Prev. Nutr. Food Sci. 2024;29(4):474-484
https://doi.org/10.3746/pnf.2024.29.4.474
ISSN 2287-8602

Protective Effects of Peanut Sprouts from a Smart Farming System on the Barrier Function of Human Epithelial Cells

Dong Yoon Kim[1], Soo-Sung Kim[1], Eun-Jin Choi[1], Hoon Kim[2], Dong Hyeon Kim[2], Seong-Min Hong[3], Sae-Byuk Lee[4,5], and Hyun-Dong Cho[1,6]

[1]Department of Food and Nutrition and [6]Glocal University Project Team, Sunchon National University, Suncheon 57922, Korea
[2]NANUMBIO Co., Ltd., Suncheon 57922, Korea
[3]College of Pharmacy, Gachon University, Incheon 21936, Korea
[4]School of Food Science and Biotechnology and [5]Institute of Fermentation Biotechnology, Kyungpook National University, Daegu 41566, Korea

ABSTRACT: Inflammatory bowel disease, including Crohn's disease and ulcerative colitis, poses an emerging threat as it can lead to colorectal cancer, thrombosis, and other chronic conditions. The present study demonstrated the protective effects of peanut sprout extracts (PSEs) prepared from day 2 to day 7 of germination against lipopolysaccharide (LPS)-induced epithelial barrier breakdown. Although the peanut sprout length increased in a time-dependent manner from day 1 to day 7, the extraction yields remained relatively consistent from day 2 to day 7. With regard to antioxidant activities, the PSE from day 6 of germination exhibited the highest oxidative radical scavenging activity and total phenolic content. Similarly, it showed remarkable anti-permeability effects in LPS-stimulated Caco-2 cells and suppressed the degradation and dissociation of junctional markers (e.g., ZO-1 and E-cadherin) at cell-cell junctions. Collectively, these data demonstrate that PSE from day 6 of germination can be used as a functional food resource to reduce inflammatory barrier dysfunction.

Keywords: antioxidants, Arachis, inflammatory bowel diseases, intestinal barrier function

INTRODUCTION

Inflammatory bowel disease (IBD) includes two main chronic inflammatory disorders: Crohn's disease and ulcerative colitis. Crohn's disease can occur anywhere in the gastrointestinal tract, but it typically affects the colon and rectum with inflammation that can be patchy and extend through the full thickness of the bowel wall. By contrast, ulcerative colitis is limited to the colon and rectum and is characterized by continuous inflammation that is confined to the mucosal layer. IBD development is believed to result from an immune response that is improperly regulated, triggered by environmental factors, and influenced by the gut microbiota, particularly in individuals with genetic susceptibility (Verstockt et al., 2022). Collectively, these factors contribute to the global prevalence of IBD, affecting an estimated 4.9 million people worldwide (Wang et al., 2023). In addition, IBD is closely associated with increasing mortality when combined with other chronic diseases, including colorectal cancer and mesenteric venous thrombosis (Wang et al., 2024). Thus, IBD prevention strategies are urgently needed (Naïk Vietti et al., 2014; Ng et al., 2017).

Reactive oxygen species (ROS), including the superoxide anion (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH), are highly reactive oxygen-containing molecules. These compounds are natural byproducts of cellular metabolism, particularly in the gastrointestinal tract. They play vital roles in cellular signaling and immune defense mechanisms. However, excessive ROS production can overwhelm the body's antioxidant defenses, resulting in oxidative stress and tissue damage (Iborra et al., 2011). The intestinal epithelial barrier, which comprises a monolayer of epithelial cells, is pivotal for maintaining gastrointestinal homeostasis by segregating the luminal contents from the underlying immune cells within the lamina propria. In IBD, the loss of junctional proteins, including ZO-1 and E-cadherin, can be increased by exposing immune cells to antigens, leading to the disruption of epithelial barrier function (Landy et al., 2016). Several studies have established correlations between elevated ROS levels and diminished antioxidant

Received 2 September 2024; Revised 28 October 2024; Accepted 30 October 2024; Published online 31 December 2024
Correspondence to Hyun-Dong Cho, E-mail: hdcho@scnu.ac.kr

defenses with IBD pathogenesis (Jarmakiewicz-Czaja et al., 2020; Jarmakiewicz-Czaja et al., 2023). Therefore, dietary antioxidants derived from natural sources and foods may present a promising strategy for mitigating the onset of IBD (Jarmakiewicz-Czaja et al., 2020).

Peanuts (*Arachis hypogaea*) are annual herbaceous plants from the Fabaceae family and have been widely consumed as food and salad ingredients in Asian countries. They are rich in proteins, carbohydrates, fiber, fats, niacin, folic acid, thiamine, arachidonic acid, flavonoids, magnesium, and phosphorus (Kang et al., 2010). They are also known for their bioactivities, including antioxidant properties, cellular protective effects (Jo et al., 2012), antitumor effects (Park et al., 2020), and ototoxicity inhibitory effects (Youn et al., 2017). Seed germination induces complex biochemical changes, and the peanut sprouting process produces leaves, stems, and roots that contain various phytochemicals. The levels of these bioactive compounds can vary depending on the germination time, culture condition, and processing and extraction methods used (Kang et al., 2010; Adhikari et al., 2018; Hong et al., 2020). Recently, the mass production of peanut sprouts under controlled conditions has become possible with the advancement of smart farming systems (Bacco et al., 2019), and these systems have expanded to the cultivation of sprout vegetables, including peanut sprouts, radish, and wheat (Sambo et al., 2019; Kim et al., 2022; Chang et al., 2023; Peladarinos et al., 2023). Furthermore, crop byproducts obtained from smart farms are being used in the development of functional food ingredients and food processing (Jeong et al., 2023). However, to the best of our knowledge, no studies have investigated the impact of germination time in peanut sprouts produced by a smart farming system on IBD.

In the present study, we compared the antioxidant activities and epithelial barrier protective properties of peanut sprout extracts (PSEs) obtained at different germination times in order to identify the most effective germination time for PSE to alleviate oxidative stress and IBD.

MATERIALS AND METHODS

Materials

Peanuts were purchased from Daeseong Agricultural Co., and the peanut sprouts used in this study were provided by Nanum Bio Co., Ltd. ZO-1, β-actin (sc-33725, sc-69879, Santa Cruz), E-cadherin (ab231303, Abcam), and Alexa Fluor anti-rabbit IgG and anti-mouse IgG (111-585-003, 111-545-003, Jackson ImmunoResearch Inc.) were purchased for this study. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Folin-Ciocalteu reagents were obtained from Sigma-Aldrich. FITC-dextran (molecular weight: 10 kDa) was provided by MedChemExpress.

Preparation of peanut sprout extracts

Peanut sprout cultivation was automatically performed in a low-carbon smart farming system using photovoltaic-thermal (PVT) technology. The smart farming system used in the present study was controlled and operated via information and communication technology (ICT) integrated with PVT technology. Data from the incubator were transmitted to mobile devices and computers for monitoring and adjustments. The peanut sprout cultivation conditions were as follows: washed peanuts were watered (36°C) for 60 s every 150 min from day 1 to day 3 and every 100 min from day 4 to day 7 using an overhead irrigation system. The peanut sprouts that were harvested daily from day 1 to day 7 were dried in a hot air dryer (Hanil GNCO Co., Ltd.) at 60°C for 48 h. Subsequently, the sprouts and roots were separated for use in PSE. To prepare the extract, 2.5 g of dried and ground peanut sprout roots, stems, and sprouts were immersed in 50 mL of 70% ethanol and extracted by stirring at 170 rpm at room temperature for 2 h. The extract was filtered and concentrated using a rotary vacuum evaporator and then redissolved in dimethylsulfoxide to a concentration of 200 mg/mL for use in antioxidant and cell experiments.

