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ORIGINAL ARTICLE

Reduced nitrite and biogenic amine concentrations and improved flavor components of Chinese sauerkraut via co-culture of *Lactobacillus plantarum* and *Zygosaccharomyces rouxii*

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Abstract The aim of this study was to investigate the effect of inoculated fermentation on the quality of Chinese sauerkraut. To this end we studied a co-culture system consisting of *Lactobacillus plantarum* Shanghai brewing 1.08 and *Zygosaccharomyces rouxii* CGMCC 3791 during inoculated sauerkraut fermentation. The nitrite concentrations in pickled cabbage and radish inoculated with starter cultures of *L. plantarum* and *Z. rouxii* were significantly lower than those in the spontaneous fermentation system during the whole fermentation process. In addition, co-culture of *L. plantarum* and *Z. rouxii* during the production of sauerkraut decreased the formation of biogenic amines in the pickled vegetables. Using gas chromatography–mass spectrometry we also compared the levels of volatile compounds in inoculated and naturally fermented Chinese sauerkraut. Sixty compounds were identified, with the sauerkraut inoculated with starter cultures containing overall higher contents of volatile compounds, including acids, alcohols, esters, and phenols. The structure of the microbial community during the production of sauerkraut was studied using phospholipid fatty-acid (PLFA) analysis. This analysis revealed that the brine of inoculated sauerkraut contained significantly higher contents of Gram-positive and fungal PLFAs and a lower content of Gram-negative PLFAs, suggesting that the improved quality of inoculated Chinese sauerkraut may be ascribed to the inhibition of the growth of Gram-positive during sauerkraut fermentation. These results may indicate a new strategy to enhance the quality of Chinese sauerkraut.

Keywords Chinese sauerkraut · Nitrite · Biogenic amines · Volatile compounds · *Lactobacillus plantarum* · *Zygosaccharomyces rouxii*

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Introduction

Sauerkraut is a fermented food that is favored by many Chinese people. Traditionally, the Chinese use cabbage, radish, ginger, and pepper as the basis for this brine-salted and lactic acid-fermented vegetable dish. The finely shredded raw vegetables are normally immersed in 6–8 % (w/v) salt solution and allowed to undergo lactic acid fermentation for 6–10 days during sauerkraut production (Xiong et al. 2012). The manufacture of Chinese sauerkraut is currently still mostly based on spontaneous fermentation, which is highly dependent on the naturally occurring lactic acid bacteria present in the raw materials (Yan et al. 2008).

More recently, the safety and quality of fermented foods have gained public attention in China and elsewhere, and scientific efforts are being focused on minimizing the risks associated with potentially unhealthy food components. Nitrate is a natural component of many vegetables, fruits, and cured meats, and it can be converted to nitrite by nitrate reductase-containing bacteria during fermentation (Du et al. 2007; Ji et al. 2009). Consumption of nitrite has been linked to methemoglobinemia and an increased incidence of cancers (Majumdar 2003). In addition, excessive nitrite could react with amines to generate N-nitroso compounds that have been shown to be carcinogenic in animal tests (Yan et al. 2008). Numerous attempts have been made in the laboratory to reduce the formation of the nitrite peak in Korean kimchi and Chinese paocai (Ji et al. 2009; Oh et al. 2004; Yan et al. 2008). Biogenic amines (tyramine, putrescine, cadaverine, histamine, β-phenylethylamine, tryptamine) are

organic basic nitrogenous compounds, and they are ubiquitous constituents of many traditional foods, such as sufu (Liu et al. 2011), miso (Kung et al. 2007), douchi (Tsai et al. 2007), sausage (Latorre-Moratalla et al. 2008, 2010), and soy sauce (Lu et al. 2009). They are primarily formed through decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. Various research groups have attempted to reduce the concentrations of biogenic amines in fermented foods. Latorre-Moratalla et al. (2010) reported various strategies, including inoculation of a decarboxylase-negative autochthonous starter culture and increased sugar content to reduce the accumulation of biogenic amines in traditional sausage manufacture. Rabie et al. (2011) reported achieving a tenfold lower content of putrescine when *Lactobacillus casei* subsp. *casei* was added to the culture during sauerkraut fermentation and that histamine and tyramine were essentially absent during storage. In another study, Latorre-Moratalla et al. (2008) evaluated the effects of the manufacturing process, including technological, physico-chemical, and microbial factors, on aminogenesis in traditional fermented food.

The use of starter cultures, such as lactic acid bacteria or yeast, may contribute towards an improved fermentation process and product quality, and the development of appropriate starter cultures is one of the prerequisites for the production of fermented foods at the industrial level (Kimaryo et al. 2000; Leroy and De Vuyst 2004). Various lactic acid bacteria or yeasts have recently been utilized in the production of traditional foods (Beganović et al. 2011; Rashad et al. 2011; Yan et al. 2008). It has been reported that a single lactic acid bacterial strain may cause serious acidification during fermentation, thereby influencing the flavor of foods (Ali and Mustafa 2009). Therefore, the co-culture of lactic acid bacteria and yeasts may be a feasible strategy to improve the quality of fermented foods (Adeniran et al. 2012; Ali and Mustafa 2009; Kedia et al. 2007; Mugula et al. 2003). This basis for this strategy is that the proliferation of yeasts in foods may be favored by the acidic environments created by lactic acid bacteria, while the growth of lactic acid bacteria may be simulated by the presence of yeasts (Kedia et al. 2007; Nout 1991). In the study reported here, we have used a novel paired starter culture system, consisting of *Lactobacillus plantarum* Shanghai brewing 1.08 and *Zygosaccharomyces rouxii* CGMCC 3791, and studied the effects of the co-culture of both strains on the accumulation of nitrite and biogenic amines and the formation of volatile compounds during sauerkraut fermentation.

