

Fig.6 ^1H NMR spectrum of sodium salt of 2-(1,3-dihydro-3-oxo-2H-indol-2-ylidene)-2,3-dihydro-3-oxo-1H-indole-5-sulfonic acid in $\text{DMSO-}d_6$ containing $\text{DSS-}d_6$

Table 2 Purity of dehydroacetic acid standard and reagent (analytical grade) determined by qNMR (n=3)

Signal (δ , ppm)	Number of protons	Standard		Reagent	
		Purity (%)	RSD (%)	Purity (%)	RSD (%)
2.22	3	99.7	0.1	99.0	0.1
2.56	3	99.7	0.1	98.9	0.2
6.03	1	99.6	0.1	98.8	0.1

Table 3 Purity of acesulfame determined by qNMR (n=3)

Signal (δ , ppm)	Number of protons	Purity (%)	RSD (%)
2.20	3	97.8	0.1
6.04	1	97.7	0.1

Table 4 Purity of Food color B2 determined by qNMR (n=3)

Signal (δ , ppm)	Number of protons	Purity (%)	RSD (%)
7.28	2	91.1	0.3
7.76	2	91.0	0.3
7.80	2	91.5	0.1
10.7	2	89.3	0.8

Table 5 Purity of sodium salt of 2-(1,3-dihydro-3-oxo-7-sulfo-2H-indol-2-ylidene)-2,3-dihydro-3-oxo-1H-indole-5-sulfonic acid determined by qNMR (n=3)

Signal (δ , ppm)	Number of protons	Purity (%)	RSD (%)
7.67	1	40.1	0.5
7.71	1	40.1	0.5

Table 6 Purity of sodium salt of 2-(1,3-dihydro-3-oxo-2H-indol-2-ylidene)-2,3-dihydro-3-oxo-1H-indole-5-sulfonic acid determined by qNMR (n=3)

Signal (δ , ppm)	Number of protons	Purity (%)	RSD (%)
6.98	1	65.4	1.4
7.35	1	63.9	0.3
7.54	1	67.3	0.1

Table 7 Comparison of purities of dehydroacetic acid determined by qNMR and neutralization titration methods

	Purity (%)	RSD (%)
qNMR	98.9	0.1
Neutralization titration	98.7	0.2

qNMR: Values represent the mean \pm standard deviation of purities obtained from three signals (δ_{H} 2.22, 2.56, and 6.03).

Titration: Values represent the mean \pm standard deviation of three independent experiments.

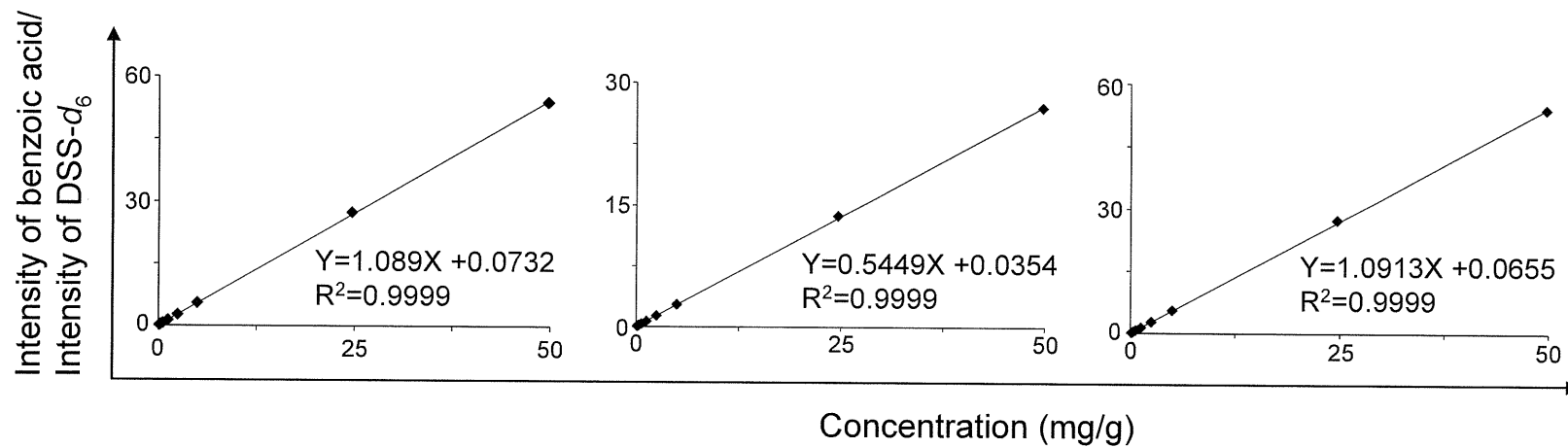


Fig.7 Relationship between concentration of benzoic acid and ratio of the integral of benzoic acid: DSS-*d*₆ signals. (a) δ_H 7.53, (b) δ_H 7.65, and (c) δ_H 7.98.

Table 8 Recoveries of benzoic acid from processed foods

Sample	Signal (δ , 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.

