Fig.14 Relationship between concentration of benzoic acid and ratio of the integral of benzoic acid: DSS-$d_6$ signals. (a) $\delta_H$ 7.53, (b) $\delta_H$ 7.65, and (c) $\delta_H$ 7.98.

Table 14 Recoveries of benzoic acid from processed foods

| Sample | Signal ($\delta$, ppm) | 0.063 g kg$^{-1}$ spiked | | 0.13 g kg$^{-1}$ spiked | | Maximum usage level spiked | | |
|---|---|---|---|---|---|---|---|---|
| | | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Level (g kg$^{-1}$) | Recovery (%) | RSD (%) |
| Caviar | 7.49 | 93.6 | 2.6 | 97.7 | 0.7 | 2.5 | 95.2 | 2.2 |
| | 7.94 | 96.5 | 1.5 | 98.7 | 0.4 | | 95.1 | 2.0 |
| Margarine | 7.49 | 86.8 | 0.2 | 86.7 | 1.9 | 1.0 | 90.6 | 1.8 |
| | 7.94 | 84.5 | 0.2 | 86.5 | 2.1 | | 90.7 | 2.0 |
| Avocado paste | 7.49 | 91.5 | 0.6 | 90.9 | 1.4 | 1.0 | 94.4 | 1.0 |
| | 7.94 | 89.6 | 0.2 | 89.9 | 2.6 | | 93.7 | 1.2 |
| Soft drink | 7.49 | 80.5 | 2.0 | 89.4 | 2.6 | 0.60 | 91.9 | 0.8 |
| | 7.94 | 81.0 | 3.0 | 89.2 | 2.7 | | 91.8 | 0.9 |
| Syrup | 7.49 | 81.9 | 3.9 | 92.3 | 1.9 | 0.60 | 96.5 | 1.3 |
| | 7.94 | 83.5 | 3.8 | 91.6 | 2.0 | | 96.4 | 1.6 |
| Soybean sauce | 7.49 | 91.4 | 1.6 | 92.5 | 0.5 | 0.60 | 91.1 | 3.6 |
| | 7.94 | 88.5 | 0.6 | 92.2 | 0.4 | | 91.1 | 3.6 |

Each recovery value represents the mean of three independent experiments on the same day.

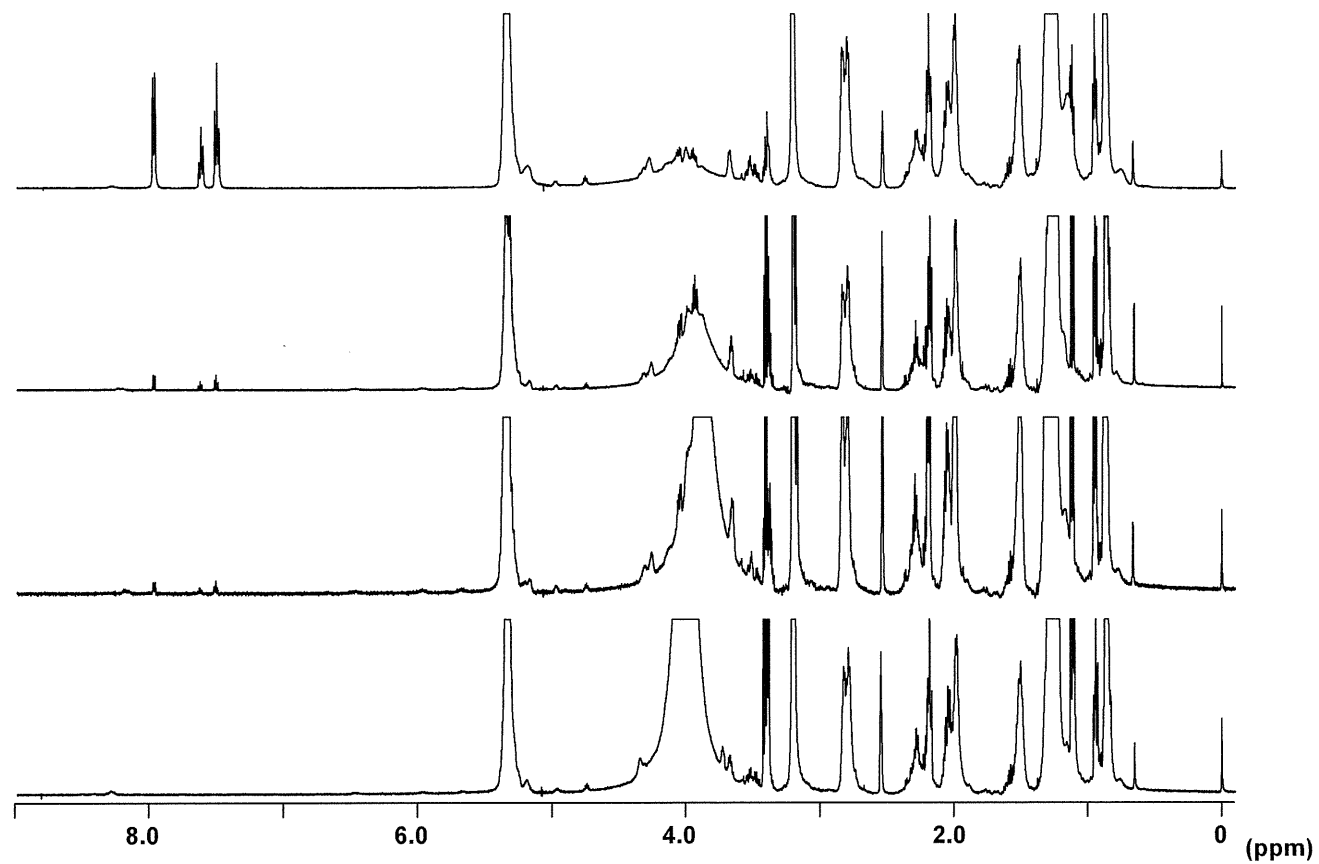RSD, intra-day relative standard deviation.

