Effects of eugenol (EG)/β-cyclodextr (β-CD) inclusion compound on the extension of the shelf-life of kimchi

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Abstract. This article aims at extending the shelf-life of kimchi by adding Eugenol (EG)/β-cyclodextr (β-CD) inclusion compound. Fermented at 10°C for 15 days, the pH, titratable acid, aspects of microbiology and perceptive changes of kimchi containing different concentrations of EG/β-CD inclusion compound (0.0-1.0%) were determined. The results showed that adding EG/β-CD inclusion compound could inhibit the growth of overall microbe and lactobacillus in kimchi, reduce the pH, titratable acid and flavor changes in kimchi during fermentation, and increase the overall acceptability. In conclusion, these results showed that adding EG/β-CD inclusion compound could prolong the expiration date of kimchi and also improve its quality.

1. Introduction
Kimchi is a traditional fermented food in East Asia [1]. It not only has many nutritional components such as vitamins [2], minerals [3], dietary fiber [4], but also presents some health functions such as anticancer [5], decrease serum total cholesterol [6], antidiabetic [7], antiobesity effects [8], and so on. Kimchi contains a variety of microorganisms [9]. During the fermentation process, lactic acid bacteria (LAB) gradually become the dominant bacteria [10], exerting a greater impact on the quality of kimchi, so that kimchi has a unique taste and flavor [11, 12]. The most important problem in the commercialization of kimchi is the over-fermentation of LAB in the storage process, which results in the excessive acidification of kimchi, i.e. softening of texture, color diffusion and weird odor [13]. Therefore, to prolong its expiration date and maintain its quality of kimchi by inhibiting the growth of LAB is the main focus of the kimchi industry [1].

At present, the factory mainly implements heating sterilization [14], high pressure [15] and traditional chemical preservatives [16] to prolong the shelf-life of kimchi. These methods not only damage the quality of kimchi [17], but also may have an influence on human health [18]. Therefore, it is particularly important to find a natural, safe and effective approach to store it for a long time.

Eugenol (EG) has broad-spectrum antimicrobial activity [19], but its application in food industry is limited because of its high volatility, poor water solubility and strong odor [20]. In this study, the EG was utilized to prohibit LAB multiplying. At the same time, making use of inclusion technology overcome the volatility of EG, thereby prolonging kimchi’s storage time.
2. Materials and methods

2.1. Materials
β-cyclodextrin (β-CD) and EG (>98% GC purity, Nature) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *L. plantarum* KCCM 11322 and *L. mesenteroides* KCCM 11324 were supplied by China Culture Center of Microorganisms (KCCM; Shanghai, China). Raw materials for kimchi were purchased in local supermarkets (Qingdao, China).

2.2. Antimicrobial test of EG
Optical density method is one of the methods to detect the growth state of microorganisms, microbial bacteria have characteristic absorption peaks at 600 nm, and the higher the OD600nm, the more the number of bacteria. The OD600nm of strains which played a major role in the fermentation of kimchi (*Lactobacillus plantarum* and *Leuconostoc mesenterica*) in MRS medium containing different concentrations of EG (0.0-1.0%, w/w) was determined to reflect the inhibition of EG on LAB. The lower the OD600nm value, the stronger the inhibition effect of EG on the growth of LAB, and vice versa, the weaker the inhibition effect.

2.3. Preparation of EG/β-CD inclusion compound
The process of preparing EG/β-CD inclusion compound depended on the saturated water solution method [21]. β-CD was dispersed in the water solution into saturated solution, EG was dissolved in appropriate amount of ethanol. The mole ratios of EG: β-CD were 1:2. At 60°C, the EG ethanol solution was quickly dropped into the β-CD saturated solution by controlling the active speed of 800 rpm, and stirred for 24 hours at constant temperature. Then, the EG/β-CD inclusion compound was gathered by vacuum filtration. In order to conduct the future research, it is necessary to keep them dry under the circumstances of the indoor temperature and an air apparatus.

2.4. Characterization of EG/β-CD inclusion compound

2.4.1. Surface characteristics of EG/β-CD inclusion compound. The surface characteristics distribution of EG/β-CD inclusion compound was conducted by microscopy and scanning electron microscope (SEM, JSM-6700F, JEOL, Japan).