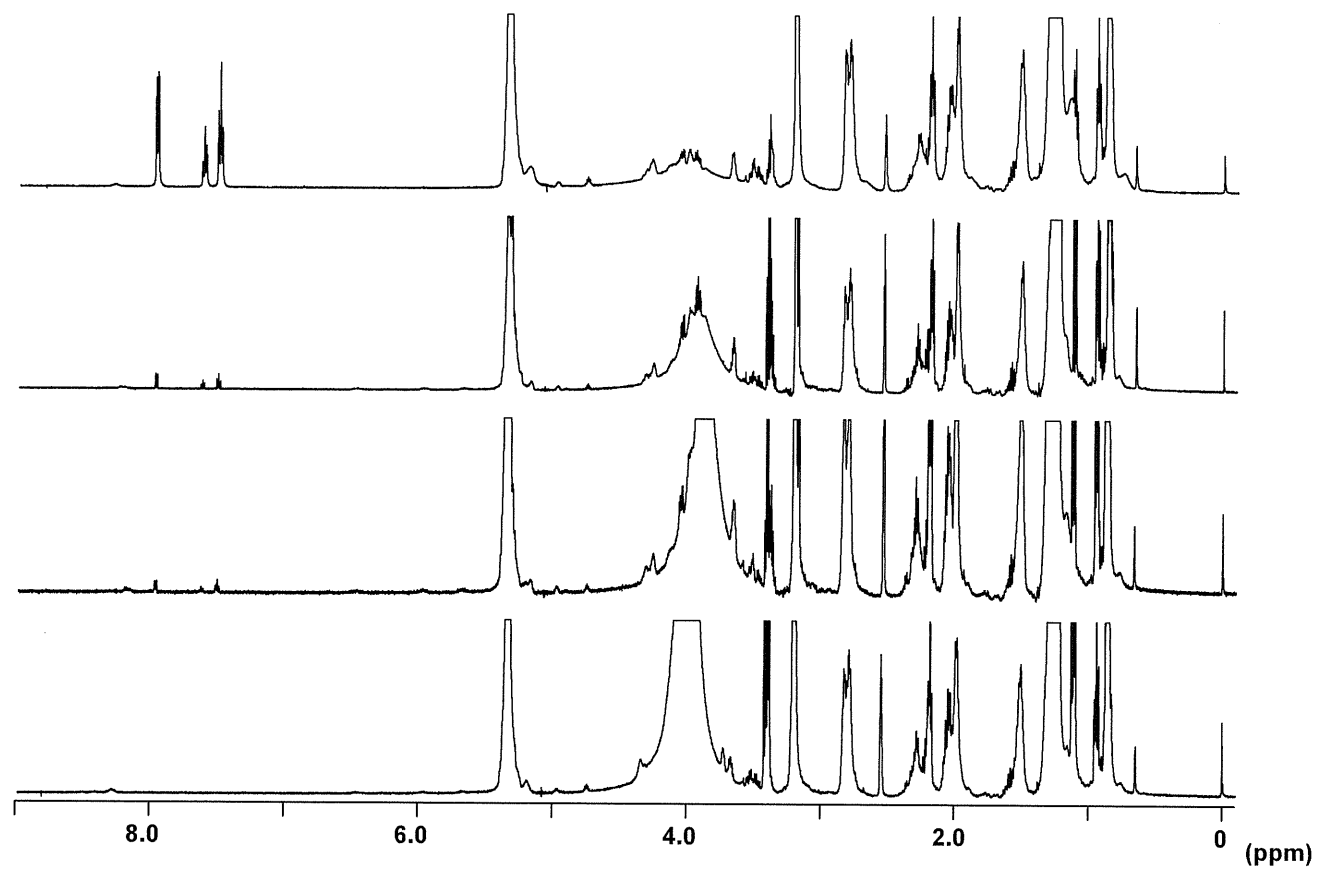


Fig.8 ^1H NMR spectrum of caviar extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg^{-1} (second), at 0.063 g kg^{-1} (third), and blank (bottom).

