



Original article

Effect of temperature on microbial changes during kimchi fermentation

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ABSTRACT

Kimchi, a salt-pickled and fermented vegetable, is one of the most popular foods in Asian countries, such as Korea, and more and more people in the world get used to its taste. In this study, Kimchi were fermented at two different temperatures. After acidification to pH=4.2, LAB were isolated, enumerated. The purpose of this study is to determine the amount of micro flora of Kimchi. The results show that the naturally occurring lactic acid bacteria load was found to vary between 1.5×10^4 cfu/gr to 1.9×10^8 cfu/gr at 20° c. The yeast counts increased from 1.13×10^4 cfu/gr to 5×10^7 cfu/gr at 20 c°. The LAB counts on MRS increased from 1.5×10^4 cfu/gr to 6.1×10^8 cfu/gr at 30 °c. The largest increase in the numbers of LAB was noted during the first 24 h of fermentation and further incubation led to decrease. The yeast counts increased from 1.13×10^4 cfu/gr to 5.1×10^7 cfu/gr. Maximum total acids produced in kimchi at 30°C. At 30°C, the optimum-ripening period was 1 day and the edible period was also 1-2 days. The results show that the temperature is effective on microbial changes during kimchi fermentation.

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1. Introduction

The word kimchi is the generic term for Korean fermented vegetables, which is derived from the Chinese characters pronounced chimchae, meaning brined vegetables Kimchi is a Korean traditional fermented vegetable. Most kimchi is characterized by its hot taste due to the use of fairly high amount of chili pepper and its visibility in

the product. However, some kimchi are made without chili pepper, but with garlic and ginger together with vegetables and other ingredients. Traditionally, kimchi was made in every household in Korean rural areas to provide the vegetables for the winter when other fresh vegetables are not readily available (hand). Kimchi contains various health-promoting components, including β -carotene, chlorophyll, vitamin C, and dietary fiber (Park et al., 1995). It has its best flavor, taste, and texture when optimally fermented at about pH 4.2; after optimum fermentation, Kimchi quality deteriorates rapidly due to formation of excessive organic acids and texture softening (Kim and Kim, 2003). Many types of kimchi are available depending on the raw ingredients used, processing methods, harvest seasons, and geographical regions. Although baechu (Korean cabbage) and radish are the most widely used in the making of kimchi, other vegetables are used depending on their seasonal availability. Because of the cold northern, and the mild southern Korean winters, the kimchi prepared in the north contains less salt, whereas the kimchi in the south requires more salt for long-term preservation. Also, people living near the sea naturally use more fish products in their kimchi (Lee et al., 1997). Therefore, the control of the fermentation process is needed to preserve quality of Kimchi and to extend its shelf-life. Kimchi fermentation occurs mainly by the microorganisms naturally present in the raw materials that contain numerous microflora including LAB. Various LABs may initiate fermentation, but hetero fermentative type LAB increased rapidly with organic acids accumulation and homo fermentative type LAB increased thereafter. Many homo and hetero fermentative LAB, such as genus *Lactobacillus*, *Leuconostoc*, *Weissella*, *Lactococcus*, *Streptococcus* and *Pediococcus* were isolated and identified from various kimchi samples, but genus *Leuconostoc*, *Weissella* and other LAB producing organic acids, carbon dioxide and bacteriocins are the most important microorganisms for the controlled fermentation of kimchi (Lee et al., 2002). Numerous chemical, physical, and biological factors may contribute directly to the growth of microorganisms and the extent of fermentation. Kimchi has a low caloric content, but it is rich in minerals, vitamins, and dietary fiber. The protein and lipid contents can be increased with various subingredients like fish, clams, oysters, and meat, which give kimchi its characteristic savor. Traditionally in Korea, large quantities of kimchi are prepared as an annual event, kimjang, for eating during the winter when the fresh vegetable supply is limited. Most of the vegetables cultivated in Korea are used as sources for making kimchi. Although 161 or 187 kinds of kimchi are currently reported (Edward, 2008). The important factors that affect kimchi fermentation are microorganisms, temperature, salt concentration, fermentable carbohydrates, other available nutrients, or any inhibitory compounds in raw materials used, as well as oxygen and pH.

The purpose of this study was to determine the amount of micro flora of kimchi. Another purpose is studying Changes in microbiological composition of kimchi during fermentation.

2. Materials and methods

In this study, the ingredients (Chinese cabbage, Leek, red pepper powder, Garlic, Ginger, Sugar, rice flour) used in kimchi preparation were purchased from local markets in Mashhad, Iran.

2.1. Production of kimchi

The preparation of kimchi was carried out based on the "baechu-kimchi" recipe. The ingredients are listed in Table 1. Previously, garlic, ginger, and leek were chopped. Sugar was weighed. The Chinese cabbage was cut into pieces 3 cm \times 3 cm in length and soaked in 15% (w/v) brine for 30 min.

