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Comparison of D- and L-Lactic Acid Contents in Commercial *Kimchi* and *Sauerkraut*

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Abstract Commercial *kimchi* and *sauerkraut* were analyzed for their D- and L-lactic acid contents. Ranges of D- and L-lactic acid contents in commercial *kimchi* were 17-57 (38.51 mean) and 25-87 (64.47 mean) mM, respectively. Ratio of D-lactic acid on L-lactic acid (D/L) was 0.50-0.80 (0.60 mean). Ranges of D- and L-lactic acid contents in commercial *sauerkraut* were 68.96-103.62 (88.97 mean) and 74.46-82.26 (78.91 mean) mM, respectively, with D/L of 0.90-1.26 (1.13 mean). Results reveal *kimchi* and *sauerkraut* contained a significant amount of D-lactic acid, with *sauerkraut* showing a higher content than *kimchi*, while L-lactic acid contents were not significantly different.

Key words: D-lactic acid, L-lactic acid, *kimchi*, *sauerkraut*, acidosis

Introduction

D- and L-lactic acids are optical isomers, which differ only in the position of the alpha-hydroxy radical. Both compounds are produced from and metabolized to pyruvate by the enzyme lactate dehydrogenase (LDH). The predominant form of lactic acid normally present in fruits, human blood, and other vertebrate is L-lactic acid, which is derived from pyruvate by the action of L-LDH. Not only has D-lactic acid recently been considered as an indicator for the reduced freshness in pork and beef (1, 2) and the degree of the microbial contamination (3, 4), but also was found to have some relation to the patho-physiology of human diseases such as encephalophacy (5) and acidosis (6), in which plasma D-lactate levels were increased.

D-lactic acidosis as a human disease was first described in 1979 (7), showing symptoms such as mental confusion disorientation, slurred speech, and delirium (8). Urirarri *et al.* (6), after reviewing clinical cases from 1979 to 1997, defined D-lactic acidosis as metabolic acidosis accompanied by increase in serum D-lactate in excess of 3 mmole/L, and L-lactic acidosis in excess of 5 mmole/L for L-lactate. The rationale for using a lower serum level for definition of D-lactic acidosis than that for L-lactic acidosis is that the renal threshold for D-lactic acid is much lower than that for L-lactic acid, such that clinically significant metabolic acidosis may occur at much lower serum levels of D-lactic acid. Thus, starting from 1974 WHO regulates the D-lactate ingestion of infants less than three months old (9).

Concerned with the cause of D-lactic acidosis, it has been thought that dietary intake with foods or intestinal absorption after microbial production may be the major source for D-lactic acid (10). Humans ingest D-lactic acid by consuming fermented vegetables and milk such as *pickle*, *sauerkraut*, *kimchi*, and *yogurt*, which contain both D- and L-lactic acids. Lactic acid bacteria (LAB) are mainly responsible for the production of D- and L-lactic acids during the fermentation of vegetables and milk. The analysis of D- and L-lactic acid contents of fermented foods, however, was only achieved in *yogurt*, a milk product (11, 12).

In this study, we analyzed and compared D- and L-lactic acid contents of popular vegetable products; *sauerkraut* and *kimchi*. *Kimchi* is a Korean traditional fermented dish, which has a sour, hot, salty, and characteristically carbonic taste from the lactic acid fermentation of salted vegetables. *Sauerkraut* is a shredded cabbage prepared by lactic acid fermentation and widely consumed preserved vegetable in many European countries.

