Hindawi
Journal of Food Biochemistry
Volume 2024, Article ID 8847548, 12 pages
https://doi.org/10.1155/2024/8847548

Research Article

Small-Molecule Peptides from *Lactiplantibacillus plantarum* SCS2 Attenuate H_2O_2-Induced Oxidative Damage in INS-1 Cells via Regulating the Keap1-Nrf2 Signaling Pathway

Xinyi Huang, Lishi Jiang, Ying Zhang, Lin Jing, Xiao Meng, and Shukun Liu

Institute of Public Health, Chengdu University of Traditional Chinese Medicine, Chengdu, China

Correspondence should be addressed to Xiao Meng; mengxiao@cdutcm.edu.cn and Shukun Liu; lsklsk_888@163.com

Received 8 June 2023; Revised 3 October 2023; Accepted 23 January 2024; Published 2 February 2024

Academic Editor: Minaxi Sharma

Copyright © 2024 Xinyi Huang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Objective. This study aimed to delve into the antioxidant potential of small-molecule peptides derived from *Lactiplantibacillus plantarum* SCS2 (*L. plantarum* SCS2), targeting hydrogen peroxide- (H_2O_2-) induced rat insulinoma (INS-1) cells. *Methods*. INS-1 cells were pretreated with small-molecule peptides of distinct molecular weights, specifically P1 (≤1 kDa), P2 (1–5 kDa), and P3 (5–10 kDa), which were isolated from *L. plantarum* SCS2, followed by H_2O_2 to induce oxidative damage in INS-1 cells. The oxidative status of the cells was assessed by measuring reactive oxygen species (ROS), malondialdehyde (MDA) concentrations, and antioxidant enzyme activities. In addition, the expression levels of proteins and genes associated with the Kelch-like ECH-associated protein 1 (Keap1)-Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway were examined to elucidate the underlying antioxidative mechanisms of the small-molecule peptides from *L. plantarum* SCS2. *Results*. The cellular activity and the activities of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, were higher after pretreatment with P1 than those after pretreatment with P2 and P3, which led to the suppression of ROS and MDA production. In addition, P1 upregulated mRNA and protein expression of Nrf2 and heme oxygenase-1, significantly decreased mRNA and protein expression of Keap1, and promoted the entry of Nrf2 into the nucleus compared with that in the model group. *Conclusion*. P1 from *L. plantarum* SCS2 could activate the Keap1-Nrf2 signaling pathway, thereby ameliorating oxidative damage in INS-1 cells.

1. Introduction

Oxidative stress, characterized by an imbalance in the pro-/antioxidant systems, is often associated with various health conditions, including cancer, cardiovascular disease, hypertension, and diabetes [1]. It is typically linked to increased intracellular levels of reactive oxygen species (ROS), causing potential harm to lipids, DNA, and proteins [2, 3]. Consequently, avenues to reduce oxidative stress may provide novel insights for disease treatment and prevention. Studies have shown that the resultant ROS activate various signaling pathways upon cellular stimulation via exogenous or endogenous oxidation [4]. In normal physiological states, a dimer is formed by Nuclear factor erythroid 2-related factor 2 (Nrf2) and Kelch-like ECH-associated protein 1 (Keap1) within the cytoplasm. A dissociation event occurs upon the onset of oxidative stress within the cell whereby Nrf2 translocates to the nucleus, thereby binding to the antioxidant response element (ARE) and subsequently facilitating the transcription of certain cytoprotective proteins such as phase II detoxification metabolism and detoxification genes [5–7]. Notably, the Keap1-Nrf2/ARE signaling pathway is considered a paramount endogenous antioxidant pathway, serving as a crucial regulator of antioxidant defense mechanisms [8]. Given this significance, the effective regulation of the Keap1-Nrf2 signaling pathway, reduction of ROS, and enhancement of antioxidant activity are essential in improving oxidative damage in cells [9, 10].