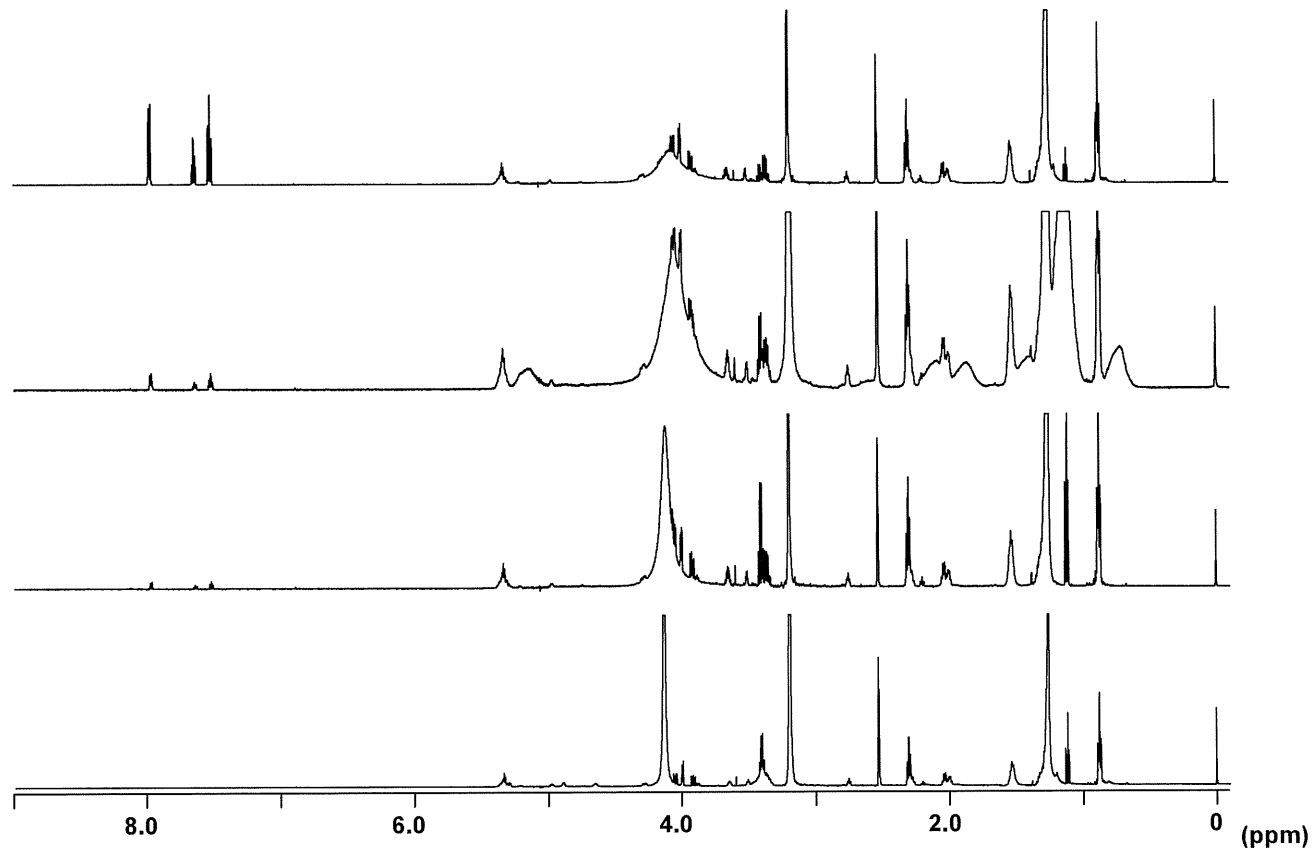


Fig.9 ^1H NMR spectrum of margarine extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg^{-1} (second), at 0.063 g kg^{-1} (third), and blank (bottom).