Fig.15 $^1$H NMR spectrum of caviar extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg$^{-1}$ (second), at 0.063 g kg$^{-1}$ (third), and blank (bottom).
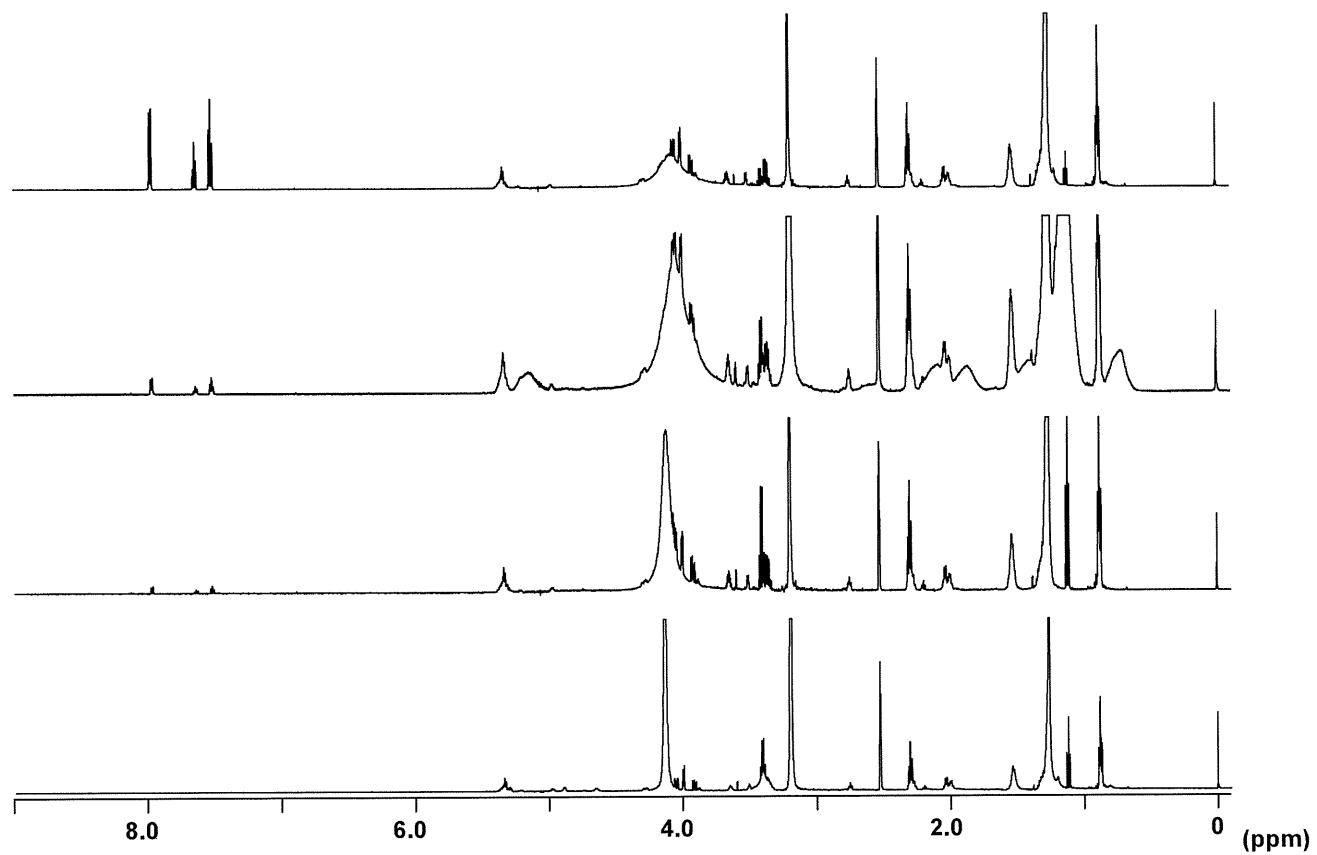
Fig.16 $^1$H NMR spectrum of margarine extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg$^{-1}$ (second), at 0.063 g kg$^{-1}$ (third), and blank (bottom).