DPPH radical scavenging activity

The scavenging effect of PSEs on DPPH radicals was measured using a modified version of Blois' method (Blois, 1958). Briefly, 50 μL of PSEs at a concentration of 500 μg/mL and 0 to 100 μM of gallic acid standard were reacted with 150 μL of 0.2 mM DPPH solution for 30 min. Then, the absorbance was measured at 517 nm using an ultraviolet/visible (UV/VIS) spectrophotometer (KLAB). The blank group was prepared by adding 100% ethanol instead of PSEs. The results were expressed as mM gallic acid equivalent (GAE) per gram of extract compared with the standard substance.

ABTS radical scavenging activity

The ABTS radical scavenging ability of PSEs was assessed using an adapted protocol from Biglari et al. (2008). To prepare the ABTS solution, 7 mM ABTS was mixed with 2.45 mM potassium persulfate and then diluted 40 times with distilled water. The absorbance was measured at 734 nm. When the value reached 0.700±0.01, it was used in the experiment. A 50 μL aliquot of PSEs at a concentration of 500 μg/mL and 0 to 100 μM of gallic acid standard were dispensed into a 96-well plate and added with 150 μL of ABTS radical solution. The mixture was reacted in the dark for 10 min, and the absorbance was measured at 734 nm using a UV/VIS spectrophotometer (KLAB). The results were expressed as mM GAE per

gram of extract compared with the standard curves.

Total phenolic content

The total phenolic content (TPC) was measured using a modified Folin-Ciocalteu method (Gao et al., 2000). A mixture comprising 20 μL of PSEs, 100 μL of 0.2 N Folin-Ciocalteu reagent, and 80 μL of 0.7 M sodium carbonate was prepared in sequence and allowed to react at room temperature for 2 h. The absorbance of the reaction mixture was measured at 760 nm using a UV/VIS spectrophotometer (KLAB). The results were expressed as mM GAE per gram of extract based on a standard curve calculated with gallic acid.

Caco-2 intestinal epithelial cell culture

The Caco-2 cells used in this study were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle medium supplemented with 100 units/mL of Antibiotic-Antimycotic (Thermo Fisher Scientific), nonessential amino acids (Sigma-Aldrich), and 10% fetal bovine serum (GenDEPOT). The cells were maintained in a 37°C, 5% CO_2 incubator (Thermo Fisher Scientific) and subcultured regularly.

Sulforhodamine B assay

To assess the cytotoxicity of PSEs, sulforhodamine B (SRB) (Sigma-Aldrich) assay was conducted in this study. SRB assay measures the total protein content in viable cells by absorbance, which indicates the extent of cell death. Caco-2 cells were diluted to a concentration of 4×10^4 cells/mL and dispensed into a 48-well tissue culture plate. After cell stabilization, PSEs from different parts of the plant were added at concentrations ranging from 0.0625 mg/mL to 1 mg/mL for 24 h. Then, 12% trichloroacetic acid solution was added to fix viable cells, which were then stained with 0.4% SRB dye. The SRB dye was dissolved using 10 mM Tris buffer, and the absorbance was measured at 540 nm using a UV/VIS spectrophotometer (KLAB). The results were normalized by the control group to determine the effect of PSEs on cell proliferation.

Morphological analysis

Caco-2 cells, which were cultured in a CO_2 incubator to form an intact monolayer, were treated with PSEs at a concentration of 1 mg/mL and incubated for 24 h. Subsequently, the morphological changes in the cells were photographed using an optical microscope at 200× magnification (Leica Microsystems).

FITC-dextran permeability

To measure the inhibitory effect of PSEs on lipopolysaccharide (LPS)-induced intestinal permeability, a transwell insert culture system was used with FITC-dextran (10 kDa). A total of 1×10^5 cells were seeded into the upper compartment of the transwell and cultured for 21 days, and the medium was regularly changed every 2 days. Then, the cells were pretreated with PSEs at a concentration of 1 mg/mL for 2 h, followed by treatment with 10 μg/mL of LPS and 1 μg/mL of FITC-dextran for 2 h. Permeabilized FITC-dextran levels were analyzed using a fluorescence spectrometer (Molecular Devices LLC) at an excitation wavelength of 490 nm and emission wavelength of 520 nm. Data were normalized by the LPS-treated group to show the relative reduction of permeabilized FITC-dextran.

Transepithelial electrical resistance analysis

To determine the inhibitory effects of PSEs on LPS-induced barrier disruption, a transwell insert culture system was used as described. Caco-2 cells were pretreated with PSEs at a concentration of 1 mg/mL for 2 h and then added with 10 μg/mL of LPS to induce damage to the intestinal epithelial cell layer for 24 h. Subsequently, the transepithelial electrical resistance (TEER) was measured using an EVOM3 resistance meter (WPI Inc.) by placing one electrode in the upper chamber and another electrode in the lower chamber of the transwell. The results were expressed in ohms (Ω)×cm^2, and changes in TEER values were quantified relative to the baseline values for each group.

Immunofluorescence

Caco-2 cells were placed on an 8-well cell culture slide (SPL Life Sciences Co., Ltd.) and incubated in a CO_2 incubator until the cells were fully confluent. The cells were pretreated with PSEs for 2 h and then treated with LPS to induce inflammatory damage. After 24 h, the cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton-X. Next, ZO-1 (sc-33725) and E-cadherin (ab231303) diluted with 4% bovine serum albumin were added to the cells overnight at 4°C and then reacted with fluorescently labeled secondary antibodies with Alexa-594 and Alexa-488. The stained cells were observed under a Nikon A1 THUNDER confocal microscope with a 40× immersion objective lens (Nikon). Image quantification was performed using ImageJ software.

Western blot analysis

Caco-2 cells cultured as a monolayer were pretreated with PSEs at a concentration of 1 mg/mL for 2 h, added with 10 μg/mL of LPS, and incubated in a CO_2 incubator for 24 h. After incubation, the cells were collected using a scraper, and the proteins were extracted with 300 μL of radioimmunoprecipitation assay buffer containing a proteinase inhibitor cocktail. The protein concentrations were normalized using a BCA protein assay kit. The protein samples were then subjected to 12% sodium do-

decyl sulfate-polyacrylamide gel electrophoresis, and the separated proteins were transferred onto a nitrocellulose membrane. The membranes were incubated with primary antibodies against ZO-1, E-cadherin, and β-actin and then treated with the corresponding secondary antibodies according to the manufacturer's protocol. The protein bands were detected using the ECL Western Blotting Substrate Kit (Thermo Fisher Scientific).

Statistical analysis

Data were statistically analyzed using GraphPad Prism software (GraphPad). Groups were independently compared by Student's *t*-test. The correlation coefficient was measured using Pearson correlation analysis. Statistical significance was indicated by $P<0.05$, $P<0.005$, and $P<0.001$.

RESULTS

Effect of peanut germination time on sprout length and extraction yield

The length and extraction yield of dried peanut sprouts were analyzed for their potential use in the food industry. The peanut sprout length increased in a time-dependent manner during germination (Fig. 1A and 1B). After 4 days of germination, the sprout length rapidly increased compared with that before 4 days. The peanut sprout lengths on days 3, 5, and 7 were significantly longer than those on days 2, 4, and 6, respectively. The extraction yield, which is defined as the percentage of dried weight in PSE compared with the initial weight of the dried peanut sprouts, ranged from 45.73% to 52.53% between day 7 and day 2 of germination (Fig. 1A and 1C). Except for PSEs obtained from day 1 of germination, the other groups showed no statistical significance. The extraction yield of PSE from day 1 of germination was only 11.73%, which was considerably lower than that in other groups. These results indicate that the extraction yield of PSE is not affected by the germination time from day 2 to day 7. However, the optimal harvest time should be determined based on the bioactive effects, phenolic compositions, and production costs.