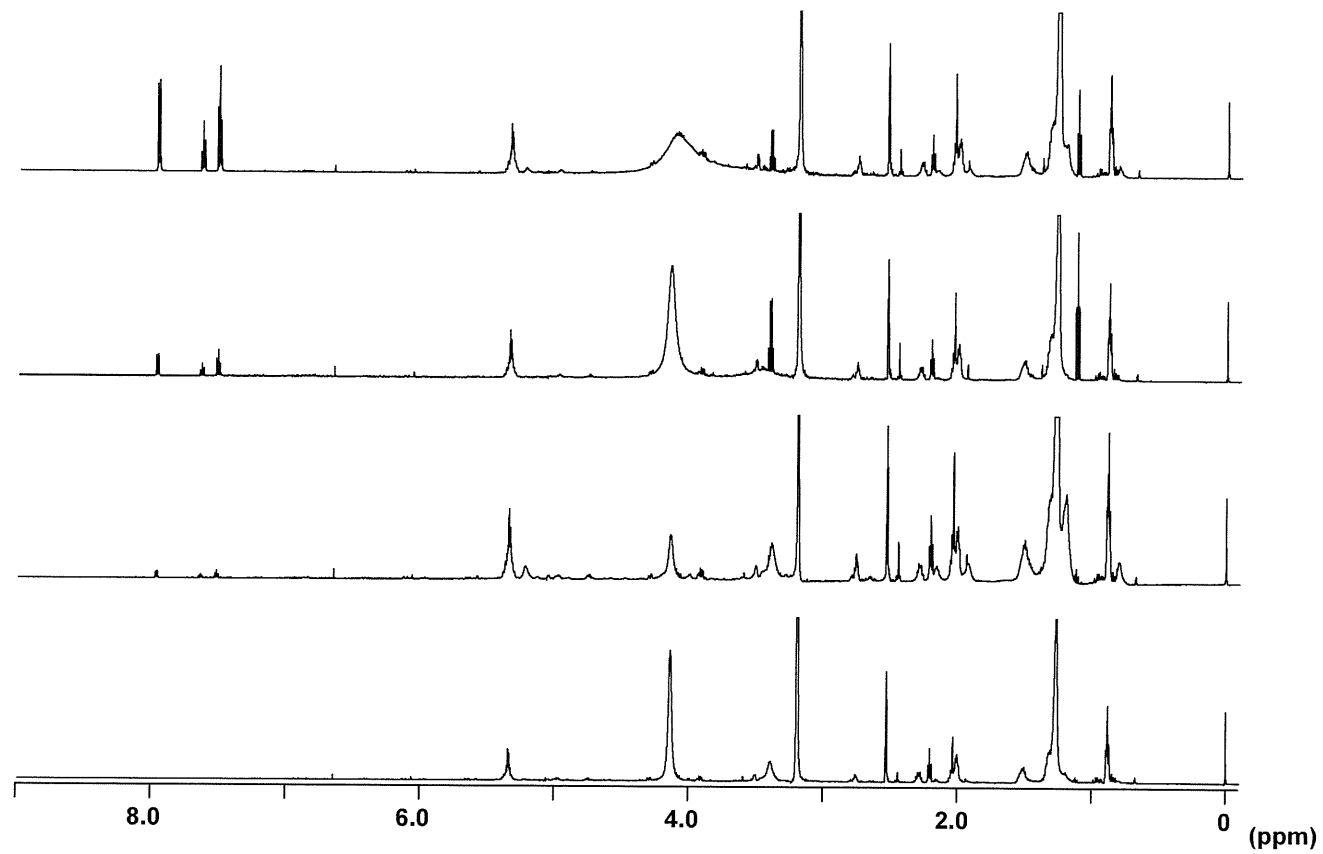


Fig.10 ^1H NMR spectrum of avocado paste extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg^{-1} (second), at 0.063 g kg^{-1} (third), and blank (bottom).

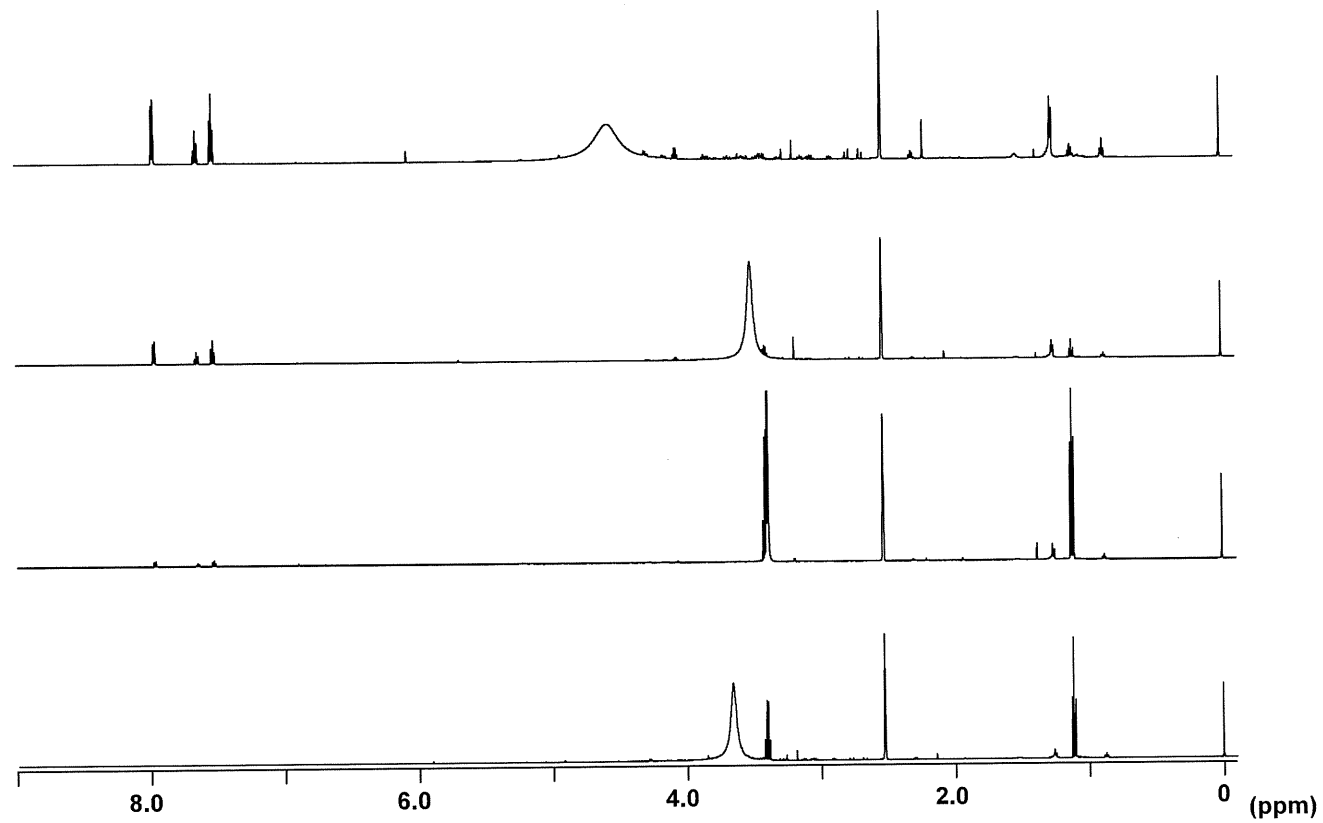


Fig.11 ^1H NMR spectrum of soft drink extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg^{-1} (second), at 0.063 g kg^{-1} (third), and blank (bottom).

