Chemical and Sensory Properties of Sauerkraut Produced with *Leuconostoc mesenteroides* Starter Cultures of Differing Malolactic Phenotypes

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ABSTRACT: Research was conducted to determine whether *Leuconostoc mesenteroides* starter cultures with and without malolactic activity (MDC$^+$ and MDC$^-$, respectively) influenced sensory and chemical properties of sauerkraut. No sensory differences were found between MDC$^+$ and MDC$^-$ sauerkraut ($P \geq 0.05$). In addition, sulfur compound profiles of the resulting sauerkraut were nearly identical. Brining at lower NaCl (0.5%) with either inoculum changed both the microbiology and chemistry of the fermenting sauerkraut, leading to decreased sauerkraut sulfur flavor. Quantification of allyl isothiocyanate (AITC), dimethyl disulfide, dimethyl trisulfide (DMTS), methyl methanethiosulfinate, and methyl methanethiosulfonate ($MMTSO_2$) by gas chromatography-mass spectrometry showed that sauerkraut sulfur flavor correlated linearly with DMTS and $MMTSO_2$ ($P \leq 0.01$).

Keywords: malolactic activity, *Leuconostoc mesenteroides*, sauerkraut, sulfur compounds

Introduction

The commercial production of sauerkraut involves the fermentation of cut and salted cabbage by naturally occurring lactic acid bacteria (LAB). Variability in the natural microflora and environmental conditions can lead to large variability in product quality (Pederson and Albury 1969; Fleming and others 1995). Proper fermentation relies heavily on the addition of sodium chloride at the correct concentration (Pederson and Albury 1969; Stamer 1983), often resulting in excess, high-chloride waste. Cabbage temperature, which is also highly variable, affects the growth and competitive situation of the naturally present microorganisms, resulting in variable product quality (Parmele and others 1927). Starter cultures have been proposed for sauerkraut to minimize the impact of these sources of variation and potentially reduce the amount of salt required for fermentation (LeFevre 1919; Pederson 1930; Fleming and McFeeters 1981; Fleming 1987; Adams and others 1990; Harris and others 1992; Corbet 1993; Breidt and others 1995).

Flavor is a key component in the quality grading of sauerkraut (Pederson and Albury 1969; Stamer 1985), and it is characterized mostly by salty, sour, and sulfur notes (Trail and others 1996). The sulfur character of sauerkraut can lend both desirable sauerkraut-like flavors, as well as objectionable "off" aromas and flavors. S-Methyl cysteine sulfoxide (SMCSO), a sulfur-containing amino acid, is present in large quantities in cabbage with reported concentrations ranging from 185 to 2218 ppm on a fresh weight basis (Morris and Thompson 1956; Synge and Wood 1956; Bradshaw and Borzucki 1982; Marks and others 1992). SMCSO is enzymatically broken down by cysteine sulfoxide lyase (C-S lyase), producing methyl methanethiosulfinate (MMTSO) and other organosulfur compounds, such as methyl methanethiosulfonate ($MMTSO_2$), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS), in a time-dependent and pH-dependent manner (Mazelis 1963; Marks and others 1992). Chin and Lindsay (1994) described MMTSO and $MMTSO_2$ as having characteristic sauerkraut aromas and reported estimated detection threshold concentrations of 550 ppb and 5 ppm for each compound, respectively.

Because the activity of C-S lyase during the early stages of sauerkraut fermentation may be influenced by the rate of pH decrease, de-acidification reactions, such as the malolactic reaction, may be important. Malic acid is a natural component of cabbage that can be converted to lactic acid and carbon dioxide with uptake of a hydrogen ion by LAB that have malolactic activity (Kunkee 1967; Radler 1986). The malolactic reaction has been found to be important in other fermentations where malic acid is also naturally present. Biological deacidification of certain wines via secondary malolactic fermentation results in less acidic wines with distinctive flavors (Henick-Kling 1995). The malolactic reaction is undesirable in cucumber fermentations due to carbon dioxide production, which contributes significantly to bloater defects in pickles (Fleming and others 1973; Fleming and Pharr 1980; McFeeters and others 1984). Because malolactic activity of *Leuconostoc mesenteroides* is variable among strains (Johanningsmeier and others 2004), it was of interest to investigate the importance of this strain characteristic in potential starter cultures for use in dry-salted and brined sauerkraut fermentations. The objective of this research was to determine sensory and chemical properties of sauerkraut inoculated with *L. mesenteroides* starter cultures with and without malolactic activity (MDC$^+$ and MDC$^-$, respectively).

Materials and Methods

Fermentations

Cabbage was prepared by removing the outer leaves, coring the heads, and slicing to 1-mm thickness. The shredded cabbage was prepared according to the treatments listed in Table 1 and packed into 46-oz jars. Sodium metabisulfite was added to brined cabbage to achieve 300 ppm sulfite (calculated as SO_2), hereafter called the sulfite treatment. This treatment served as a nonfermented, brined, and acidified control. Starter cultures were obtained from the United Stated Department of Agriculture-Agricultural Research

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Table 1—Experimental design for sauerkraut inoculated with *L. mesenteroides* with (MDC$^+$) and without (MDC$^-$) malolactic activity