Materials and methods

Strains and growth conditions

The strains used in this study were *Lactobacillus plantarum* Shanghai brewing 1.08 and *Zygosaccharomyces rouxii* CGMCC 3791. The *L. plantarum* strain was purchased from Difa Biological Products Co., Ltd. (China), and the *Z. rouxii* strain was isolated from horsebean chili paste, a traditional Chinese condiment. Both strains were stored in a sterile vial containing 30 % (v/v) glycerol at −80 °C for use. To obtain the precultures needed for inoculation, the *L. plantarum* cells were grown statically in MRS broth (Oxoid, Basingstoke, Hampshire, UK) supplemented with 9 % NaCl at 30 °C for 48 h and the *Z. rouxii* cells were grown statically in malt extract broth (Oxoid) supplemented with 9 % NaCl at 30 °C for 48 h. The precultures were washed twice with saline and resuspended in 0.9 % saline to produce cell suspensions.

Preparation of Chinese sauerkraut

The cabbage and radish used as the raw materials for the manufacture of Chinese sauerkraut were purchased from a local supermarket in Chengdu, China. The two vegetables were first washed and then cut into strips (3×3 cm). Portions of each vegetable (250 g) was then placed into separate 2-l ceramic containers equipped with a sink on the upper part of the jar and 750 ml of a sterile NaCl solution (9 %) was then added. For inoculated fermentation, 50 ml of cell suspensions of *L. plantarum* (10^7 CFU/ml) and *Z. rouxii* (10^6 CFU/ml) were inoculated into the pickled vegetables. The containers were sealed with water, and the pickled vegetables were incubated at ambient temperature for 10 days. Three individual fermentations were performed for each kind of sauerkraut.

Measurement of nitrite concentration of Chinese sauerkraut

Nitrite content of the sauerkraut was determined using a colorimetric nitrite assay as described by Yan et al. (2008).

Determination of biogenic amine concentration of Chinese sauerkraut

The concentration of biogenic amines in the sauerkraut was determined using acid extraction according to Saarinen (2002), followed by the dansyl chloride derivatization reaction according to Dugo et al. (2006) with slight modifications. Briefly, 0.5 ml internal standard (0.1 mg/ml heptyl amine in 0.4 M $HClO_4$) and 20 ml of 0.4 M perchloric acid were added to 5 g of sample. The sample was shaken for 60 min and centrifuged (4,000 *g*) for 10 min. The supernatant was collected and the residue re-extracted according to the same procedure as described. The two supernatant extracts were combined and diluted to 50 ml with 0.4 M perchloric acid solution. For the derivatization of the samples, an aliquot of 1 ml of the acid extracts was added to 1.5 ml of saturated sodium bicarbonate, followed by 1 ml of derivatization reagent (1 % dansyl chloride in acetone, prepared daily). The sample was mixed and incubated at 60 °C for 30 min, 100 μl

of ammonia was added, and the sample was mixed and incubated at 40 °C for 45 min. After the reaction, the acetone was removed in a slight stream of nitrogen with a pressure of 0.05 MPa. The solution was then extracted twice with 3 ml diethyl ether. The upper organic layers were transferred to a plastic tube and then evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1 ml acetonitrile and filtered through a 0.22-μm membrane. Samples were quantified with high-performance liquid chromatography (model 1100; Agilent Technologies, Santa Clara, CA) according to Dugo et al. (2006).

Determination of titratable acidity

Titratable acidity was determined by titrating the brine with 0.1 M sodium hydroxide with phenolphthalein [0.1 % (m/v) in 95 % ethanol] as the indicator.

Ethanol concentration measurement

Determination of ethanol concentration was carried out according to Mcfeeters et al. (1984).

PLFA analysis

Phospholipid fatty acids were extracted using a modified procedure according to the protocols previously reported (Bligh and Dyer 1959; Frostegård and Bååth 1996). In brief, 20 ml brine was harvested and centrifuged at 10,000 *g* for 10 min; the pellets were washed twice with citrate buffer (0.15 M, pH 4.0) and then collected for lipid extraction. Lipid extraction was carried out according to Bligh and Dyer (1959). In brief, the phospholipids were methylated to fatty acid methyl esters (FAMEs) using methanol–toluene (1:1, v/v) and 0.2 M methanolic KOH. FAMEs were analyzed on a gas chromatography–mass spectrometry (GC-MS) system (Trace GC Ultra-DSQII; Thermo Electron Corp, Thermo Fisher Scientific, Waltham, MA). Separations were carried out in a TR-5MS capillary column (30.0 m×0.32 mm i.d., d_f: 0.25 μm) operated at a constant helium flow of 1 ml/min. The injector temperature was 250 °C. The oven temperature was held at 40 °C for 5 min, then increased at 5 °C/min to 200 °C and held for 10 min. The ionization energy was set equal to 70 eV. MS spectra were performed in scan mode (45~400 amu). Identification of each peak was based on a comparison of the mass spectrum to the NIST05 MS database and the retention time of standard samples (C4~C24; AccuStandard, New Haven, CT). The concentration of the PLFAs was quantified relative to the internal standard material (IS), methyl nonadecanoate fatty acid (19:0, Fluka, St. Gallen, Switzerland).

Standard nomenclature was used to describe FAMEs in accordance with Moore-Kucera and Dick (2008). The PLFAs characteristic of Gram-positive bacteria, Gram-negative bacteria, and fungi are listed in Table 1.