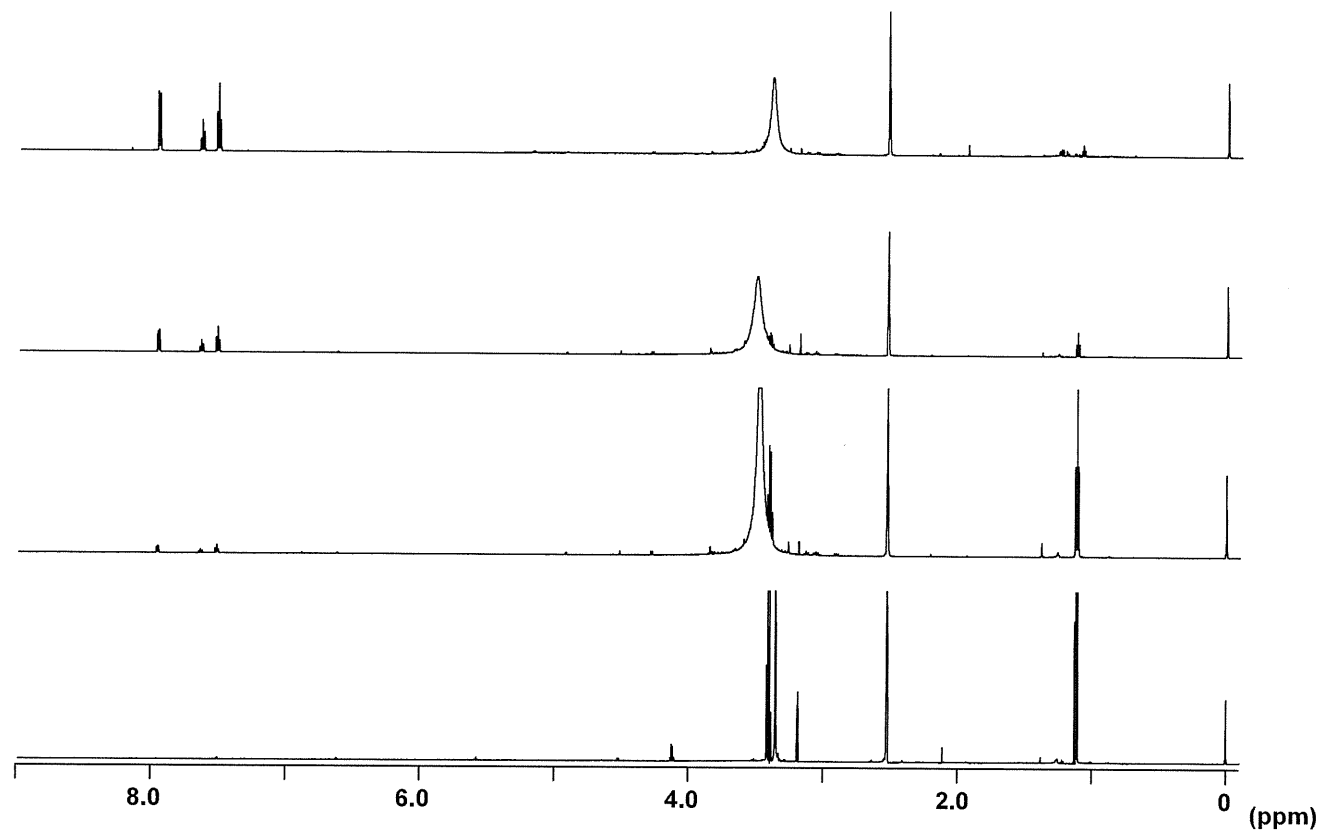


Fig.12 ^1H NMR spectrum of syrup extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg^{-1} (second), at 0.063 g kg^{-1} (third), and blank (bottom).