Treatment	NaCl	Method of salting	Inoculation
Natural fermentation	2%	Dry-salted	None
Sodium metabisulfite (300 ppm calculated as SO_2), pH 3.5 (HCl)	0.5%	Brined (60:40 cabbage-brine)	None
LA 81 (MDC$^+$)	2%	Dry-salted	*L. mesenteroides*[a] strain LA 81
LA 10 (MDC$^-$)	2%	Dry-salted	*L. mesenteroides* strain LA 10
LA 81 (MDC$^+$)	0.5%	Brined (60:40 cabbage-brine)	*L. mesenteroides* strain LA 81
LA 10 (MDC$^-$)	0.5%	Brined (60:40 cabbage-brine)	*L. mesenteroides* strain LA 10
LA 81 (MDC$^+$), 20 m*M* malic acid	2%	Dry-salted	*L. mesenteroides* strain LA 81
LA 10 (MDC$^-$), 20 m*M* malic acid	2%	Dry-salted	*L. mesenteroides* strain LA 10
Canned commercial sauerkraut	2%	Dry-salted	None

[a]*Leuconostoc mesenteroides*.

Service (USDA-ARS) Food Fermentation Laboratory Culture Collection (Raleigh, N.C., U.S.A.). *Leuconostoc mesenteroides* strain LA 81 (MDC$^+$, ATCC 8293, type strain) or *L. mesenteroides* strain LA 10 (MDC$^-$, C33, J. R. Stamer, Dept. of Food Science, Cornell Univ.) was inoculated onto sliced cabbage at approximately 10^6 colony-forming units (CFU)/g. Natural microflora were not removed or intentionally suppressed but were present in significantly lower numbers. Duplicate fermentation jars were packed for each treatment and incubated at 18 °C for 9 mo.

Fermentors for studying sauerkraut fermentation (Fleming and others 1988) were packed with the following treatments: 2% dry-salted cabbage inoculated with *L. mesenteroides* LA 81 or LA 10, and brined cabbage (0.5% NaCl, equilibrated) inoculated with each of the 2 test cultures. Each of the 4 fermentors was monitored during the 1st 14 d by aseptically sampling the brine and analyzing it for *Enterobacteriaceae* (CFU/mL), LAB (CFU/mL), total aerobes (CFU/mL), pH, and fermentation products, as described by Fleming and others (1988). Ten LAB isolates from each fermentor were randomly selected at 2, 5, and 14 d of fermentation and tested for malolactic activity in MD medium (Daeschel and others 1984).

Sensory analysis

Twelve individuals from the Dept. of Food Science at North Carolina State Univ. (NCSU) in Raleigh, N.C., were selected based on availability, prior panel experience, and ability to distinguish and scale the basic tastes. The panel was trained to evaluate sauerkraut by category scaling using several commercial and experimental sauerkrauts as examples. A scale of 0 = not detectable to 14 = very strong was used for aroma and flavor attributes of sauerkraut as shown in Table 2. Firmness was scored using a scale from 0 = very soft to 14 = very firm. A 6-member expert panel individually evaluated several coded commercial samples for use as a reference sample. One canned commercial sauerkraut of a specific lot number was clearly identified as being free of off-flavors, balanced in salt and acidity, and characteristic of kraut sulfur flavor. The chosen reference sample (canned commercial sauerkraut produced from naturally fermented salted cabbage) was scored, and a consensus on intensities of each attribute was reached (Table 3). The reference sample was then presented to the panelists during training for identification and scaling of kraut sulfur flavor. Panelists completed 4 h training before evaluating samples. Experimental sauerkraut treatments and commercial sauerkraut were evaluated in a randomized complete block design with 2 sensory replications per jar and 2 jars per treatment. Each sample was coded with its own random 3-digit number. A maximum of 3 samples was presented to each panelist in a random order at each tasting session. The commercial reference sample (Table 3) was provided at each tasting session along with water and unsalted soda crackers for palate cleansing between samples. Analysis of variance (ANOVA) was used to determine statistically significant differences among treatments using SAS statistical software (SAS Inst., Cary, N.C., U.S.A.).

Table 2—Defined attributes for sensory analysis of sauerkraut using category scaling

Attribute[a]	Definition
Kraut sulfur	The strong sulfur note that is characteristic of properly fermented sauerkraut. Example: Aroma and flavor of reference sample
Raw cabbage	Green, vegetative aroma and flavor of raw cabbage
Saltiness	Basic taste associated with sodium chloride in solution
Acid flavor	Sour taste associated with organic acids in solution. Example: lactic acid
Musty/dirty	Aroma and flavor associated with soil. Example: experimental sauerkraut sample determined by the expert panel to have this note
Paint/latex	Aroma associated with latex paint or gloves
Metallic	Flavor associated with metal substances in the mouth. Example: sulfite solutions
Cheesy/butyric	Aroma and flavor characteristic of dilute butyric acid
Other	An open scale with space allotted for a write-in descriptor to be used when an off-note is observed that is not anticipated
Firmness	The amount of force or effort required for masticating the sample

[a]Scale from 0 = not detectable to 14 = very strong for flavor and aroma attributes; 0 = very soft to 14 = very firm for "Firmness."

