



Potential of phytic acid in synergy with sodium chloride as a natural-borne preservative to inactivate *Escherichia coli* O157:H7 and inhibit natural microflora in fresh noodles at room temperature

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ABSTRACT

The increase in consumer demand and the high cost of maintaining a cold chain during distribution emphasize the need for preservative technology to ensure the microbiological quality of fresh noodles with a moisture content of 32–40%. However, few studies have been conducted to increase the storage stability of fresh noodles by using a preservative with a significant inhibitory effect against microorganisms and/or minimizing the use of synthetic antimicrobial agents. This study aimed to propose a synergistic natural-borne antimicrobial that could interact with NaCl, an essential component of noodles, for extended preservation of fresh noodles at room temperature. NaCl (0–1.6% (w/w) based on the total weight of the noodle dough) and phytic acid (0–1.0% (v/w)) were applied to fresh noodles. The bactericidal effect on *Escherichia coli* O157:H7 and the inhibitory effect on the indigenous microflora were assessed within 21 days at 30 °C. After cooking fresh noodles, physicochemical/textural and sensory characteristics (whiteness, pH, water activity; hardness, adhesiveness, springiness, chewiness; appearance, odor, overall acceptance) were further evaluated as objective and subjective quality parameters. In fresh noodles preserved with 0.6% phytic acid and 1.6% NaCl, the *E. coli* O157:H7 population was eliminated below the detection limit (>5.8 log reduction; $P < 0.05$) within 4 days of storage. This preservative significantly inhibited ($P < 0.05$) the mesophilic bacterial and total yeast/mold counts naturally present in fresh noodles for 12 days, while the largest antimicrobial activity was observed in noodles supplemented with 1.0% phytic acid combined with 1.0–1.6% NaCl. Although the objective parameters were significantly affected by the preservatives, analysis of the subjective parameters demonstrated that all samples were slightly or moderately favored by the panelists ($P > 0.05$). Considering the normal range of objective parameters for fresh noodles, the optimal preservative was determined to be 0.6% phytic acid and 1.6% NaCl. This study suggests the potential use of phytic acid as a natural-borne preservative that combines with NaCl in fresh noodles and exerts a synergistic effect. The developed method is expected to be applicable to extending the shelf life of other grain-based foods containing NaCl as an essential ingredient.

1. Introduction

Wheat noodles are one of the foods mostly consumed in Asian countries such as China, Indonesia, Japan, Vietnam, and Korea and have gained popularity beyond Asia (Gulia et al., 2014; Lierheimer, 2022). They are composed of wheat flour, water, and salt/alkali and can be classified into four types (raw/fresh, wet/boiled, dry, and fried noodles) depending on the processing method (Adejuwon et al., 2020; Tan et al., 2018). While the consumption of fried noodles is decreasing because of changes in consumer awareness of the health and well-being, demand

for non-fried noodles has steadily increased in the global market. Especially, the fresh noodle market has achieved a steady growth since the market for processed fresh noodles emerged (Choi et al., 2003; Veeman et al., 2002).

Fresh noodles are produced without an additional process of frying, parboiling, or drying after the formation of the dough. As fresh noodles have a relatively high moisture content of 32–40%, their shelf life is short compared to that of other noodles. Thus, the distribution or storage of fresh noodles at room temperature can promote the growth of microorganisms and enzyme activity, which can lead to the deterioration

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of fresh noodles (Fu et al., 2008; Rezaei and VanderGheynst, 2010). Previous studies have reported quality or safety issues of flour and wheat noodles caused by spoilage and foodborne microorganisms (Akhigbemidu et al., 2015; Berghofer et al., 2003; Fang et al., 2003; Guo et al., 2022; Ma et al., 2020). Additionally, food safety agencies have recommended or restricted noodles/pasta to meet microbiological limits for hygienic indicators (*Escherichia coli*, coliforms, aerobic plate counts, and yeasts/molds) and certain foodborne pathogens (*Salmonella* spp., *Staphylococcus aureus*, and *Bacillus cereus*) (FDA RSSC, 2019; ICMFSF, 1986, 2005; MFDS, 2015).

To prevent or slow microbial growth and extend the shelf life of fresh noodles, their production process involves the use of preservatives. Recently, as consumers' preference for natural-borne ingredients has increased, research on natural-borne substances that can be used as preservatives for noodles is being conducted. For instance, organic acids (lactic acid, malic acid, acetic acid, etc.) and alcohols (ethanol) are currently added to fresh noodles and pasta (Hou, 2001; Jeong, 1998; Lee et al., 2009). Other substances such as herb and spice extracts, tea and tea extracts, essential oils, monoacylglycerols, glycine, and chitosan are also considered antimicrobials for the preservation of pasta and noodles (Li et al., 2014; Tiwari et al., 2009). However, most preservation techniques have been tested in previous studies against a limited range of microorganisms (e.g., mesophilic/psychrotrophic bacteria or artificial inoculation of specific fungal strains) or storage conditions (e.g., refrigeration temperature or duration less than 5 days) (Del Nobile et al., 2009; Li et al., 2011; Xu et al., 2008), which were not expected to be effectively applicable to noodle processing. Considering the excessive cost to maintain cold chain distribution/storage (Shashi et al., 2018), it is necessary to develop a natural-borne technology that can inhibit a wide spectrum of microorganisms naturally present in noodles, even for a long time at room temperature.

Phytic acid is a natural component of grains and legumes. Although the chelating ability of phytic acid is known to inhibit nutrient utilization in a dose-dependent manner, recent studies have recognized its beneficial effects on human health by inactivation of iron-driven oxidation (Chen and Xu, 2023). It is also readily biodegradable in water and is considered to have no or low ecotoxicological effects on aquatic invertebrates and algae, respectively (ECHA, 2024). Moreover, it showed antibacterial/antifungal efficacy against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Rhodotorula mucilaginosa* *in vitro* (Bloot et al., 2023; Feizollahi et al., 2021), demonstrating its potential application in the food industry. In fact, phytic acid is widely used as an antioxidant or preservative to extend the shelf life of fruits/vegetables, meat products, and seafood products, etc. (Wang and Guo, 2021).