Lactic acid bacteria (LAB), frequently used in probiotic formulations, have a long history of their remarkable roles in food safety [11]. LAB exhibit advantageous host interactions, which include modulating intestinal microflora balance, reducing cholesterol level, and demonstrating antioxidant

properties [12]. Hence, LAB are acknowledged as safe and nontoxic potential antioxidants and have emerged as a prominent research hotspot. Currently, research efforts dedicated to understanding the antioxidant impacts of LAB mainly focus on live LAB, heat-inactivated LAB, LAB-produced peptides from fermentation processes, and LAB extracellular polysaccharides. It has been reported that *Lactobacillus strains* TSP05, TSF331, and TSR332 have been shown to alleviate alcohol-triggered oxidative stress and inflammatory responses in mouse liver, primarily by amplifying the activities of antioxidant enzymes and thereby forestalling liver damage [13]. Heat-killed *Lactobacillus rhamnosus* ATCC 7469 has demonstrated the capacity to increase the expression of Nrf2 in the nucleus and superoxide dismutase (SOD) activity, decrease ROS concentrations, and confer protection against oxidative damage induced by ultraviolet radiation in cells [14]. Peptides derived from rice fermentation by *Lactiplantibacillus plantarum* have been observed to modulate oxidative cell damage via modulating the Keap1-Nrf2 pathway [15]. In addition, the exopolysaccharides of *Lactobacillus reuteri* SJ-47 further contribute to the antioxidant defense system by reducing ROS and malondialdehyde (MDA) levels and upregulating antioxidant enzyme expression, thereby exerting a protective effect on human skin fibroblasts under oxidative stress [16]. However, Luo et al. [17] identified intracellular peptides isolated from *Lactobacillus rhamnosus* that also demonstrated antioxidant capacity.

Meanwhile, natural peptides are recognized for their multifaceted beneficial effects, including antioxidative, antihypertensive, antiobesity, and anticancer properties [18–20]. Recently, the peptides extracted from food and biological waste have emerged as a prominent focus in antioxidant research. For instance, researchers have successfully extracted peptides from the by-products of skipjack tuna, which have exhibited outstanding antioxidative activity, thereby helping alleviate the cell damage caused by oxidative stress [21–23]. Similarly, the peptides extracted from the swim bladders of monkfish have shown significant antioxidative potential, reducing the risk of potential diseases triggered by oxidative stress [24]. Furthermore, peptides with bioactive properties derived from dandelions and purified from watermelon seed hydrolysates have demonstrated antioxidative capabilities [25, 26].

Initial investigations into *Lactiplantibacillus plantarum* SCS2 (*L. plantarum* SCS2) have unveiled the capacity of its intracellular protein to mitigate oxidative damage in hyperglycemic model mice [27]. However, the precise constituents of *L. plantarum* SCS2 intracellular proteins and whether they exert their antioxidant effects via the Keap1-Nrf2 pathway remain unexplored. Therefore, we hope to delve deeper into the molecular mechanisms by which the intracellular small-molecule peptides of *L. plantarum* SCS2 with different molecular weights alleviate oxidative damage in cells. In conclusion, this study aimed to investigate the potential of small-molecule peptides derived from *L. plantarum* SCS2 to mitigate cellular oxidative damage and provide a scientific basis for the subsequent development of foods with antioxidant functions.

2. Materials and Methods

2.1. Isolation of Peptides from Lactiplantibacillus plantarum SCS2 by Ultrafiltration. L. plantarum SCS2, derived from Sichuan fermented sausage, was stored at the Laboratory Center of Public Health Institute of Chengdu University of Traditional Chinese Medicine. Strains were revived in De Man, Rogosa, and Sharpe broth medium (Biosharp, Beijing, China) at a 0.5% volume ratio, followed by a 24 h incubation at 37°C. After centrifugation at 10,000 rpm and 4°C for 10 min, the supernatant was decanted, and the bacteria were washed three times with 0.1 mol/L sterile phosphate-buffered saline (PBS) (Solarbio, Beijing, China). The bacterial content was collected and resuspended in an adequate volume of PBS. This solution was subjected to ultrasonic disruption for 20 min, followed by additional centrifugation at 10,000 rpm and 4°C for 10 min to obtain the supernatant. The supernatant was subsequently processed using ultrafiltration centrifuge tubes (Pall, USA; Sartorius, Germany; Millipore, USA) equipped with membranes of molecular weight cutoff values of 1, 5, and 10 kDa. The collected permeate fractions, such as ≤1 kDa (referred to as P1), 1–5 kDa (referred to as P2), and 5–10 kDa (referred to as P3), were freeze-dried and stored at −20°C for further analysis [28].