Chemical analysis

Brine samples from each fermentation jar were collected in 15-mL vacutainer tubes at the time of sensory testing and stored at –83 °C. Samples were analyzed for pH, NaCl, sugars, and acids. Glucose, mannitol, ethanol, glycerol, lactic acid, acetic acid, malic acid, succinic acid, butyric acid, and propionic acid were measured by high-performance liquid chromatography (HPLC) using 3 m*M* heptafluorobutyric acid as eluent on an organic acid column at 65 °C with conductivity and refractive index (RI) detectors (McFeeters 1993). NaCl concentration was determined by titration with $AgNO_3$ (Fleming and others 2001). A pH meter (Fisher Accumet pH meter model 825MP, Pittsburgh, Pa., U.S.A.) was calibrated with pH 4.01 and pH 7.00 buffers and used for brine pH determinations.

Sauerkraut extract preparation for gas chromatography-mass spectroscopy (GC-MS) and HPLC with integrated pulsed amperometry (IPAD)

Ten milliliters of filtered sauerkraut brine were vortexed with 2.5

Table 3—Sauerkraut reference sample[a] attributes and intensities

Attribute	Intensity[b]
Sauerkraut sulfur aroma	12
Raw cabbage aroma	0
Firmness	11
Sauerkraut sulfur flavor	12
Raw cabbage flavor	0
Saltiness	7
Acid flavor	11

[a]Canned commercial sauerkraut.
[b]Scale from 0 = not detectable to 14 = very strong for flavor and aroma attributes; 0 = very soft to 14 = very firm for "Firmness."

g NaCl in a glass screw-cap test tube. Dichloromethane (0.5 mL) containing 20 ppm butyl isothiocyanate (BITC) internal standard was added. The mixture was vortexed on high speed for 5 min to extract volatile sulfur compounds. The extraction mixture was then centrifuged at 3800 rpm for 15 min for separation of the liquid layers. The bottom dichloromethane layer was removed with a glass Pasteur pipette and dried with Na_2SO_4. This dichloromethane extract was decanted into a small screw-cap vial ready for GC-MS analysis or dilution with eluent (1:80) for HPLC-IPAD analysis.

Quantitative analysis of sulfur compounds

Dimethyl disulfide (DMDS), allyl isothiocyanate (AITC), dimethyl trisulfide (DMTS), MMTSO, and methyl methanethiosulfonate ($MMTSO_2$) were quantified using GC-MS with single ion monitoring (GC-MS SIM) in selected samples. Published methods for analysis of volatile sulfur compounds (Marks and others 1992; Kyung and Fleming 1994) were modified to obtain maximum sensitivity without degradation of the selected analytes. GC-MS conditions were as follows: Hewlett Packard (Houston, Tex., U.S.A.) 5890 Series II Plus gas chromatograph; J&W Scientific (Folsom, Calif., U.S.A.) DB-5MS column (30 m × 0.25 mm; 0.25-μm film thickness); 0.8 mL/min helium carrier gas; 0 °C to 110 °C at 5 °C/min then 110 °C to 150 °C at 15 °C/min; 2 μL splitless injection at 150 °C; Hewlett Packard 5972 Series Mass Selective Detector in electron ionization mode (EI) 70 eV; 250 °C detector inlet temperature; SIM. Analytes were quantified using the method of standard additions.

Qualitative analysis of sulfur compounds

Chromatograms from HPLC-IPAD were obtained for the following treatments: commercial sauerkraut, sulfite-preserved cabbage (nonfermented control), and dry-salted (2% NaCl) MDC$^+$-inoculated and MDC$^-$-inoculated fermentations. Electrochemical waveforms (LaCourse and Owens 1995; Shofran 1997; Hanko and others 2001) for selective and sensitive detection of sulfur compounds were modified to give adequate electrode cleaning for detection of AITC, MMTSO, $MMTSO_2$, dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) under typical reverse-phase chromatographic conditions. Limits of detection for standards in solution were estimated to be 2 ppb, 4 ppb, 6.5 ppb, 4 ppb, and 5 ppb, respectively. Reverse-phase chromatography was developed for separation of these compounds using an acetonitrile-phosphate buffer eluent. HPLC-IPAD conditions were as follows: Alltech Platinum EPS C8 column (150 × 4.6 mm; 3-μm particle size) (Alltech Associates, Inc., Deerfield, Ill., U.S.A.) with guard column and in-line filter; 17.5% CH_3CN, 50 m*M* $NaPO_4$, pH 3.0 eluent; ambient temperature; 10-μL injection loop, 5-s injection; Dionex ED40 detector with gold (Au) working electrode (Dionex Corp., Sunnyvale, Calif., U.S.A.) using the optimized integrated amperometry waveform shown in Table 4.

Results and Discussion

All LAB isolates from the *L. mesenteroides* strain LA 81 inoculated fermentors were MDC$^+$, corresponding correctly to the phenotype of the added starter culture. In *L. mesenteroides* strain LA 10 (MDC$^-$) inoculated fermentors, 59/60 LAB isolates were MDC$^-$. Furthermore, Plengvidhya and others (2004) showed that *L. mesenteroides* strain LA 81 starter culture was the predominant LAB in the corresponding fermentors at 2, 5, and 14 d using a random amplified polymorphic deoxyribonucleic acid (DNA) polymerase chain reaction (RAPD-PCR) method. Although *L. mesenteroides* strain LA 10 constituted the majority of the LAB population at 2 d and 5 d, it was no longer predominant at 14 d. This gives us evidence that both of the added starter cultures were able to express their malolactic phenotype and predominate in the early phase of fermentation, believed to be most critical in flavor development in sauerkraut.