Extraction and analysis of aromatic compounds

The extraction of aromatic compounds was carried out according to Qian and Reineccius (2002) with some modifications. Briefly, 50 ml anhydrous ether and IS (2-octanol and octanoic acid; Sigma-Aldrich, St. Louis, MO) were added to 50 ml brine in 250-ml round-bottomed flasks. The mixture was adjusted to pH 10.0 with 1 M NaOH, 10 g NaCl was added to the solution, and the mixture was stirred for 20 min. The ether phase was then separated out in a separatory funnel, and the aqueous phase was re-extracted twice according the above procedure. All of the extracts in ether phase were combined and labeled as "extraction 1". All of the extracts in the aqueous phase were combined and adjusted to pH 1.7 with 1 M H_2SO_4 and subsequently extracted with 100 ml anhydrous ether. The ether extracts were combined and labeled as "extraction 2". Extract 1 and extract 2 were dried with 10 g anhydrous $MgSO_4$ overnight and then filtered through a Whatman 1PS filter paper. The filtrates were concentrated to 0.5 ml slowly under a gentle stream of nitrogen in an ice bath and labeled as the "basic fraction" and "acidic fraction", respectively, for the GC–MS analysis.

Samples were analyzed in triplicate on a Trace GC Ultra gas chromatograph-DSQ II mass spectrometer (Thermo Electron Corp.) equipped with a TR-5MS capillary column (30.0 m×0.25 mm i.d., 0.25 μm film thickness; Thermo Electron Corp.). GC analyses were performed under the following conditions: an inlet temperature of 250 °C, split ratio of 10:1, and a helium (purity: 99.999 %) carrier gas flow of 1 ml/min. The oven temperature was held at 40 °C for 3 min, followed by an increase of 5 °C/min to 100 °C, and then programmed at 6 °C/min until 220 °C and held for 10 min. For the mass spectrometry, the temperatures of the transfer

Table 1 Phospholipid fatty acid markers used for taxonomic microbial groups

Taxonomic group	PLFA group	Specific PLFA markers	Reference
Bacteria	Multiple groups	a14:0, i16:0, 16:1ω9	Frostegård and Bååth (1996)
Gram-positive bacteria	Branched PLFAs	a14:0, i16:0	Moore-Kucera and Dick (2008)
Gram-negative bacteria	Cyclopropyl and monosaturated PLFAs	16:1ω9	Moore-Kucera and Dick (2008)
Fungi	Polyunsaturated PLFAs	18:1ω9, 18:2ω6,9	Federle et al. (1986)

PFLA, Phospholipid fatty acid

line, quadruple and ionization source were 250, 150, and 230 °C, respectively. The mass spectrum was generated in the electron impact (EI) mode at 70 eV. The spectra was taken over the m/z range of 40~400. Volatile compounds were identified by comparing their mass spectrum with the NIST05 library database. Quantitative data were obtained by calculating the peak areas in relation to that of the IS.

Statistical analysis

The Student's *t* test was employed to investigate statistical differences. Differences between samples with *p* values of ≤0.05 were considered to be statistically significant.

Results

In this study, a co-culture of *L. plantarum* and *Z. rouxii* was inoculated into the fermenting vegetables during the production of Chinese sauerkraut. The quality of the sauerkraut produced by spontaneous fermentation and by fermentation with the inoculated co-culture was compared by measuring the concentrations of nitrite, biogenic amines, and volatile compounds.

Changes in nitrite concentration during sauerkraut fermentation

Changes in the nitrite concentration during sauerkraut fermentation of cabbage and radish are shown in Fig. 1. The nitrite concentration accumulated rapidly from 7.9 to 14.2 mg/kg during the first 3 days of spontaneous fermentation of cabbage (Fig. 1a), with the peak concentration appearing on the third day followed by a slight decline during the following 5 days of fermentation. On day 8 of spontaneous fermentation, the nitrite content had reached 12.2 mg/kg. In comparison, during the inoculated fermentation of *L. plantarum* Shanghai brewing 1.08 and *Z. rouxii* CGMCC 3791, the nitrite content decreased sharply after the first day of cultivation, and on day 8 of fermentation, the nitrite content had fallen to 2.9 mg/kg. The concentrations of nitrite in the inoculated fermentation system were significantly lower than those in the spontaneous fermentation system during the whole cultivation process (Fig. 1a). Similarly, a reduced content of nitrite in pickled radish was observed with co-culture of *L. plantarum* and *Z. rouxii* (Fig. 1b). On day 8 following the inoculation of the fermentation system with starter cultures, the nitrite content in pickled radish was 4.4-fold lower than that of the spontaneous fermentation. These results suggest that co-culture of *L. plantarum* and *Z. rouxii* during sauerkraut fermentation may effectively inhibit the formation of nitrite and decrease nitrite content in pickled vegetables.

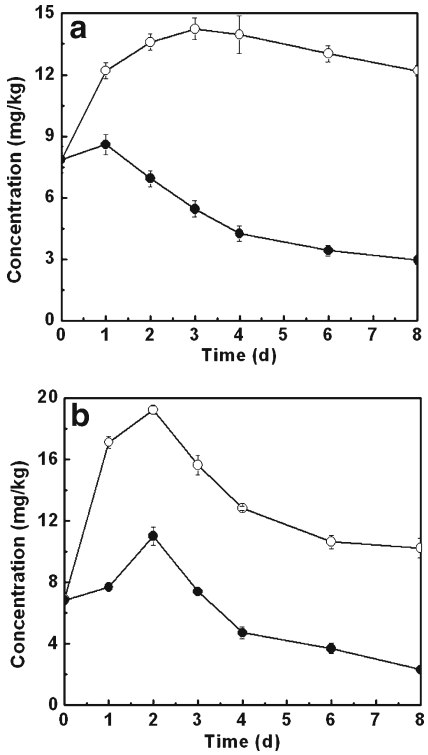

Fig. 1 Changes in nitrite concentration in pickled cabbage (**a**) and radish (**b**) during sauerkraut fermentation. *Open circles* Spontaneous fermentation, *filled circles* inoculated fermentation of *Lactobacillus plantarum* Shanghai brewing 1.08 and *Zygosaccharomyces rouxii* CGMCC 3791. *Error bars* Standard deviation (SD) ($n=3$)