Herein, we aimed to establish a method of preservation for fresh noodles by adding phytic acid, which exhibits synergistic antimicrobial activity with NaCl. The bactericidal and inhibitory effects of the natural-borne preservative against artificially-inoculated *E. coli* O157:H7 and indigenous microorganisms (mesophilic bacterial count and total yeast/mold counts) in fresh noodles were investigated by simulation of two steps in the manufacturing process (noodle formation and storage at 30 °C). Changes in the physicochemical, textural, and sensory properties of cooked fresh noodles were further examined after treatment with the antimicrobials.

2. Materials and methods

2.1. Experimental overview

This study was conducted using the following experiments: The antimicrobial effects of phytic acid and NaCl in fresh noodles were evaluated against target pathogens. The preservatives for eradicating *E. coli* O157:H7 were selected by artificial inoculation of the bacteria on untreated/treated fresh noodles. In addition, the applicability of the preservatives was verified without inoculation. The microbiological

quality and storability of fresh noodles treated with the preservatives were confirmed by changes in the natural microflora and in physicochemical, textural, and sensory qualities of the noodles.

2.2. Bactericidal efficacy test of phytic acid and NaCl in fresh noodles against *Escherichia coli* O157:H7

2.2.1. Bacterial cell suspensions

E. coli O157:H7 (ATCC 35150, 43889, and 43895) were obtained from the Food Microbiology Culture Collection at Korea University (Seoul, Korea) and used in this study. The target pathogen was selected based on previous studies involving microbiological analysis of fresh noodle products or wheat flour, suggesting *E. coli* as one of the major foodborne bacteria frequently detected in foods. Each strain was stored at -20 °C in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) containing 20% glycerol and resuscitated prior to use in the experiment. The three strains were separately inoculated into 3 ml of TSB, followed by incubation at 37 °C for 24 h. The enriched culture was centrifuged (Varispin 4; Cryste Novapro Co., Ltd, Seoul, Korea) at 3000×g for 15 min. The supernatant was discarded, and the pellet was washed twice with sterile 0.85% saline. The final pellet was resuspended in 10 ml of sterile 0.85% saline.

2.2.2. Preparation and inoculation of fresh noodles

Wheat flour (Daehan Flour Mills Co., Ltd. Seoul, Korea) was purchased from a local retail market (Seoul, Korea). It was stored at room temperature without opening the package and used for the experiment within one week of purchase. Before the use in the experiment, the wheat flour was heat-treated in a microwave oven (LG MW208GB; LG Electronics Co., Seoul, Korea) for 1 min and then immediately cooled at room temperature. The absence of *E. coli* O157:H7 was confirmed by microbial analysis. A salting solution for noodles was prepared with 0, 1, 3, 5% NaCl and/or 0, 0.5, 1.0% phytic acid (50% w/w solution in H₂O; Sigma-Aldrich, St. Louis, MO, USA) in sterile distilled water. Based on the total weight of the noodle dough, the concentrations of NaCl and phytic acid corresponded to 0, 0.3, 1.0, 1.6% (w/w) and 0, 0.3, 0.6, 1.0% (v/w), respectively. Fresh noodle samples were prepared according to the "AACCI approved methods technical committee report on the guidelines for laboratory preparation of Japanese Udon noodles," published by the American Association of Cereal Chemists International (Hou et al., 2015), with some modifications. Briefly, salting water and wheat flour were mixed in a sampling bag (3M, St. Paul, MN, USA) at a ratio of 47 g:100 g (w/w). The noodle dough was kneaded by hands for 10 min until it became a cohesive and evenly-textured form and then rested in a plastic bag for 30 min at room temperature. After preparation of the noodle dough for each treatment, the bacterial suspension was inoculated to a final concentration of ca. 7 log CFU/g. The inoculated dough was sheeted three times with roll gaps of 3.0, 2.0, and 1.0 mm, and then cut into noodle strands with a width of 2 mm using a noodle rolling machine (YT 150; Yafeng Glass Products Co. Ltd., China). A sample (25 g) was sealed in a sterile PE bag and stored in an incubator (VS-2103P1; Vision Scientific Co. Ltd., Korea) at 30 °C for 4 days.

2.2.3. Microbiological analysis for *E. coli* O157:H7

Populations of *E. coli* O157:H7 in untreated/treated fresh noodles were analyzed on Days 0, 2, and 4. Samples (25 g) were placed in a stomacher bag containing 225 ml of sterile 0.85% saline and homogenized at 230 rpm for 2 min (400 Circulator; Seward, London, UK). An aliquot (0.1 ml) of a 10-fold dilution in 0.85% saline was inoculated in duplicate on eosin methylene blue agar (EMB; Difco, Becton Dickinson, Sparks, MD, USA). Additionally, 1 ml of the undiluted homogenate was spread-plated in duplicate on two plates of EMB agar to achieve a low detection limit (1.0 CFU/g). The plates were incubated at 37 °C for 24 h, and typical colonies of *E. coli* O157:H7 formed on EMB agar, which are distinguished by their green metallic sheen, were enumerated. The experiments were conducted in six replicates per treatment.

2.3. Inhibitory efficacy test of phytic acid and NaCl in fresh noodles against natural microflora

2.3.1. Preparation of fresh noodles

We used the microflora naturally present in wheat flour to investigate changes in mesophilic bacterial and total yeast/mold counts in untreated/treated fresh noodles. The wheat flour was not heat-treated in a microwave oven, unlike the method described in 2.2.2. Based on the antibacterial effect on *E. coli* O157:H7, NaCl (0, 0.3, 1.0, 1.6%) and phytic acid (0, 0.3, 0.6, 1.0%) were selected and dissolved in sterile distilled water. In this experiment, a phytic acid concentration of 1.0% was additionally included considering the higher resistance of indigenous microflora than that of single-species pathogens. The process of making noodles after preparation of flour and the salt solution was mostly the same as described in 2.2.2. The only difference was that the noodle samples (25 g) were stored at 30 °C for up to 21 days without bacterial inoculation.

