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Effects of microencapsulated Allyl isothiocyanate (AITC) on the extension of the shelf-life of *Kimchi*

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ABSTRACT

Allyl isothiocyanate (AITC) is a well-recognized antimicrobial agent but, application of AITC to food systems is limited due to its high volatility and strong odor. This study was performed to overcome the volatility of AITC by encapsulation using gum Arabic and chitosan and to investigate the effect of microencapsulated AITC as a natural additive on the shelf-life and quality of *Kimchi*. AITC loaded microparticles were prepared using gum Arabic and chitosan and were added to *Kimchi* at various concentrations (0–0.02%, w/w). The titratable acidity, pH, microbial changes, and sensory test of *Kimchi* were examined for 15 days at different fermentation temperatures (4 and 10 °C). The pH of *Kimchi* containing AITC microparticles was significantly higher than that of control and the higher the quantity of added AITC, the higher the pH became. The titratable acidity of *Kimchi* increased during storage especially, titratable acidity of control increased significantly higher than those of *Kimchi* with added AITC microparticles. The number of *Leuconostoc* and *Lactobacillus* species in *Kimchi* decreased with an increase in the concentration of AITC. The addition of AITC induced reduction of sour taste and improvement of the texture of *Kimchi* during fermentation. However, as the content of AITC increased, the scores of overall acceptability decreased due to the odor of AITC. These results indicate that addition of AITC (less than 0.1%) to *Kimchi* is an effective way of enhancing the shelf-life of *Kimchi* without reducing quality.

1. Introduction

Kimchi is a traditional Korean fermented vegetable food whose increased consumption worldwide is attributable to various functional characteristics, such as anti-carcinogenic (Choi et al., 1997), antioxidative activity (Lee et al., 2004; Ryu et al., 2004) and immune stimulatory activity (Kim and Lee, 1997). The magazine Health mentioned *Kimchi* in its list of the top five "World's Healthiest Foods" (http://eating.health.com/2008/02/01/worlds-healthiest-foods-kimchi-korea/#). There are many fermentative microorganisms in *Kimchi*, especially lactic acid bacteria such as *Lactobacillus plantarum* and *Leuconostoc mesenteroides*. These bacteria are beneficial inhabitants of the intestinal tract of humans and animals and produce the characteristic taste and flavor of *Kimchi*. As fermentation progresses, the lactic acid bacterial count increases, resulting in an increase in acidity and formation of CO_2 gas along with production of the sour and characteristic carbonic taste of *Kimchi* (Shin et al., 2002). Factory manufactured *Kimchi* is usually packaged in glass jars, plastic trays or plastic pouches without pasteurization, and therefore lactic acid fermentation still continues during its storage and marketing. After it reaches a well-aged step, these microbiological and enzymatic activities continue and result in a sour and bitter taste, off-odor, and softening due to the deterioration of *Kimchi* (Lee et al., 2000). Therefore, control of the fermentation process is needed to preserve the quality of commercial *Kimchi* and to extend its shelf-life. Several studies have been conducted to extend the shelf-life of *Kimchi* using heat treatment (Kang et al., 1991), gamma radiation (Kim et al., 2008), antiseptic treatment (Park and Woo, 1988), antimicrobial agents (Moon et al., 1995), herbal medicines (Jung et al., 2002; Kim et al., 2008), oyster shell powder (Choi et al., 2006), and pH adjusters (Lee and Kim, 2003). The best way to overcome the problem of over-ripening is to control the growth of lactic acid bacteria without damaging the product, thus suggesting the use of the natural antimicrobial allyl isothiocyanate (AITC).

AITC ($CH_2{=}CHCH_2N{=}C{=}S$), the majority pungent compound in plants, belongs to the *Cruciferae* family, which includes cabbage, broccoli, horseradish, and mustard (Delaquis and Sholberg, 1997). AITC is known to possess strong antimicrobial activity, capable of killing fungal and bacterial pathogens on plant seeds, fresh produce, bread, meat, and cheese (Lee et al., 2009; Li et al., 2007; Nadarajah et al., 2005; Nielsen and Rios, 2000). Therefore, it might be a potential

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natural antimicrobial agent for food preservation. However, any application of AITC to food systems is limited due to its high volatility, strong odor, poor water solubility, and reactivity with natural food nucleophiles (Chacon et al., 2006; Kim et al., 2008). To solve these problems, a microencapsulation method was considered. Microencapsulation can provide protecting sensitive food components, masking taste and odor as well as promoting controlled lease of core materials (Ko et al., 2008).

Gum Arabic (gum acacia) is a negatively charged polyelectrolyte that is commonly used as a flavor-encapsulating material due to its high water solubility, low viscosity in concentrated solution relative to other hydrocolloid gums, and good retention of volatile compounds (Ko et al., in press; Shiga et al., 2001). Chitosan [poly(ß -(1→4)-2-amino-2-deoxy-Dglucose)], a natural cationic polysaccharide derived from chitin, has been studied by the food industry since it has film-forming properties, nutritional quality, and antimicrobial agents (Shahidi et al., 1999). Therefore, the combined use of gum Arabic with chitosan could provide an inter-biopolymer electrostatic complex that would form strong viscoelastic films around AITC and provide good barrier properties against its unique flavor.

The objective of this study was to overcome the volatility of AITC by encapsulation using gum Arabic and chitosan and to evaluate the effects of AITC microparticles as a natural additive on the shelf-life and quality of packaged *Kimchi* during the storage.