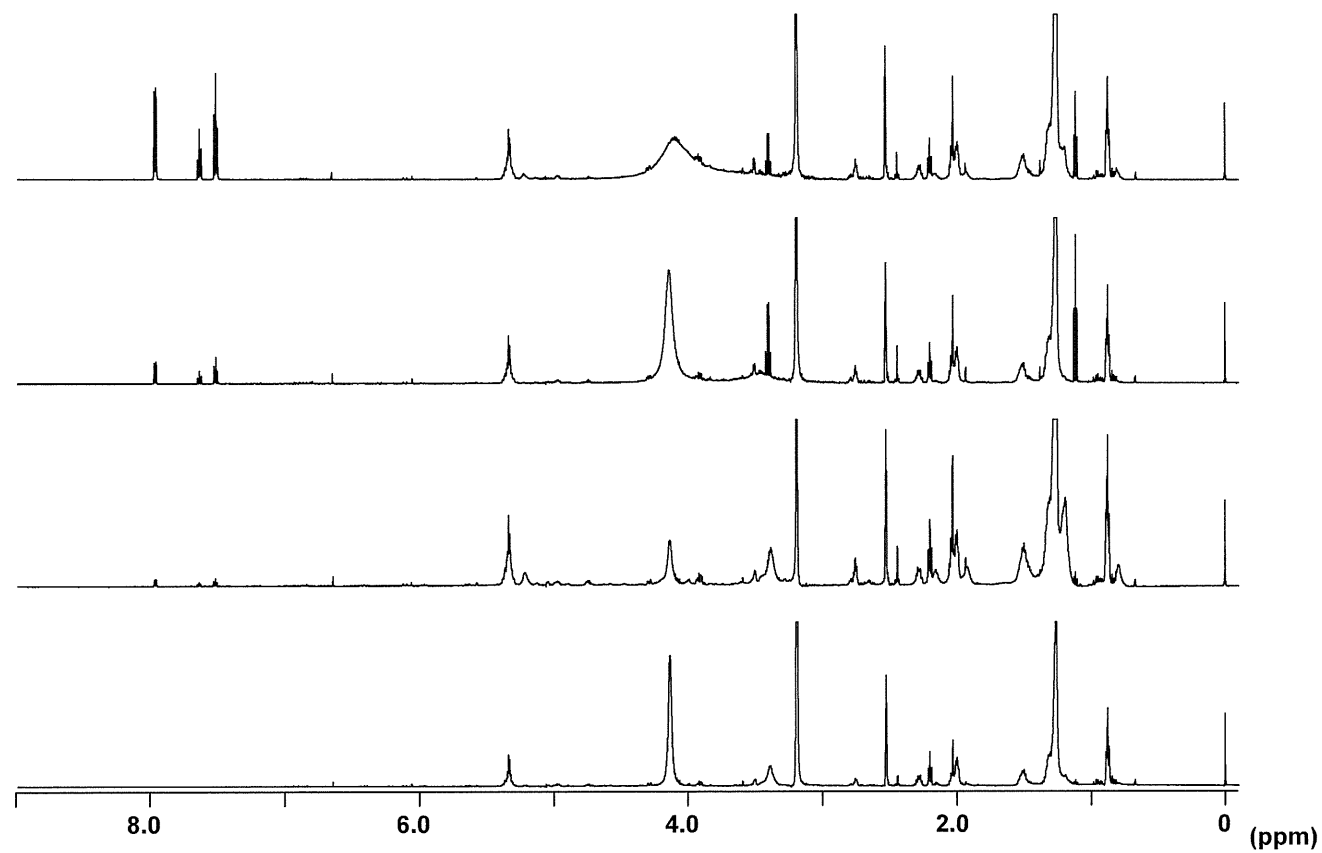
Fig.17  $^1$H NMR spectrum of avocado paste extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg$^{-1}$ (second), at 0.063 g kg$^{-1}$ (third), and blank (bottom).
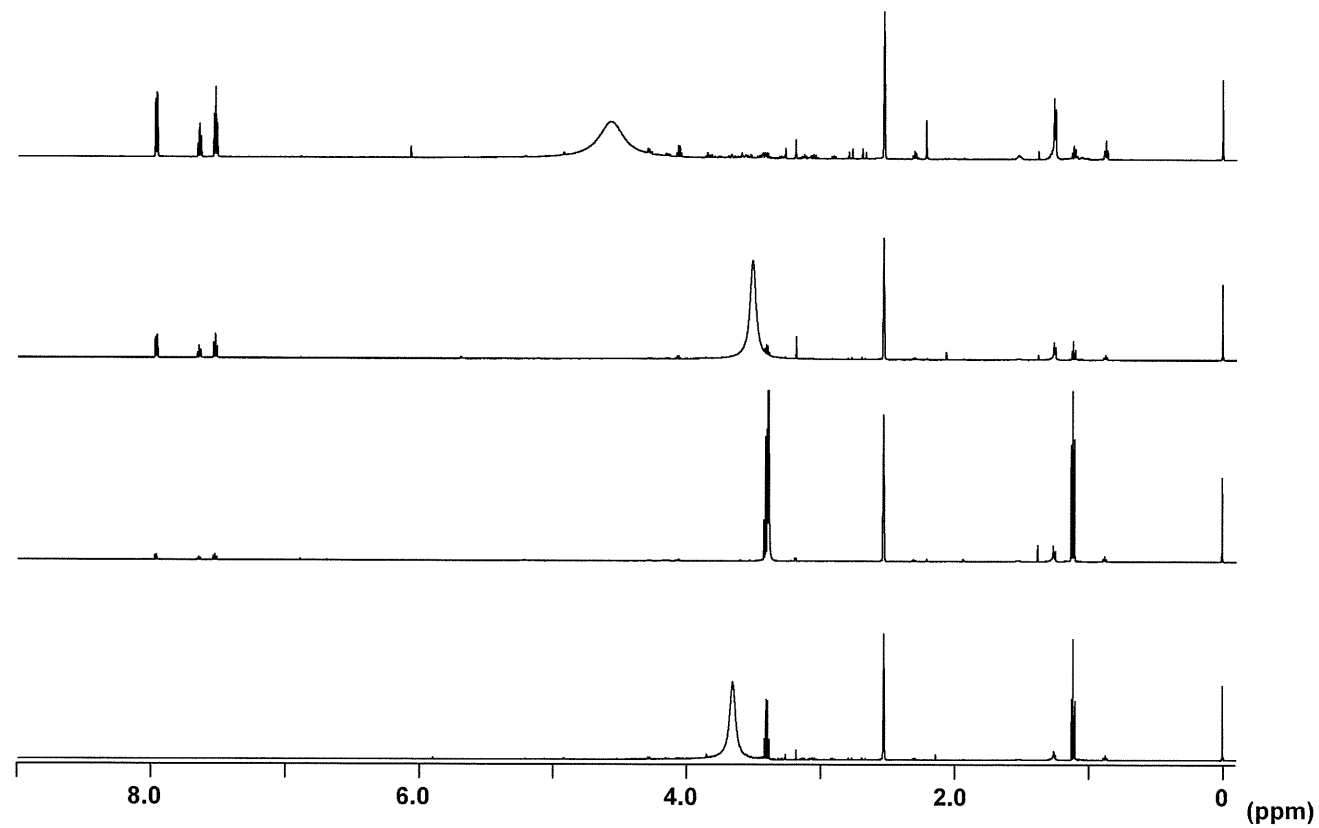
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Fig.18  $^1$H NMR spectrum of soft drink extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg$^{-1}$ (second), at 0.063 g kg$^{-1}$ (third), and blank (bottom).
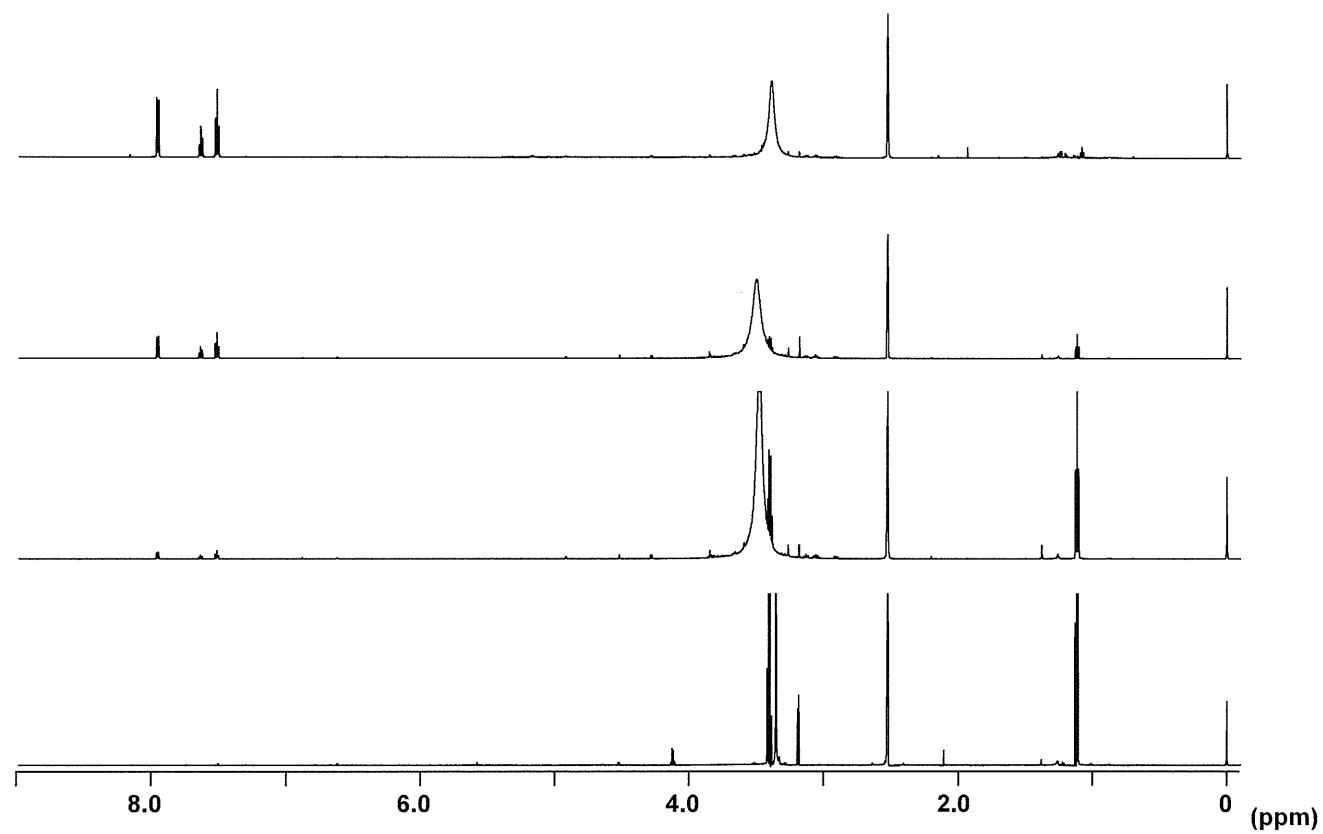
Fig.19  $^1$H NMR spectrum of syrup extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg$^{-1}$ (second), at 0.063 g kg$^{-1}$ (third), and blank (bottom).
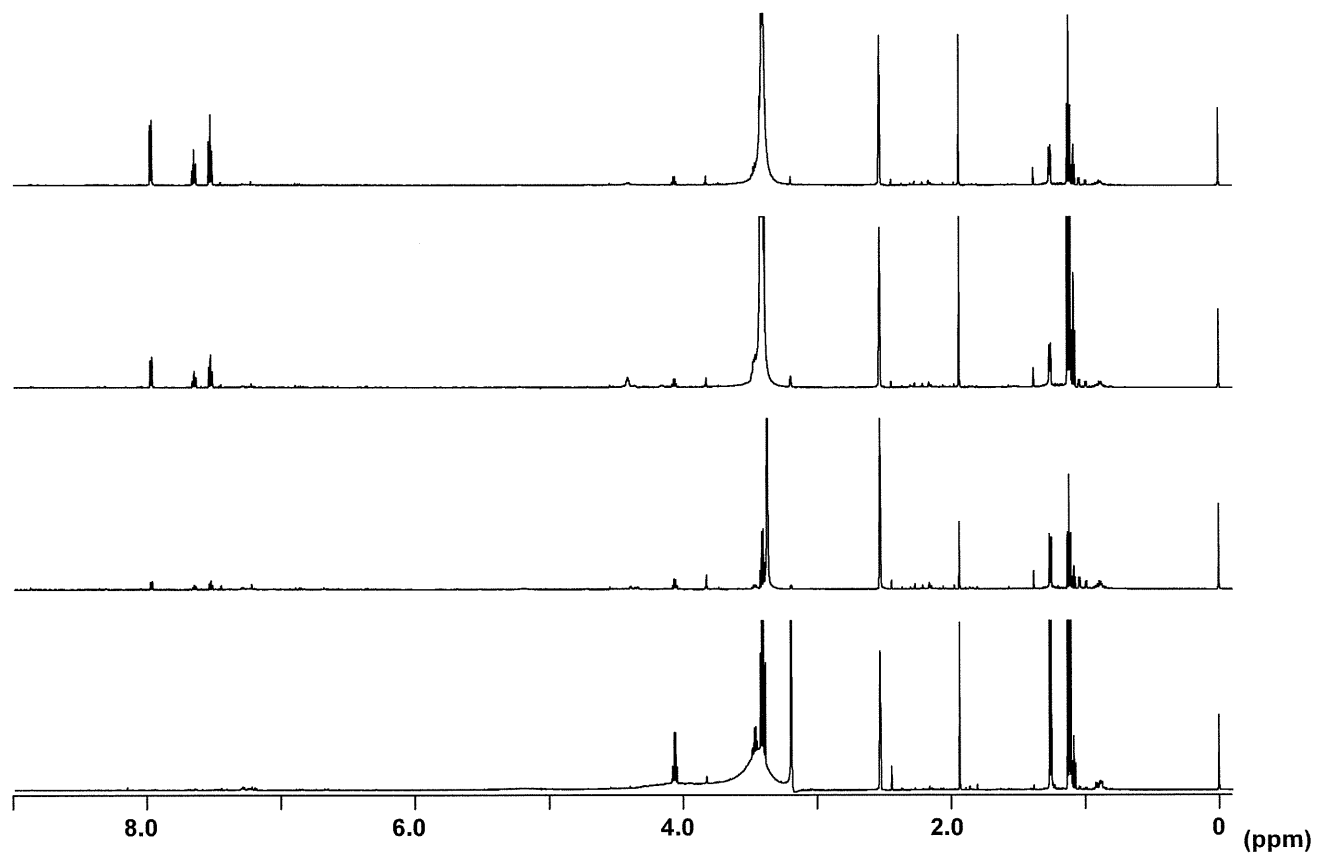
45