Inoculating sliced, salted cabbage with *L. mesenteroides* strain LA 10 (MDC$^-$) produced more DMDS ($P \geq 0.05$) during fermentation than inoculating with LA 81 (MDC$^+$), 77.9 versus 51.2 ppb, respectively (Table 5). However, DMDS concentration did not correlate well ($r^2 = 0.374$) with sauerkraut sulfur flavor sensory scores (Figure 1), and no sensory differences were found between MDC$^+$-inoculated and MDC$^-$-inoculated sauerkrauts for all flavor attributes and salting treatments ($P > 0.05$). MDC$^+$ and MDC$^-$ treatments were similar in production of AITC, DMTS, and $MMTSO_2$, and contained no MMTSO (Table 5). HPLC-IPAD detection of sulfur compounds in MDC$^+$-inoculated and MDC$^-$-inoculated sauerkraut showed that they were nearly identical in sulfur compound composition (Figure 2). These results indicate that malolactic activity in strains of *L. mesenteroides* was not sufficient to modulate the formation of sulfur flavor compounds. A side-by-side experiment replicating selected treatments in larger fermentors (approximately 9 kg cabbage) showed no significant difference in the rate of pH decrease between MDC$^+$-inoculated and MDC$^-$-inoculated sauerkraut (Figure 3), which reasonably explains why there was no noticeable change in the concentrations of C-S lyase breakdown products, MMTSO, $MMTSO_2$, DMDS, and DMTS. Even an additional 20 m*M* malic acid added to the fermentations did not result in substantial sensory differences between MDC$^+$ and MDC$^-$ treatments. Malic acid addition resulted in decreased sauerkraut sulfur flavor (Figure 4) for both MDC$^+$-inoculated and MDC$^-$-inoculated sauerkraut and increased "off–flavor" in the MDC$^-$ inoculated treatment (Figure 5). Additionally, the final pH was higher in both of the malate

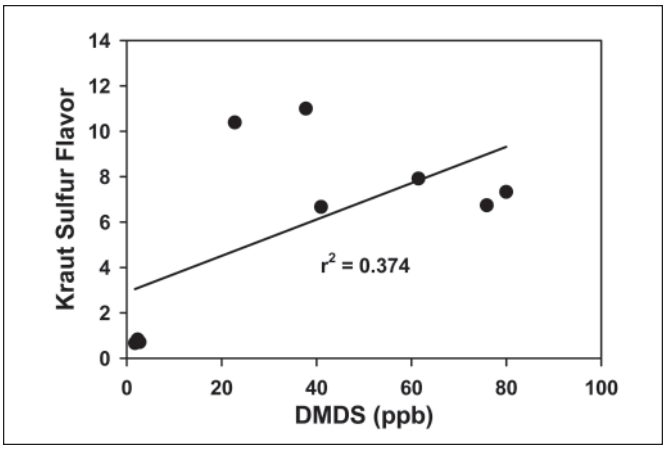

Figure 1—Correlation of sauerkraut sulfur flavor to dimethyl disulfide (DMDS)

Table 4—Waveform for high-performance liquid chromatography with integrated pulsed amperometry (HPLC-IPAD) analysis of sulfur compounds in sauerkraut

Time (s)	Potential (V)
0.00	+0.10
0.10 Begin integration	+0.10
0.40	+1.50
0.70	+0.10
0.80 End integration	+0.10
0.81	–0.70
0.83	–0.70
0.84	+1.60
1.10	+1.60
1.11	–0.70
1.40	–0.70

Table 5—Sulfur compounds in selected sauerkraut extracts measured by gas chromatography-mass spectroscopy (GC-MS)

Treatment	DMDS[a] (ppb)	AITC (ppb)	DMTS (ppb)	MMTSO (ppb)	$MMTSO_2$ (ppb)
Sulfite	2.3a	352.1a	0.5a	1.5a	0a
LA 81 (MDC^+)					
2% NaCl	51.2b	85.2b	21.7b	0b	0.5a,b
LA 10 (MDC^-)					
2% NaCl	77.9c	78.6b	29.9b	0b	0.8b
Commercial	30.3d	54.6b	30.6b	1.3a	1.6c

[a]Letters within columns designate different means, statistically significant at $P < 0.05$. AITC = allyl isothiocyanate; DMDS = dimethyl disulfide; DMTS = dimethyl trisulfide; MDC^+ = with malolactic activity; MDC^- = without malolactic activity; MMTSO = methanethiosulfinate; $MMTSO_2$ = methyl methanethiosulfonate.

added treatments (Table 6), indicating that the buffering action of malic acid itself may have influenced flavor development.