Effect of PSEs on the antioxidant activities and TPC

To evaluate the impact of PSEs on the antioxidant activities and TPC, we assessed the ROS scavenging efficiency using gallic acid as a reference compound. As shown in Table 1, the PSEs obtained from day 2 to day 7 of germination demonstrated DPPH radical scavenging efficacy ranging from 20.7 mM GAE/g to 62.9 mM GAE/g of PSEs. The ABTS free radicals were also neutralized by PSEs, with values ranging from 30.9 mM GAE/g to 45.1 mM GAE/g. The TPC in the PSEs ranged from 266.1 mM GAE/g to 445.1 mM GAE/g. Considering the radical scavenging properties and TPC, the highest antioxidant activity in the PSEs was observed at 6 days of germination.

Effect of PSEs on Caco-2 cell viability

To evaluate the cytotoxicity of PSEs on intestinal epithelial cells, we performed an SRB assay. Caco-2 intestinal epithelial cells were treated with PSEs at concentrations ranging from 0.25 mg/mL to 2 mg/mL for 24 h. The results showed that incubation with 1 mg/mL of PSEs did not cause significant changes in cell morphol-

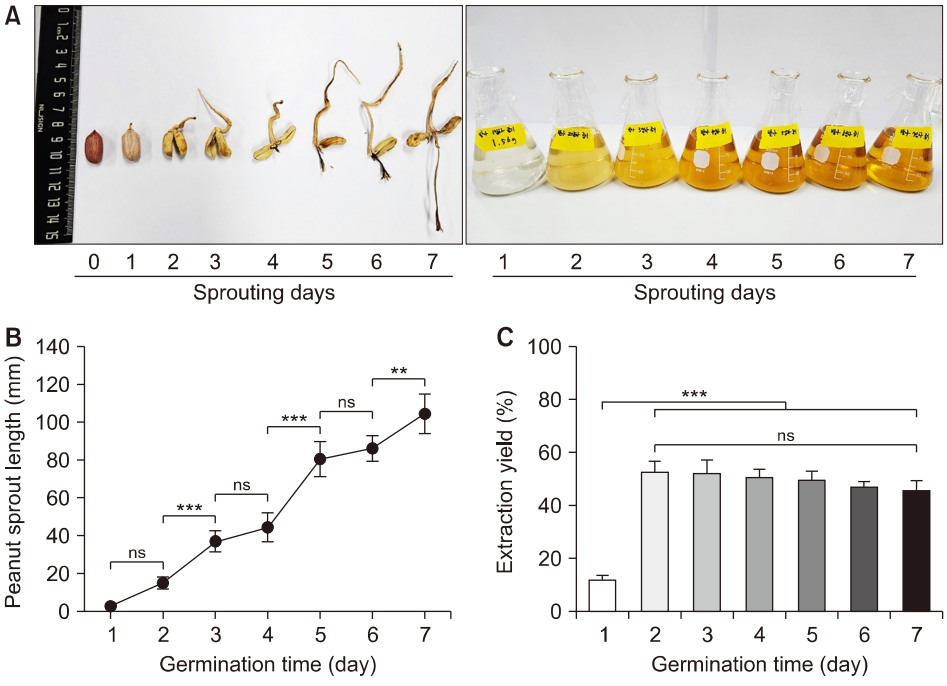

Fig. 1. Effect of germination time of peanut sprout extracts (PSEs) on sprout length and extraction yield. (A) Morphological changes in peanut sprouts and PSEs with germination time. (B) Peanut sprout length. (C) Extraction yield in PSEs obtained from day 1 to day 7 of germination. Statistical significance was analyzed by one-way analysis of variance with Tukey's multiple comparison and marked as ***$P<0.001$ (n=3). ns, not significant.

Table 1. Antioxidant activity and total phenolic content of peanut sprout extracts

	PSEs (mM GAE/g)					
	2 days	3 days	4 days	5 days	6 days	7 days
DPPH radical scavenging activity	23.8±3.9	38.2±6.6	33.0±0.4	20.7±3.3	62.9±3.1	40.4±3.0
ABTS radical scavenging activity	34.2±0.8	31.8±0.7	39.1±0.5	30.9±0.7	45.1±2.0	33.2±1.0
Total phenolic content	296.3±15.8	266.1±14.0	390.9±8.3	306.2±7.5	445.1±27.7	309.2±14.4

Values are presented as mean±SE.
PSE, peanut sprout extracts; GAE, gallic acid equivalent/g of PSEs; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

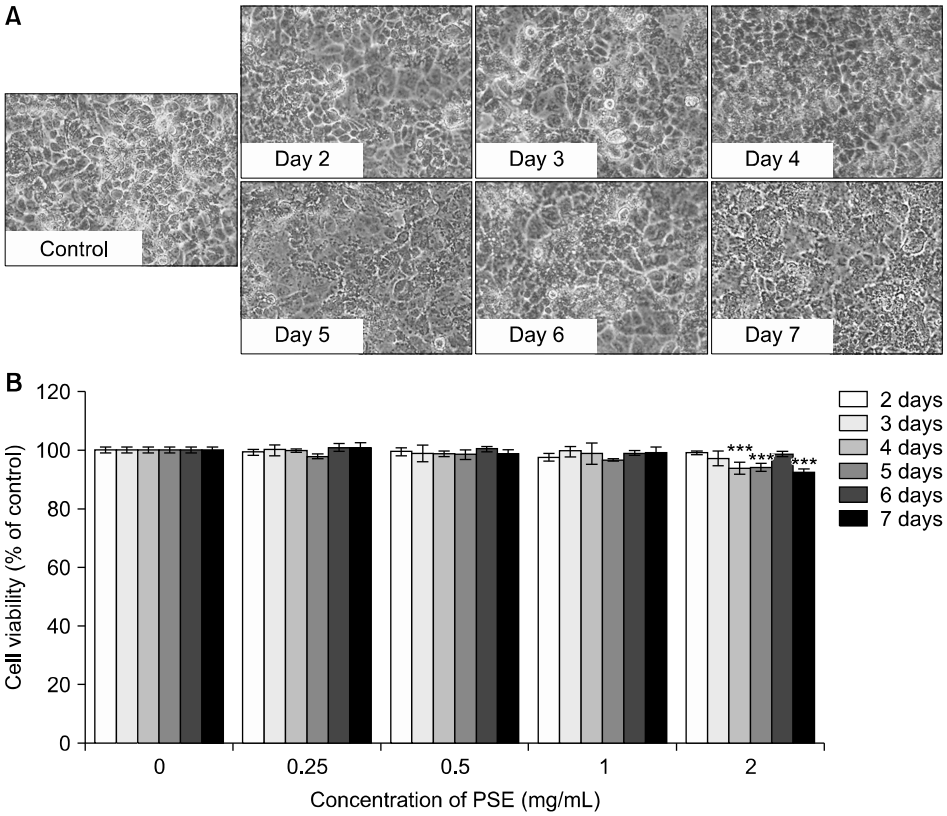

Fig. 2. Effect of peanut sprout extracts (PSEs) on the growth of Caco-2 intestinal epithelial cells. (A) Morphological differences in Caco-2 cells treated with 1 mg/mL of PSEs for 24 h. (B) Cell viability according to treatment with PSEs at concentrations ranging from 0.25 to 2.0 mg/mL. Statistical significance was analyzed by Student's *t*-test and marked as ***P<0.001 compared with the control group (n=3).

ogy or cytotoxic effects compared with the control group (Fig. 2). Furthermore, no significant alterations were observed in PSEs from day 2 to day 7 at a concentration of 1 mg/mL. These results indicate that 24-h exposure of Caco-2 cells to 1 mg/mL of PSEs does not affect cell viability and cytotoxicity.