Materials and Methods

Preparation of samples Four kinds of *kimchi* products from five producers, shredded Chinese cabbage *kimchi* (*mak-kimchi*), whole cabbage *kimchi* (*pogi-kimchi*), white cabbage *kimchi* (*baek-kimchi*), and small radish *kimchi* (*chonggak-kimchi*), were purchased from retail stores. All *kimchi* samples had been delivered to stores without sterilization just after production and kept at 4°C for 3 to 8 days. Five brands of cabbage *sauerkraut* made in the United States, packed in bottles or cans after sterilization during the production, were purchased from three retail stores in Chicago, USA and delivered to the laboratory within 2 days of purchase. The liquid samples from *kimchi* and *sauerkraut* were decanted, centrifuged at 10,000 × g, and analyzed immediately after filtering using a 0.45-μm pore size filter.

HPLC quantitation The analysis method of Okubo *et al.* (13) was used to quantitate L- and D-lactic acids after modification. The normal phase HPLC system consisted of a Spectra SYSTEM P4000 pump with a UV1000 spectro-

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photometer, a SpectraSeries AS1000 autosampler (Thermo Separation Products Inc., Muskegon, Michigan, USA) and a 100×4.6 mm Shodex ORpac CRX853 4C column (Showa Denco, Tokyo, Japan). Lithium salts of racemic L- and D-lactic acids were obtained from Sigma (St. Louis, MO, USA), and stock standard solutions were 10.0 mmol/L in distilled water.

A twenty microliter portion of the samples was injected into the HPLC. The mobile phase was a mixture of 9 volume 1.00 mmol/L $CuSO_4$ in H_2O and 1 volume methanol. The flow rate of the mobile phase was 0.3 mL/min, and the temperature of the column was maintained at room temperature. The analytes were monitored at 250 nm with a UV spectrophotometric detector. The concentration of each analyte was obtained from the ratio of its peak area against that of the working standard solution.

Results and Discussion

Method validation Based on the chromatograms of D- and L-lactic acids for standards and *kimchi*, retention times of D- and L-lactic acid were determined to be 23.521 and 19.423 min, respectively. When standard plots were made using standard solution, linear lines were between 0.10 and 5 mM for D-lactic acid, and 0.11 and 10 mM for L-lactic acid. The correlations of peak area and concentration were expressed by the following equations:

D-lactic acid: $y=4\times10^{-7}x + 0.0052$ (R^2=0.9998) (mM)
L-lactic acid: $y=3\times10^{-7}x + 0.1785$ (R^2=0.999) (mM)

The analytical method validation parameters such as precision, accuracy, limit of detection, and limit of quantitation were calculated to further ensure the validity of the method. Table 1 shows precision and accuracy. Precision is made up of two components: repeatability (intra-day assay) and reproducibility (inter-day assay). Intra-assay precision was established through five consecutive assays of liquids from *kimchi* and *sauerkraut*. The coefficients of variation (the mean values) were 5.6% (1.06 mmol/L) for D-lactic acid and 5.7% (1.27 mmol/L) for L-lactic acid. Inter-day precision was established through a 5-day consecutive assay of *kimchi*. The coefficients of variation (the mean values) were 9.7% (0.92 mmol/L) for D-lactic acid and 9.8% (1.59 mmol/L) for L-lactic acid. Relative recovery was expressed as a percentage. The limit of detection (LOD) and limit of quantitation (LOQ) were determined based on the detector' signal-to-noise (S/N) ratio. The standard deviation of the S/N ratio was calculated and multiplied by a factor of 3, then this value was added to the average of the S/N ratio to obtain the LOD. For LOQ, 10 was chosen as a factor (14). The LOD in mmol/L were 0.0056 and 0.1788, and the LOQ in mmol/L were 0.0061 and 0.1792 for D- and L-lactic acids, respectively. The results of validation parameters were reliable and satisfactory.