Canned commercial sauerkraut scored the highest in sauerkraut sulfur flavor, followed by dry-salted treatments (MDC^+, MDC^-, and naturally fermented), which had a "moderate" amount of sauerkraut sulfur flavor (Figure 4). Experimental treatments were not heated, as is the case with commercially canned sauerkraut, perhaps accounting for flavor differences between experimental and canned commercial sauerkraut. Brining at a reduced salt level (0.5%) significantly decreased sauerkraut sulfur flavor and aroma for both MDC^+ and MDC^- treatments ($P < 0.05$). Brining cabbage at 0.5% NaCl resulted in delayed acid production (data not shown) and therefore, a slower initiation of pH decrease than dry-salted (2% NaCl) treatments (Figure 3). Although the treatments were inoculated with a high concentration of LAB (10^6 CFU/g), brining at lower salt concentration allowed outgrowth of the naturally present *Enterobacteriaceae* (Figure 6a), which subsequently delayed activity of the LAB inoculum (Figure 6b) compared with the dry-salted (2% NaCl) sauerkraut treatments. It is not presently clear whether the decrease in kraut sulfur flavor was due to the change in microbiology associated with the brining treatment or simply the dilution of constituents as a result of the brining process. It has been shown that growth of Gram-negative bacteria initially present on cabbage results in off-flavors and darker kraut color (Fulde and Fabian 1953). However, no increase in off-flavor was detected ($P \leq 0.05$) in the low-salt, brined treatments (Figure 5), where *Enterobacteriaceae* was shown to increase in numbers early in the fermentation (Figure 6a).

Sulfite-preserved (pH 3.5, nonfermented) cabbage had almost no

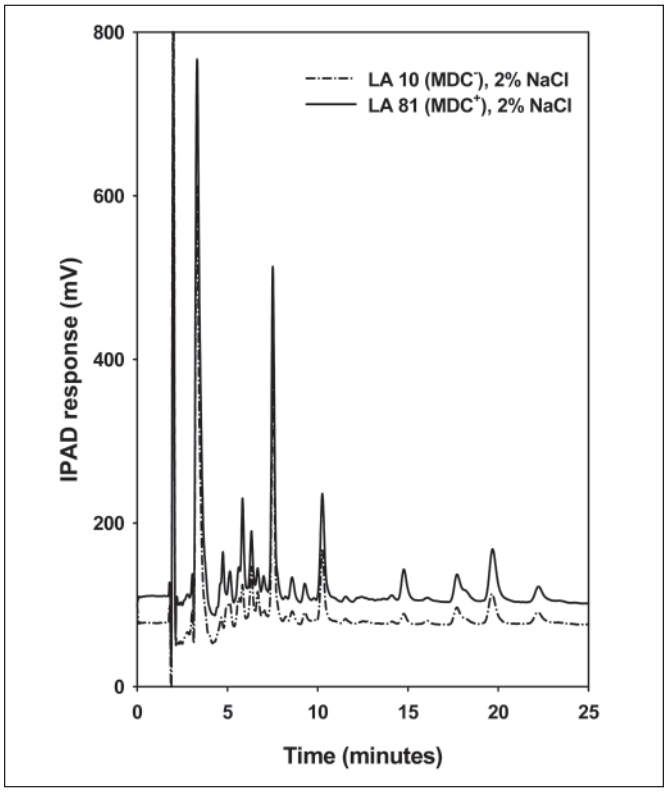

Figure 2—Chromatography of extracts from sauerkraut inoculated with *Leuconostoc mesenteroides* cultures with (MDC^+) and without (MDC^-) malolactic activity using HPLC with integrated pulsed amperometric detection

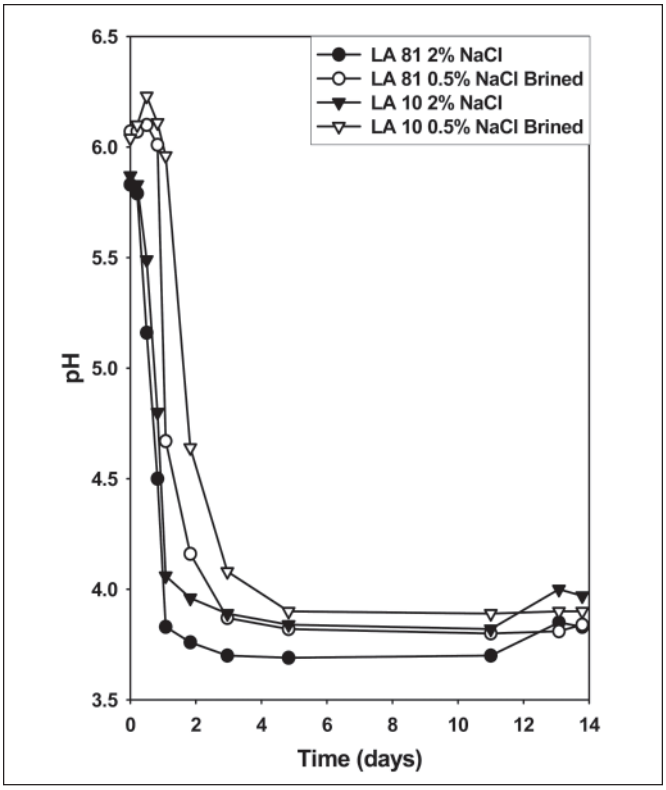

Figure 3—pH changes during sauerkraut fermentation

Table 6—Organic acid concentrations in *L. mesenteroides* - inoculated sauerkraut