Protective effect of PSEs against LPS-induced epithelial barrier disruption

We next assessed whether treatment with PSEs could protect against LPS-induced disruption of the Caco-2 epithelial monolayer. We previously observed that LPS treatment at 10 μg/mL reduced ZO-1 protein expression, a key marker of tight junctions, in a time-dependent manner, indicating damage to the epithelial junctions (data not shown). The epithelial barrier function was evaluated by measuring FITC-dextran permeability and TEER in Caco-2 cell monolayers on transwells. Compared with LPS-treated cells, permeabilized FITC-dextran levels were significantly decreased by treatments with PSEs obtained from day 4 to day 7 of germination (Fig. 3A). However, PSEs obtained from day 2 and day 3 of germination did not show a protective effect against inflammatory stimulation.

To demonstrate the effect of PSEs on LPS-induced barrier disruption in a time-dependent manner, the TEER was measured in Caco-2 cells. LPS-stimulated Caco-2 cells showed a considerable reduction in electrical resistance (Ω/cm^2) compared with the control group (Fig. 3B). However, pretreatment with PSEs for 2 h markedly increased the electrical resistance compared with LPS treatment. Notably, PSEs from day 4 to day 7 of germination showed the most significant protective effect against LPS-induced TEER reduction, whereas those from day 2 and day 3 of germination did not significantly influence the barrier function (Fig. 3B). These results suggest that

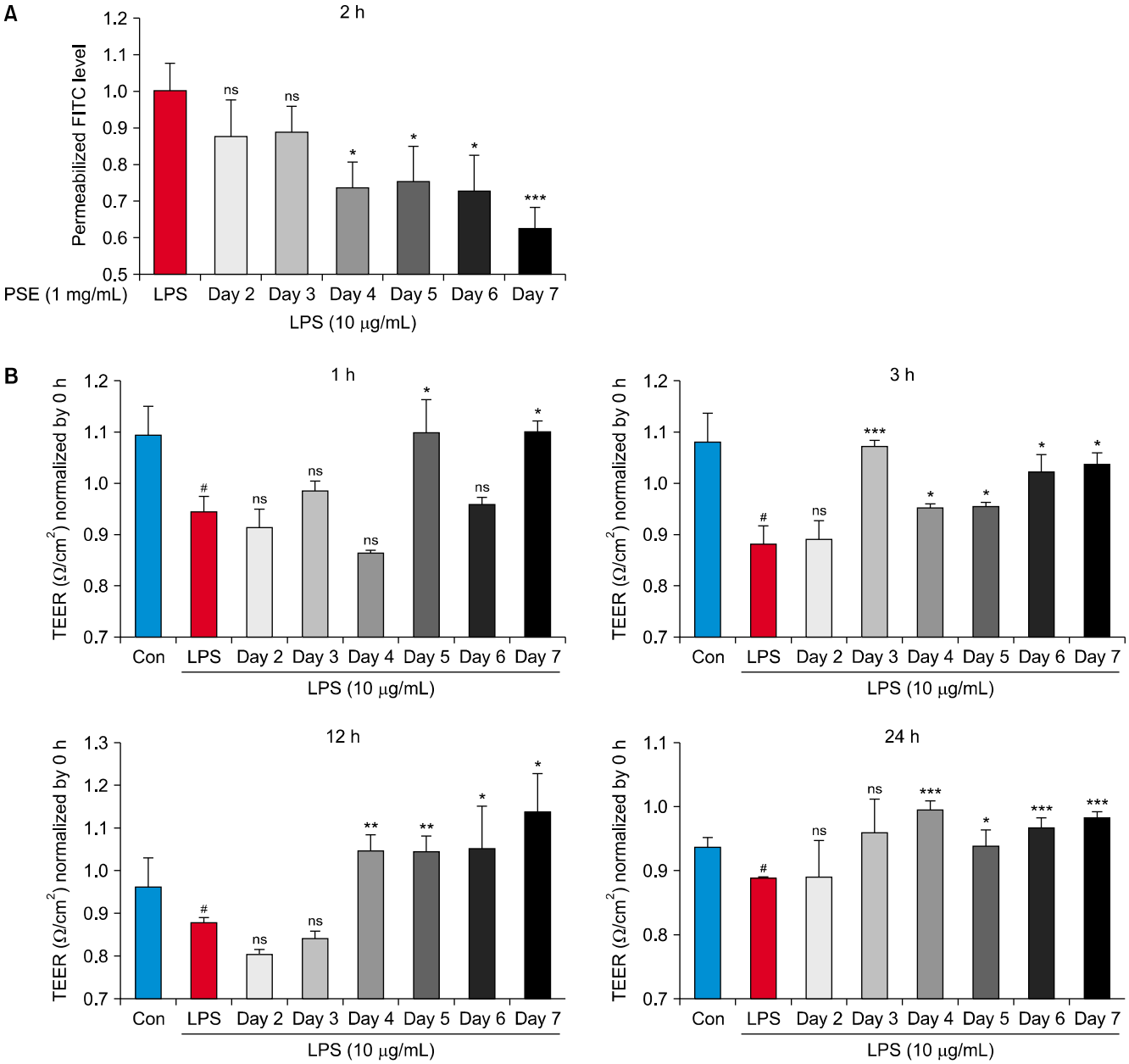

Fig. 3. Effect of peanut sprout extracts (PSEs) on lipopolysaccharide (LPS)-induced intestinal barrier disruption. (A) Permeabilized FITC-dextran levels in the Caco-2 cell monolayer. The monolayer permeability was identified by the leakage of the FITC-dextran level through the Caco-2 monolayer on the transwell. The relative FITC-dextran intensity was normalized by the LPS group. (B) Transepithelial electrical resistance levels in the Caco-2 cell monolayer. Monolayer permeability was identified by a reduction in the TEER level (Ω/cm^2). Statistical significance was compared with the control group at $^{\#}P<0.05$ and with the LPS group at $^{***}P<0.001$, $^{**}P<0.005$, and $^{*}P<0.05$ using Student's *t*-test (n=3). ns, not significant.

treatments with PSEs obtained from day 4 to day 7 of germination effectively mitigated LPS-induced epithelial barrier disruption.

Effect of PSEs on the levels of proteins involved in tight and adherens junctions

Maintaining a stable epithelium in the intestine relies on tight and adherens junctions, which are supported by ZO-1 and E-cadherin proteins at cell-cell junctions. To identify the localization of ZO-1 and E-cadherin, we stained confluent cells with antibodies against ZO-1 (red) and E-cadherin (green) (Fig. 4A). The control cells displayed an intact and linear arrangement of ZO-1 at cell-cell junctions. However, LPS treatment reduced the total ZO-1 level and its distribution at the plasma membrane, even under fully confluent conditions (Fig. 4A and 5A). The LPS-induced breakdown of the ZO-1 protein was significantly suppressed by pretreatment with PSEs from day 4 to day 7 of germination (Fig. 4B). Moreover, the total ZO-1 levels were significantly preserved in groups treated with PSE from day 6 to day 7 of germination compared with LPS-treated cells (Fig. 5B). Although LPS treatment notably decreased E-cadherin fluorescence at the cell junctions, only PSEs from day 6 of germination

