



Effects of salt concentration on Chinese sauerkraut fermentation



Tao Xiong^{a, b, *}, Junbo Li^{a, b}, Fan Liang^c, Yanping Wang^{a, b}, Qianqian Guan^{a, b}, Mingyong Xie^a

^a State Key Laboratory of Food Science & Technology, No. 235 Nanjing East Road, Nanchang, Jiangxi, 330047, PR China

^b College of Food Science, Nanchang University, Nanchang, 330047, PR China

^c College of Food Science and Technology, Hunan Agricultural University, Changsha, 410128, PR China

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ABSTRACT

The aim of the study was to determine the effects of salt concentration on traditional sauerkraut fermented spontaneously. Lactic acid bacteria (LAB), fungi and *Escherichia coli* (*E. coli*) in the brine were analyzed in the three kinds of sauerkraut. The contents of sugars (sucrose, glucose, fructose) and organic acids (lactic acid, acetic acid) in the brine and inside the cabbage were monitored by high-performance liquid chromatography (HPLC). In addition, the pH value was monitored in the brine. Results demonstrated that sucrose and glucose were consumed and fructose was accumulated gradually during fermentation. The whole fermentation process was dominated by LAB and a considerable accumulation of lactic acid was observed both in cabbage and brine at the end of fermentation. Salt concentration had a significant effect on sauerkraut fermentation at early stage. The LAB population and metabolic rate was reduced and the yield of lactic acid decreased with the increase of salt concentration. Suitable salt concentration can effectively inhibit the reproduction of fungi and *E. coli*. In comparison, high salt concentration delayed the maturation of sauerkraut and inhibited the metabolism of LAB.

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1. Introduction

It was recorded that Chinese sauerkraut (also known as “Pao Cai”) originated from Zhou Dynasty 3000 years ago (Zhang, 1994) and spread to South Korea in the 5th century (Ma, 2010). Chinese sauerkraut, as a typical brine-salted vegetable fermented by lactic acid bacteria (LAB), is widely consumed in China (Yan, Xue, Tan, Zhang, & Chang, 2008). Unlike kimchi which uses direct salting to withdraw juice from the cabbage (dry salting), traditional Chinese sauerkraut is anaerobic, fermented in brine with a low salt concentration (2%–10%) by the indigenous microorganism on the raw cabbage (Chen, 2007). LAB plays an important role during fermentation, because they contribute to sensory characteristics and preservation (Holzapfel, 1995). Fermentation of sauerkraut can be divided into hetero-fermentation and homo-fermentation phase, and species and quantity of LAB varies with fermentation stage. The initial phase was hetero-fermentation dominated by *Leuconostoc citreum*, *Leuconostoc mesenteroides* and *Weissella*

koreensis et al. (Park et al., 2010; Wiander & Ryhänen, 2008), and then gradually transitioned to homo-fermentation phase, dominated by *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus curvatus*, and *Lactobacillus sakei* et al. (Kim et al., 2002; Plengvidhya, Breidt, Lu, & Fleming, 2007). It was also reported that the fermentation process of Chinese sauerkraut with 4% salt also experienced hetero-fermentative and successively homo-fermentative phase (Xiong, Guan, Song, Hao, & Xie, 2012). Salt concentration of sauerkraut had an effect on saline taste and microbial structure of brine so as to directly or indirectly affect the quality and flavor of sauerkraut. High salt concentration can better inhibit the growth of spoilage bacteria in brine, meanwhile, the first hetero-fermentative phase was absent, due to the intolerance towards salt of *Leuconostocs* (Cagno et al., 2009; Wouters et al., 2013). Now, many researches about the effect of salinity on utilization ratio of sugar and acid production during olive and cucumber fermentation have been reported (Efstathios & Constantinou, 2006; Frederico et al., 2005; Lu, Fleming, & Mcfeeters, 2001; Mcfeeters & Pérez-Díaz, 2010). The water activity and osmotic pressure of brine with different combinations of salt in Spanish olive fermentation were described in detail (Panagou, Hondrodinou, Mallouchos, & Nychas, 2011). However, there is little information regarding the influence of salt concentration on the fermentation of Chinese

* Corresponding author. State Key Laboratory of Food Science & Technology, No. 235 Nanjing East Road, Nanchang, Jiangxi, 330047, PR China.

E-mail address: xiongtao0907@163.com (T. Xiong).

sauerkraut.

The aim of this study was to determine the effect of salt concentration on sauerkraut fermentation. The changes of pH, LAB, fungi and *Escherichia coli* in brine as well as the contents of organic acid, sugar, and sucrose in cabbage and brine under different salt concentrations were examined.

2. Materials and methods

2.1. Materials

Fresh cabbage and other auxiliary materials were purchased from a local supermarket in Nanchang, Jiangxi Province, China.

2.2. Preparation of sauerkraut

Cabbages were cut into small pieces (2–3 cm × 6–8 cm), then the pieces of cabbage were washed and drained as the raw material of sauerkraut. Fermentation were carried out in 5 L ceramic jars, each containing 1 kg sliced cabbage pieces and auxiliary materials, including crystal sugar (4%), hot red pepper (4%), garlic (3%), ginger (2%) and Chinese prickly ash (1.5%) (All percentages were calculated by the volume of sterile water). Salt (2%, 5%, and 8%, w/v) and sugar (4%) were dissolved in 2000 mL sterile water, saline solutions at the three concentrations were prepared as mentioned above. Finally cold sterile water was added into sauerkraut jar that was then water sealed. The sauerkraut jars were kept at ambient temperature (20–25 °C) during experiments.