Treatment	Final pH	Lactic acid (m*M*)	Acetic acid (m*M*)	Malic acid (m*M*)
Natural fermentation	3.63	87.1 ±20.9	66.0 ±7.5	Trace
Sulfite (300 ppm, pH 3.5)	3.44	0	0.9 ±0.1	10.3 ± 0.7
LA 81 (MDC^+) 2% NaCl	3.61	80.3 ±17.5	64.1 ±1.3	0
LA 10 (MDC^-) 2% NaCl	3.47	124.4 ±6.1	61.8 ±7.1	0
LA 81 (MDC^+) 0.5% NaCl brined	3.27	117.0 ±25.4	40.9 ±1.9	0
LA 10 (MDC^-) 0.5% NaCl brined	3.22	128.3 ±8.1	37.6 ±1.3	0
LA 81 (MDC^+) + 20 m*M* malic acid	3.87	102.2 ±5.2	65.9 ±2.8	0
LA 10 (MDC^-) + 20 m*M* malic acid	4.01	61.7 ±7.5	59.8 ±1.8	21.1 ±7.0
Canned commercial sauerkraut	3.43	142.4 ±9.2	65.8 ±4.2	0

[a]Sauerkraut was stored for 9 mo at 18 °C before analysis.
MDC^+ = with malolactic activity; MDC^- = without malolactic activity

sauerkraut sulfur flavor and aroma (Figure 4), a slight raw cabbage flavor, and the greatest amount of "off-flavor" (Figure 5). Sulfite-preserved cabbage contained significantly more AITC (352 ppb) than fermented treatments (Table 5). AITC has been reported as an important component in fresh cabbage flavor by several researchers. Chin and Lindsay (1993) found that AITC appeared to be important in fresh cabbage flavor because this was the main volatile sulfur compound produced shortly after disruption of the cabbage tissues reaching near maximum concentrations at that time. The current research found a good correlation ($r^2 = 0.919$) between raw cabbage flavor and AITC concentration (data not shown). Previous studies showed directly acidified cabbage had less AITC than fermented treatments (Corbet 1993). However, Daxenbichler and others (1980) analyzed sauerkraut for glucosinolates and their breakdown products and found no AITC. A slight (panel mean = 1.68, $P < 0.05$) metallic off-note was found by the panelists in the sulfite-preserved cabbage, which may be due to sulfite. However, most of the off-flavors were described as paint/latex and onion-like off-notes. Previous taste panel studies have also shown that directly acidified cabbage products did not resemble sauerkraut in flavor or aroma (Lonergan and Lindsay 1979; Corbet 1993).

Parmele and others (1927) found that the rate of acidification during natural sauerkraut fermentation depended on temperature and was important to sauerkraut quality. In the current study, temperature was controlled at the optimum for quality (18 °C) established by Parmele and others (1927). However, treatments that affected the rate of acidification and/or the microbiology of the fermentation, such as sulfite preservation at pH 3.5, brining at lower

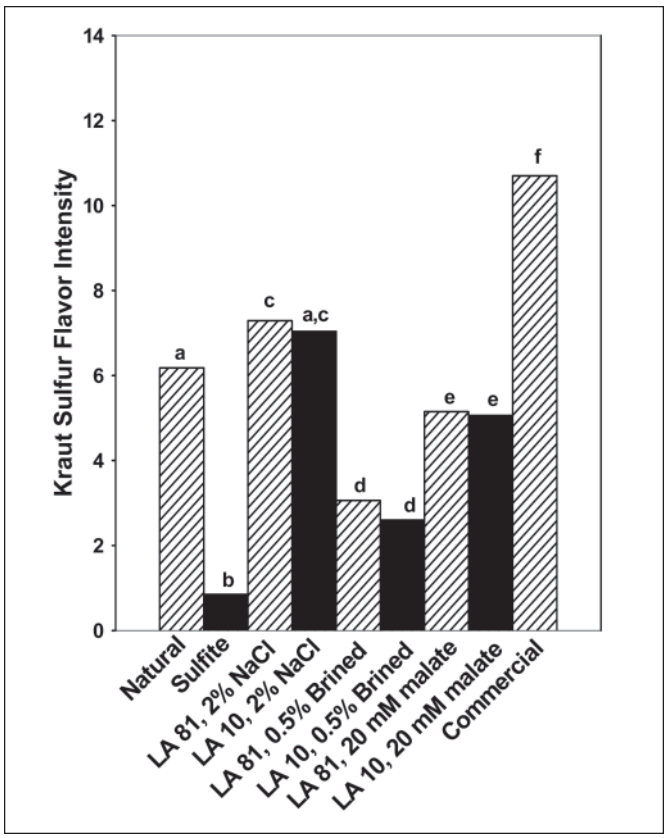

Figure 4—Sauerkraut sulfur flavor in *L. mesenteroides* - inoculated sauerkraut (lowercase letters above each bar designate different means, statistically significant at $P < 0.05$)