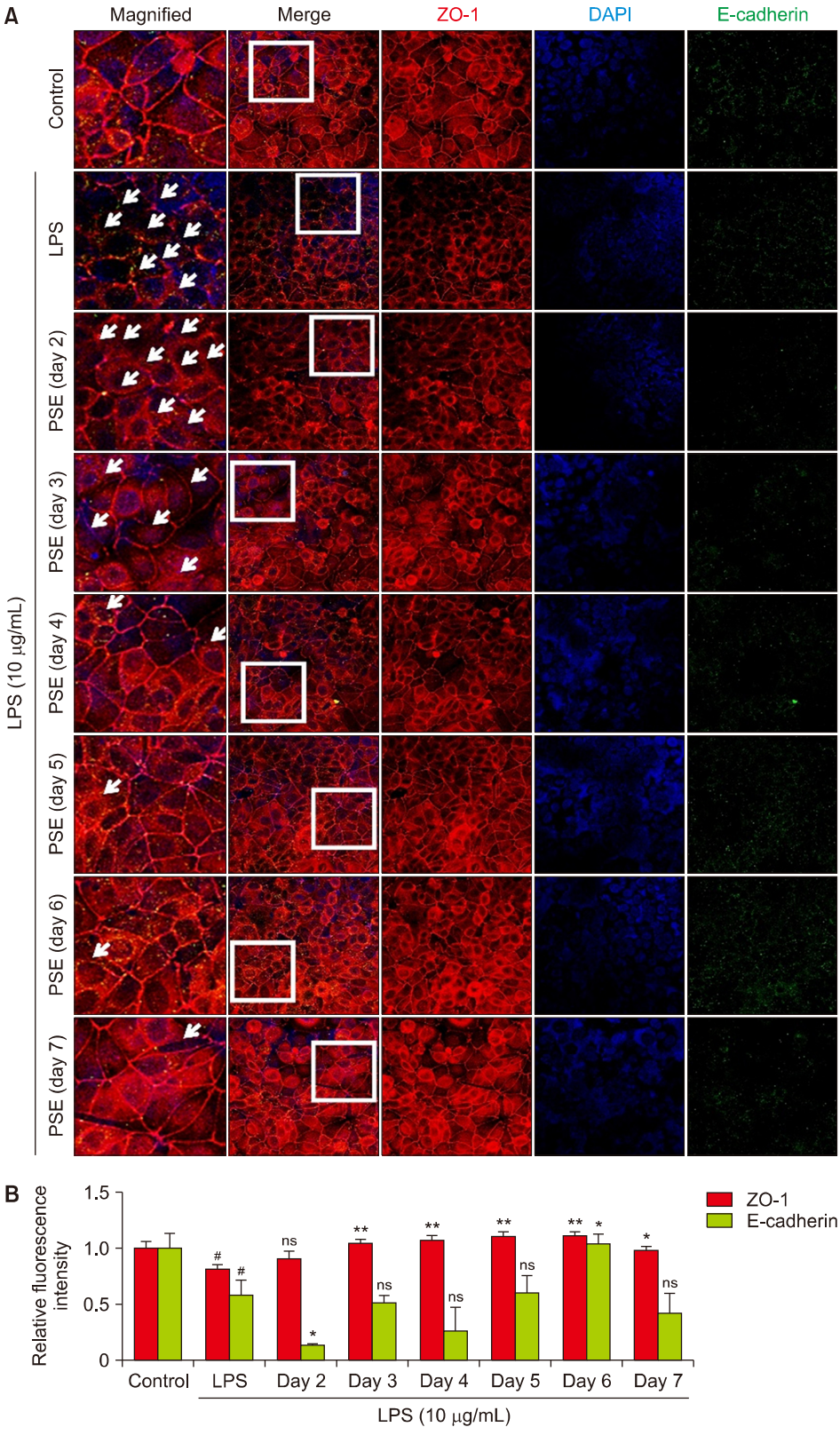

Fig. 4. Effect of peanut sprout extracts (PSEs) on lipopolysaccharide (LPS)-induced junctional damage. (A) Immunostaining for ZO-1 (red) and E-cadherin (green). White arrows indicate the abnormal morphological shapes of ZO-1 at cell-cell junctions. (B) Fluorescence intensity in ZO-1 and E-cadherin. Caco-2 cells were pretreated with PSEs and stimulated with LPS for 24 h. Statistical significance was compared with the control group at $^{\#}P<0.05$ and with the LPS group at $^{**}P<0.005$ and $^{*}P<0.05$ using Student's *t*-test (n=3). ns, not significant.

maintained a substantial amount of E-cadherin under LPS-stimulatory conditions (Fig. 4B). Furthermore, the total E-cadherin protein level was significantly affected by LPS for 24 h, whereas PSEs from day 6 to day 7 of germination significantly upregulated E-cadherin levels in LPS-stimulated Caco-2 cells. These results indicate that PSEs from later germination stages (days 6–7) are more effective in mitigating the LPS-induced degradation of ZO-1 and E-cadherin compared with those from earlier stages.

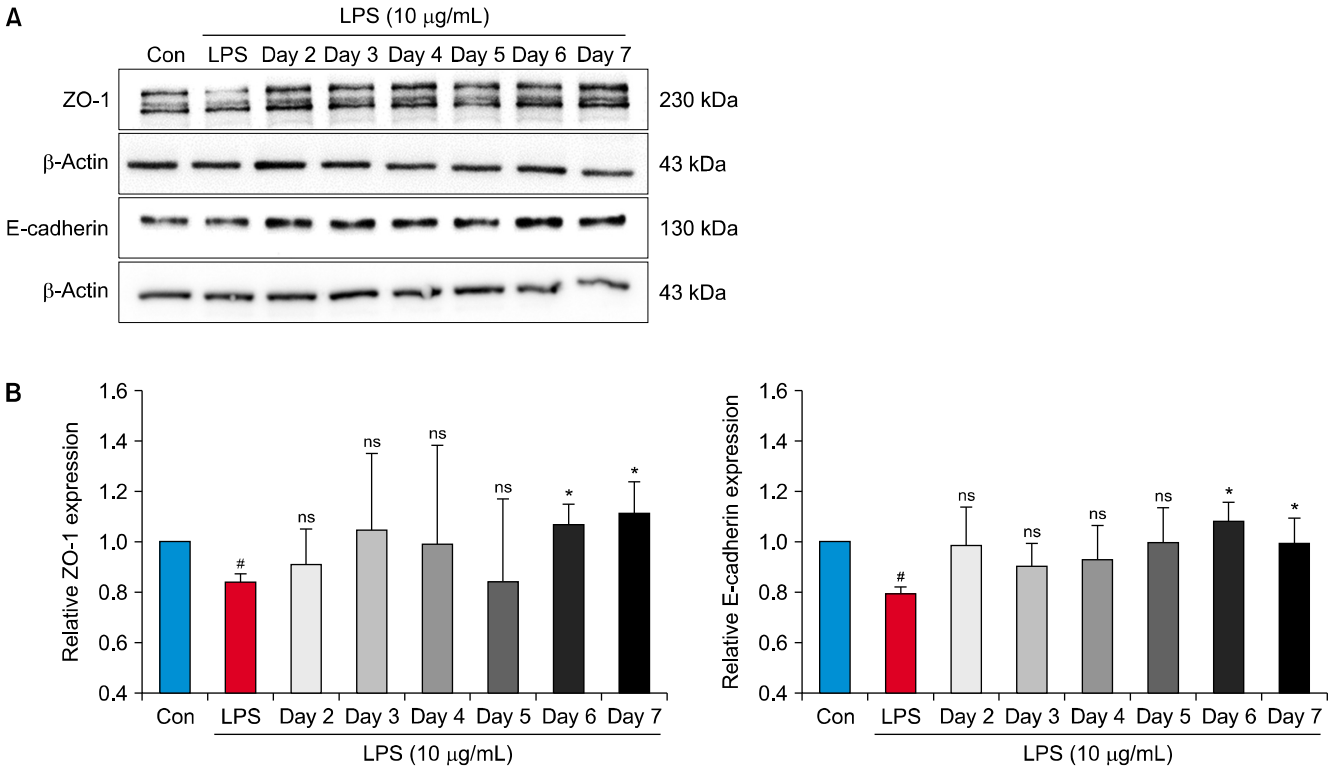

Fig. 5. Effect of peanut sprout extracts (PSEs) on ZO-1 and E-cadherin protein expression. (A) Protein band against ZO-1 and E-cadherin. (B) Relative protein levels normalized by the control group. Caco-2 cells were pretreated with PSEs and stimulated with lipopolysaccharide (LPS) for 24 h. The protein expression was determined using Western blot analysis and quantified by β-actin. Statistical significance was compared with the control group at $^{\#}P<0.05$ and with the LPS group at $^{*}P<0.05$ using Student's *t*-test (n=3). ns, not significant.