2. Materials and methods

2.1. Materials

Gum Arabic HPS Powder (Food grade, IRX 50924) was supplied by CNI (France). AITC (>98% GC purity, Nature) was purchased from Hyangwon-Spice (Seoul, Korea). Chitosan (MW: 30,000 and DOD 90%) was purchased from BioTech Inc. (Mokpo, Korea). *L. plantarum* KCCM 11322 and *L. mesenteroides* KCCM 11324 strains were obtained from the Korean Culture Center of Microorganisms (KCCM; Seoul, Korea). Other chemicals were reagent grade and used without further purification. *Kimchi* was purchased from Pungmi Foods Co., Ltd (Suwon, Korea).

2.2. Antimicrobial test of AITC

The antimicrobial activity of encapsulated AITC against *L. plantarum* and *L. mesenteroides* was evaluated using liquid culture test methods. Microorganisms were grown in 20 mL of Tryptic soy broth (TSB; Difco, St. Louis, USA) in an incubator at 37 °C. *L. plantarum* and *L. mesenteroides* (0.3 mL) was inoculated into 30 mL of MRS broth (Difco, St. Louis, USA) supplemented with 0 to 0.50% (w/w) of AITC, respectively and incubated at 30 °C for 24 h. Each culture was sampled (1.5 mL) periodically every 4 h during the incubation period. The optical density of each culture sample was measured at $\lambda = 600$ nm using an UV/visible spectrophotometer to represent the cell concentrations of the microorganisms in the media.

2.3. Preparation of microparticles

An aqueous solution of gum Arabic (25% w/w) was prepared and stirred overnight at room temperature. Emulsions were prepared by adding AITC oil to the gum Arabic solution with tween 20 (0.5%), and the mass ratio of gum Arabic to AITC was 3:1. The mixture was then homogenized using a high-speed mixer (T25-basic, IKA, Germany) set at 11,000 rpm for 3 min. Then, the chitosan solution (2.5%, w/v), which was dissolved in 50 mM lactic acid solution, was added to this mixture. The final mixture was homogenized at 11,000 rpm for 10 min. The AITC emulsion was fed through a spray dryer (Lab Plant-SD05, Huddersfield, UK) equipped with a centrifugal atomizer. The operation conditions of spray drying were as follows: inlet air temperature of 200 °C, outlet temperature of 115 °C, feed rate of 5 mL/min, air flow rate of 73 m^3/h, and atomizer rotational speed of 30,000 rpm. Finished powder was stored in a hermetically-sealed bottle at −20 °C.

2.3.1. Characterization of microparticles

The particle size distribution of the spray-dried microparticles was determined using a particle size analyzer (CILAS-1064, Orleans, France) fitted with a small volume sample presentation unit and integration software. Calculation of the particle size distribution was based on a relative particle refractive index of 1.1500 and particle absorption of 1.0000. Powder particle size distributions were determined following their dispersion in propan-2-ol.

The external structures and microcapsule morphology were assessed using a cold stage scanning electron microscope (JEOL, JSM-5300, Tokyo, Japan) at an accelerating voltage of 15 kV. Microcapsules were placed on stubs using double-sided sticky carbon tape. Stubs were then coated with a gold-palladium layer using a SEM magnetron sputter coater.

2.3.2. Quantification of encapsulated AITC

The total content of AITC in the powder was measured according to the solvent extraction method. One gram of powder was weighed into a 50 mL glass bottle and mixed with 4 mL of distilled water, after which 4 mL of diethyl ether with phenyl isothiocyanate as an internal standard was added. This was followed by violent shaking and then centrifugation at 3000 rpm for 10 min to separate the organic phase from distilled water. The flavor content in the organic phase was measured using a gas chromatograph (HP 5890 series, Hewlett-Packard Co. Avondale, PA). The column used was a HP-1 cross linked methyl siloxane column (30 m × 0.25 mm inner diameter, 0.25 µm film thickness, Agilent Technologies, Inc., Palo Alto, CA, USA). The oven temperature was programmed from 50 °C for 1 min to 200 °C for 3 min at 10 °C/min. The injection port temperature and detector temperature were kept at 160 °C and 250 °C, respectively. The carrier gas was nitrogen at a split ratio of 1:30. Peaks areas were recorded and calculated using HP Chemstation software.

2.3.3. Release of AITC from microparticles

In other to evaluate release of AITC from microparticles, 0.1 g of powder was put in 5 mL vial capped with septum. The volume was up to 1 mL with sodium phosphate citrate buffers adjusted to pH 4, and the vial capped with Teflon lined cap equipped septum. Then, the mixture was stored at 10 °C for 15 days. The amount of AITC retained in the powder was quantified by the method described above and expressed as a percentage of the initial amount.

2.4. Characteristics of packaged Kimchi during storage

2.4.1. Preparation of Kimchi

Kimchi was prepared on the basis of the following composition (%, w/w) — radish (7.5%), red pepper (2.8%), leaf mustard (0.7%), garlic (1.2%), ginger (0.4%), onion (0.7%), green onion (0.5%), liquefied fish sauce (0.8%), salted shrimps (1.0%), white sugar (0.6%), and refined salt (0.38%). Each quarter head of blended cabbage was anaerobically packaged in a polyethylene vinyl pouch (500 mL). AITC microparticles (0–0.2% against the weight of *Kimchi*) were added to the condiment and spread onto the cabbage for preparation of *Kimchi*. AITC 0% means only gum Arabic–chitosan microparticles without core material, AITC. The prepared *Kimchi* was fermented in a refrigerator for 15 days at 4 and 10 °C, respectively. The temperature of a showcase of *Kimchi* at the market is about 10 °C.

2.4.2. Measurement of pH and titratable acidity

Kimchi juice was centrifuged at 3100 rpm for 10 min. The pH of the supernatant was determined using a pH meter. Titratable