Changes in biogenic amine concentration during sauerkraut fermentation

The accumulation of biogenic amines, including putrescine, cadaverine, and tyramine, was monitored throughout the manufacture of sauerkraut (Fig. 2). In the spontaneous fermentation system, the content of putrescine in pickled cabbage increased with the increasing fermentation time, with the highest amount of putrescine (45.9 mg/kg) detected on the fourth day. This was followed by a slight decline in putrescine level during the three subsequent days (Fig. 2a). In comparison, the putrescine content in pickled radish remained relatively stable during 8 days of spontaneous cultivation (Fig. 2b). However, during the inoculated fermentation, the putrescine levels in both the fermented cabbage and radish decreased sharply during the whole cultivation process. After 8 days of cultivation, the level of putrescine in both pickled vegetables in the spontaneous fermentation system was 13-fold higher than that obtained in the inoculated fermentation system.Similar change trends were observed during sauerkraut fermentation for cadaverine and tyramine (Fig. 2c–f). A detailed analysis of the data reported in Fig. 2 revealed that the disparity in the content of the various biogenic amines between the spontaneous fermentation and inoculated fermentation system increased with increasing cultivation time.

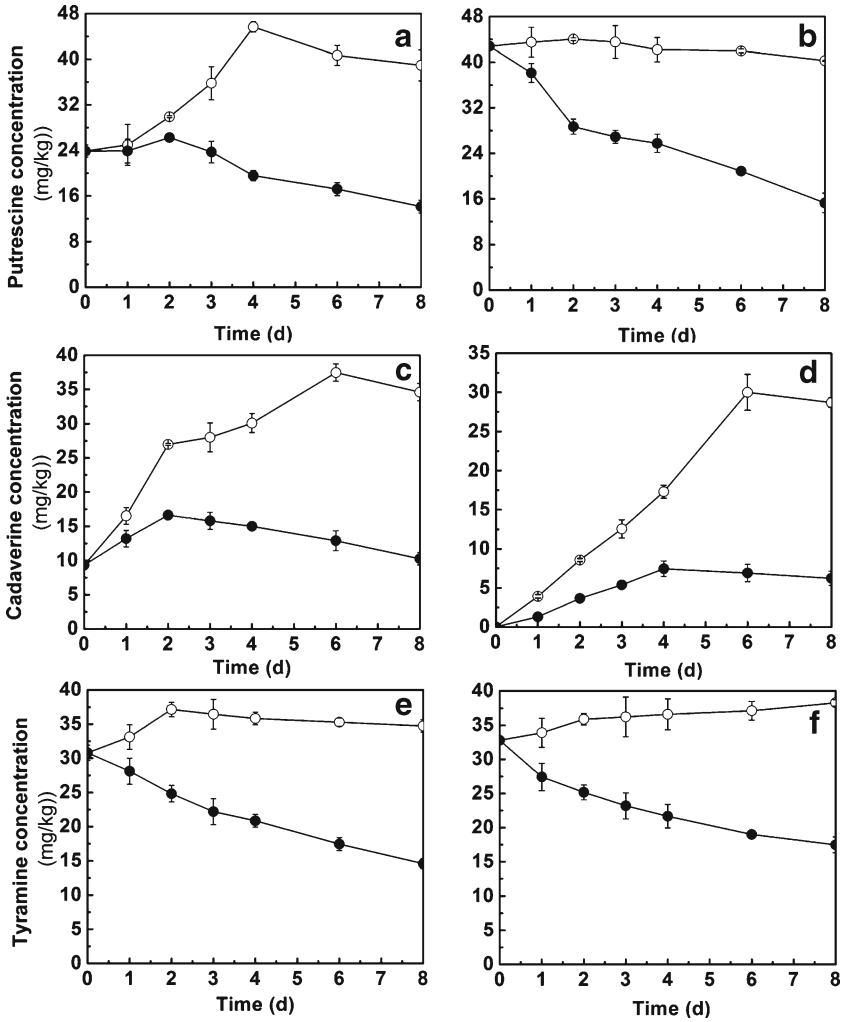

Fig. 2 Changes in the contents of putrescine (**a**, **b**), cadaverine (**c**, **d**), and tyramine (**e**, **f**) in pickled cabbage (**a**, **c**, **e**) and radish (**b**, **d**, **f**). *Open circle* Spontaneous fermentation, *closed circle* inoculated fermentation of *L. plantarum* Shanghai brewing 1.08 and *Z. rouxii* CGMCC 3791. *Error bars* SD ($n=3$)

These results suggest that co-culture of *L. plantarum* and *Z. rouxii* during the manufacture of sauerkraut may lower the formation of biogenic amines in pickled vegetables.

Comparison of the volatile compounds by GC-MS analysis

Representative GC–MS chromatograms of inoculated and naturally fermented sauerkraut are shown in Fig. 3. While similar volatile compounds were identified in various samples, differences in the peak intensity of individual volatiles and total volatiles between the naturally fermented and inoculated fermented sauerkraut were observed, (Fig. 3; Table 2). A total of 60 compounds were identified, including 22 acids, four alcohols, 20 esters, nine phenols, one pyrazine, and four furans. Among these compounds, acids and esters formed the largest groups (Table 2).