Fig.20 $^1$H NMR spectrum of soybean sauce extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg$^{-1}$ (second), at 0.063 g kg$^{-1}$ (third), and blank (bottom).

Table 15  Comparison of benzoic acid contents in commercial food determined by two methods.

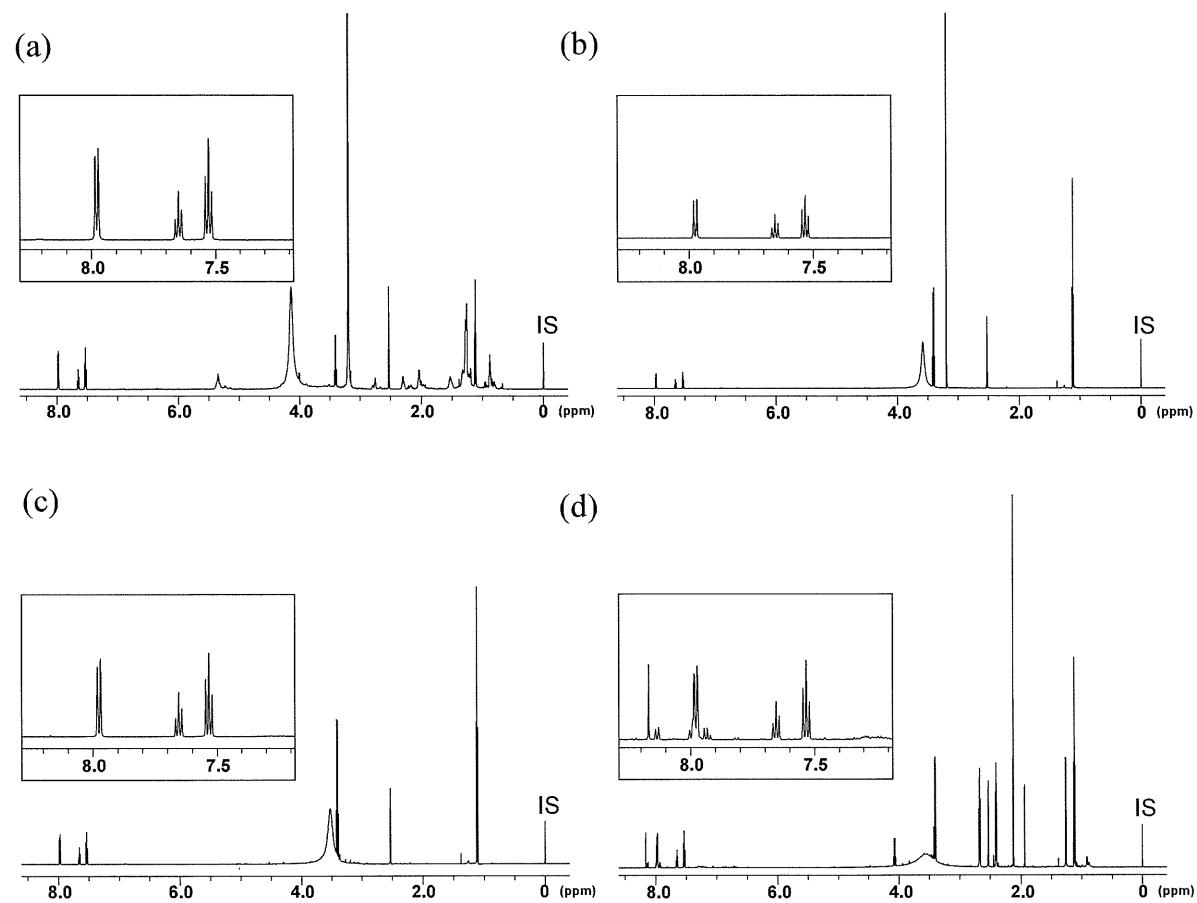| Sample | Proposed method (Solvent extraction/qHNMR) | | | Conventional method (Steam distillation/HPLC) | |
|---|---|---|---|---|---|
| | Signal ($\delta$, ppm) | Content (g kg$^{-1}$) | RSD (%) | Content (g kg$^{-1}$) | RSD (%) |
| Margarine | 7.49 | 0.46 | 4.0 | 0.47 | 1.1 |
| | 7.94 | 0.46 | 4.0 | | |
| Soft drink | 7.49 | 0.26 | 4.5 | 0.25 | 0.1 |
| | 7.94 | 0.26 | 5.0 | | |
| Syrup | 7.49 | 0.48 | 2.1 | 0.45 | 0.9 |
| | 7.94 | 0.48 | 2.2 | | |
| Soybean sauce | 7.49 | 0.45 | 4.9 | 0.47 | 0.5 |
| | 7.94 | — | — | | |

