

Supporting Information for:

Real-Time Monitoring of Chemical Changes in Three Kinds of Fermented Milk Products During Fermentation Using Quantitative Difference Nuclear Magnetic Resonance Spectroscopy

Yi Lu, Hiroto Ishikawa, Yeondae Kwon, Fangyu Hu, Takuya Miyakawa, and Masaru Tanokura*

Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

*Corresponding author (Tel: +81-3-5841-5165; Fax: +81-3-5841-8023; E-mail: amtanok@mail.ecc.u-tokyo.ac.jp)

Table S1. Assignments of ^1H and ^{13}C signals of compounds in Bulgarian yogurt (pH 5.4).

Compound	Assignment	^1H (ppm)	Multiplicity J (Hz)	^{13}C (ppm)
D-lactose	CH-1'	4.33	d(7.70)	103.57
	CH-2'	3.43		71.79
	CH-3'	3.54		73.31
	CH-4'	3.80		69.38
	CH-5'	3.61		76.15
	CH ₂ OH-6'	3.64		61.94
	CH- α 1	5.11 ^a	d(3.05)	92.62
	CH- α 2	3.47	t(8.85)	71.98
	CH- α 3	3.71		72.24
	CH- α 4	3.54		78.89
	CH- α 5	3.82		70.86
	CH ₂ OH- α 6	3.76		60.66
	CH- β 1	4.55	d(7.90)	96.55
	CH- β 2	3.16 ^a	t(7.83)	74.66
	CH- β 3	3.53		75.17
	CH- β 4	3.54		78.75
	CH- β 5	3.52		75.56
CH ₂ OH- β 6	3.82		60.80	
D-galactose	CH- α 1	5.14 ^a	d(3.35)	93.06
	CH- α 2	3.69		69.18
	CH- α 3	3.72		69.99
	CH- α 4	3.85		70.11
	CH- α 5	3.96		71.25
	CH ₂ OH- α 6	3.66		62.02
	CH- β 1	4.46 ^a	d(7.80)	97.23
	CH- β 2	3.36		72.70
	CH- β 3	3.53		75.17
	CH- β 4	3.82		69.56
	CH- β 5	3.60		75.94
	CH ₂ OH- β 6	3.63		61.84

(To be continued)

Table S1 (Continued)

Compound	Assignment	¹ H (ppm)	Multiplicity [J (Hz)]	¹³ C (ppm)
citric acid	CH ₂	2.48 ^a , 2.64	AB	44.93
	C			75.80
	CH ₂ -COOH			177.70
	COOH			181.40
lactic acid	CH ₃	1.21	d(6.60)	20.84
	CH	4.03 ^a	dd	68.95
	COOH			182.50
creatine	CH ₃	2.90 ^a	s	37.40
	CH ₂	3.81	s	54.78
	C			158.30
	COOH			175.40
lecithin	trimethylamine	3.09 ^a	s	54.28
	CH ₂ -3	3.73, 3.79		
	CH ₂ -4	4.17		60.22
	CH ₂ -5	3.53		67.09
acyl chains of fatty acids	CH ₃ -ω1	0.76 ^a		14.74
	CH ₂ -ω2	1.17		23.54
	CH ₂ -Δ3	1.45		25.60
	oleate			
	CH ₂ -ω8,11	1.89		27.93
	CH ₂ (ω4-n)	1.17		29.78–30.90
	CH ₂ -ω3	1.14		32.79
	CH ₂ -Δ2	2.11		34.37
oleate HC=CH	5.19		130.18	
glycerol backbone of fats	glycerol-1,3	3.98, 4.16		62.54
	glycerol-2	5.10		69.59

^aProton signals were chosen for the quantitative analysis.

^bThe symbol “ω” indicates the position from the methyl group, the symbol “Δ” indicates the position from the ester group.

Table S2. Assignments of ^1H and ^{13}C signals of compounds in Caspian Sea yogurt (pH 4.8).