2.3.2. Microbiological analysis of the natural microflora

Mesophilic bacterial and total yeast/mold counts in untreated/treated fresh noodles were examined at 0, 1, 3, 5, 7, 14, and 21 days of storage. As explained in 2.2.3, samples were transferred to a stomacher bag with 0.85% saline and homogenized. One milliliter of the homogenate and 0.1 ml of the diluent were spread-plated in duplicate on both plate count agar (PCA; Difco) for mesophilic bacteria counting and on dichloran rose bengal chloramphenicol agar (DRBC; Difco) for total yeast/mold counting. The PCA and DRBC plates were incubated at 37 °C for 48 h and at 25 °C for 7 days, respectively. All microbial colonies formed on PCA and DRBC plates were counted. The experiments were carried out in three replicates per treatment.

2.3.3. Observation of the appearance

In this experiment, the dough was used instead of noodles to clearly show the trend of visual changes according to the days of storage. For all treatment groups, 6 dough samples (10 g) were prepared per treatment as mentioned in 2.3.1. The untreated/treated dough was formed into a cylindrical shape using a sterile stainless steel mold with a diameter of 36 mm and a height of 10 mm. One sample was taken from each treatment on 6 days (1st, 3rd, 5th, 7th, 14th, and 21st days) during storage at 30 °C, and changes in the appearance were observed.

2.4. Measurements of physicochemical, textural, and sensory qualities of cooked fresh noodles

2.4.1. Cooking procedure

In this experiment, four representative combinations (NaCl 1.6% and phytic acid 0, 0.3, 0.6, 1.0%), including the optimal combination, were selected for the following cooking steps. Fresh noodles were cooked according to previous studies (Cao et al., 2021; Luo et al., 2015; Shao et al., 2019) with some modifications. Briefly, fresh noodle strands were cooked in boiling water (10 g/500 ml; w/v) for 5 min and stirred with a stick during the process. The cooked noodles were rinsed under running ice-cold water for 30 s and rested for 15 min. All measurements on cooked noodles were performed in 10 replicates per treatment.

2.4.2. Color, pH, and water activity analysis

The color of the cooked noodles was examined at room temperature using a chroma meter (CR-400; Konica Minolta Sensing, Inc., Osaka, Japan). Color results were recorded using three parameters, lightness (L^*), greenness/redness (a^*), and blueness/yellowness (b^*), according to the Commission International de l'Éclairage (C.I.E). Based on the values of a^* and b^* , the whiteness index was calculated using the following equations: $Whiteness = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$. The pH and water activity of the cooked noodles was analyzed using an S20 SevenEasy pH meter (Mettler Toledo, Greifensee, Switzerland) and LabMaster-aw (Novasina, Lachen, Switzerland) at room temperature.

2.4.3. Texture profile analysis

The texture profile analysis of cooked noodles was performed using a TA-XT plusC texture analyzer (Stable Micro Systems, Ltd., Godalming, UK) equipped with a cylinder probe with a diameter of 36 mm (P/36R). The samples were individually compressed twice to 70% strain at the pre-test, test, and post-test speeds of 1 mm/s. Force versus time was recorded by software and calculated as the area under the curve, peak forces, and distances on each graph. The parameters of hardness, adhesiveness, springiness, and chewiness were obtained from the graph.

2.4.4. Sensory evaluation

The sensory properties of cooked noodles were evaluated by 20 trained panelists (7 males and 13 females) who gave informed consent to participate. After placing samples in Petri dishes separately, the panelists were asked to rank the appearance and odor of the samples based on a nine-point hedonic scale. On this scale, 1, 2, 3, 4, 5, 6, 7, 8, and 9 represent “dislike extremely”, “dislike very much”, “dislike moderately”, “dislike slightly”, “neither like nor dislike”, “like slightly”, “like moderately”, “like very much”, and “like extremely”, respectively. The all procedures were performed in accordance with appropriate protocols of relevant laws and institutional guidelines regarding human subject experimentation (Reference number KUIRB-2023-0303-01).

2.5. Statistical analysis

The counts of mesophilic bacteria and total yeasts/molds in samples were converted to a logarithmic unit of log CFU/g. The log reduction of *E. coli* O157:H7 was expressed as the difference between the initial population and that after storage [Log reduction = Initial population - Population after storage]. Mean values of microbial populations and physicochemical, textural, and sensory parameters of untreated/treated samples were assessed by analysis of variance using the SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). The significance of the results ($P < 0.05$) was determined using Tukey's studentized range test.

3. Results

3.1. Bactericidal efficacy of phytic acid and NaCl in fresh noodles against *Escherichia coli* O157:H7

The *E. coli* O157:H7 populations in untreated/treated fresh noodles stored at 30 °C for up to 4 days are presented in Fig. 1. In the untreated sample (negative control), the *E. coli* population significantly increased on Day 2 compared to the initial population (6.7 log CFU/g), resulting in a final concentration of 9.0 log CFU/g ($P < 0.05$). It was maintained at 9.1 log CFU/g until Day 4. Similar trend were observed in samples treated with NaCl only (0.3, 1.0, 1.6%) without phytic acid (positive controls), showing an average count of 8.9 log CFU/g on both days. There were clear differences between 0.3% NaCl + phytic acid and 1.0–1.6% NaCl + phytic acid at the same concentrations of phytic acid (0.3–0.6%). This indicated that an optimal combination is required to achieve a significant reduction of *E. coli* O157:H7 growth in noodles. When 0.3% phytic acid + 0.3% NaCl was added to noodles, there was no bactericidal effect on the growth of *E. coli*. However, as the NaCl concentration was increased to 1.0–1.6%, the growth of *E. coli* was reduced by 3.3 and 3.6 log CFU/g on Days 2 and 4, respectively. Similarly, the elimination rate of *E. coli* in fresh noodle samples was the highest in 0.6% phytic acid + 1.0–1.6% NaCl treatments, exhibiting a 5.7 log CFU/g reduction on Day 2 and an 8.5 log CFU/g reduction on Day 4. In particular, the treatment with 0.6% phytic acid + 1.6% NaCl eradicated *E. coli* from the fresh noodles to a level below the detection limit on the 4th day.