Fig. 21 $^1$H NMR spectra of each sample solution from commercially produced food containing benzoic acid (BA). The BA signals shown on the top is highlighted. (a) Margarine. (b) Soft drink. (c) Syrup. (d) Soybean sauce. IS, internal standard (DSS-$d_6$).
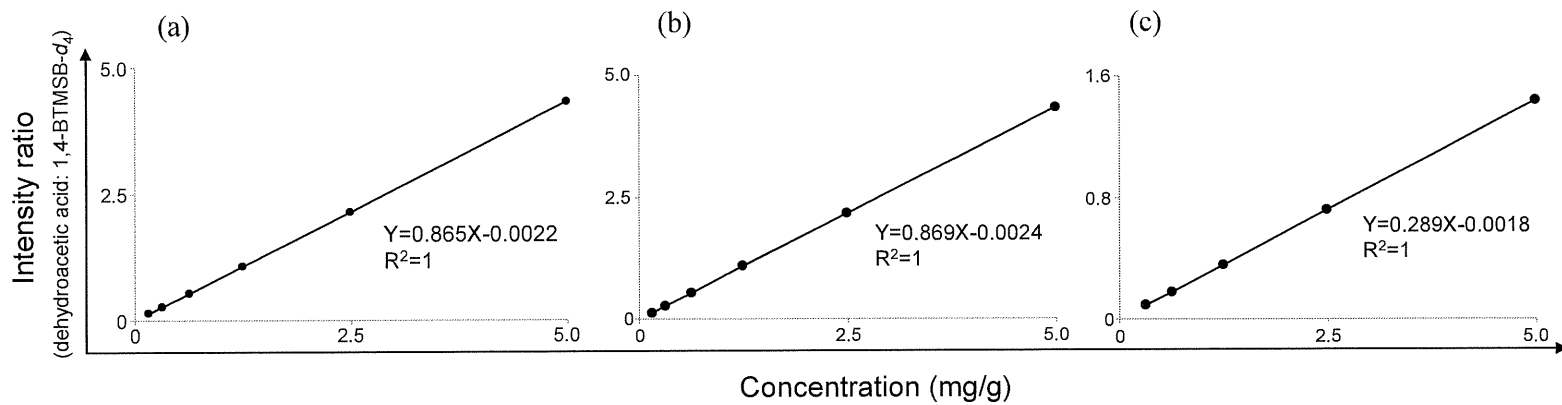
Fig.22    Relationship between concentration of dehydroacetic acid (DA) and ratio of the integral of DA: 1,4-BTMSB-$d_4$ signals. (a) $\delta_H$ 2.22, (b) $\delta_H$ 2.56, and (c) $\delta_H$ 6.03.