Compound	Assignment	^1H (ppm)	Multiplicity J (Hz)	^{13}C (ppm)
D-lactose	CH-1'	4.33	d(7.70)	103.57
	CH-2'	3.43		71.79
	CH-3'	3.54		73.31
	CH-4'	3.80		69.38
	CH-5'	3.61		76.15
	CH ₂ OH-6'	3.64		61.94
	CH- α 1	5.11 ^a	d(3.05)	92.62
	CH- α 2	3.47	t(8.85)	71.98
	CH- α 3	3.71		72.24
	CH- α 4	3.54		78.89
	CH- α 5	3.82		70.86
	CH ₂ OH- α 6	3.76		60.66
	CH- β 1	4.55	d(7.90)	96.55
	CH- β 2	3.16 ^a	t(7.83)	74.66
	CH- β 3	3.53		75.17
	CH- β 4	3.54		78.75
	CH- β 5	3.52		75.56
CH ₂ OH- β 6	3.82		60.80	
citric acid	CH ₂	2.53 ^a , 2.68	AB	44.93
	C			75.80
	CH ₂ -COOH		177.70	
	COOH		181.40	
lactic acid	CH ₃	1.21	d(6.60)	20.84
	CH	4.03 ^a	dd	68.95
	COOH			182.50
creatine	CH ₃	2.90 ^a	s	37.40
	CH ₂	3.81	s	54.78
	C			158.30
	COOH			175.40

(To be continued)

Table S2 (Continued)

Compound	Assignment	¹ H (ppm)	Multiplicity [J (Hz)]	¹³ C (ppm)
lecithin	trimethylamine	3.09 ^a	s	54.28
	CH ₂ -3	3.73, 3.79		
	CH ₂ -4	4.17		60.22
	CH ₂ -5	3.53		67.09
acyl chains of fatty acids	CH ₃ -ω1	0.76 ^a		14.74
	CH ₂ -ω2	1.17		23.54
	CH ₂ -Δ3	1.45		25.60
	oleate CH ₂ -ω8,11	1.89		27.93
	CH ₂ (ω4-n)	1.17		29.78–30.90
	CH ₂ -ω3	1.14		32.79
	CH ₂ -Δ2	2.11		34.37
	oleate HC=CH	5.19		130.18
glycerol backbone of fats	glycerol-1,3	3.98, 4.16		62.54
	glycerol-2	5.10		69.59

^aProton signals were chosen for the quantitative analysis.

^bThe symbol “ω” indicates the position from the methyl group, the symbol “Δ” indicates the position from the ester group.

Table S3. Assignments of ^1H and ^{13}C signals of compounds in kefir (pH 5.8).

Compound	Assignment	^1H (ppm)	Multiplicity J (Hz)	^{13}C (ppm)
D-lactose	CH-1'	4.33	d(7.70)	103.57
	CH-2'	3.43		71.79
	CH-3'	3.54		73.31
	CH-4'	3.80		69.38
	CH-5'	3.61		76.15
	CH ₂ OH-6'	3.64		61.94
	CH- α 1	5.11 ^a	d(3.05)	92.62
	CH- α 2	3.47	t(8.85)	71.98
	CH- α 3	3.71		72.24
	CH- α 4	3.54		78.89
	CH- α 5	3.82		70.86
	CH ₂ OH- α 6	3.76		60.66
	CH- β 1	4.55	d(7.90)	96.55
	CH- β 2	3.16 ^a	t(7.83)	74.66
	CH- β 3	3.53		75.17
	CH- β 4	3.54		78.75
	CH- β 5	3.52		75.56
	CH ₂ OH- β 6	3.82		60.80
D-galactose	CH- α 1	5.14 ^a	d(3.35)	93.06
	CH- α 2	3.69		69.18
	CH- α 3	3.72		69.99
	CH- α 4	3.85		70.11
	CH- α 5	3.96		71.25
	CH ₂ OH- α 6	3.66		62.02
	CH- β 1	4.46 ^a	d(7.80)	97.23
	CH- β 2	3.36		72.70
	CH- β 3	3.53		75.17
	CH- β 4	3.82		69.56
	CH- β 5	3.60		75.94
	CH ₂ OH- β 6	3.63		61.84

(To be continued)

Table S3 (Continued)