2.4.2. Drug-loading rate of EG/β-CD inclusion compound. The drug-loading rate of EG/β-CD inclusion compound was determined by GC [22]. 0.5g EG/β-CD inclusion compound powder was precisely weighed and placed in a 25 mL capacity bottle. Set the volume with n-hexane. Ultrasound treatment (800w) for 15min, compensated for loss. Measure out moderate amount of solution, the flavor content was measured using a gas chromatograph (GC, HP 5890 series, Hewlett Packard Co. Avondale, PA). The GC conditions were as follows.

The chromatographic column stationary phase was PEG-20M, the coating concentration was 10%, 2m×2mm, the FID detector, the column temperature was 190°C, the detector temperature was 230°C, the inlet temperature was 230°C, the carrier gas was nitrogen, and the flow rate was 40 mL/min.

2.4.3. Release of EG from inclusion compound. The release behavior of EG was also determined by GC [22]. The release medium was lactic acid-sodium lactate buffer with pH 4.0.

2.5. The process of preparing kimchi
The process of preparing kimchi involved several aspects (weight compositions): Chinese cabbage 80%, sauce 20%. More details about the preparation of sauce: star anise 1.4%, Sichuan pepper 2.0%, sugar 1.2%, ginger 0.8%, garlic 2.4%, spirits 3.2%, salt 0.76%, vinegar 1.0%, scallion 1.0%, onion 1.4%, red pepper 5.6%. With EG/β-CD inclusion compound (0.0-1.0% against the weight of kimchi),...
sauce and Chinese cabbage mixed and stirred together, it is almost a whole process for preparing kimchi, which was stored in 1000mL of glass bottles and fermented for 15 days at 10°C [11].

2.6. Characteristics of kimchi during storage

2.6.1. Measurement of pH and titratable acidity. The kimchi solids and kimchi juice were dealt with homogeneously and quickly by employing Handheld High Speed Dispersion Homogenizer (SZ-FJ 150, China), and went through the freezing centrifugation process of 3000 rpm for 20 min [23]. Determined of pH value of supernatant by pH meter, titratable acidity was titrated to pH 8.3 with 0.1N sodium hydroxide titration solution, and total acidity was counted and converted to a certain proportional lactic acid [24].

2.6.2. The analysis of microbiology. The above epipelagic liquid of kimchi required to go through the process of dilution by 0.1% peptone water, MRS agar (Basebio, Hangzhou, China) was utilized for the determination of lactic acid bacteria [25], and plate count agar (Basebio, Hangzhou, China) was employed for total viable counts [26]. All plates were triplicated, cultivated at the temperature of 30°C for 48 hours, and the numbers of living cell were considered as colony forming units (CFU) per mL [27].

2.6.3. Sensory evaluation. The development of perceptive evaluation of kimchi could be traced back to an approach depicted by Ko et al [24], employing a 15-point hedonic scale method. Organoleptic evaluation of kimchi on configuration, sourness, color, flavor, and overall acceptability were prepared for three copies to a panel of judges. There has the ranking appraisal system for assessing and judging texture and sourness like very low (1-3 points), low (4-6 points), medium (7-9 points), intensive (10-12 points), and extreme intensive (13-15 points). The color, flavor, and overall acceptability would be described like so poor (1-3 points), poor (4-6 points), soft (7-9 points), great (10-12 points) and so great (13-15 points).

2.7. Statistical analysis
All analysis based on statistic was performed by OriginPro 2017 (OriginLab, Northampton, MA). One-way analysis of variance (ANOVA) was utilized to compare means of EG/β-CD inclusion compound of kimchi. Those means with variance were analyzed by Fisher LSD test. P<0.05 is considered to be significant difference.

3. Results and discussion

3.1. Antimicrobial test of EG
The inhibition results were shown in Fig. 1.

![Figure 1](image_url)

Figure 1. Changes in the growth of (a) Leuconostoc mesenteroides and (b) Lactobacillus plantarum in medium containing Eugenol (EG). □: control, △: 0.1%, ▼: 0.2%, ○: 0.5%, ■: 1.0%.
Optical density experiments showed that different concentrations of EG had inhibitory effect on the growth of LAB, and the inhibitory effect was positively correlated with the amount of EG added.