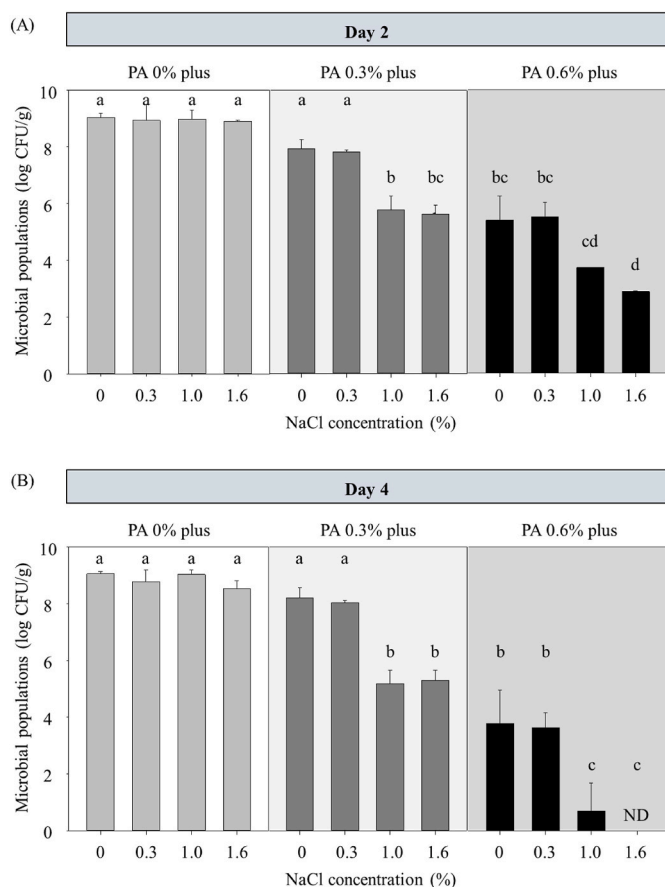


Fig. 1. Microbial reductions in *Escherichia coli* O157:H7 in fresh noodles by storage time of (A) Day 2 and (B) Day 4. Data are expressed as the mean \pm standard deviation for each treatment (initial population: 6.8 ± 0.3 log CFU/g). Values assigned to different alphabetical letters (a–d) are significantly different ($P < 0.05$). *ND: Not detected (Detection limit: 1.0 log CFU/g; $n = 6$).

3.2. Inhibitory efficacy of phytic acid and NaCl in fresh noodles against natural microflora

3.2.1. Mesophilic bacterial counts

Figs. 2 and 4 show changes in the mesophilic bacterial counts from the natural microflora during storage of untreated/treated fresh noodle samples in an incubator at 30 °C for 21 days. The initial bacterial load in the samples was 2.0 log CFU/g. On Day 3, the bacterial counts exceeded 6 log CFU/g in the negative control, single-treatment groups of 0.3–1.6% NaCl or 0.3% phytic acid, and combined treatment groups of 0.3% phytic acid + 0.3–1.6% NaCl (Fig. 2A and B). The counts increased to 7.2–7.4 log CFU/g on the 21st day of storage.

The results obtained in the presence of 0.6% phytic acid alone and its combination with 0.3–1.6% NaCl showed that the growth rate of bacteria from the natural microflora was significantly different, depending on the concentration of NaCl added (Fig. 2C). Bacterial populations increased to more than 6 log CFU/g on Days 5–7 in samples treated with 0.6% phytic acid alone or in combination with 0.3% NaCl, whereas the growth of bacteria in the noodles treated with 0.6% phytic acid + 1.0–1.6% NaCl was suppressed to less than 6 log CFU/g (3.4 and 4.9 log CFU/g, respectively). After 14 days of storage, microbial growth in these treatment groups reached 6 log CFU/g or more. In addition, the inhibitory effect on bacterial growth according to the NaCl concentration added was more clearly observed in the 1.0% phytic acid treatment groups (Fig. 2D). These treatments maintained bacterial counts in the noodles at less than 6 log CFU/g until Day 7. However, from the 14th day of storage, the treatments with 1.0% phytic acid alone and in

combination with 0.3% NaCl showed bacterial growth higher than 6 log CFU/g, while the mesophilic bacterial counts in combination treatments with 1.0–1.6% NaCl were close to the initial concentration (< 2.9 log CFU/g).

3.2.2. Total yeast/mold counts

Changes in the total yeast/mold counts from the natural microflora during storage of untreated/treated fresh noodles at 30 °C for 21 days are provided in Figs. 3 and 4. The initial yeast and mold load in the samples was 2.5 log CFU/g on average, which was similar to the initial mesophilic bacterial count. In Fig. 3A, the overall growth patterns were similar between the negative and positive controls, with the populations increasing to levels of 6.3–6.7 log CFU/g on Day 21, although initial growth of yeasts/molds was slightly faster in the samples treated with 0.3% NaCl only. On Day 21, the yeast and mold growth in the noodles with 0.3% phytic acid alone and with its combinations with 0.3–1.0% NaCl increased to a higher level than that in the negative control, whereas 0.3% phytic acid + 1.6% NaCl showed a similar level to that of the negative control (Fig. 3B).

When 1.0–1.6% NaCl was added with 0.6% or 1.0% phytic acid to noodles, the growth of yeasts/molds proceeded significantly slower ($P < 0.05$) during storage than that in the treatments with phytic acid and its combination with 0.3% NaCl (Fig. 3C and D). On the 7th day of storage, the total yeast and mold counts increased to 7.2 log CFU/g in the single treatment with 0.6% phytic acid and its combined treatment with 0.3% NaCl, while the growth was inhibited in the treatments with 0.6% phytic acid + 1.0–1.6% NaCl (4.5 and 2.4 log CFU/g, respectively). The final populations of yeasts/molds with 0.6% phytic acid alone and in combination with 0.3% NaCl reached 8.3–8.9 log CFU/g by 21 days of storage, while they were only 6.4–6.5 log CFU/g in the combination treatment groups with 1.0–1.6% NaCl. The results obtained in the groups treated with 1.0% phytic acid alone and its NaCl combination showed that the total yeast and mold counts decreased on Day 3 and were maintained in all treatment groups below 4 log CFU/g until Day 7. However, the yeast/mold counts in the noodles on Day 21 were 4.2, 2.1, and 2.4 log CFU/g, when 0.3%, 1.0%, and 1.6% NaCl was added with 1.0% phytic acid, respectively. This pattern contrasted with the final concentration in the treatment group with 1.0% phytic acid alone (9.3 log CFU/g), suggesting that the combined treatments with 1.0% phytic acid and 1.0–1.6% NaCl can control the growth of yeasts/molds in noodles with similar initial population levels.