Compound	Assignment	¹ H (ppm)	Multiplicity [J (Hz)]	¹³ C (ppm)
citric acid	CH ₂	2.44 ^a , 2.60	AB	44.93
	C			75.80
	CH ₂ -COOH			177.70
	COOH			181.40
lactic acid	CH ₃	1.21	d(6.60)	20.84
	CH	4.03 ^a	dd	68.95
	COOH			182.50
ethanol	CH ₃	1.03 ^a		17.45
	CH ₂	3.48		58.13
creatine	CH ₃	2.90 ^a	s	37.40
	CH ₂	3.81	s	54.78
	C			158.30
	COOH			175.40
lecithin	trimethylamine	3.09 ^a	s	54.28
	CH ₂ -3	3.73, 3.79		
	CH ₂ -4	4.17		60.22
	CH ₂ -5	3.53		67.09
acyl chains of fatty acids	CH ₃ -ω1	0.76 ^a		14.74
	CH ₂ -ω2	1.17		23.54
	CH ₂ -Δ3	1.45		25.60
	oleate	1.89		27.93
	CH ₂ -ω8,11	1.89		
	CH ₂ (ω4-n)	1.17		29.78–30.90
	CH ₂ -ω3	1.14		32.79
	CH ₂ -Δ2	2.11		34.37
oleate HC=CH	5.19		130.18	
glycerol backbone of fats	glycerol-1,3	3.98, 4.16		62.54
	glycerol-2	5.10		69.59

^aProton signals were chosen for the quantitative analysis.

^bThe symbol “ω” indicates the position from the methyl group, the symbol “Δ” indicates the position from the ester group.