Among the 22 acidic compounds identified in Chinese sauerkraut, butanoic acid, phenylacetic acid, pelargonic acid, myristic acid, 9-hexadecylenic acid, and hexadecanoic acid were the major acids in pickled cabbage, while nonanoic acid, palmitoleic acid, and stearic acid were the main acids in pickled radish; these two groups accounted for 67.50 and 88.72 % of the total acids in natural fermented cabbage and radish, respectively. In addition, it should be noted that the contents of many acid compounds increased in the inoculated fermented sauerkraut. In particular, the content of hexanoic acid and palmitoleic acid increased by 94.20- and 11.35-fold, respectively, in pickled cabbage and radish in the inoculated fermentation system. Hexanoic acid confers a rancid, fatty, and cheese odor to food, with an odor perception threshold of 3 mg/l (Muñoz et al. 2007). Therefore, the odor activity value (OAV) obtained based on the ratio of the observed concentration of the compound to its threshold was higher than 1 (OAV >1), suggesting that hexanoic acid makes a major contribution to the sensory profiles of Chinese sauerkraut.

A total of four alcohols were detected in our study, and 3-methyl-1-butanol was the most abundant compound. The threshold value of 3-methyl-1-butanol (4 μg/l) (Giri et al. 2011) was reached in both inoculated fermented samples. Alcohols give pleasant aromas and sweet flavors (Steinhaus

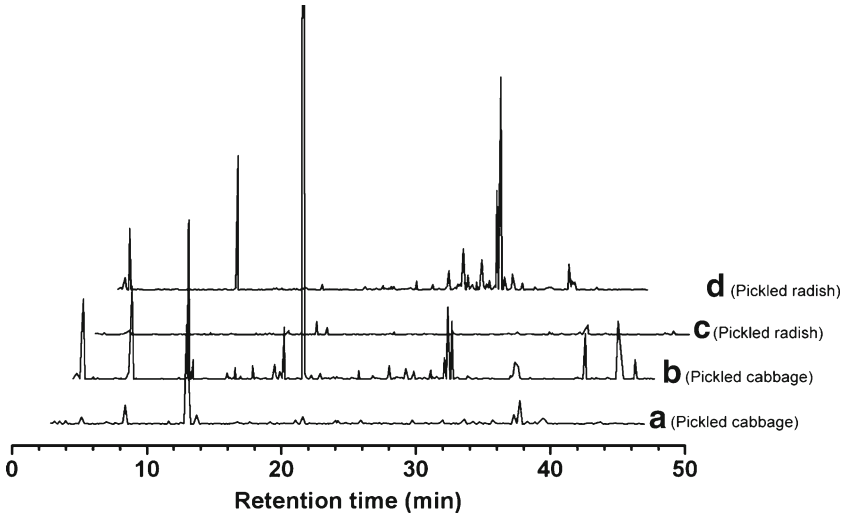

Fig. 3 Representative chromatograms of the gas chromatography–mass spectrometry analysis of brine in pickled cabbage and radish during sauerkraut fermentation. *A*, *C* Spontaneous fermentation, *B*, *D* inoculated fermentation of *L. plantarum* Shanghai brewing 1.08 and *Z. rouxii* CGMCC 3791

et al. 2009), and the natural fermented samples contained many fewer alcohols than the inoculated fermented samples. All of the alcohols detected were present at relatively higher amounts in both inoculated fermented samples (Table 2).

Esters are one of the most important flavor compounds in fermented foods as they volatilize quickly and human nose is sensitive to them (Lee and Ahn 2009; Raghavendra et al. 2010). Most of the esters detected in this study had been previously found in soybean paste, miso, or soy sauce (Fan et al. 2011; Lee and Ahn 2009; Sun et al. 2010; Zhao et al. 2011). Nine esters in pickled cabbage and nine esters in picked radish were present at significantly increased levels during inoculated sauerkraut fermentation. In addition, the content of total esters in inoculated cabbage and radish increased by 2.06- and 2.67-fold, respectively. An interesting observations was that most of the esters detected were ethyl esters, which is in agreement with previous research on soybean paste (Zhao et al. 2011). The concentration of ethyl esters in naturally fermented cabbage and radish was 28.83 and 37.07 mg/l, respectively, representing 86.86 and 84.07 % of the total esters; in inoculated fermented cabbage and radish, these values were 57.09 and 85.38 mg/l, respectively, representing 83.60 and 72.41 % of the total esters. Analysis of the OAVs of methyl palmitate and ethyl hexadecanoate showed that they were unlikely to be important compounds due to their high threshold values (>2 mg/l) (Buttery et al. 1988).

We also identified a total of nine phenols. Two phenols in pickled cabbage and four phenols in pickled radish were found to significantly increase in content during the inoculated sauerkraut fermentation. Only one pyrazine, tetramethylpyrazine, was detected in samples, at a relatively low content. Four furans were also detected in the samples, and the level of 2-furfuralcohol was much higher in pickled radish during the inoculated fermentation. However, the threshold value of 2-furfuralcohol (4.5 mg/l) (Giri et al. 2011) was not reached in the inoculated sample, as the concentration was relatively low.

Changes in the structure of the microbial community during the production of Chinese sauerkraut based on PLFA analysis

To clarify the effect of inoculated fermentation on the distribution of the microbial community in Chinese sauerkraut, we investigated the structure of the microbial community structure based on PLFA analysis (Fig. 4). As shown in Fig. 4a, b, a total of nine fatty acids were identified in the brine of both pickled vegetables, including saturated fatty acids (14:0, 15:0, 16:0, and 18:0), branched fatty acids (a14:0 and i16:0), monounsaturated fatty acids (16:1ω9, 18:1ω9), and polyunsaturated fatty acids (18:2ω6,9). Higher levels of PLFAs 14:0, a14:0, i16:0, 18:1ω9, 18:2ω6,9, and 18:0 were detected in both samples of the inoculated sauerkraut fermentation compared with the natural fermentation. However, we found a markedly lower concentration of 16:1ω9 in inoculated sauerkraut (Fig. 4a, b). Figure 4c, d shows the total abundances of PLFAs, bacterial PLFAs and fungi PLFAs. The total PLFA concentration, which is an indicator of active living biomass, in sauerkraut produced by inoculated fermentation was higher than that produced by spontaneous fermentation. In addition, significantly higher contents of Gram-positive bacterial and fungal PLFAs and a lower content of Gram-negative bacterial PLFAs were observed in inoculated fermented sauerkraut (Fig. 4c, d). These results suggest that the addition of *L. plantarum* and *Z. rouxii* during the manufacture of sauerkraut may inhibit the growth of Gram-negative bacteria.