3.3. Quality changes of cooked fresh noodles treated with phytic acid and NaCl

Table 1 shows the physicochemical and textural characteristics of cooked noodles. The samples treated with NaCl alone had higher whiteness (62.8) than samples treated with phytic acid and NaCl (57.5–58.0; $P < 0.05$). As the phytic acid concentration increased, the pH of the samples decreased from 7.2 to 6.5, 5.2, and 4.1 ($P < 0.05$). For water activity, there was no significant difference between samples with different concentrations of phytic acid ($P > 0.05$). The results of texture profile analysis included the hardness, adhesiveness, springiness, and chewiness of cooked noodles. A slight increase was observed in the hardness, which ranged from 3046 g to 3569 g with different phytic acid concentrations ($P < 0.05$). Regardless of the presence or absence of phytic acid, there were no significant changes in the adhesiveness, with an average of -70.8 g ($P > 0.05$). The addition of phytic acid increased both springiness (0.11) and chewiness (35.8) (control: 0.08 and 23.4, respectively; $P < 0.05$).

The consumer perception of the cooked noodles was also evaluated (Table 2). The sensory scores for the appearance, odor, and overall acceptance ranged in 6.1–6.7, 6.0–6.4, and 6.4–7.0, respectively. Between the treatments, no significant differences in sensory scores were observed ($P > 0.05$). This suggests that the antimicrobial combinations consisting of 0.3–1.0% phytic acid and 1.6% NaCl did not affect the

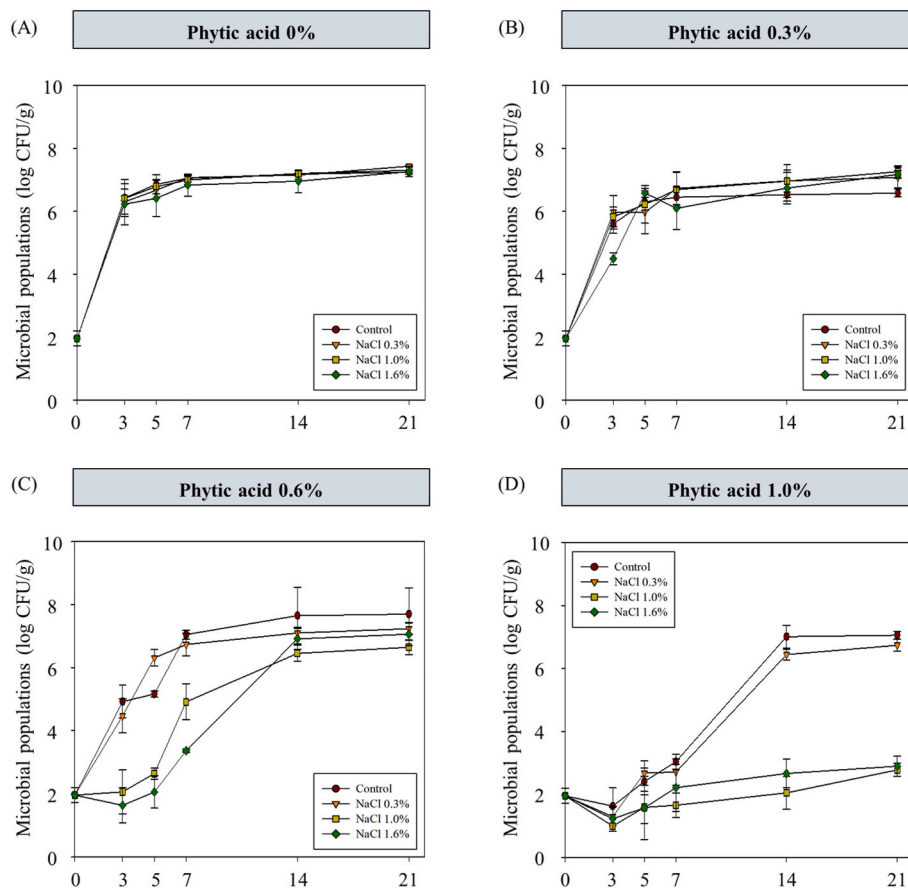


Fig. 2. Changes in mesophilic bacterial count of fresh noodles by combined treatments of 0, 0.3, 1.0, 1.6% NaCl with (A) 0%, (B) 0.3%, (C) 0.6%, and (D) 1.0% phytic acid. Data represent the mean and standard deviation per treatment (initial population: 2.0 ± 0.2 log CFU/g; $n = 3$).

sensory qualities of the samples compared to the control ($P > 0.05$).

4. Discussion

In the present study, a preservative method for fresh noodles was developed by the addition of a natural-borne substance, and its effectiveness and applicability were evaluated. We screened various natural-borne substance candidates that were expected to have synergistic effects with an essential component of fresh noodles, NaCl (within the concentration range of use of $<6\%$). In preliminary experiments, phytic acid was selected from the library of natural-borne antibacterial substances of our research team and applied to improve the storage stability of fresh noodles. When combined with NaCl, phytic acid exhibited significantly different ($P < 0.05$) antibacterial effects in cell suspensions compared to those of other organic acids (lactic and acetic acids) (data not shown). The bactericidal/inhibitory efficacies of this preservative in fresh noodles were validated in this study by designing two experiments with and without microbial inoculation.

The major finding was that phytic acid showed synergistic potential with NaCl added to fresh noodles against a wide range of microorganisms, which may be naturally present in raw materials or cross-contaminated from the environment. After 4 days of storage of fresh noodles containing 0.6% phytic acid and 1.6% NaCl, the counts of the pathogenic strains of a hygienic indicator bacterium, *E. coli* O157:H7, in the noodles were reduced below the detection limit (Fig. 1). Also, the antimicrobial effects of the preservatives (0.6% phytic acid + 1.0–1.6% NaCl, 1.0% phytic acid + 0–1.6% NaCl) were expanded against a spectrum of the natural microflora for 21 days (Figs. 2–4). Based on the antimicrobial efficacy and quality parameters of each treatment, 0.6% phytic acid combined with 1.6% NaCl was determined to be the optimal

preservative in this study. The bactericidal and inhibitory effects of the combined treatments involving synergy were in accordance with the results from our earlier studies (Kim and Rhee, 2015; Moon et al., 2017; Moon and Rhee, 2016), which proposed the applicability of the preservative in various food matrices with respect to ingredients that can be added to foods. Since the antimicrobial activity of NaCl itself is not effective at the concentrations added to noodles, this study developed a preservative method by the addition of phytic acid that can show a synergistic effect in combination with NaCl while replacing the existing acidity regulators.