Table 1. Precision and recovery for *kimchi* and *sauerkraut*

	Intra-day assay (n=5)	Inter-day assay (n=5)	Relative recovery (%) (n=5)
Kimchi			
L-lactic acid	1.27 mmol (5.7%)	1.59 mmol (9.8%)	98.3±5.5
D-lactic acid	1.06 mmol (5.6%)	0.92 mmol (9.7%)	98.7±4.0
Sauerkraut			
L-lactic acid	1.10 mmol (6.1%)	1.17 mmol (6.7%)	101.1±7.3
D-lactic acid	1.38 mmol (6.2%)	1.40 mmol (7.0%)	100.4±3.6

D- and L- lactic acid contents of commercial *kimchi* D- and L-lactic acid contents of four kinds of *kimchi* were analyzed (Table 2). The D- and L-lactic acid contents were

Table 2. Levels of D-, L-lactic acid of commercial *kimchi*[1)]

Kinds	Maker code[2)]	pH	D-lactic acid (mM)		L-lactic acid (mM)		D/L
			Mean±SD	CV[3)] (%)	Mean±SD	CV (%)	
Shredded cabbage *kimchi*	A	3.74	39.92±0.13	0.33	77.75±3.52	4.52	0.51
	B	3.99	39.66±0.79	2.00	74.99±1.23	1.64	0.52
	C	3.95	51.15±4.30	8.41	87.61±1.27	1.44	0.58
	D	4.09	42.63±1.73	4.07	74.64±3.07	4.11	0.57
	E	3.85	45.00±3.03	6.73	76.75±3.38	4.39	0.58
Whole cabbage *kimchi*	A	3.77	45.54±1.59	3.50	70.71±8.17	11.56	0.64
	C	4.04	43.51±1.72	3.95	71.32±2.0	2.89	0.61
	D	4.02	43.15±0.60	1.38	86.96±0.66	0.76	0.50
	E	3.87	38.73±3.60	9.28	76.01±3.27	4.30	0.51
Small radish *kimchi*	A	5.19	22.61±0.73	3.25	44.15±1.89	4.29	0.51
	B	4.00	56.63±3.46	6.11	79.46±2.20	2.76	0.71
	C	3.98	54.11±1.35	2.50	81.68±2.53	3.10	0.66
White cabbage *kimchi*	A	3.44	17.01±2.04	11.97	24.94±1.70	6.80	0.57
	B	3.45	19.48±1.30	6.67	34.79±0.32	0.93	0.56
	C	3.67	35.85±3.52	9.82	43.53±1.38	3.17	0.82
	D	3.69	21.15±1.81	8.53	26.24±0.81	3.09	0.81

1)All values are expressed as means±SD for 3 samples.
2)Maker code: A-E, the *kimchies* were remarked irrespective of their own brand. Same letters in the same column indicate the same maker.
3)CV: Coefficient of variables (%)

Table 3. Levels of D-, L-lactic acid of commercial *sauerkraut* (n=3)[1)]

Maker code[2)]	D-lactic acid (mM)		L-lactic acid (mM)		D/L
	Mean±SD	CV[3)] (%)	Mean±SD	CV (%)	
A	103.62±6.42	6.20	82.26±5.00	6.07	1.26
B	82.68±0.55	0.66	79.83±0.31	0.39	1.04
C	100.94±3.16	3.13	81.34±2.00	2.46	1.24
D	88.63±0.53	0.60	74.46±0.88	1.17	1.19
E	68.96±6.82	9.88	76.67±3.89	5.08	0.90

[1)]All values are expressed as means±SD for three samples.
[2)]Maker code: A-E, the *sauerkrauts* were remarked irrespective of their own brand.
[3)]CV: Coefficient of variables (%)

in the range of 17.01-56.63 (38.51 mean) and 24.94-87.61 (64.47 mean) mM, respectively. The ratios of D-lactic acid on L-form (D/L) were between 0.50-0.82 (0.60 mean). These results reveal that the levels of D-lactate concentration in commercial *kimchi* were lower than those of L-form. ANOVA results using SAS software (SAS Inc. USA) (15) show significant differences ($P<0.01$) in the L-lactic acid content and D/L ratio between white cabbage *kimchi* and the others, whereas no difference in D-lactic acid between shredded cabbage *kimchi* and whole cabbage *kimchi*, possibly due to the difference of ingredients between white cabbage *kimchi* and other *kimchi* types. When the lactic acid content of *kimchi* was compared to that of *yogurt* with [D-lactic acid (64.4 mM) and L-lactic acid (41.7 mM) as reported by Olieman (12)], *kimchi* showed lower D/L ratio.