Correlation between the TPC, DPPH, ABTS, TEER, FITC-dextran permeability, and ZO-1 and E-cadherin levels in PSEs

The Pearson correlation coefficient measures the strength and direction of the linear relationship between two datasets, with values ranging from −1 to 1. A positive value indicates that as one variable increases, the other tends to increase as well, whereas a negative value suggests that an increase in one variable is associated with a decrease in the other (Sedgwick, 2012). To explore the relationship between the TPC and antioxidant activities of PSE, as well as its protective effects on the epithelial barrier, we performed Pearson's correlation analysis and visualized the results in a heatmap (Fig. 6). The TPC showed positive correlations with DPPH, TEER (12 and 24 h), ZO-1 (fluorescence, protein expression), and E-cadherin (fluorescence, protein expression) while exhibiting negative correlations with ABTS, TEER (1 and 3 h), and FITC-dextran permeability. Moreover, the DPPH radical scavenging activity displayed similar correlations to those of TPC with TEER, FITC-dextran permeability, and ZO-1 and E-cadherin levels. Notably, the correlations between TPC-DPPH, TPC-ZO-1 (fluorescence), DPPH-E-cadherin (fluorescence), and DPPH-ZO-1 (protein) were statistically significant ($P<0.05$). These results suggest that the TPC of PSE influences its antioxidant activity and protective effects against LPS-induced epithelial barrier disruption.

DISCUSSION

In the present study, we found that PSEs derived from different germination time points can suppress oxidative stress and LPS-induced epithelial barrier dysfunction. Notably, the higher antioxidant effect and TPC of PSEs was correlated with a protective effect against LPS-induced monolayer disruption in Caco-2 cells.

Peanut sprout germination was precisely controlled through ICT and monitoring within a low-carbon smart farming system utilizing PVT technology. The analysis results showed that the peanut sprout lengths increased over time under controlled conditions. Notably, the peanut sprout length observed on day 5 of germination increased by 181.7% compared with that at day 4 of germination, suggesting that the optimal harvest time might be after 5 days of germination. These data were partly consistent with the gradual increase in the TPC and antioxidant activities of PSEs during germination (Fig. 1B). Interestingly, they peaked on day 6 of germination, and their values started to decline on day 7 (Table 1). According to a previous study, 7 days of peanut sprout germination using the manual system resulted in an average sprout length of 19.7±3 cm (Pae et al., 2011), and 9 days

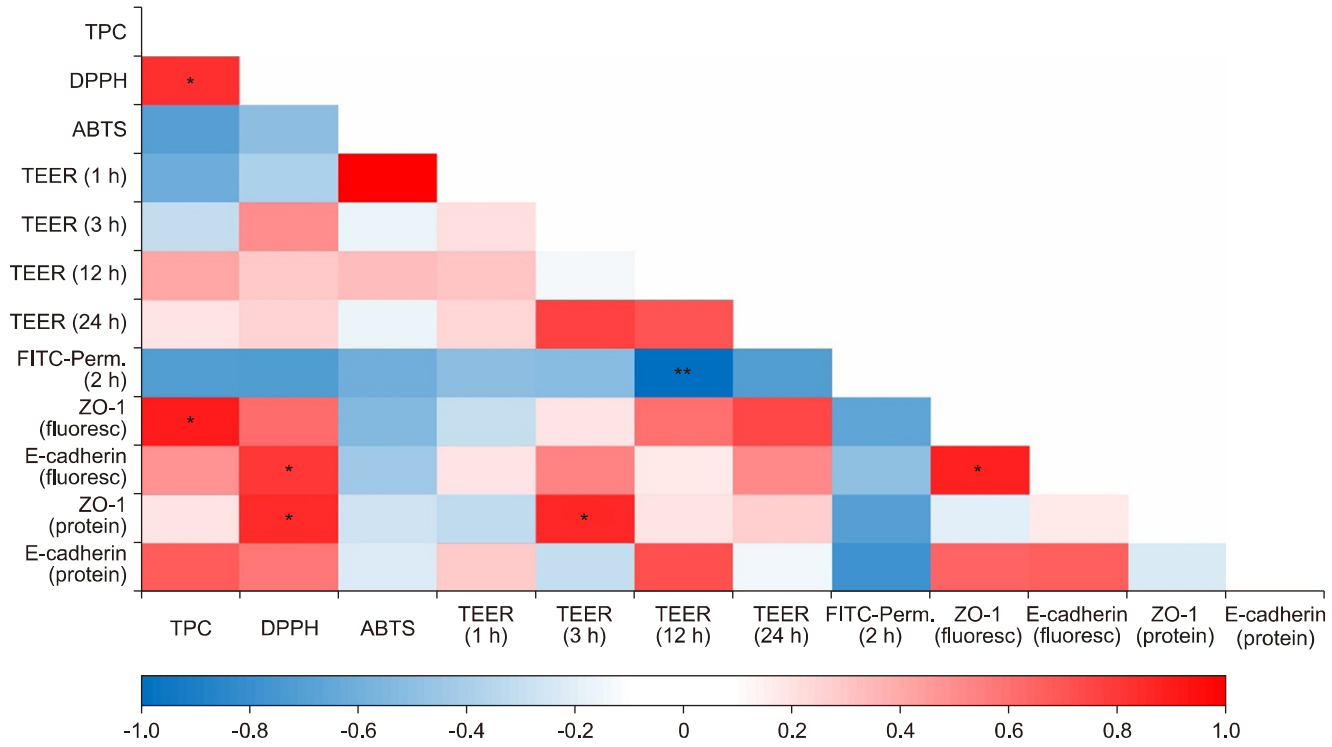

Fig. 6. Correlation heatmap of TPC, DPPH, ABTS, TEER, FITC-permeability, and ZO-1 and E-cadherin levels in peanut sprout extracts. Pearson's correlation coefficients were used for the analysis. Red indicates a positive correlation, whereas blue indicates a negative correlation. Statistical significance is denoted by $^{*}P<0.05$ and $^{**}P<0.01$. TPC, total phenolic content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TEER, transepithelial electrical resistance; FITC-Perm, FITC-dextran-permeability; fluoresc, fluorescence.

of germination yielded a TPC ranging from 9.4 mg GAE/g to 22.4 mg GAE/g (Kang et al., 2010). In the present study, we found that the peanut sprout length on day 6 of germination was 10.46 cm after drying, and the TPC was 75.65 mg GAE/g. Although further systematic research is needed to fully understand the benefits of peanut sprout germination under smart farming systems, our findings suggest that smart farming systems can enhance the phenolic contents of peanut sprouts compared with traditional methods. Furthermore, Limmongkon et al. (2017) reported that the antioxidant activity, TPC, and resveratrol content in peanut sprouts peaked at 3 days of germination and began to decrease after 4 days (Limmongkon et al., 2017). Similarly, peak levels of phenolics, flavonoids, vitamin C, and anthocyanins were observed at 6 days of germination and then started to decrease in some cultivars of peanut sprouts harvested in China (Zhou et al., 2021). These results imply that different cultivars, incubation conditions, and pretreatments in peanut sprouts affect the phenolic content and antioxidant potential. Considering the antioxidant and phenolic compounds of peanut sprouts produced using smart farm technology, 6 days of germination is an appropriate and suitable time point for harvest.