Discussion

Lactic acid bacteria and yeasts are widely used in the production of fermented food due to their quality-promoting efficacy. In particular, during the manufacture of pickled foods, the strains which exhibit a high tolerance to salt stress have been

Table 2 Gas chromatography–mass spectrometry analysis of volatile compounds of naturally fermented and inoculated fermented Chinese sauerkraut

Retention time (min)	Volatile compound	Concentration[a] (mg/l)			
		Cabbage		Radish	
		Spontaneous fermentation (SF)	Inoculated fermentation (IF)	Spontaneous fermentation (SF)	Inoculated fermentation (IF)
Acids (22)					
5.07	2-Methyl- propanoic acid	ND[b]	ND	0.19	0.79*
7.47	Butanoic acid	5.27	14.32*	0.25	6.56*
8.47	3-Methyl-butanoic acid	0.30	0.35	0.56	0.51
8.74	2- menthyl-butanoic acid	0.04	0.36*	0.55	1.37*
9.99	Pentanoic acid	0.55	0.59	0.13	0.40*
14.5	Hexanoic acid	0.05	4.71*	ND	2.66*
16.27	Heptanoic acid	0.33	0.14	0.26	1.04*
16.73	Furoic acid	0.89	0.90	TR	2.39*
21.59	Phenylacetic acid	5.45	13.14*	0.38	1.26*
21.76	Pelargonic acid	3.44	10.18*	14.83	40.46*
23.8	Phenylpropionic acid	0.33	0.50	0.23	0.82*
24.28	Capric acid	0.70	0.52	1.38	1.34
29.09	Dodecanoic acid	2.47	2.91	0.21	1.71*
33.38	3-Methoxy-4-hyd roxy-phenylpropionic acid	0.60	0.95	0.27	1.06*
33.54	Myristic acid	8.06	16.49*	0.48	3.09*
35.6	Pentadecanoic acid	0.94	1.09	0.39	3.38*
37.24	9-Hexadecylenic acid	3.12	7.83*	0.54	6.13*
37.85	Hexadecanoic acid	4.13	37.21*	40.23	92.78*
40.17	15-Methyl- heptadecylic acid	0.28	0.52	0.22	0.74*
42.9	Linolenic acid	2.37	10.88*	1.17	7.27*
43.14	Oleic acid	1.38	6.32*	3.35	21.41*
43.81	Stearic acid	2.96	8.29*	27.98	48.24
Alcohols (4)					
3.46	3-Methyl-1-butanol	0.21	2.00*	0.79	15.73*
5.22	2,3-Butanediol	ND	ND	0.76	1.96*
8.01	1-Hexanol	0.01	0.08	0.01	0.03
12.34	1,2,3-Butantriol	0.01	0.05	ND	0.18*
Esters (20)					
9.95	Methyl hexanoate	0.21	0.28	0.19	0.15
36.75	Methyl palmitate	2.12	6.42*	2.65	24.62*
5.11	Ethyl butyrate	4.71	4.07	4.08	6.04
5.69	Ethyl lactate	0.73	0.59	0.17	0.28
9.01	Ethyl valerate	0.49	0.49	0.33	0.35
9.95	Ethyl hexanoate	3.57	7.82*	4.13	5.35
14.82	2-Hydroxy-4-methyl-ethyl valerate	0.01	0.02	0.01	0.31*
16.14	Ethyl heptylate	0.21	0.32	0.02	0.58*
18.86	Diethyl succinate	0.01	0.01	0.01	0.12
19.2	Ethyl caprylate	1.32	2.71*	1.18	1.36
22.03	Ethyl pelargonate	1.12	2.56*	0.01	3.15*
24.47	Hexyl hexanoate	2.02	4.48*	4.18	7.46
24.7	Ethyl caprate	2.13	1.36	0.24	0.25
34.03	Ethyl myristate	0.01	0.22*	0.02	0.24*
35.49	Ethyl pentadecanoate	0.21	0.26	11.67	22.78*

Table 2 (continued)

Retention time (min)	Volatile compound	Concentration[a] (mg/l)			
		Cabbage		Radish	
		Spontaneous fermentation (SF)	Inoculated fermentation (IF)	Spontaneous fermentation (SF)	Inoculated fermentation (IF)
38.21	Ethyl hexadecanoate	14.22	28.30*	15.05	33.61*
43.32	Ethyl linoleate	0.08	4.82*	ND	5.72*
43.59	Ethyl oleate	ND	3.48*	ND	5.37*
44.61	Ethyl octadecanoate	0.02	0.08	0.18	0.18
Phenols (9)					
8.55	Ortho-xylene	0.01	ND	0.09	0.17
11.7	Benzaldehyde	0.19	0.11	0.05	1.27*
14.49	Phenylcarbinol	0.01	0.01	0.01	0.13
16.97	Phenylethyl alcohol	0.04	0.50*	0.02	2.17*
18.62	Ethyl benzenecarboxylate	0.14	0.11	0.19	0.25
20.75	Ethyl phenylacetate	0.25	5.70*	0.25	4.54*
30.9	Phenyl ethyl butyrate	0.02	0.07	0.05	0.20*
32.03	6-Ethyl-3-octyl ester- phthalic acid	0.04	0.10	21.36	38.08
Pyrazines (1)					
15.93	Tetramethylpyrazine	0.01	0.07	0.01	0.06
Furans (4)					
6.70	3-Fur(fur)aldehyde	0.14	0.14	ND	0.08
7.69	2- Furfuralcohol	0.23	0.26	0.05	0.42*
31.81	5-Methyl −2- carboxylic acid furan formaldehyde	0.06	0.11	0.27	0.50
34.41	5-Methyl-2-furan formaldehyde	0.01	0.02	0.80	0.65