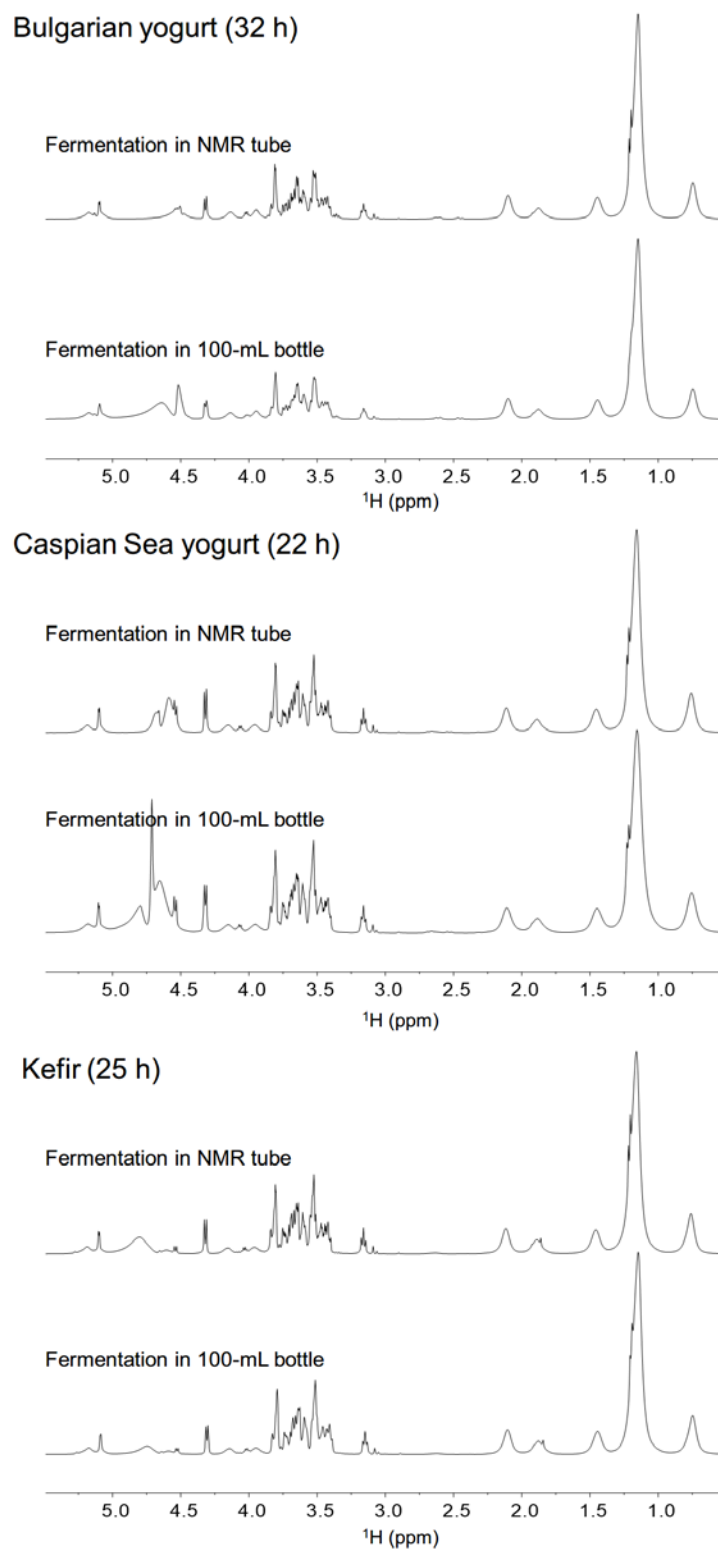


Figure S1. Comparison of ^1H NMR spectra of the three fermented milk products that were prepared in different scale.

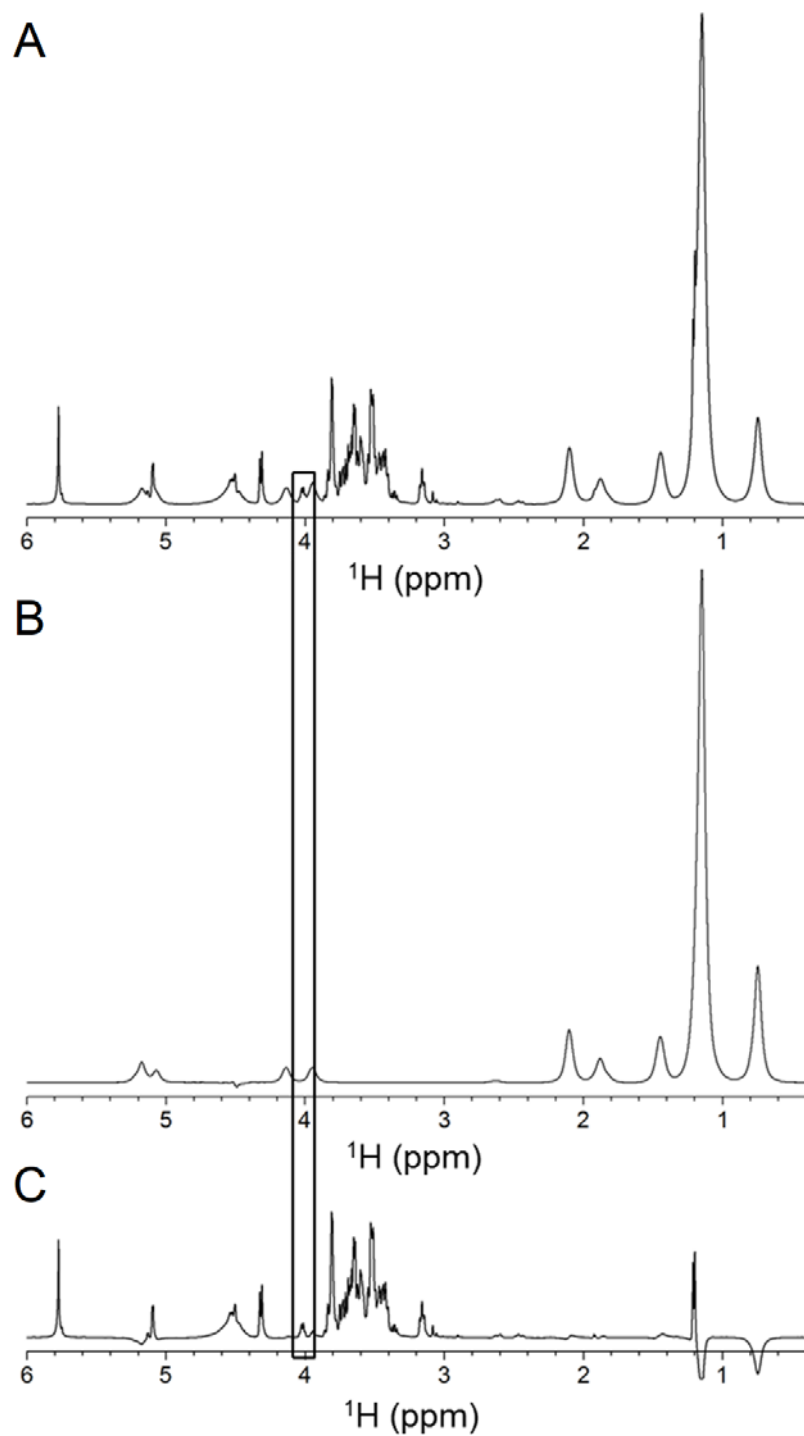


Figure S2. (A) Quantitative ^1H NMR spectrum of Bulgarian yogurt. (B) DOSY NMR spectrum of Bulgarian yogurt. (C) The difference spectrum for quantitating the integral of the signal at 4.03 ppm between (A) and (B).