In inflammatory disorders such as IBD, chronic inflammation is a key contributor to disease exacerbation, resulting in increased intestinal permeability and carcinogenesis. Elevated gut permeability and epithelial barrier impairment are usually associated with upregulated levels of pro-inflammatory cytokines, including tumor necrosis factor, interleukin-6, and interferon-γ, which are produced by immune cells near the intestine (Semin et al., 2021). In the present study, we evaluated the protective effect of PSEs on IBD using an LPS-stimulatory inflammation model in intestinal epithelial cells. Treatment with 1 mg/mL of PSEs did not induce significant cytotoxicity in intestinal epithelial cells for 24 h. However, pretreatment with PSEs markedly suppressed the FITC-dextran leakage and maintained the TEER in an epithelial cell monolayer model for 1 to 24 h. Notably, PSEs obtained from day 4 to day 7 of germination showed a significant barrier protective effect compared with LPS-treated cells for 2 to 24 h (Fig. 3). Interestingly, PSEs obtained from day 3 of germination considerably suppressed the LPS-induced reduction of TEER at 3 h (Fig. 3B). These findings partly support the hypothesis that the specific phenolic compositions in PSEs are more important than the TPC in mitigating LPS-induced epithelial permeability (Table 1). Although further investigations are required to identify the predominant phenolic compound in PSEs that is related to the anti-permeability activities, these results suggest that the PSEs harvested from day 4 to day 7 of germination show strong anti-permeability activities in LPS-stimulated epithelial cells.

Toll-like receptor 4 (TLR4) is a plasma membrane receptor that recognizes bacterial and viral stimuli, activating the innate immune system (Hayashi and Nakase, 2022). LPS is known as a major ligand that stimulates TLR4 in intestinal epithelial cells (Caco-2) (Latorre et al., 2014). The activation of TLR4 with LPS immediately initiates downstream signaling, including the recruitment of Myd88, phosphorylation of IKK-β, and nuclear translocation of NF-κB protein, followed by the production of pro-inflammatory cytokines (Latorre et al., 2014). In the intestinal epithelium, ZO-1 and E-cadherin proteins directly form tight and adherens junctions between epithelial cells, which primarily suppress the leakage of hazardous chemicals, macronutrients, and pathogens into the extracellular area and blood vessels (Semin et al., 2021). Thus, the expression and localization of ZO-1 and E-cadherins are essential regulators in IBD. In the present study, treatment with PSEs significantly improved their localization at cell-cell junctions and recovered their expressions compared with LPS-treated cells. In particular, PSE obtained from day 6 of germination showed the highest levels of ZO-1 and E-cadherin at the plasma membrane of Caco-2 cells (Fig. 4). Similarly, significant differences in ZO-1 protein expression were observed in PSEs from day 6 and day 7 of germination compared with the LPS group (Fig. 5). Several studies have reported that various phenolic compounds with antioxidant potential are promising therapeutic options for IBD, both in combination with classic medication or as independent treatments (Dziąbowska-Grabias et al., 2021). He et al. (2020) revealed that ferulic acid promotes AKT pathway activation, maintaining ZO-1 proteins and tight junctions in LPS-treated Caco-2 cells. Similarly, Suzuki and Hara (2009) showed that quercetin enhances barrier function by maintaining ZO-1 in Caco-2 cells. Interestingly, treatment with caffeic acid and *p*-coumaric acid markedly suppressed LPS-induced barrier dysfunction, resulting in ROS inhibition in Caco-2 cells (Song et al., 2022). The protection of the ZO-1 protein at cell-cell junctions was primarily attributed to caffeic acid in LPS-treated Caco-2 cells (Song et al., 2022). These findings are consistent with those observed in the present study, which showed improved TEER in PSEs obtained from day 4 to day 7 of germination. In particular, PSE obtained from day 6 of germination showed an anti-permeability effect in LPS-stimulated Caco-2 cells with notably preserved ZO-1 and E-cadherin levels and the highest TPC and antioxidant activity compared with other PSEs. These results indicate that the protective effect of PSEs on LPS-induced barrier disruption is dependent on their phenolic contents and specific composition.

The present study demonstrated that PSEs produced within smart farming systems contributed to IBD prevention by maintaining and localizing ZO-1 and E-cadherin at cell-cell junctions. Notably, PSEs obtained from day 6 of germination exhibited the highest antioxidant activity and TPC and preserved protein levels, as well as barrier function in LPS-stimulated Caco-2 cells. Although further research is needed to clarify the role of the major bioactive compounds in the anti-permeability effect of PSEs, these findings suggest that PSEs could be used as a functional food ingredient to alleviate IBD symptoms.

ACKNOWLEDGEMENTS

We appreciate NANUMBIO Co., Ltd. for providing dried peanut sprouts at different stages of germination.

FUNDING

This paper was supported by Sunchon National University Research Fund in 2023 (Grant number: 2023-0313).

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: HDC, DHK, HK. Analysis and interpretation: DYK, SSK, EJC. Data collection: DYK, SSK, EJC, DHK, HK. Writing the article: HDC, SMH, SBL. Critical revision of the article: SBL. Final approval of the article: all authors. Statistical analysis: HDC. Obtained funding: HDC. Overall responsibility: HDC.

REFERENCES

Adhikari B, Dhungana SK, Ali MW, Adhikari A, Kim ID, Shin DH. Resveratrol, total phenolic and flavonoid contents, and antioxidant potential of seeds and sprouts of Korean peanuts. Food Sci Biotechnol. 2018. 27:1275-1284. https://doi.org/10.1007/s10068-018-0364-7

Bacco M, Barsocchi P, Ferro E, Gotta A, Ruggeri M. The digitisation of agriculture: a survey of research activities on smart farming. Array. 2019. 3-4:100009. https://doi.org/10.1016/j.array.2019.100009

Biglari F, AlKarkhi AFM, Easa AM. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem. 2008. 107:1636-1641. https://doi.org/10.1016/j.foodchem.2007.10.033

Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958. 181:1199-1200. https://doi.org/10.1038/1811199a0

Chang B, Bae J, Yun S, Kim Y, Park S, Kim S. Wheat sprouts (*Triticum aestivum* Linn.) cultured by a smart farm system amelio-

rate NAFLD through the AMPK-mediated SREBP signaling pathway. Food Sci Biotechnol. 2023. 32:1585-1594. https://doi.org/10.1007/s10068-023-01289-y

Dziąbowska-Grabias K, Sztanke M, Zając P, Celejewski M, Kurek K, Szkutnicki S, et al. Antioxidant Therapy in Inflammatory Bowel Diseases. Antioxidants (Basel). 2021. 10:412. https://doi.org/10.3390/antiox10030412

Gao X, Björk L, Trajkovski V, Uggla M. Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. J Sci Food Agric. 2000. 80:2021-2027. https://doi.org/10.1002/1097-0010(200011)80:14<2021::AID-JSFA745>3.0.CO;2-2

Hayashi Y, Nakase H. The molecular mechanisms of intestinal inflammation and fibrosis in Crohn's disease. Front Physiol. 2022. 13:845078. https://doi.org/10.3389/fphys.2022.845078

He S, Guo Y, Zhao J, Xu X, Wang N, Liu Q. Ferulic acid ameliorates lipopolysaccharide-induced barrier dysfunction via microRNA-200c-3p-mediated activation of PI3K/AKT pathway in Caco-2 Cells. Front Pharmacol. 2020. 11:376. https://doi.org/10.3389/fphar.2020.00376

Hong SJ, Cho JJ, Boo CG, Youn MY, Lee SM, Shin EC. Comparison of physicochemical and sensory properties of bean sprout and peanut sprout extracts, subsequent to roasting. J Korean Soc Food Sci Nutr. 2020. 49:356-369.