*Significant difference between SF and IF at $p<0.05$ by the Student's t test

[a] The concentration of each compound was calculated on the basis of the ratio of peak area of the respective compounds and that of the internal standard (2-octanol; Sigma-Aldrich, St. Louis, MO). Three individual fermentations were performed, and the data were the mean value of triplicate measurements

[b] ND, Not determined or peak area of <0.05 %

found to contribute to the formation of volatile compounds. Therefore, in this study, we studied a novel paired starter culture system consisting of *Lactobacillus plantarum* and *Zygosaccharomyces rouxii* and investigated the effect of inoculated fermentation on the quality of Chinese sauerkraut.

During the manufacture of Chinese sauerkraut, we observed that the co-culture of *L. plantarum* and *Z. rouxii* decreased the content of nitrite and biogenic amines in pickled vegetables (Figs. 1, 2). Nitrate is a natural component of many vegetables, and the main mechanism involved in nitrite depletion is enzymatic degradation (Dodds and Collins-Thompson 1984; Oh et al. 2004). Under anaerobic conditions, nitrate may be reduced to nitrite by nitrate-reducing bacteria. At the initial stage of spontaneous fermentation, the number of lactic acid bacteria was low, and some Gram-negative bacteria (such as Enterobacteria) were more prominent, which led to the rapid growth of nitrate-reducing bacteria (Yan et al. 2008). Therefore, a sudden rise in nitrite concentration was observed (Fig. 1). However, during inoculated fermentation, *L. plantarum* and *Z. rouxii* grew quickly, and higher contents of Gram-positive bacterial and fungal PLFAs and lower content of Gram-negative bacterial PLFA were observed (Fig. 4). The growth of *L. plantarum* and *Z. rouxii* resulted in higher contents of total titratable acid and ethanol (Fig. 5), which may effectively inhibit the growth of Gram-negative bacteria. A low pH and other antimicrobial substances produced by the dominant lactic acid bacteria have been reported to inhibit *Enterobacteria* and other Gram-negative bacteria (Nout 1991). Previous research has demonstrated that some lactobacilli are capable of enzymatically reducing nitrite, and the production of lactic acid and the consequent decrease in pH are partly responsible for the depletion of nitrite (Dodds and Collins-Thompson 1984; Yan et al. 2008). In our study, the co-culture of *L. plantarum* and *Z. rouxii* decreased the content of nitrite, which is in agreement with previous observations.

Another intriguing finding is the reduction in the content of biogenic amines during inoculated fermentation. Three