Table 16  Intra- and Inter-day recoveries, repeatability, and intermediate precisions of dehydroacetic acid in butter, cheese, and margarine.

| Sample | Intra-day | | | | Inter-day | | | | | |
| | 0.13 g kg$^{-1}$ spiked | | 0.50 g kg$^{-1}$ spiked | | 0.13 g kg$^{-1}$ spiked | | | 0.50 g kg$^{-1}$ spiked | | |
| | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD$_r$ (%) | RSD$_{ip}$ (%) | Recovery (%) | RSD$_r$ (%) | RSD$_{ip}$ (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Butter | 92.4 | 4.6 | 90.4 | 3.1 | 88.3 | 6.6 | 8.2 | 89.5 | 5.3 | 5.4 |
| Cheese | 91.2 | 2.1 | 93.8 | 3.8 | 86.8 | 4.1 | 7.1 | 89.4 | 3.3 | 4.4 |
| Margarine | 92.1 | 1.8 | 96.9 | 1.3 | 93.1 | 3.1 | 5.4 | 94.6 | 2.1 | 6.0 |

Intra-day recovery value, the mean of analysis results in triplicate on same one day. Inter-day recovery value, the mean of analysis results in duplicate on five different days. RSD$_r$ (repeatability) and RSD$_{ip}$ (intermediate precision) are calculated by one-way analysis of variance of the recovery values obtained in duplicate on five different days.
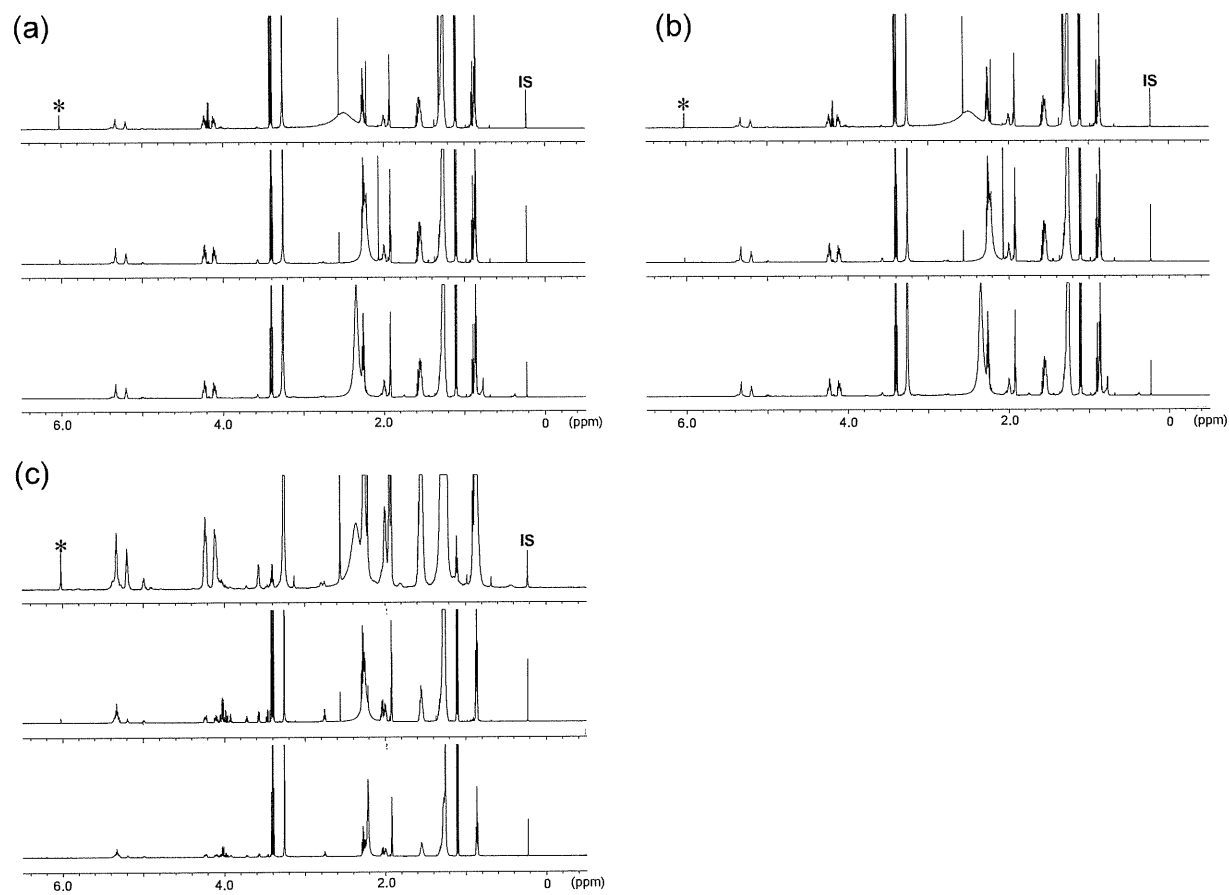
Fig. 23    $^1$H NMR spectra of each sample extract spiked with dehydroacetic acid at the maximum usage level (0.5 g kg$^{-1}$) (top), 0.13 g kg$^{-1}$ (middle), and blank (bottom). Signals marked with asterisks were used for quantification. (a) Butter. (b) Cheese. (c) Margarine. IS, internal standard (1,4-BTMSB-$d_4$).