Fresh noodles or pasta are known for their vulnerability to spoilage and pathogenic microorganisms, and previous studies have been conducted to suggest intervention strategies to prolong the shelf lives of these products using natural-borne preservatives. Han et al. (2022) and Lin et al. (2016) described the effects of acidity regulators on the microbiological quality of fresh noodles. In these studies, organic acids such as phosphoric acid, monosodium fumarate, fumaric acid, citric acid, and lactic acid inhibited the mesophilic bacterial population, extending the shelf life of noodles at 4–5 °C by 4–10 days compared to that of untreated noodles. Furthermore, a variety of studies have focused on substances of plant origin (tea polyphenols, flaxseed flour, thymol, lemon extract, grapefruit seed extract, and curcumin) or animal origin (chitosan and Maillard reaction products) as preservatives of fresh noodles (Cato and Li, 2020; Hafsa et al., 2021). For instance, chitosan and Maillard reaction products prevented mesophilic bacterial growth at 4 °C and extended the shelf life of fresh noodles by 6 and 14 days, respectively (Huang et al., 2007). During storage at 25 °C, the shelf life of millet fresh noodles was prolonged from 22 h to 30 h with the addition of 0.05% curcumin (Wang et al., 2020). In addition to the use of chemical additives, research has been conducted to enhance the storage

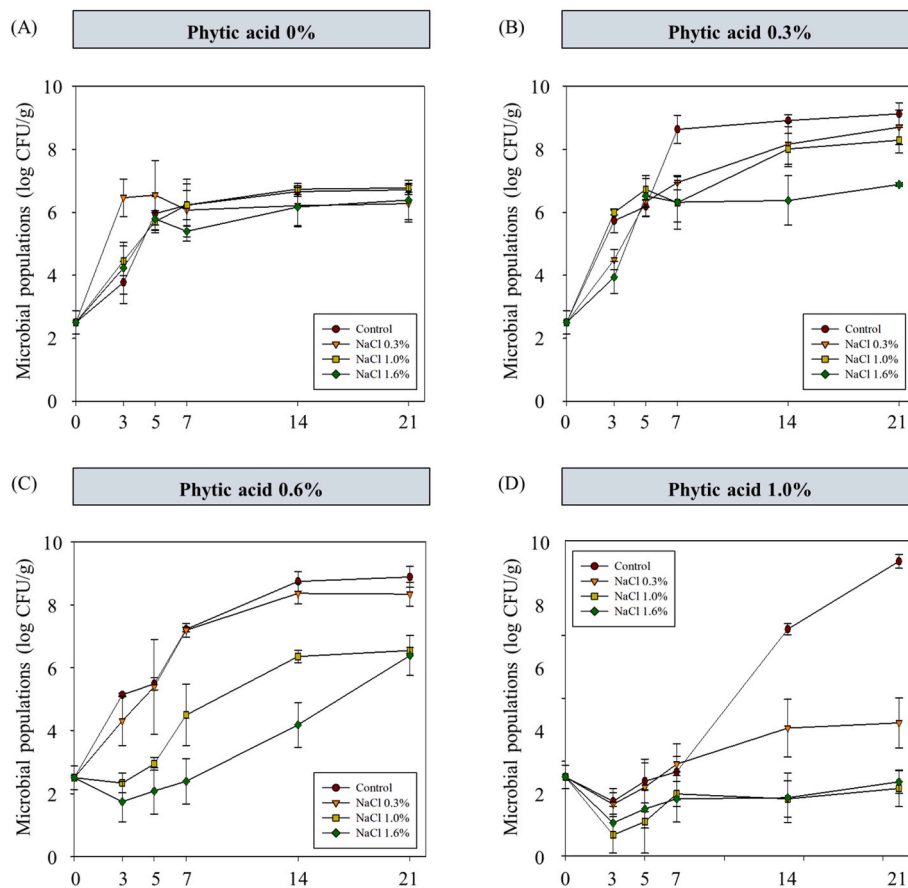


Fig. 3. Changes in total yeasts/molds count of fresh noodles by combined treatments of 0, 0.3, 1.0, 1.6% NaCl with (A) 0%, (B) 0.3%, (C) 0.6%, and (D) 1.0% phytic acid. Data represent the mean and standard deviation per treatment (initial population: 2.5 ± 0.4 log CFU/g; $n = 3$).

stability of fresh noodles using physical treatments (microwaves, superheated steam, and electron beam irradiation) (Huang et al., 2022; Li et al., 2017; Wang et al., 2021). Most of the relevant studies consider a mesophilic bacterial count exceeding 6 log CFU/g as the standard limit, and the extension of the shelf life is determined by whether or not the microbial growth rate can be slowed down even slightly compared to that in the control. Few preservative methods are capable of preventing the growth of both mesophilic bacteria and yeasts/molds from the indigenous microflora.

Given the relatively low bactericidal and inhibitory effects of a single treatment of fresh noodles, combined antimicrobial treatments have been proposed. Tantala et al. (2022) developed a combination of 0.1% chitosan and 0.1% potassium sorbate showing a synergistic activity against microorganisms in fresh rice noodles during storage at 30 °C. These results are in agreement with the results of this study in that antimicrobial synergy could efficiently inhibit the growth of yeasts/molds as well as bacteria during extended storage of noodles at high temperature. Moreover, recent approaches have focused on the development of active food packaging with incorporated effective substances, as opposed to previous research targeting only vacuum or modified atmospheric packaging (Cruz et al., 2006; Rachtanapun and Tangnonthaphat, 2011; Shahid et al., 2021). Several research groups have reported sodium benzoate, potassium sorbate, and monolaurin as antimicrobial substances that can be added to fresh noodles/pasta via an edible film or in a microencapsulated form to extend the shelf life (de Camargo Andrade-Molina et al., 2013; Wangprasertkul et al., 2021; Xu et al., 2008).

The strong bactericidal and inhibitory effects of the preservative method developed in this study were assumed to be induced by the synergistic antimicrobial action of phytic acid combined with NaCl.