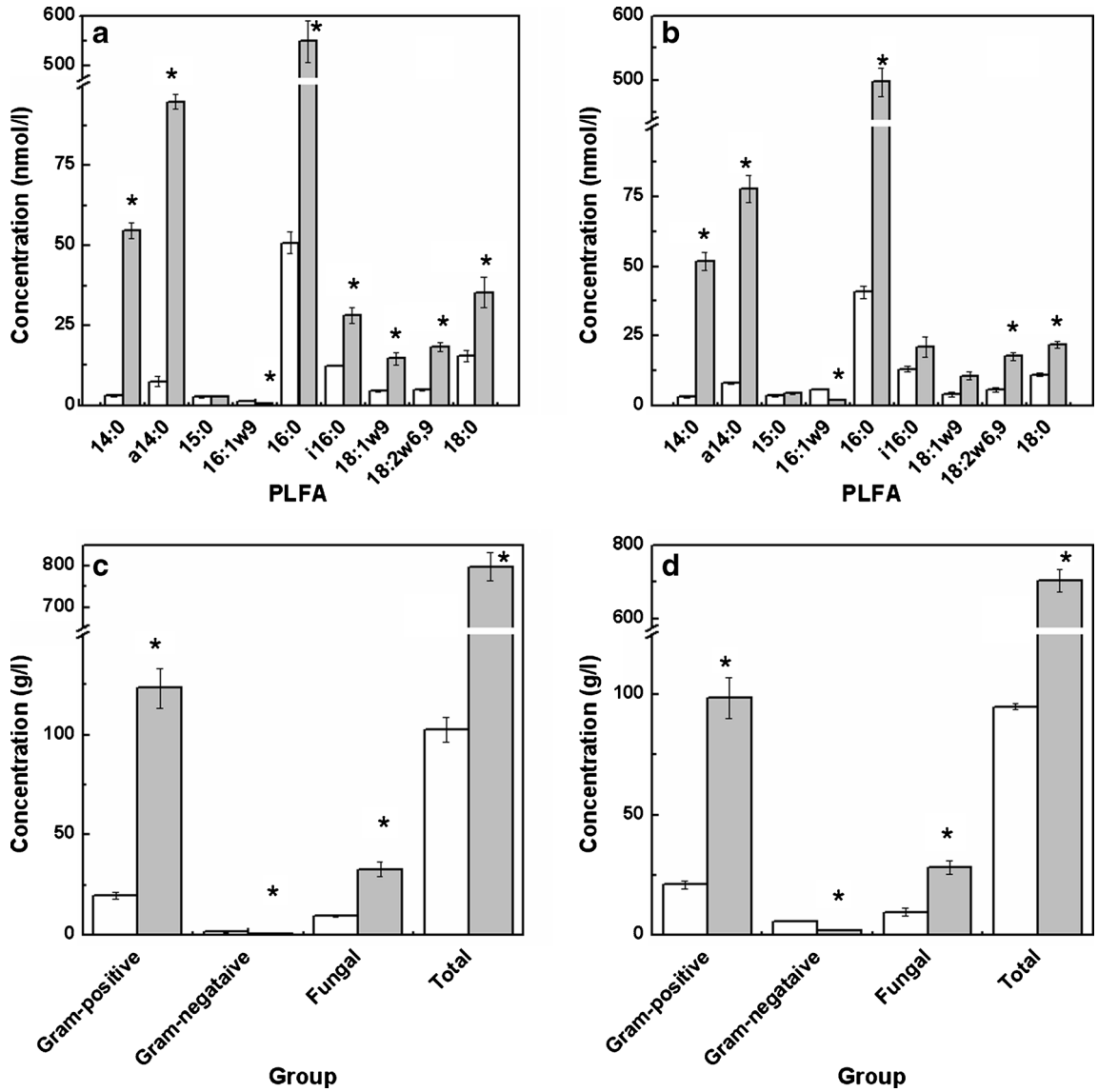

Fig. 4 **a**, **b** Concentrations of different groups of phospholipid fatty acids (*PFLAs*) in brine of cabbage (**a**) and radish (**b**) during Chinese sauerkraut manufacture. **c**, **d** content of bacterial, fungal and total PLFAs in brine of cabbage (**c**) and radish (**d**) during Chines sauerkraut manufacture. *White bars* Spontaneous fermentation, *gray bars* Inoculated fermentation of *L*. *plantarum* Shanghai brewing 1.08 and *Z*. *rouxii* CGMCC 3791. *Error bars* SD ($n=3$). Statistically significant differences ($p<0.05$) were determined by Student's *t* test and are indicated with an *asterisk*

dominant biogenic amines, including putrescine, cadaverine, and tyramine, were monitored throughout the manufacture of sauerkraut. An obvious difference in biogenic amines contents between spontaneous fermentation and inoculated fermentation was observed, and the inoculated fermentation exhibited lower contents of biogenic amines. Many strains, such as *Lactobacillus sakei*, *Staphylococcus equorum*, and *Lactobacillus casei* subsp. *casei*, have been shown to be effective in the degradation of biogenic amines in traditional fermentation foods (Latorre-Moratalla et al. 2010; Rabie et al. 2011). In our study, we also demonstrated that a combination of *L*. *plantarum* and *Z*. *rouxii* resulted in the reduction of biogenic amines in Chinese sauerkraut.

Comparison of the volatile compounds of the inoculated and naturally fermented Chinese sauerkraut was carried out based on GC–MS analysis. Higher contents of volatiles were detected in the inoculated fermented sauerkraut in comparison with spontaneously fermented sauerkraut (Table 2). These results demonstrate that co-culture of *L*. *plantarum* and *Z*. *rouxii* improved the flavor of Chinese sauerkraut. The enhanced production of volatiles in co-culture may be taken to be indicative of an interaction between lactic acid bacteria and yeasts (Mugula et al. 2003). There were also higher contents of acids and alcohols (Fig. 5), which were produced by lactic acid bacteria and yeast, in the mixed culture system which increased the formation of various esters and, consequently,

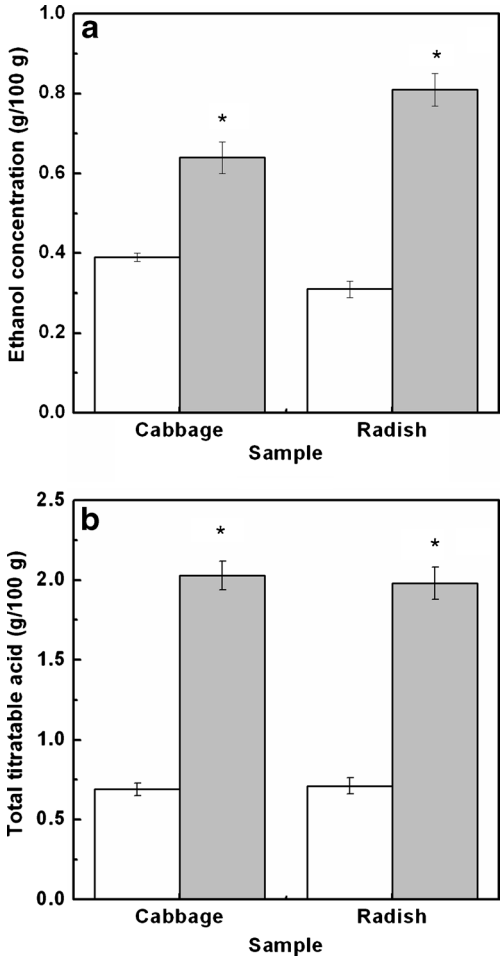

Fig. 5 Influence of inoculated fermentation on total titratable acid (**a**) and ethanol concentration (**b**) during Chinese sauerkraut manufacture. *White bars* Spontaneous fermentation, *gray bars* inoculated fermentation of *L. plantarum* Shanghai brewing 1.08 and *Z. rouxii* CGMCC 3791. *Error bars* SD ($n=3$). Statistically significant differences ($p<0.05$) were determined by Student's *t* test and are indicated with an *asterisk*

conferred a stronger flavor to sauerkraut. The contribution of yeasts to flavor acceptability in lactic acid-fermented products has been verified in earlier studies, which reported that the enhanced production of flavor compounds could be obtained via co-culture of lactic acid bacteria and yeasts (Kedia et al. 2007; Mugula et al. 2003).

In conclusion, in this study we investigated the effect of co-culture of *L. plantarum* and *Z. rouxii* on the quality of Chinese sauerkraut. The results presented in this study demonstrate that sauerkraut samples inoculated with combined microbial starters exhibit decreased contents of nitrite and biogenic amine and improved flavor compounds. These results may revolutionize the manufacturing process of Chinese sauerkraut manufacture by producing a product of improved quality.

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