Iborra M, Moret I, Rausell F, Bastida G, Aguas M, Cerrillo E, et al. Role of oxidative stress and antioxidant enzymes in Crohn's disease. Biochem Soc Trans. 2011. 39:1102-1106. https://doi.org/10.1042/bst0391102

Jarmakiewicz-Czaja S, Ferenc K, Filip R. Antioxidants as protection against reactive oxidative stress in inflammatory bowel disease. Metabolites. 2023. 13:573. https://doi.org/10.3390/metabo13040573

Jarmakiewicz-Czaja S, Piątek D, Filip R. The influence of nutrients on inflammatory bowel diseases. J Nutr Metab. 2020. 2020:2894169. https://doi.org/10.1155/2020/2894169

Jeong H, Huh CK, Ha HK, Kim J, Oh I. Development of an emulsion gel containing peanut sprout oil as a fat replacer in muffins: Physicochemical, tomographic, and texture properties. Gels. 2023. 9:783. https://doi.org/10.3390/gels9100783

Jo NR, Park CI, Park CW, Shin DH, Hwang YC, Kim YH, et al. Cellular protective effects of peanut sprout root extracts. Appl Chem Eng. 2012. 23:183-189.

Kang HI, Kim JY, Park KW, Kang JS, Choi MR, Moon KD, et al. Resveratrol content and nutritional components in peanut sprouts. Korean J Food Preserv. 2010. 17:384-390.

Kim S, Noh S, Kim D, Park J. Germination, growth, and secondary metabolite contents of radish sprouts (*Raphanus sativus* L.) as affected by plasma activated water process time. Research Square [Preprint]. [posted 2022 Sep 6; cited 2024 Oct 5]. Available from: https://doi.org/10.21203/rs.3.rs-1993356/v1

Landy J, Ronde E, English N, Clark SK, Hart AL, Knight SC, et al. Tight junctions in inflammatory bowel diseases and inflammatory bowel disease associated colorectal cancer. World J Gastroenterol. 2016. 22:3117-3126. https://doi.org/10.3748/wjg.v22.i11.3117

Latorre E, Mendoza C, Layunta E, Alcalde AI, Mesonero JE. TLR2, TLR3, and TLR4 activation specifically alters the oxidative status of intestinal epithelial cells. Cell Stress Chaperones. 2014. 19:289-293. https://doi.org/10.1007/s12192-013-0461-8

Limmongkon A, Janhom P, Amthong A, Kawpanuk M, Nopprang P, Poohadsuan J, et al. Antioxidant activity, total phenolic, and resveratrol content in five cultivars of peanut sprouts. Asian Pac J Trop Biomed. 2017. 7:332-338. https://doi.org/10.1016/j.apjtb.2017.01.002

Naïk Vietti N, Vietti Violi N, Schoepfer AM, Fournier N, Guiu B, Bize P, et al. Prevalence and clinical importance of mesenteric venous thrombosis in the Swiss Inflammatory Bowel Disease Cohort. AJR Am J Roentgenol. 2014. 203:62-69. https://doi.org/10.2214/ajr.13.12447

Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet. 2017. 390:2769-2778. https://doi.org/10.1016/s0140-6736(17)32448-0

Pae SB, Ha TJ, Lee MH, Hwang CD, Shim KB, Park CH, et al. Evaluation of characteristics of peanut sprout using Korean cultivars. Korean J Crop Sci. 2011. 56:394-399. https://doi.org/10.7740/kjcs.2011.56.4.394

Park H, Song JH, Hwang B, Moon B, Yun SJ, Kim WJ, et al. Evaluation of the in vitro and in vivo antitumor efficacy of peanut sprout extracts cultivated with fermented sawdust medium against bladder cancer. Appl Sci. 2020. 10:8758. https://doi.org/10.3390/app10238758

Peladarinos N, Piromalis D, Cheimaras V, Tserepas E, Munteanu RA, Papageorgas P. Enhancing smart agriculture by implementing digital twins: A comprehensive review. Sensors. 2023. 23:7128. https://doi.org/10.3390/s23167128

Sambo P, Nicoletto C, Giro A, Pii Y, Valentinuzzi F, Mimmo T, et al. Hydroponic solutions for soilless production systems: Issues and opportunities in a smart agriculture perspective. Front Plant Sci. 2019. 10:923. https://doi.org/10.3389/fpls.2019.00923

Sedgwick P. Pearson's correlation coefficient. BMJ. 2012. 345:e4483. https://doi.org/10.1136/bmj.e4483

Semin I, Ninnemann J, Bondareva M, Gimaev I, Kruglov AA. Interplay between microbiota, toll-like receptors and cytokines for the maintenance of epithelial barrier integrity. Front Med. 2021. 8:644333. https://doi.org/10.3389/fmed.2021.644333

Song L, Wu T, Zhang L, Wan J, Ruan Z. Chlorogenic acid improves the intestinal barrier by relieving endoplasmic reticulum stress and inhibiting ROCK/MLCK signaling pathways. Food Funct. 2022. 13:4562-4575. https://doi.org/10.1039/d1fo02662c

Suzuki T, Hara H. Quercetin enhances intestinal barrier function through the assembly of zonula occludens-2, occludin, and claudin-1 and the expression of claudin-4 in Caco-2 cells. J Nutr. 2009. 139:965-974. https://doi.org/10.3945/jn.108.100867

Verstockt B, Bressler B, Martinez-Lozano H, McGovern D, Silverberg MS. Time to revisit disease classification in inflammatory bowel disease: Is the current classification of inflammatory bowel disease good enough for optimal clinical management?. Gastroenterology. 2022. 162:1370-1382. https://doi.org/10.1053/j.gastro.2021.12.246

Wang R, Li Z, Liu S, Zhang D. Global, regional and national burden of inflammatory bowel disease in 204 countries and territories from 1990 to 2019: a systematic analysis based on the Global Burden of Disease Study 2019. BMJ Open. 2023. 13:e065186. https://doi.org/10.1136/bmjopen-2022-065186

Wang Z, Chang Y, Sun H, Li Y, Tang T. Advances in molecular mechanisms of inflammatory bowel disease–associated colorectal cancer (Review). Oncol Lett. 2024. 27:257. https://doi.org/10.3892/ol.2024.14390

Youn CK, Jo ER, Sim JH, Cho SI. Peanut sprout extract attenuates cisplatin-induced ototoxicity by induction of the Akt/Nrf2-mediated redox pathway. Int J Pediatr Otorhinolaryngol. 2017. 92:61-66. https://doi.org/10.1016/j.ijporl.2016.11.004

Zhou Z, Fan Z, Meenu M, Xu B. Impact of germination time on resveratrol, phenolic acids, and antioxidant capacities of different varieties of peanut (*Arachis hypogaea* Linn.) from China. Antioxidants. 2021. 10:1714. https://doi.org/10.3390/antiox10111714