Phytic acid is one of the organic acids found in grain and legume seeds and is generally recognized as safe according to the U.S. Food and Drug Administration (Kumar et al., 2021). It is also known for its strong chelating ability with high affinity for cations (Fe^{2+} , Zn^{2+} , Mg^{2+} , K^+ , and Ca^{2+}) due to its six negatively charged ions (Perera et al., 2018; Pramitha et al., 2021). Chelating agents can affect cell membrane integrity by binding divalent cations of lipopolysaccharides in the outer membrane of gram-negative bacteria (Nassar et al., 2021). There is a report that the membrane permeability of gram-positive bacteria can also be disrupted by chelating agents (Zhou et al., 2019). The coexistent properties of phytic acid both as an organic acid and a chelating agent can result in antimicrobial effects. However, the latter is presumed to further contribute to a distinct antimicrobial pattern of phytic acid which differs from those of other organic acids, and this has been consistently demonstrated in our previous studies. When compared to other organic acids (acetic, citric, lactic, and malic acids) at the same concentrations, phytic acid showed a significantly higher ($P < 0.05$) antibacterial effect against *E. coli* O157:H7 and a significantly higher acidity. In addition, the combination of 3–4% NaCl and 0.4% phytic acid completely disintegrated the bacterial cell membrane, although 3–4% NaCl adjusted with HCl to the same pH value did not cause cell membrane degradation (Kim and Rhee, 2016a). The chelating characteristic was also effective in destroying bacterial biofilms formed by *E. coli* O157:H7 without recovery, as shown in the study by Kim and Rhee (2016b). Considering the earlier studies mentioned above, the mechanism underlying the synergism between phytic acid and NaCl combined in fresh noodles can be elucidated as follows: phytic acid reacts with and destabilizes positively charged ions bound to the bacterial outer membrane, and the Na^+ , Cl^- , H^+ ions penetrate the disrupted cell membrane, resulting in cell damage or death (Kim et al., 2017; Nassar et al., 2023;

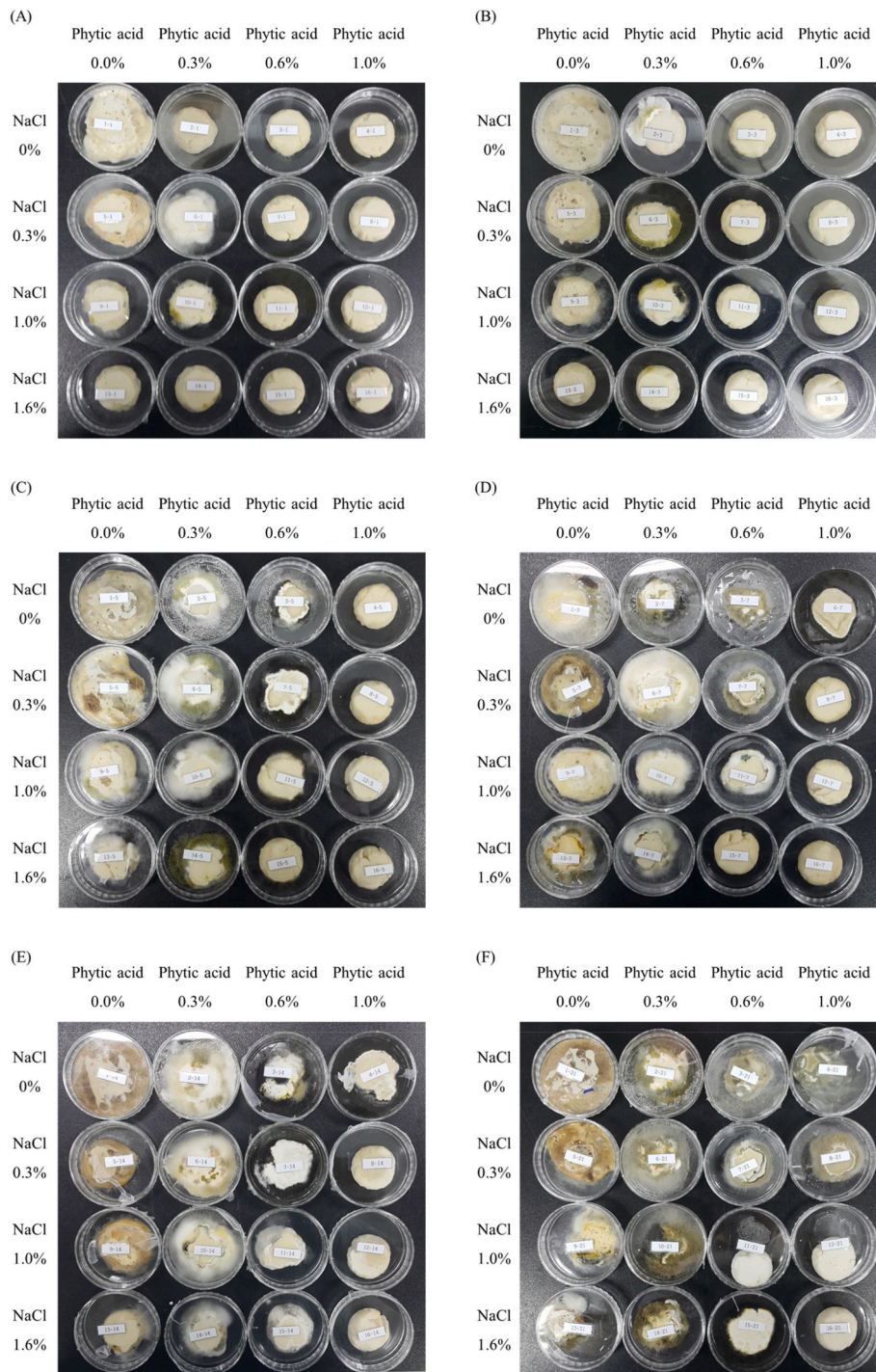


Fig. 4. Changes in the appearance of fresh noodles by combined treatments of 0, 0.3, 1.0, 1.6% NaCl with 0, 0.3, 0.6, 1.0% phytic acid during the storage period of (A) Day 1, (B) Day 3, (C) Day 5, (D) Day 7, (E) Day 14, and (F) Day 21 (n = 3).