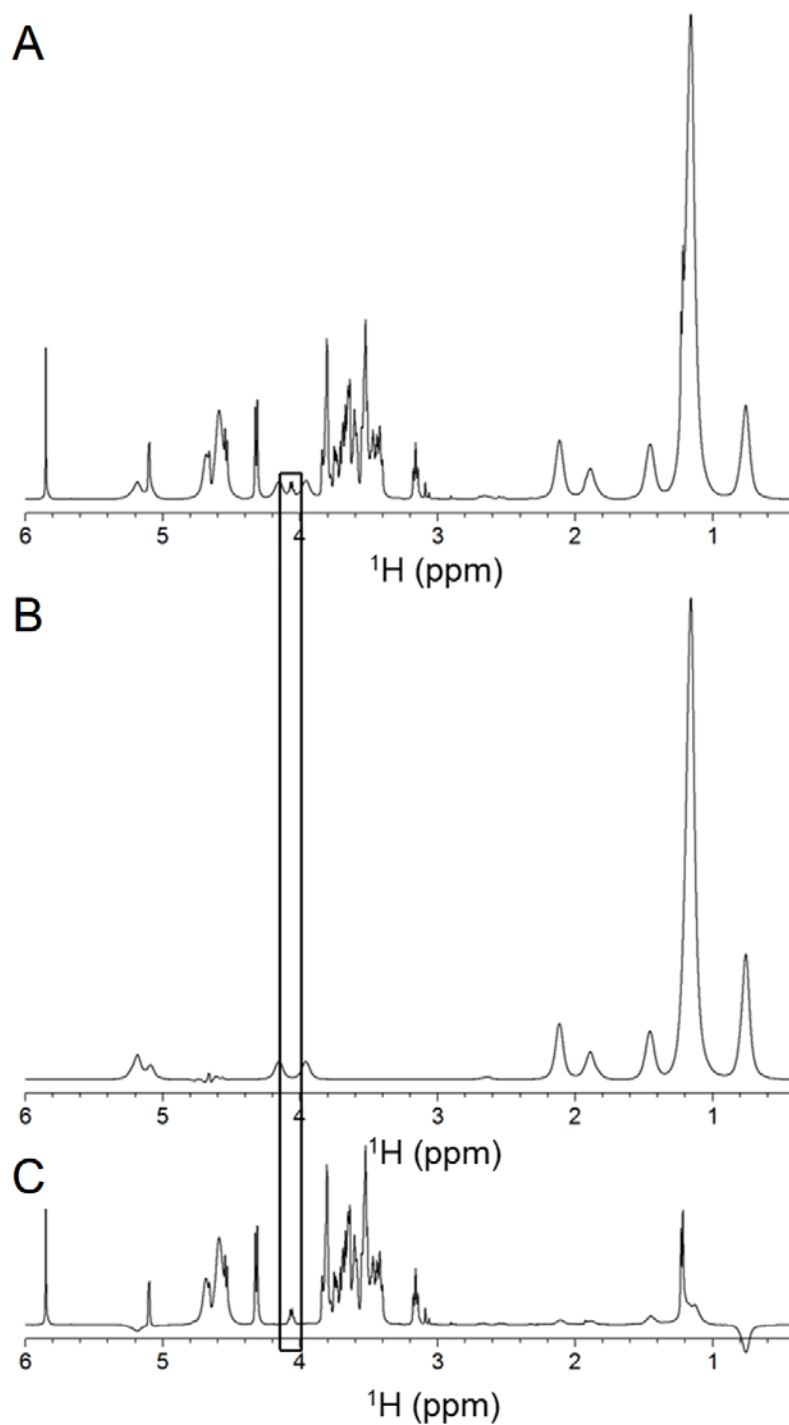


Figure S3. (A) Quantitative ^1H NMR spectrum of Caspian Sea yogurt. (B) DOSY NMR spectrum of Caspian Sea yogurt. (C) The difference spectrum for quantitating the integral of the signal at 4.03 ppm between (A) and (B).