Table 1

Composition of kimchi

Materials and ingredients	Percentage share
Salted Chinese cabbage	90
Leek	4
Red Pepper powder	2
Garlic 2	2
Ginger 1	1
Sugar	1

The soaked cabbage was washed twice with fresh water and then drained for 30 min. The prepared ingredients were mixed well and then distributed evenly on the Chinese cabbage. The kimchi mixture was put into a jar. It was fermented at 20°C for 2 days and 30°C for 1 day.

2.2. Samples

In the present study, samples of kimchi were prepared. Then samples were transported to the laboratory and analyzed (Kacem et al., 2005). 25g samples were homogenized with 225mL sterile sodium citrate solution 2% (w/v), in a Stomacher 400 (Seward Medical, London, UK). Serial decimal dilutions (10^{-2} to 10^{-5}) were made in 0.1% (w/v) peptone solution. (Abdi et al., 2006) Decimal dilution of these samples were mixed with MRS medium (AEB, France) and incubated at 37°C for 48-72 h under anaerobic conditions. (Lengkey et al., 2009) The numbers of LAB were measured by the plate count on MRS agar (Difco Laboratories, Detroit, USA) mold and yeast were counted on Potato Dextrose agar (Nissui) incubated for 24 h at 30°C. Each LAB colony was purified twice by streaking on MRS agar. Colonies were counted as viable numbers of Microorganisms (colony forming unit (CFU) g^{-1} of kimchi) (Duan et al., 2008) 0.1 mL of the diluents were streaked on Nutrient agar for total bacteria counts.

2.3. Determination of pH Values and acidity of the Test Samples

The pH values of each samples were determined at 25C using a pH meter (WTW-Inolab Level 3 Terminal, Weilheim, German). (Yilmaz et al., 2010). Acid content was determined by titrating with 0.1N NaOH with the indicator of phenolphthalein until it's colour changes to pink.

3- Results and discussion

The fermentation of kimchi is carried out through a naturally controlled brining process. During kimchi fermentation, microorganisms should be tolerant to salt, acidity, anaerobic conditions, and endogenous antimicrobial compounds in the ingredients. The main microorganisms involved in kimchi fermentation are LABs, which are facultative anaerobes, microaerobes, or anaerobes. Several factors, such as salt concentration, temperature, pH, microorganism population, and air exposure control the kimchi fermentation process. The most important factor affecting kimchi fermentation is temperature, since the kimchi fermentation occurs mainly by the microorganisms naturally present in raw materials. Kimchi is now available in all year round but the quality of kimchi differs depending on localities and seasons. Ambient temperature is applied for making kimchi at household level. Kimchi fermentation and over-acidification occurs simultaneously at ambient temperature (Yoon et al., 2000). Optimum ripening period and eatable period of Kimchi were investigated through panel test. Ripening time of Kimchi varied of panel test, it was evaluated that the pH of optimum ripening period of Kimchi was 4.2 respectively. The microbial changes in during laboratory fermentation of kimchi are shown in (Figure1, 2 and Table2). The LAB counts on MRS increased from 1.5×10^4 to 1.9×10^8 cfu/gr at 20 c. The largest increase in the numbers of LAB was noted during the first 24 h of fermentation and further incubation led to decrease. The yeast counts increased from 1.13×10^4 to 5×10^7 cfu/gr. The pH and total acid changes of kimchi are shown in (Figure3 and 4). In the present investigation, the pH of kimchi ranged between 4.2 and 4.5. The LAB counts on MRS increased from 1.5×10^4 to 6.1×10^8 cfu/gr at 30 c. The largest increase in the numbers of LAB was noted during the first 24 h of fermentation and further incubation led to decrease. The yeast counts increased from 1.13×10^4 to 5.1×10^7 cfu/gr. The pH changes of kimchi are shown in (Figure3 and Table3), shows the changes of pH and total acids during kimchi fermentation at various temperatures. Ripening time of kimchi depending on fermentation temperature, accordingly the changes in pH and acidity, showed notable differences. At 20°C and 30 °C, pH dropped sharply with increasing acidity. Maximum total acid produced in kimchi at 30°C. At 30°C, the optimum-ripening period was 1 day and the edible period was also 1-2 days. But at lower temperature, the optimum ripening time and the edible period were longer than those of at higher temperature.

Gobbetti et al. (1994) proposed that lactic acid bacteria create an acidic environment conducive to yeast proliferation while the yeasts provide vitamins and other growth factors such as amino acids for the lactic acid bacteria. The simultaneous increase in numbers of both LAB and yeasts may therefore be attributed to their symbiotic association the results are in agreement with those reported in study. The decrease in pH and increase in lactic acid followed the same trend as reported for other natural fermented foods (Mohammed et al., 1991; Choi et al., 1994). Another reason for the difference of microflora appeared among various fermented products in

different regions was probably related to chemical and physical factors, including substrates, NaCl concentration and fermentation temperature (Cho et al., 2006; Wood, 1998).