D- and L- lactic acid contents of commercial *sauerkraut* D- and L-lactic acid contents of five commercial *sauerkraut* were measured, and D/L ratios were calculated (Table 3). The D- and L-lactic acid contents were in the ranges of 68.96-103.62 (88.97 mean) and 74.46-82.26 (78.91 mean) mM, respectively. The D/L ratios were in the range of 0.90-1.26 (1.13 mean), showing higher D-lactic acid content than L-form in four *sauerkraut* samples.

Comparison of *kimchi* and *sauerkraut* *Kimchi* and *sauerkraut* are representative vegetables made by lactic acid fermentation and have common features in raw material (cabbage vs. Chinese cabbage), preparation method (salting procedure), and microorganisms involved in fermentation (*Leuconostoc* spp. and Lactobacilli). We analyzed the content of D- and L-lactic acids in both *kimchi* and *sauerkraut* to estimate the daily intake of D-lactic acid. Results revealed that both *kimchi* and *sauerkraut* contain D-lactic acid at significant levels, with D-lactic acid content (88.97 mM) of *sauerkraut* being significantly higher than that of *kimchi* (38.51 mM), while the L-lactic acid contents were not much different (78.91 mM vs 64.47 mM). The D/L ratios of *sauerkraut* and *kimchi* (1.13 vs 0.60) clearly confirms this result. The higher content of D-lactic acid in *sauerkraut* could be due to either difference in raw materials or microbials during fermentation.

The preparation procedure for *sauerkraut* is not much different from that of *kimchi* except for raw materials; for *kimchi*, Chinese cabbage or radish is generally used, and hot pepper and garlic are added as spices. For *sauerkraut* and *kimchi*, *Leuc. mesenteroides* (16, 17) is one of the major fermenting strains during fermentation, and the bacterium solely produces D-lactic acid; thus, higher D-lactic acid content of *sauerkraut* could be derived from the compo-sition of lactic acid bacteria. *L. delburueckii, L. lactis, L. bulgaricus,* and *L. leichmanii* synthesize only D-lactic acid, whereas *L. casei, L. salivarius,* and *B. bifidum* only L-form. On the other hand, *L. plantarum, L. brevis, L. fermentum, L. acidophilus,* and *L. jugurii* synthesize both D- and L-lactic acids. *L. acidophilus* is known to produce equal amounts of D- and L-form (18). In *yogurt*, in which lactobacilli are major strains with most of them producing both D- and L-lactic acids, D/L ratio was low (0.1-0.81) (11).

Initially, D-lactic acidosis was thought to be due to over-ingestion of D-lactic acid through meals or overproduction by intestinal bacteria, because humans were presumed not able to metabolize D-lactic acid (10). Recently, mammals including humans were found to have D-lactate dehydrogenase (D-LDH) gene and metabolize D-lactate by secreting D-LDH in the liver (19). This finding suggests that the development of D-lactic acidosis requires impaired ability to metabolize D-lactic acid in addition to excessive intake. Thus, although the healthy adults have sufficient ability to metabolize D-lactic acid after intake, excessive absorption through foods may cause a metabolic stress in infants and patients. The analysis of D- and L-lactic acid contents of fermented foods was achieved only in *yogurt* (11, 12). This article provides the first report on the D- and L-lactic acid contents of fermented vegetables, *kimchi* and *sauerkraut*. For a better understanding of the influence of microbials on the content of D-lactic acid in lactic acid-fermented vegetables or *yogurts*, further experiments under controlled conditions are needed using starter microorganisms.

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