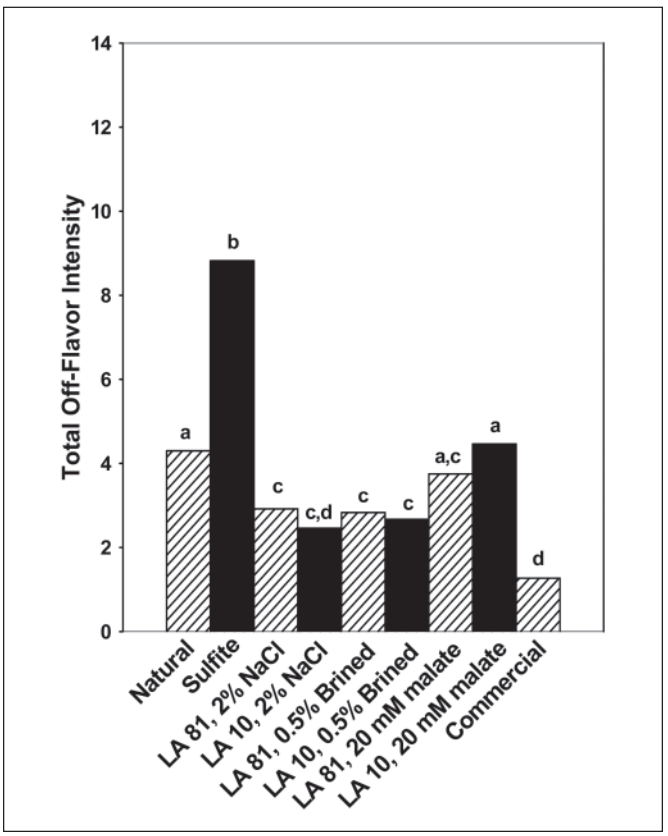

Figure 5—Total "off-flavor" in *L. mesenteroides* - inoculated sauerkraut (lowercase letters above each bar designate different means, statistically significant at $P < 0.05$)

NaCl concentration, or the addition of 20 m*M* malic acid, impacted the sensory scores for sauerkraut sulfur flavor and "off" flavors dramatically. It appears from these data that even at the optimum temperature for sauerkraut fermentation, other factors can influence the fermentation and the resulting sauerkraut flavor characteristics. Catabolism of sulfur-containing amino acids by LAB during cheese ripening has been found to contribute significantly to cheese flavor (Weimer and others 1999; Williams and others 2001; Yvon and Rijnen 2001). Therefore, it is possible that the LAB involved in sauerkraut fermentation are not only metabolizing sugars into acids, but also metabolizing sulfur-containing amino acids into important flavor compounds.

DMDS, DMTS, and $MMTSO_2$ were produced in greater quantities in fermented (MDC^+, MDC^-, and commercial) than in nonfermented (sulfite, pH 3.5) treatments (Table 5). Detection of sulfur compounds in sulfite-preserved (nonfermented, pH 3.5) cabbage compared with commercial canned sauerkraut (Figure 7) showed a very different pattern of sulfur-containing components. Corbett (1993) also observed major differences between acidified and fermented cabbage in the relative amounts of a group of highly volatile sulfur compounds. Among the 5 sulfur compounds measured by GC-MS, DMTS and $MMTSO_2$ correlated linearly with the sensory sauerkraut sulfur flavor scores ($P \leq 0.01$; Figure 8). Increasing concentrations of DMTS correlated with increasing sauerkraut sulfur flavor scores (Figure 8a). DMTS has been previously reported in sauerkraut by many researchers. Caraway spiced commercial sauerkraut, known for being less sulfurous and milder in flavor than traditional sauerkraut, had no DMTS compared with 150 ppb DMTS in unspiced commercial sauerkraut (Chin and Lindsay 1994b). DMDS was also lower in caraway-spiced sauerkraut than in unspiced commercial sauerkraut (25 versus 63 ppb). However, the current study, as well as headspace analysis by Corbet (1993), did not establish a correlation between DMDS and sauerkraut sulfur flavor or aroma. $MMTSO_2$ concentration correlated well with sauerkraut sulfur flavor scores (Figure 8b). However, all measured values were below the reported 5 ppm threshold value (Chin and

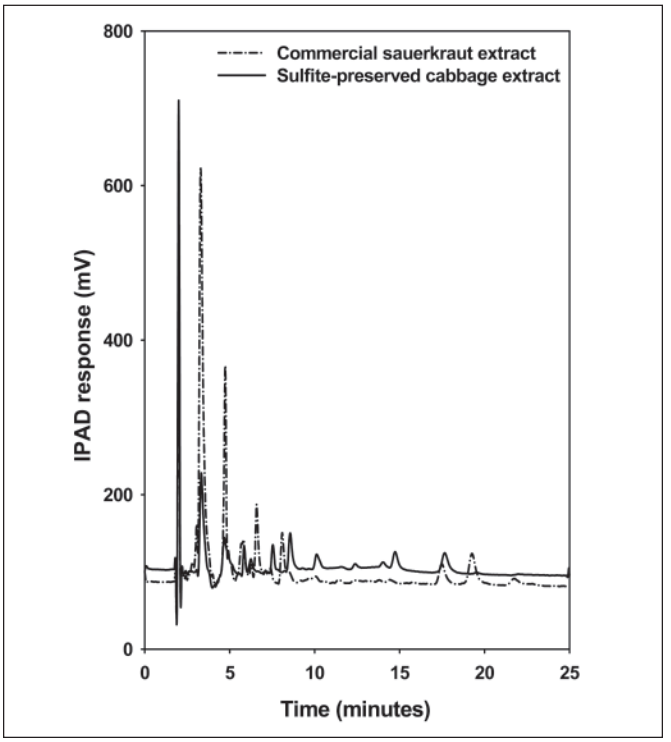

Figure 7—High-performance liquid chromatography with integrated pulsed amperometry (HPLC-IPAD) chromatograms of sauerkraut and sulfite-preserved cabbage extracts