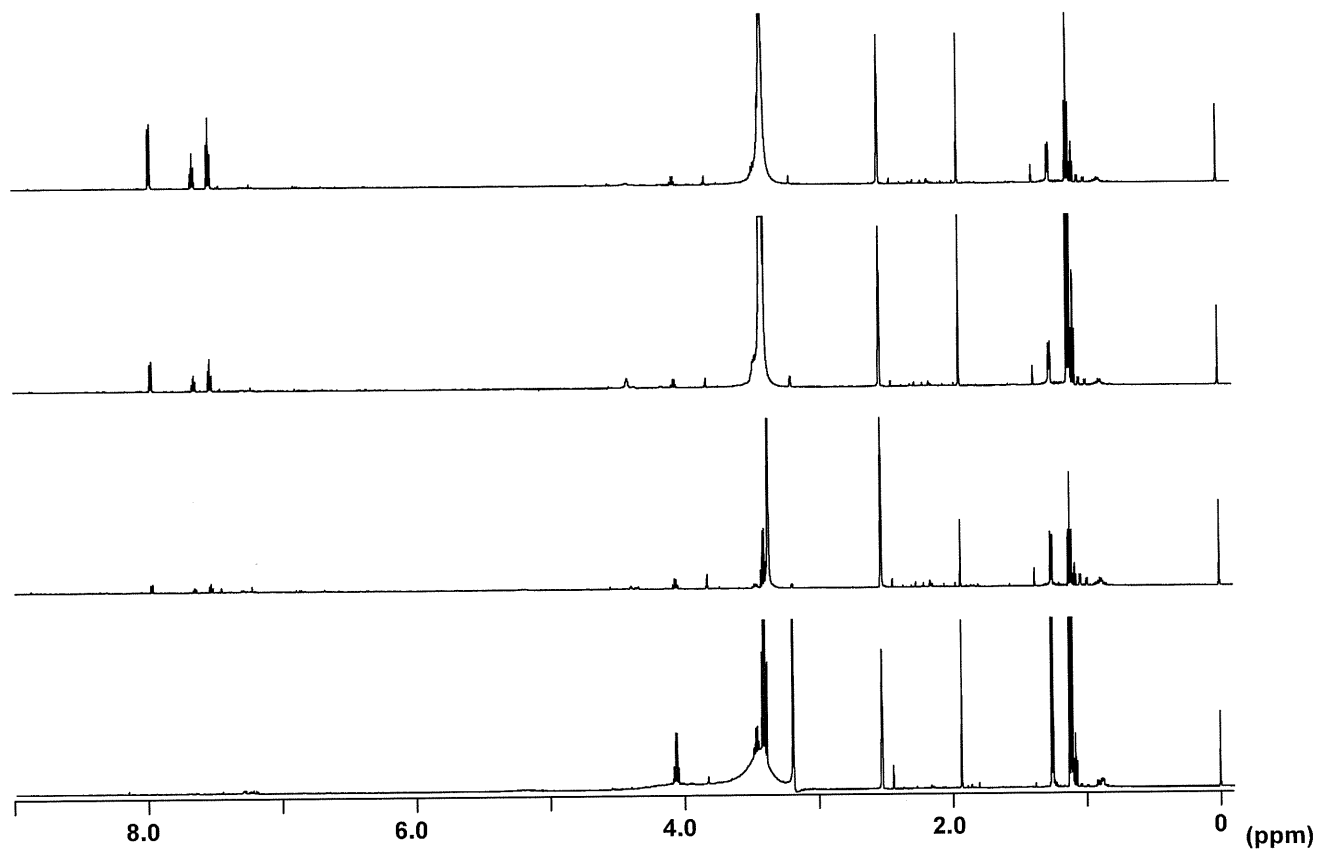


Fig.13 ^1H NMR spectrum of soybean sauce extract spiked with benzoic acid at the maximum usage level of each processed food (top), at 0.13 g kg^{-1} (second), at 0.063 g kg^{-1} (third), and blank (bottom).

Table 9. Comparison of benzoic acid contents in commercial food determined by two methods.

Sample	Proposed method (Solvent extraction/qNMR)			Conventional method (Steam distillation/HPLC)	
	Signal (δ , ppm)	Content (g kg ⁻¹)	RSD (%)	Content (g kg ⁻¹)	RSD (%)
Margarine	7.53	0.46	4.0	0.47	1.1
	7.98	0.46	4.0		
Soft drink	7.53	0.26	4.5	0.25	0.1
	7.98	0.26	5.0		
Syrup	7.53	0.48	2.1	0.45	0.9
	7.98	0.48	2.2		
Soybean sauce	7.53	0.45	4.9	0.47	0.5
	7.98	—	—		

