Food Control 91 (2018) 390–396

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Inactivation of norovirus surrogates by kimchi fermentation in the presence of black raspberry

Garam Bae [a, 1], Jeongwon Kim [a, 1], Hyojin Kim [a], Jong Hyeon Seok [b], Dan Bi Lee [b], Kyung Hyun Kim [b], Mi Sook Chung [a, *]

[a] Department of Food and Nutrition, Duksung Women's University, Seoul 01369, South Korea
[b] Department of Biotechnology & Bioinformatics, Korea University, Sejong 30019, South Korea

ARTICLE INFO

Article history:
Received 23 January 2018
Received in revised form
9 April 2018
Accepted 11 April 2018
Available online 17 April 2018

Keywords:
Murine norovirus
Feline calicivirus
Baechu kimchi
Black raspberry seed extract
Alliin
Capsaicin

ABSTRACT

Kimchi, a food made of seasoned vegetables is probiotic-rich when fermented. Recent increases in the consumption of commercially-made kimchi have resulted in norovirus outbreaks in schools where freshly-prepared kimchi is served. We previously showed that black raspberry (*Rubus coreanus*) seed extract (RCS), inactivated murine norovirus (MNV) and feline calicivirus (FCV). The aim of this study was to evaluate the antiviral effects of RCS on MNV and FCV in kimchi fermentation for 50 days, during which fresh *baechu* kimchi became optimally ripened and acidic (~pH 4.3). During the fermentation period, recovered FCV titers were significantly reduced by 1.7 log PFU/ml at day 0, to undetectable levels at day 30, primarily due to the effects of RCS and the acidic pH produced by lactic acid bacteria. In contrast, recovered MNV titers were reduced by 1.1 and 2.1 log PFU/ml at days 0 and 50, respectively, by kimchi seasonings with RCS. Two seasoning ingredients, red pepper and garlic, showed significant antiviral effects on MNV, and their major components, capsaicin and alliin at 10–200 μM, inhibited MNV in a concentration-dependent manner. Optimally ripened kimchi in the presence of RCS can thus provide the potential to reduce norovirus outbreaks in catering service settings.

© 2018 Elsevier Ltd. All rights reserved.

1. Introduction

Norovirus (NoV) which belongs to the *Caliciviridae* family is the major pathogen of epidemic acute gastroenteritis and the leading cause of approximately 90% of non-bacterial outbreaks worldwide (Lindesmith et al., 2003). It is associated with severe complications in immunocompromised individuals, the elderly and young children (Siebenga et al., 2008). NoV is subdivided into six genogroups designated GI through GVI based on phylogenetic analysis of its capsid gene, while a tentative genogroup VII was proposed (Vinje, 2015). Specific genogroup/genotype viruses (e.g., GII.2, GII.3, GII.4, and GII.6) have caused recent large outbreaks of gastroenteritis, and the GII.4 genotype has been the most common cause of human NoV (HuNoV) outbreaks for at least 20 years. A novel GII.P17-GII.17 virus emerged in 2013 has spread very rapidly and has been responsible for causing an increasing number of outbreaks (Kobayashi et al., 2016).

HuNoV is transmitted via the fecal-to-oral route and infections primarily spread through person-to-person contacts. Transmission also occurs from contaminated foods such as seafood, fresh fruits, and vegetables. HuNoV was recently discovered to grow in B cells (Jones et al., 2014) and stem cell-derived human enteroids (Ettayebi et al., 2016). However, murine norovirus (MNV), feline calicivirus (FCV), and Tulane virus are still used as HuNoV surrogates for elucidating the molecular modes of NoV replication and pathogenicity, due to the availability of a robust cell culture system (Predmore et al., 2015; Steinmann, 2004; Wobus, Thackray, & Virgin, 2006).