2.3. Sampling

During the fermentation, brine (10 mL) and cabbage (10 g) samples were withdrawn aseptically every 12 h for 7 days, the sauerkraut jars were shaken before each sampling. A part of the brine was used for measurements of pH value and analysis of microbiological changes, the other part was stored at –20 °C for HPLC analysis. The cabbage samples were pulped and diluted 10 times, 5 mL diluent was taken and then stored at –20 °C for HPLC analysis (McFeeters & Pérez-Díaz, 2010).

2.4. Analytical methods

2.4.1. Microbiological analysis

1 mL brine was aseptically added into 9 mL sterile saline (0.85% NaCl, w/v), appropriately diluted, 100 µL brine of 3 suitable gradient dilutions were respectively coated on the following flat plate, each with a parallel coating. Violet Red Bile Dextrose agar (VRBDA) for Enterobacteriaceae, the agar media were incubated at 37 °C for 24 h, LAB on MRS incubated at 37 °C for 48 h (Efstathios & Constantinou, 2006; Wang, Ren, Liu, Zhu, & Wang, 2013), Fungi on Yeast Extract Peptone Dextrose Medium agar (YPD) incubated at 25 °C for 72 h. To avoid bacterial growth, YPD was supplemented with chloramphenicol (0.1 g L⁻¹; Sigma-Aldrich) (Wendy, Ilenys, & Pérez, 2012).

2.4.2. Determination of organic acids, sugar and pH value

HPLC (Model 1200, Agilent, USA) was used to determine the concentration of sugars and organic acids. For HPLC analysis, the brine and cabbage slurry samples were thawed and centrifuged at 10000 × g for 10 min, then filtered through 0.22 µm membrane (Xiong, Li, Guan, Peng, & Xie, 2014). The pH value of the brine samples was measured using a pH meter (PHS-25, Shanghai Precision Scientific Instruments Company, China).

2.4.3. Statistical analysis

Data was represented as mean values (n = 4) ± standard deviation of means. Analysis of variance (ANOVA) was performed on the data obtained every 12 h, followed by Student's *t* test using SPSS 20. Differences were considered significant at *p* < 0.05. Origin8.6 software was used for mapping.

3. Results and discussion

3.1. Changes of pH value during Chinese sauerkraut fermentation

T, F, E represent for 2%, 5%, 8% salt concentration sauerkraut, further referred to as T, F, E. Error bars represent the standard deviation.

The pH is a critical indicator of fermentation progress, and its drop occurred mainly due to lactic acid, the metabolism by LAB (Adams, 1990; Kandler, 1983). As shown in Fig. 1, the initial pH values of sauerkraut with different salt concentrations (2%, 5%, 8%, w/v) were between 6.35 and 6.50. The pH values decreased sharply at first and followed by a slowly decrease to a stable level, reducing to 4 in the 1st, 2nd and 3.5th day, respectively. Salt had a significant (*P* < 0.05) influence on pH values at the first 48 h in fermentation, the lower the salt concentration was, the faster the pH decreased, which may be because the acid producing ability of LAB was restrained gradually with the increasing of salt concentration (Rodríguez-Gómez et al., 2012). In contrast, salt had no significant (*P* > 0.05) effect on the changes of pH at the end of fermentation, but the lower salt concentration resulted in the lower pH.

3.2. Microbiological changes during fermentation

As shown in Fig. 2A, the original population of LAB were 3.14–3.34 log CFU/mL and then sharply increased at the first day in three fermentation, the concentration of T and F exceeded log8.0 CFU/mL at 1st and 1.5th day respectively. However, the growth of LAB in E was relatively slow, only reaching to 8.02 log CFU/mL in the 3rd day, probably due to the initiating strain of fermentation mainly was hetero-fermentative LAB with short metabolic cycle, poor salt tolerance and acid resistance (Wouters et al., 2013; Xiong et al., 2012). In the mid-late fermentation, the amount of LAB remained stable above 8.0 log CFU/mL, probably because the dominant LAB was homo-fermentative with strong salt-tolerance and acid-resistance (Chorianopoulos, Bozariis, Stamatidou, & Nychas, 2005; McFeeters & Pérez-Díaz, 2010). Therefore, salt had a significant inhibitory effect on the growth of LAB in brine at early stage of fermentation, but the effect was not

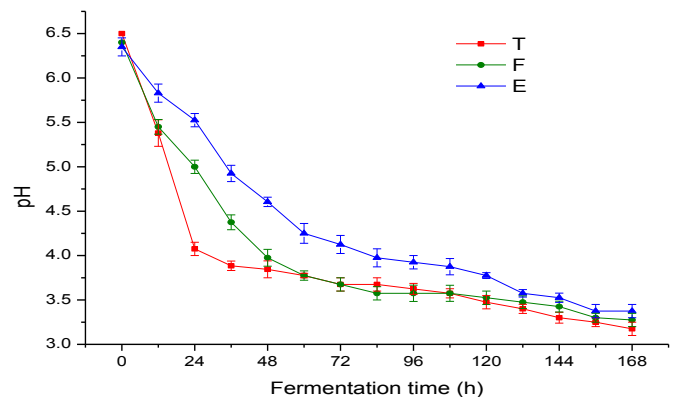


Fig. 1. Changes of pH during the fermentation of sauerkraut with three different salt concentrations.

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