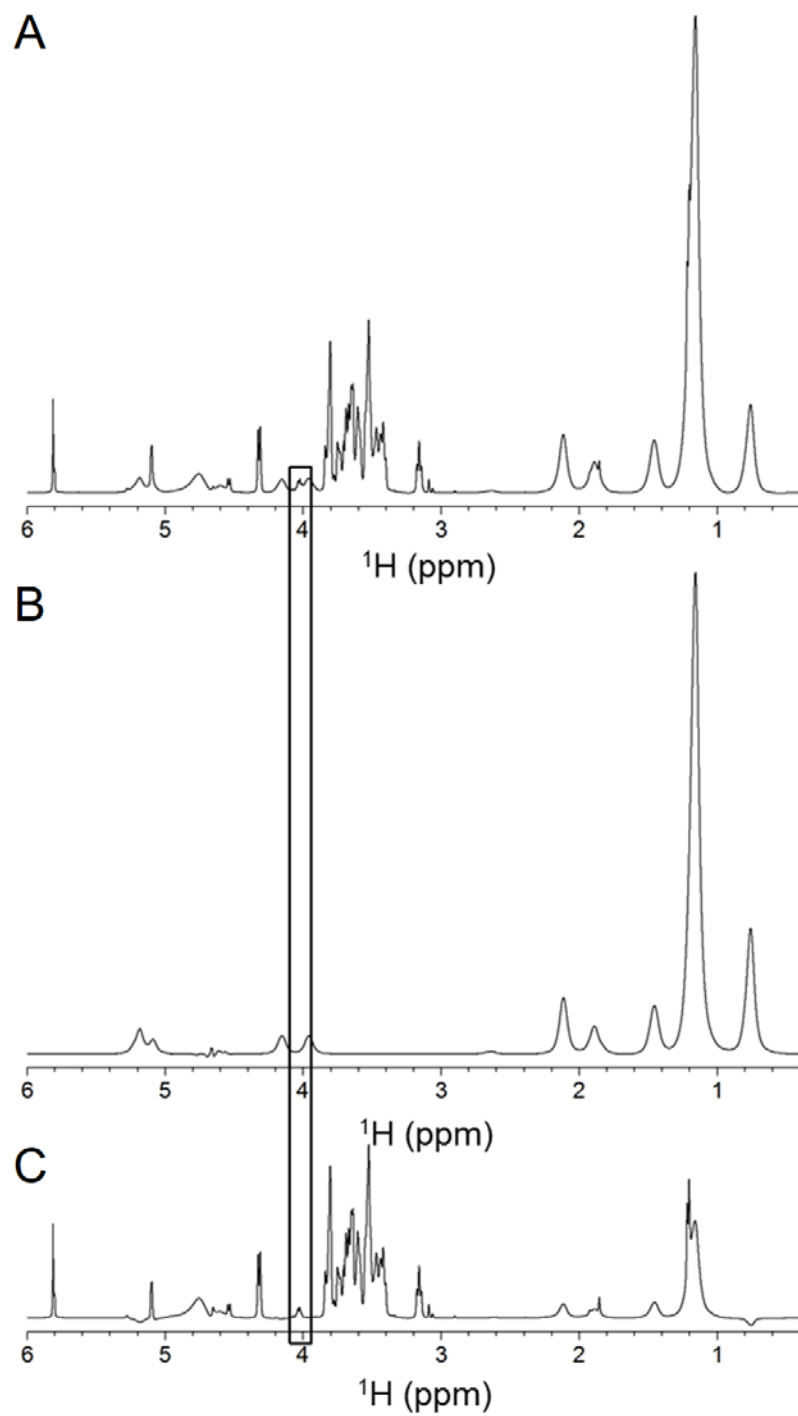


Figure S4. (A) Quantitative ^1H NMR spectrum of kefir. (B) DOSY NMR spectrum of kefir. (C) The difference spectrum for quantitating the integral of the signal at 4.03 ppm between (A) and (B).