Kimchi, a traditional salad of fermented vegetables, is classified into hundreds of varieties, based on the type of vegetables from different seasons and regions. The most common type is *baechu* kimchi (Patra, Das, Paramithiotis, & Shin, 2016) which is prepared with cabbage as a main ingredient. It is trimmed, cut, salted, and mixed with seasonings that consist of red pepper, garlic, ginger, and edible allium species including green onion. It is then fermented to create a favourable environment for the growth of lactic acid bacteria (LAB), giving the best flavor and taste (FAO, 2001). However, increased consumption of kimchi as a healthy fermented food

* Corresponding author.
E-mail address: mschung@duksung.ac.kr (M.S. Chung).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.foodcont.2018.04.025
0956-7135/© 2018 Elsevier Ltd. All rights reserved.

worldwide has raised a serious issue of microbiological safety. Commercialized kimchi products are distributed to market mostly fresh and unripened in most catering services. Foodborne disease outbreaks have occurred in school catering settings due to the consumption of contaminated kimchi, of which the cabbage was rinsed with HuNoV-contaminated underground water (Moon et al., 2014; Park et al., 2015).

Kimchi fermentation is known to inactivate foodborne microbial pathogens such as *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Salmonella typhimurium* (Kim, Zheng, & Shin, 2008; Lee et al., 2009). Recently, several inactivation studies of NoV surrogates by fermented foods have been reported; and these have shown inactivation of MNV or FCV by *dongchimi*, a kind of kimchi product in water, fermented oyster, sauerkraut, oyster kimchi, and cabbage kimchi at different salinities (Gagné, Barrette, Savard, & Brassard, 2015; Kang et al., 2016; Lee, Yoo, Ha, & Choi, 2012; Seo, Lee, Seo, Ha, & Choi, 2014; Shin et al., 2010). Evaluations of HuNoV-contaminated cabbage kimchi also showed that HuNoV from food processing was gradually reduced but not completely removed during kimchi fermentation (Lee, Lee, Kim, Eun, & Ha, 2017). LAB were reported to inhibit FCV, MNV and HuNoV virus like particles (VLP) (Aboubakr, El-Banna, Youssef, Al-Sohaimy, & Goyal, 2014; Li, Breiman, le Pendu, & Uyttendaele, 2016). Nevertheless, the antiviral effect of kimchi ingredients against MNV or FCV during fermentation is not yet fully explored. In addition, we have recently shown that black raspberry seed extract (RCS) and its polyphenol compounds, cyanidin-3-glucoside and cyanidin-3-rutinoside, were effective in both preventing noroviral replication and disrupting viral particles (Lee et al., 2016a, 2014). In light of this, RCS can be developed as a potential antiviral supplement in kimchi seasoning. We thus evaluated the antiviral effects of kimchi combined with RCS, against MNV and FCV. The inactivation of MNV and FCV by kimchi seasonings, individual ingredients, and the major compounds of each ingredient were further examined. *Baechu* kimchi, a major kimchi product, was prepared with kimchi seasonings mixed with RCS, and virus titers were monitored over a period of 50 days, during which the freshly prepared *baechu* kimchi became optimally ripened.

2. Materials and methods

2.1. Viruses and cells

RAW 264.7 cells (mouse leukemic macrophage cell line; RAW), Crandell Reese feline kidney (CRFK) cells, and FCV-F9 (FCV) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). MNV-1 (MNV) was kindly provided by Dr. Herbert Virgin, Washington University School of Medicine, USA. CRFK and RAW cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Karlsruhe, Germany) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO, USA) and 1% penicillin streptomycin (PS) (Invitrogen, Grand Island, NY, USA) at 37 °C in 5% CO_2. FCV and MNV were propagated in CRFK and RAW 264.7 cells with the DMEM-FBS-PS medium for 24 and 48 h, respectively, and stored at −80 °C.

