectrum in Fig. 6 to that of betaine hydrate in The Sigma Library[6] indicates that the bulk powder contained predominantly hydrate. Because the water content generally varies from one betaine product to another, the IR spectrum of a single product cannot serve as a standard IR spectrum. Another bulk powder also gave a similar IR spectrum to that shown in Fig. 6.

We then examined suitable drying conditions for obtaining the anhydride in a reproducible manner. Drying commercial betaine at 105℃ for 3 hours did not affect the IR spectrum according to the General Method of "Loss on Drying Test" in the Voluntary Specifications of Existing Food Additives: 3rd Ed.[1] Thus, drying was conducted over phosphorus pentoxide at 105℃ for 3 hours. The IR spectrum of this dried betaine is shown in Fig. 7. The IR spectrum in Fig. 7 agreed with that of betaine anhydride provided by The Sigma Library[6]. Further, the spectrum in Fig. 7 was in agreement with that of betaine anhydride measured by the KBr method[2], except that the former also contains absorption bands due to liquid paraffin.

When drying was conducted at different temperatures, 120, 140, 170 or 200℃, over phosphorus pentoxide for three hours, the IR spectra of the dried betaine samples were identical. We, therefore, concluded that the IR spectrum shown in Fig. 7 could be available as a standard IR spectrum. Needless to say, when this spectrum is used as a standard in the identification of a betaine product, the spectra must be compared in the region other than that containing the absorption bands marked by "*", which are due to liquid paraffin.

As mentioned above, the IR spectra of betaine hydrate measured by the KBr and paste methods were different. On the other hand, the IR spectrum of betaine anhydride measured by the KBr disk method at low humidity (relative humidity; less than 12%)[2] was in agreement with that of betaine anhydride measured by the paste method. Accordingly, when using the IR spectrum measured by the KBr method in the identification test of betaine, it is necessary to confirm that the spectrum is identical to the spectrum measured by the paste method in order to ensure the reliability of the test results. The identification method based on the IR spectrum has various advantages, including energy saving, and wide applicability, and is a convenient alternative to chemical identification methods. It is therefore important to ensure good reproducibility of the spectrum.

Conclusion

We examined suitable conditions and methods to obtain a reliable and reproducible IR spectrum applica-

ble to identification of betaine as a standard spectrum. It was found that a reproducible spectrum can be obtained by drying betaine at 105℃ for 3 hours over phosphorus pentoxide and then measuring the IR spectrum by the paste method. The IR spectrum thus obtained is suitable to be used as a standard reference IR spectrum.

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