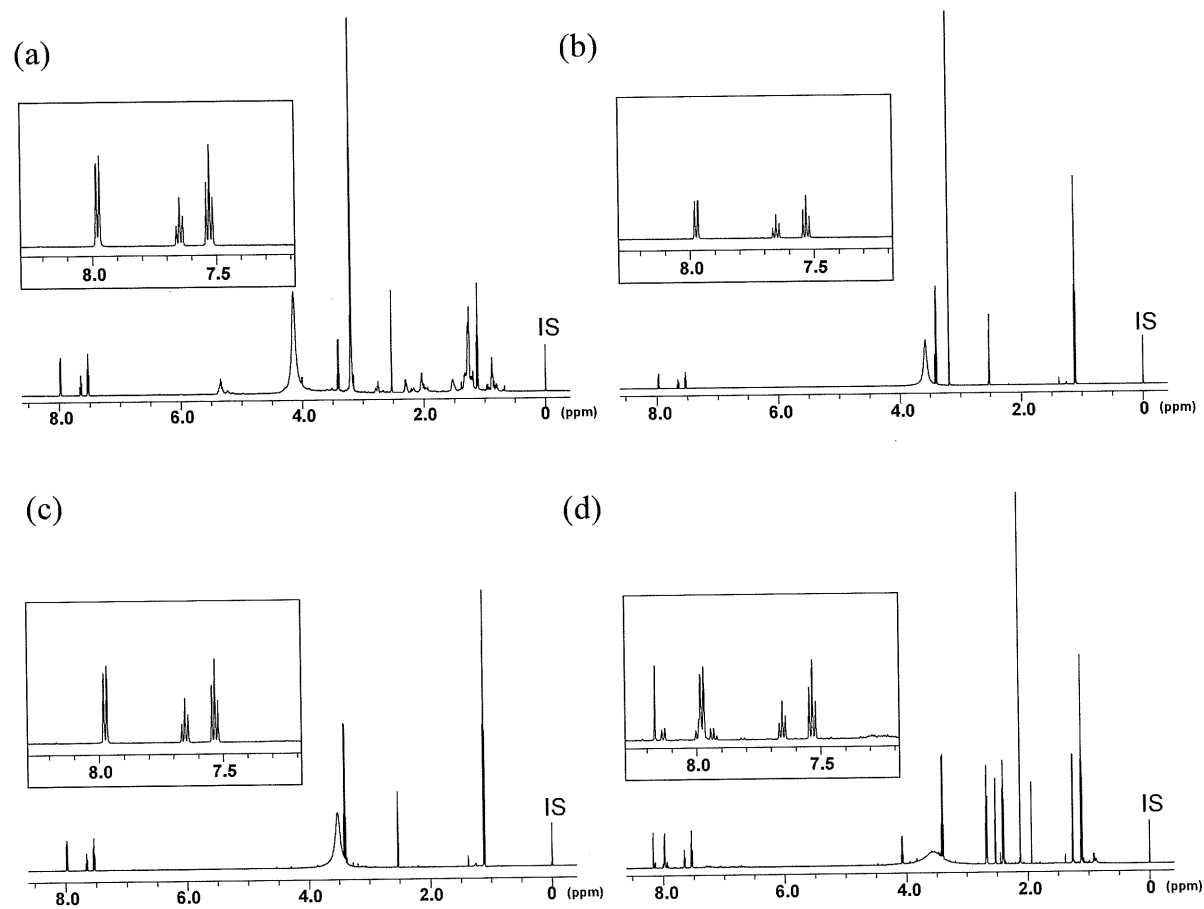


Fig. 14 ^1H NMR spectra of each sample solution from commercially produced food containing benzoic acid (BA). The BA signals shown on the top is highlighted. (a) Margarine. (b) Soft drink. (c) Syrup. (d) Soybean sauce. IS, internal standard ($\text{DSS-}d_6$).

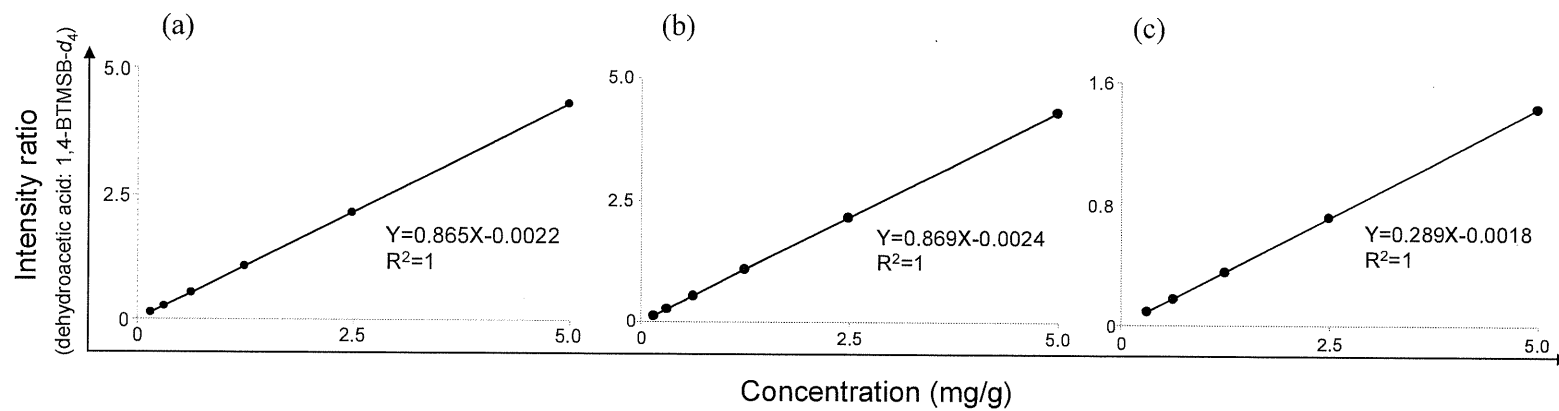


Fig.15 Relationship between concentration of dehydroacetic acid (DA) and ratio of the integral of DA: 1,4-BTMSB- d_4 signals. (a) δ_H 2.22, (b) δ_H 2.56, and (c) δ_H 6.03.

Table 10 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.