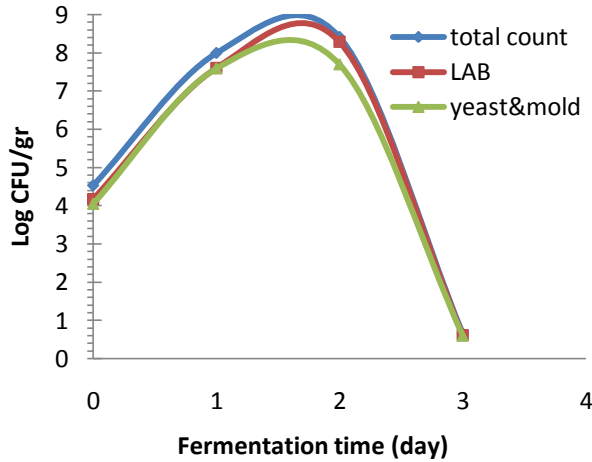


Fig. 1. Changes in M.O. during fermentation 20°C

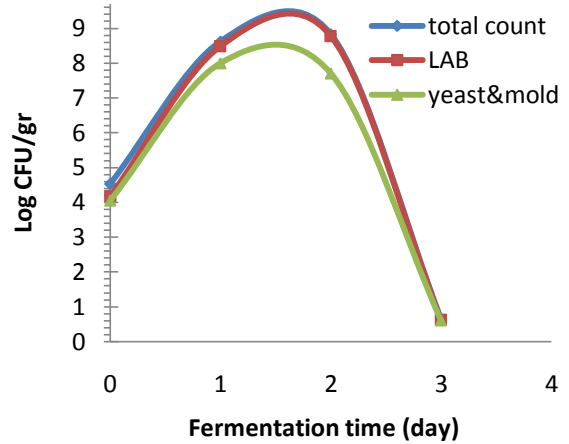


Fig. 2. Changes in M.O. during fermentation 30°C

Table 2

Change in microorganism during fermentation at 20°C and 30°C

Yeast and mold		LAB		Total count		day
30°C	20°C	30°C	20°C	30°C	20°C	
1.13×10^4	1.13×10^4	1.5×10^4	1.5×10^4	3.4×10^4	3.4×10^4	0
1×10^8	3.9×10^7	3.12×10^8	4×10^7	4.18×10^8	9.9×10^7	1
5.1×10^7	5×10^7	6.11×10^8	1.92×10^8	6.74×10^8	2.6×10^8	2

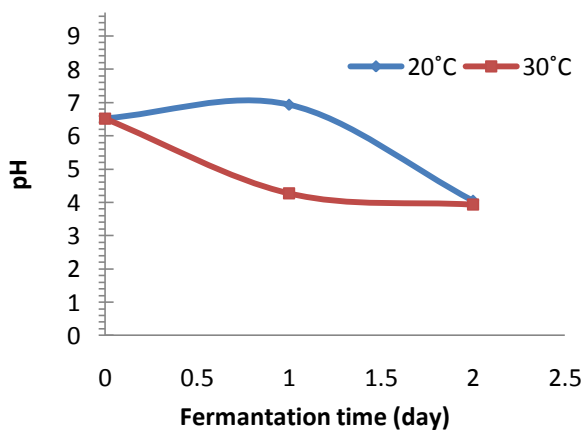


Fig. 3. Changes of total pH during Kimchi fermentation at various temperatures.

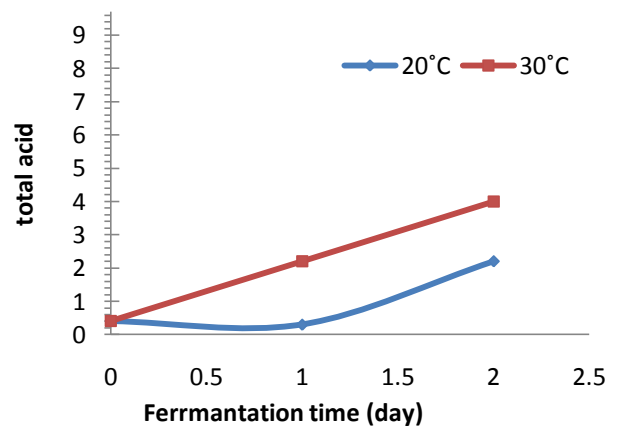


Fig. 4. Change of total acid during Kimchi fermentation at various temperatures.

Table3

Change of total acid and PH during Kimchi fermentation at 20°C and 30°C.

Total acid		pH		Day
30°C	20°C	30°C	20°C	
0.4	0.4	6.52	6.52	0
2.2	0.3	4.27	6.93	1
4	2.2	3.93	4.05	2

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