Zhou et al., 2019).

In this study, fresh noodle samples also showed distinct patterns of total yeast/mold counts with and without NaCl (Fig. 3). Overall, the growth of the total yeast/mold population was suppressed through a synergistic effect of phytic acid and NaCl. On the other hand, the total yeast/mold population growth was facilitated when phytic acid was added without NaCl, and this trend was attenuated with increasing concentrations of phytic acid. Since the antifungal effect of phytic acid has rarely been studied, further studies should be conducted to identify the antifungal mechanism of phytic acid alone or in the presence of NaCl. However, this mechanism can be inferred from research on

antifungal agents and their modes of action, which suggests the possible mechanisms to involve resistance reversion of fungi to antifungal substances. Previous studies highlighted the contribution of synergistic effects to a significant increase in antifungal activity. For instance, one of the chelating agents, tetracycline, is known to form pores in the fungal plasma membrane and alter iron homeostasis. This can lead to increased membrane fluidity and allow additional entry of antimicrobial agents into the cell (Rossato et al., 2021). This finding was in agreement with another report showing that the addition of a synergistic agent could make resistant fungal strains susceptible or more sensitive (Campbell et al., 2012).

Table 1
Physicochemical and textural qualities of cooked fresh noodles treated by NaCl and phytic acid.

Treatment		Physicochemical and textural quality					
		Whiteness	pH	Texture			
Phytic acid (%)	NaCl (%)			Hardness (g)	Adhesiveness (g-sec)	Springiness	Chewiness
0.0	1.6	62.8 ± 0.9 ^a	7.2 ± 0.0 ^a	3569.7 ± 268.3 ^a	-77.5 ± 33.4 ^a	0.08 ± 0.01 ^a	23.4 ± 4.0 ^a
0.3	1.6	58.0 ± 0.6 ^b	6.5 ± 0.0 ^b	3434.6 ± 184.3 ^{ab}	-68.3 ± 24.2 ^a	0.11 ± 0.00 ^b	36.0 ± 2.4 ^b
0.6	1.6	57.5 ± 0.7 ^b	5.2 ± 0.1 ^c	3171.7 ± 169.6 ^{bc}	-59.4 ± 30.8 ^a	0.11 ± 0.01 ^b	37.6 ± 3.3 ^b
1.0	1.6	57.9 ± 0.5 ^b	4.1 ± 0.1 ^d	3049.5 ± 309.2 ^c	-77.9 ± 18.5 ^a	0.11 ± 0.01 ^b	33.8 ± 4.8 ^b

The water activity of the samples was 0.89 ± 0.01 ($P > 0.05$).

Results are expressed as the mean ± standard error per treatment (n = 10).

a to d values in the same column are significantly different ($P < 0.05$).

Table 2
Sensory qualities of cooked fresh noodles treated by NaCl and phytic acid.

Treatment		Sensory quality		
Phytic acid (%)	NaCl (%)	Appearance	Odor	Overall acceptance
0.0	1.6	6.1 ± 1.4	6.3 ± 1.5	6.4 ± 1.4
0.3	1.6	6.7 ± 1.3	6.4 ± 1.2	7.0 ± 1.0
0.6	1.6	6.7 ± 1.6	6.3 ± 1.4	6.5 ± 1.3
1.0	1.6	6.2 ± 1.4	6.0 ± 1.6	6.4 ± 1.4

Results are expressed as the mean ± standard error per treatment (n = 20).

The values in the same column are not significantly different ($P > 0.05$).

The nine-point hedonic scale ranges from 1 to 9 (1 = Dislike extremely, 2 = Dislike very much, 3 = Dislike moderately, 4 = Dislike slightly, 5 = Neither like nor dislike, 6 = Like slightly, 7 = Like moderately, 8 = Like very much, 9 = Like extremely).

It has been reported that the quality of noodle products is related to their objective parameters (Niu and Hou, 2019; Wang et al., 2018). In this study, the cooked noodles treated with phytic acid and NaCl tended to be less white than the control, since phytic acid has a light yellow to light brown color. Additionally, changes in pH and some textural parameters (hardness, springiness, and chewiness) were attributed to the addition of phytic acid. However, these changes were positively supported by subjective quality parameters. According to a nine-point hedonic scale, the scores for the appearance, odor, and overall acceptance of all samples indicated that the sensory properties assessed in this study might be at least slightly or moderately preferred by the panelists. Some panels did not perceive differences in the appearance (e.g., color and firmness) and pungent odor that were affected by phytic acid, suggesting the possibility of applying the developed preservative technology to fresh noodles.

Moreover, the optimal preservative for fresh noodles (0.6% phytic acid and 1.6% NaCl) was selected considering the normal pH range of fresh noodles. Except for alkaline fresh noodles, the pH of fresh noodles is generally in the range of 5.0–7.0 (Davis, 2016; Han et al., 2022; Li et al., 2022; Schebor and Chirife, 2000). The acidity of cooked noodles pre-treated with organic acids as acid regulators might vary depending on the type and concentration of organic acids (Jeong, 1998; Lin et al., 2016). Certain types of fresh noodles are subjected to treatments such as boiling, cooling, dipping in acidulants (pH < 4.8), or pasteurization to suppress spore germination and increase storage stability (Low et al., 2021; Shamsudin et al., 2022; Yu et al., 2023). For the practical application of the developed preservative, combination with vacuum or modified atmospheric packaging can be considered, and this method is expected to further enhance the preservative effect.

In conclusion, we suggest a potential preservative consisting of phytic acid and NaCl that can be used at the dough-making stage of fresh noodle production. Practical application in a real food scenario proved that this method could be an alternative to currently-used preservatives of fresh noodles for the following reasons: (1) fresh noodles can contain natural-borne ingredients that replace chemically synthesized ingredients; (2) the growth of a wide range of microorganisms (pathogenic

E. coli, indigenous mesophilic bacteria and total yeasts/molds) can be inhibited; and (3) fresh noodles can be distributed or stored at room temperature for an extended period of time. The developed method can contribute to the prevention of microbial growth, thereby ensuring the storage stability of fresh noodles/pasta or other grain-based foods that include salt as the main ingredient.

CRediT authorship contribution statement

Hary Yu: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Visualization. **Min Suk Rhee:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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