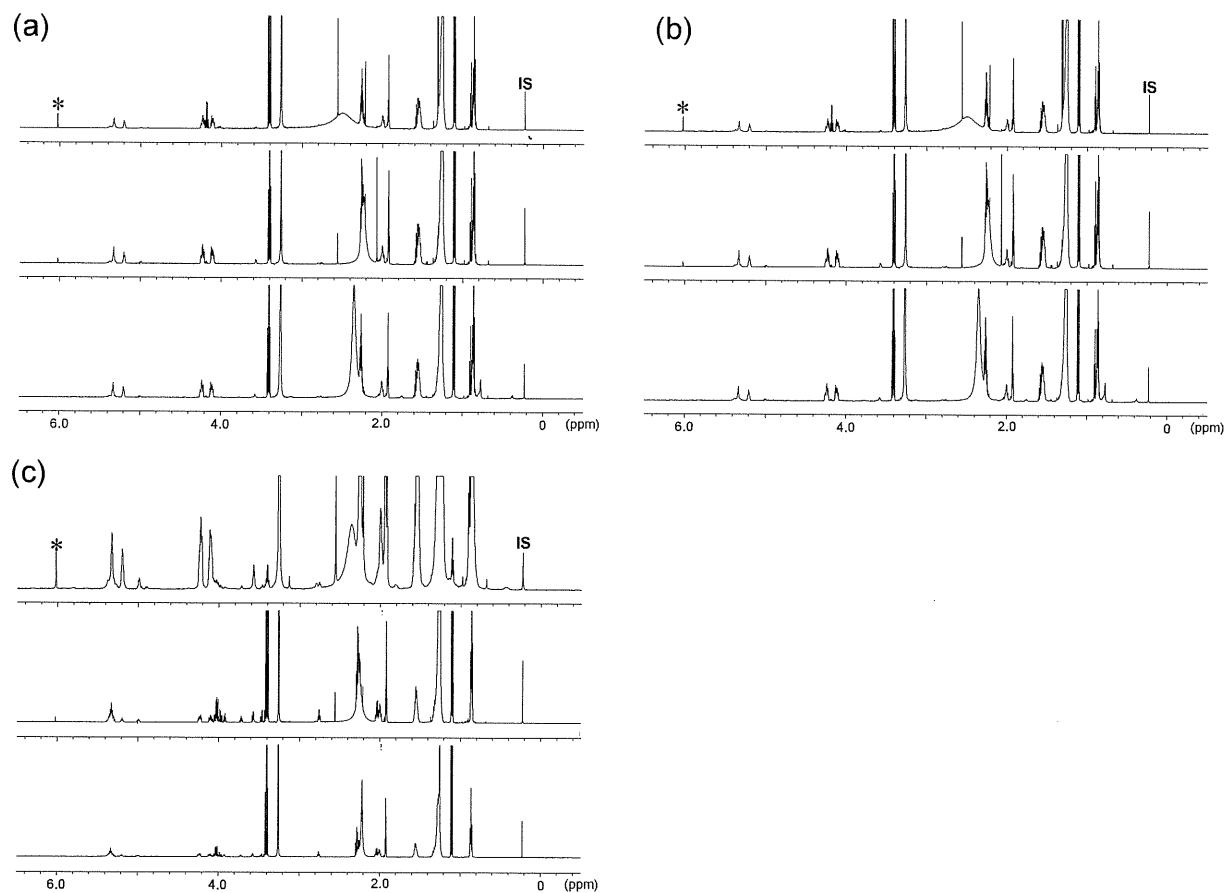


Fig. 16 ^1H NMR spectra of each sample extract spiked with dehydroacetic acid at the maximum usage level (0.5 g kg^{-1}) (top), 0.13 g kg^{-1} (middle), and blank (bottom). Signals marked with asterisks were used for quantification. (a) Butter. (b) Cheese. (c) Margarine. IS, internal standard (1,4-BTMSB- d_4).

Table 11 Comparison of dehydroacetic acid contents in commercial foods determined by two methods.

Sample	Proposed method (Solvent extraction/qNMR)		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

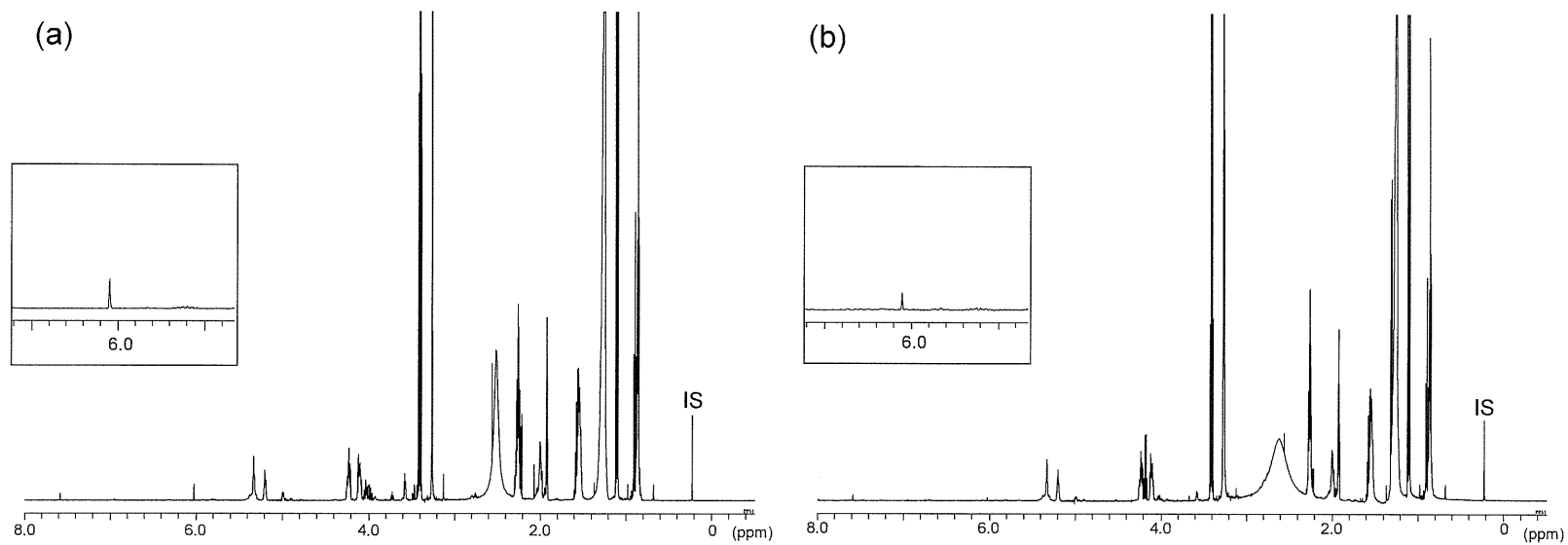


Fig. 17 ^1H NMR spectra of each sample solution from commercially produced food containing dehydroacetic acid (DA). The DA signals at $\delta_{\text{H}} 6.03$ shown on the top is highlighted.

(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表

雑 誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
	なし				