3.2. Characterization of EG/β-CD inclusion compound

The surface characteristics of inclusion complexes of β-CD and β-CD were observed by microscopy and SEM. The results are shown in Fig. 2. The surface of β-CD is rough and its structure is different. The surface of EG/β-CD inclusion compound is smooth and crystalline. Microscopic and SEM images of inclusion complex show that EG has been combined with β-CD. The results of particle size measurement show that the particle size is mainly distributed in 80-120 nm, and the average particle size is about 100 nm.

![Figure 2. Microscopes and SEM of β-CD (a,c) and EG/β-CD inclusion compound(b,d).](image)

In Fig. 3, the release behavior of inclusion complexes of β-CD to EG was studied. Around 30% loaded EG was released within 15 days. During the process of fermentation, EG in clathrate compound can be released continuously, thus prolonging kimchi’s shelf-life.
3.3. Kimchi’s characteristics during storage

3.3.1. pH and titratable acidity. During fermentation at 10°C, the change of the pH of kimchi samples which were treated with different concentrations of EG/β-CD inclusion compound is shown in Fig. 4a. The results show that the pH value of kimchi increased with the addition of EG/β-CD inclusion compound. After 15 days of fermentation, the control group’s pH reduced to 3.81 from 5.8, and there was a significant difference between the control group and the 1% EG/β-CD inclusion compound treatment group (p<0.05).

During fermentation at 10°C, the change of kimchi’s titratable acidity is indicated in Fig. 4b. The results showed that kimchi’s titratable acidity decreased with the increase of EG/β-CD inclusion compound. After 15 days of fermentation, the acidity of the control group rose from 0.3% to 1.22%, and there was significant difference between the 1.0% EG/β-CD inclusion compound group and the blank control group (p<0.05).

The pH and acidity of kimchi during shelf-life should be 4.1-4.5 and 0.28%-1.00% [28]. When compared with the control group, the experimental group could significantly delay the change of pH and the acidity of kimchi. Therefore, adding EG/β-CD inclusion compound can prolong the shelf-life of kimchi.
3.3.2. Microbial analysis. Figure 5a indicates LAB’s growth in kimchi under fermentation at 10°C. LAB are the main bacteria in the fermentation of kimchi. During the entire fermentation period, the number of LAB increased continuously, which could induce excessive acidification of kimchi. With the rising of the concentration of EG/β-CD inclusion compound in kimchi, the number of LAB decreased, which could delay the excessive acidification of kimchi and prolong their shelf-life.

Figure 5b presents the growth of total microorganisms in kimchi fermented at 10°C. At the beginning of fermentation, the total microbial biomass of the control group was 4.8 log CFU/mL. Within 9 days before fermentation, the total microbial biomass of kimchi samples increased significantly, and then increased slightly. On the 12th day, the total microbial biomass of kimchi samples reached the maximum.

3.3.3. Sensory evaluation. Figure 6 shows the result of the fermentation of kimchi with different concentrations of EG/β-CD inclusion compound at 10°C for 15 days. The control group’s overall acceptability score greatly differed from the experimental group’s score ($p<0.05$). Generally, the overall acceptability of kimchi is good, which indicated that kimchi still has unique aroma. Thus, extending kimchi’s shelf-life can be positively affected by adding EG/β-CD inclusion compound.

Figure 6. Changes in sensory of kimchi during fermentation process. (a): control, ●:blank inclusion compound, △:0.1%, ▼:0.2%, ○:0.5%, ■:1.0%.
Flavor study showed that there was significant difference between the control group and the 0.5% experimental group \( (p<0.05) \). As the content of EG/β-CD inclusion compound increased, the scores for flavor taste decreased. Furthermore, 1.0% experimental group affected the sensory qualities of kimchi, its might due to excessive eg release from inclusion complex. Thus, the treatment with 0.5% is recommendable for kimchi.

4. Conclusion
So as to extend shelf-life, the research was conducted to study the impacts of EG/β-CD inclusion compound as a natural preservative on kimchi’s quality and fermentation. The addition of EG/β-CD inclusion compound to kimchi led to positive transformations in microbial analysis, pH and titratable acidity in comparison to the control group. Nonetheless, about sensory analysis, EG/β-CD inclusion compound of 0.5% are suggested to producing kimchi. The results can lead to a basis for the usage of EG/β-CD inclusion compound as an additive in the industry of fermented food.

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