Table 17    Comparison of dehydroacetic acid contents in commercial foods determined by two methods.

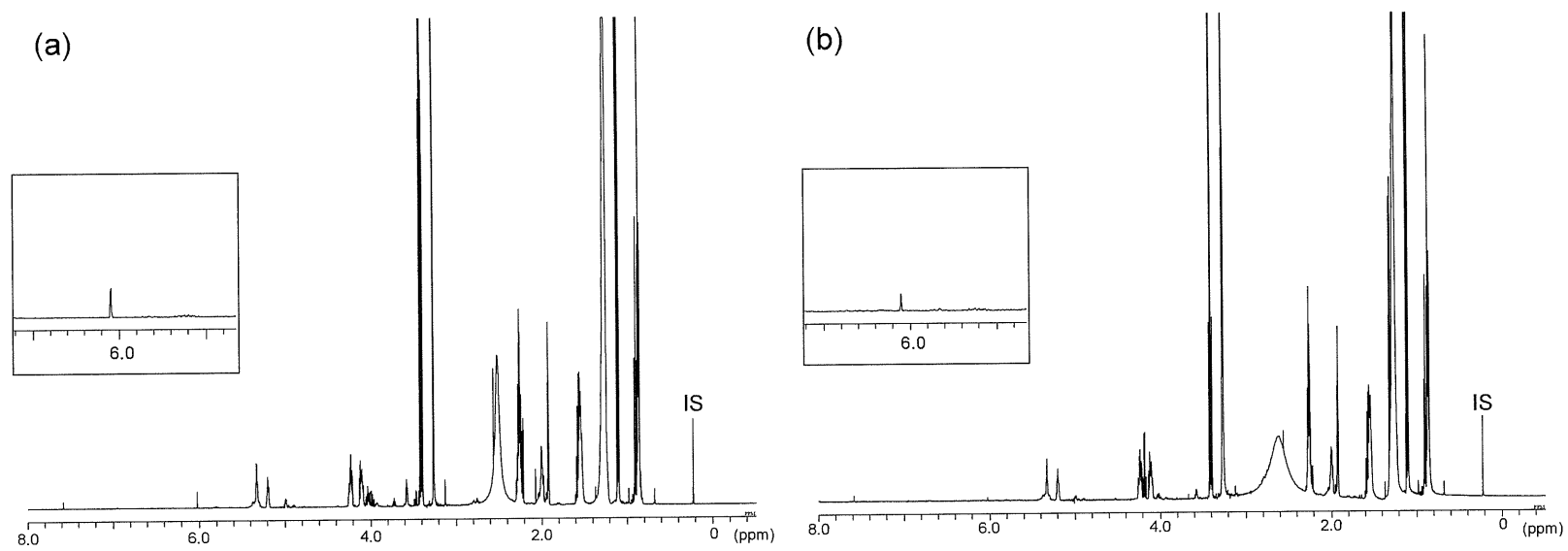| Sample | Proposed method (Solvent extraction/qHNMR) | | Conventional method (Steam distillation/HPLC) | |
|---|---|---|---|---|
| | Content (g kg$^{-1}$) | RSD (%) | Content (g kg$^{-1}$) | RSD (%) |
| Butter | 0.30 | 4.6 | 0.26 | 6.8 |
| Cheese | 0.13 | 6.6 | 0.12 | 2.4 |

Fig. 24  $^1$H NMR spectra of each sample solution from commercially produced food containing dehydroacetic acid (DA).   The DA signals at $\delta_H$ 6.03 shown on the top is highlighted.

(a) Butter.   (b) Cheese. IS, internal standard (1,4-BTMSB-$d_4$).

研究成果の刊行に関する一覧

雑誌

1. Ohtsuki, T.; Sato, K.; Sugimoto, N.; Akiyama, H.; Kawamura, Y. "Absolute quantitative analysis for sorbic acid in processed foods using proton nuclear magnetic resonance spectroscopy", *Analytica Chimica Acta* in press.