2.2. RCS preparation and polyphenol analysis

RCS was prepared as previously described (Lee et al., 2016a). Briefly, the black raspberry seeds were ground to fine powder and extracted in 70% ethanol using ultrasound (40 kHz, Powersonic 420, Hwashin Instrument Co. Ltd., Seoul, Korea) for 20 min at 20 °C, then centrifuged (9,500 g, 60 min, 4 °C). The supernatant was fractionated through an ultrafiltration membrane with a molecular weight cut-off of 1 kDa (Millipore Corp., MA, USA), and then subjected to rotary evaporation to remove solvent, before lyophilization for subsequent assays. The final yield of RCS was typically 1.5%. Total polyphenols were determined using Folin-Ciocalteu method with absorbance measured at 765 nm using a microplate reader (SpectraMax M2, Molecular Devices Corp. USA). Gallic acid was used as a standard compound and the total phenolic content of RCS was 110 mg/g as gallic acid equivalents. Polyphenols in RCS were quantitatively analyzed using LCMS-8040 spectrometer and Nexera UHPLC system (Shimadzu, Kyoto, Japan) equipped with a Shimpack XR-ODSII column (2.0 × 75 mm, 2.2 μm). Gallic acid, caffeic acid, ellagic acid, quercetin, cyanidin-3-glucoside, cyanidin- 3-rutinoside, *trans*-resveratrol, 3,4-dihydroxybenzoic acid, *p*-coumaric acid, catechin, rutin, *trans*-ferulic acid, and chlorogenic acid (Sigma-Aldrich) were used as standard compounds (Lee et al., 2016b). RCS polyphenols present in decreasing order were; catechin, ellagic acid, cyanidin-3-rutinoside, 3,4-dihydroxybenzoic acid, and gallic acid, in the range of 1.3–34.6 mg/g, and other minor compounds including cyanidin-3-glucoside (Supplementary Table S1).

2.3. Kimchi preparation and sampling

Baechu kimchi was prepared as previously reported with minor modification (Lee, Jung, & Jeon, 2015). Briefly, all containers and utensils used for kimchi preparation were treated with hot water and 70% aqueous ethanol to achieve microbial sterility. Salted cabbage was purchased from Jong-ga-jip Co. (Daesang NFN, Seoul, Korea) and cut into pieces of approximately 2 cm by 2 cm. Red pepper powder, garlic, ginger, and green onion were purchased from local markets and garlic and ginger were homogenized individually. Kimchi seasonings were prepared by mixing ground garlic, ground ginger, chopped green onion, red pepper powder, and water (14:4:28:15:39 by weight). Kimchi was prepared by mixing salted cabbage with seasonings at a ratio of 80:20 (w/w), which was distributed to six polyethylene plastic bags (630 g each). RCS in 70 ml phosphate buffered saline (PBS) was then added to achieve a final concentration of 10 mg/100 g kimchi (w/w), corresponding to ~0.6 mg/ml in kimchi soup which was adopted from our previous studies (Lee et al., 2016a). They were stored with gentle rocking (Vision Scientific Co, Daejeon, Korea) at 4 °C during fermentation. The experimental groups were (1) kimchi as a control, (2) FCV or MNV-inoculated kimchi (FCV + kimchi or MNV + kimchi), and (3) FCV or MNV-inoculated kimchi mixed with RCS (FCV + kimchi + RCS or MNV + kimchi + RCS). FCV or MNV in PBS with titers of 5.5 or 4.6 log PFU/ml, respectively, was inoculated to kimchi soup (the liquid fraction of kimchi) and mixed with kimchi vegetables so that the virus was distributed homogeneously. Immediately following inoculation of virus, FCV and MNV titers were 5.3 or 4.4 log PFU/ml in the absence of seasonings, respectively (data not shown). Sensory evaluation of the kimchi and kimchi + RCS group was carried out prior to viral inoculation. There was no difference in the flavour, taste, and color between the kimchi and kimchi + RCS groups at a concentration of 10 mg/100 g at day 0. Although *baechu* kimchi became optimally ripened at day 50, RCS did not affect the flavor, taste, and color of kimchi. A group containing each virus in acetate buffer (pH 4.3) or PBS (pH 7.5) was used as a control. A volume of 3 ml kimchi soup was then periodically sampled from each group at days 0, 3, 6, 12, 18, 24, 30, and 50 during fermentation at 4 °C. The samples were centrifuged at 4,000 g and 4 °C for 20 min to remove large particles, and the supernatant was used for plaque assays of MNV or FCV and analyzed for LAB and pH.

Download English Version:

https://daneshyari.com/en/article/8887928

Download Persian Version:

https://daneshyari.com/article/8887928

Daneshyari.com