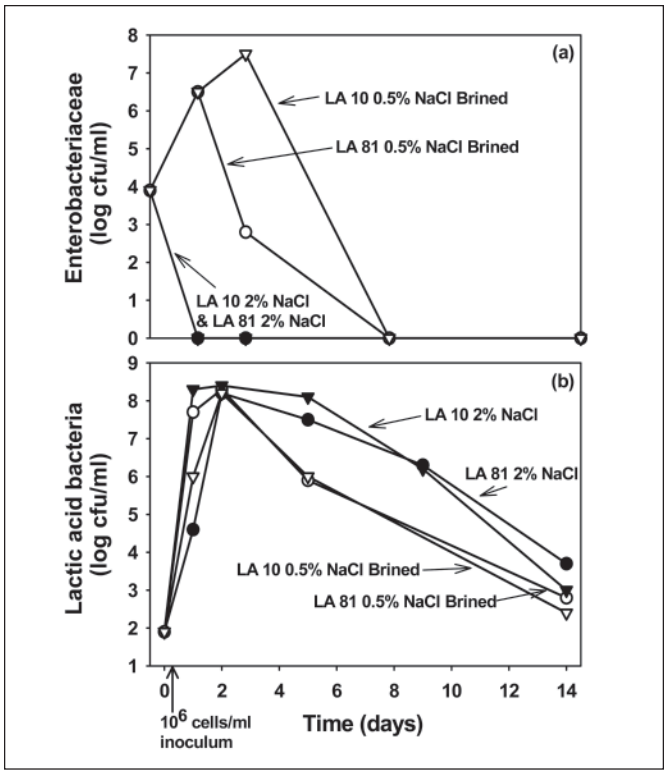

Figure 6—Growth of *Enterobacteriaceae* and lactic acid bacteria during sauerkraut fermentation

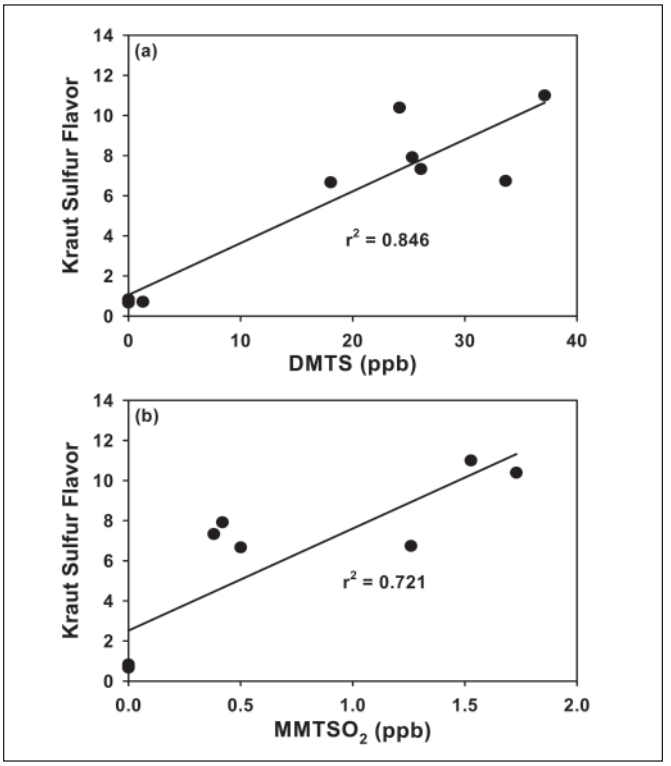

Figure 8—Correlation of sauerkraut sulfur flavor to dimethyl trisulfide (DMTS) and methyl methanethiosulfonate ($MMTSO_2$)

Lindsay 1994a). Among the samples tested, commercial sauerkraut had the greatest concentration of $MMTSO_2$ at 1.6 ppb (Table 5) and the most sauerkraut sulfur flavor (Figure 4). MMTSO was found in trace levels, well below the reported threshold value of 550 ppb (Chin and Lindsay 1994a), in sulfite-preserved cabbage and commercial sauerkraut samples, but not in MDC^+-inoculated and MDC^--inoculated sauerkraut (Table 5). MMTSO has been observed in freshly prepared cabbage juice by GC-MS. The maximum MMTSO concentration was reached at 24 h after preparation, and it then decreased when stored at 30 °C (Kyung and Fleming 1994). The transient nature of thiosulfinates, such as MMTSO, has been well established by other authors (Ostermayer and Tarbell 1960; Moore and O'Conner 1966; Block and others 1992; Marks and others 1992; Chin and Lindsay 1994a). Therefore, it was not surprising that little or no MMTSO was found in the sauerkraut treatments.

Conclusions

Malolactic activity of *L. mesenteroides* strains used for sauerkraut fermentation did not significantly influence the production of DMDS, AITC, DMTS, MMTSO, and $MMTSO_2$. No difference in flavor was found between MDC^+-inoculated and MDC^--inoculated sauerkraut, indicating that malolactic activity in starter culture strains is not important for sauerkraut fermentation. Brining cabbage at 0.5% NaCl resulted in significant changes in the microbiology and chemistry during the early stages of sauerkraut fermentation leading to undesirable changes in the resulting sauerkraut flavor. Sulfite-preserved cabbage was distinctly different from fermented cabbage in both sensory properties and sulfur compound composition, giving further evidence that fermentation is essential for generating sauerkraut-like flavors, due to the initial rate of acidification, sulfur metabolism by LAB, or a combination of these factors.

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