2.2. INS-1 Cell Culture. The rat insulinoma (INS-1) cells were kindly provided by Feng Hui Life Science and Technology Co., Ltd. (Hunan, China). The cells were incubated in Roswell Park Memorial Institute 1640 (RPMI 1640) medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 100 μg/mL streptomycin (HyClone, UT, USA), 100 U/mL penicillin (HyClone, UT, USA), 1 mmol/L sodium pyruvate, and 50 μmol/L β-mercaptoethanol (Gibco, Grand Island, NY, USA). The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 [29].

2.3. H_2O_2-Induced Oxidative Damage Model. INS-1 cells in their logarithmic growth phase were seeded at a density of 5×10^3 cells per well and cultured in a 96-well plate for a duration of 24 h. INS-1 cells were treated with hydrogen peroxide (H_2O_2) dissolved in RPMI 1640 medium at various concentrations (20–100 μmol/L) in an incubator at 37°C and in the presence of 5% (v/v) CO_2 for 24 h to establish a model of oxidative injury. Simultaneously, the control group cells were incubated with an equivalent volume of the medium. The medium was discarded after treatment, and the cells in all groups were washed twice with PBS. Subsequently, 10 μL of the cell counting kit-8 (CCK-8) (Biosharp, Beijing, China) solution was introduced into each well, followed by a further 2 h incubation in the dark. The absorbance of each well was detected at the wavelength of 450 nm using a microplate reader (BioTek Instruments, Inc., USA) [30].

2.4. Viability of INS-1 Cells. INS-1 cells were cultured in 96-well plates (5×10^3 cells per well) and incubated overnight in a CO_2 incubator. The cells were then exposed to different

concentrations of *L. plantarum* SCS2 small-molecule peptides with a molecular weight of P1, P2, and P3. These were dissolved in RPMI 1640 medium at concentrations ranging from 0.025 to 0.4 mg/mL and administered to the cells for 24 h. The medium was discarded following treatment, and the cells were rinsed twice with PBS. Each well was then treated with 10 μL of the CCK-8 solution and incubated at 37°C for 2 h. Then, the cells were pretreated with different concentrations of *L. plantarum* SCS2 small-molecule peptides, followed by a 24 h exposure to the modeling concentration of H_2O_2 determined in Section 2.3. The oxidative damage model was established as described earlier. The medium was removed after the treatment, the cells were washed twice with PBS, and 10 μL of CCK-8 was added to each well, followed by a 2 h incubation at 37°C. The absorbance was assessed at a 450 nm wavelength using an enzyme marker [31].

2.5. ROS Level Measurement. ROS levels were assessed using the 2′,7′-dichlorofluorescin diacetate (DCFH-DA) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) method. Prior to analysis, INS-1 cells were pretreated with small-molecule peptides for 24 h and rinsed three times with PBS. The cell damage was induced by H_2O_2. The cells were incubated with 10 μmol/L DCFH-DA at 37°C for 30 min in the dark. The medium containing the DCFH-DA probe was then discarded, and the cells were rinsed with PBS. After collecting cells, perform analysis using a flow cytometer (excitation wavelength: 488 nm, emission wavelength: 525 nm) (Beckman Coulter, Inc., Brea, CA) [32].

2.6. Biochemical Analyses. INS-1 cells were inoculated in six-well plates and damaged by oxidation with the modeling concentration of H_2O_2determined in Section 2.3 after pretreatment with small-molecule peptides for 24 h. They were washed with PBS, lysed with cell lysis solution (Solarbio, Beijing, China), and centrifuged at 10,000*g* and 4°C for 10 min. The supernatant was collected. MDA, SOD, catalase (CAT), and glutathione peroxidase (GPx) activities were measured using the corresponding diagnostic kits following the manufacturer's protocols (Shanghai Enzyme-Linked Biotechnology Co., Ltd., Shanghai, China) [31].

2.7. Real-Time Quantitative Polymerase Chain Reaction. The experiments were performed based on the methods used in previous studies [33]. The extraction of total RNA from the cells was accomplished using a Total Cell RNA Isolation Kit (Chengdu Foregene Biological Technology Co., Ltd., Chengdu, China). After confirmation of RNA concentration and purity, the samples were subsequently reverse transcribed into complementary DNA (cDNA) using a Revert Aid First-Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA). The quantitative analysis of gene expression was conducted via real-time quantitative polymerase chain reaction (RT-qPCR). The primer sequences are presented in Table 1 (Chengdu Foregene Biological Technology Co., Ltd., Chengdu, China). This procedure was performed using a SYBR Green PCR Master Mix (Chengdu Foregene Biological Technology Co., Ltd., Chengdu, China) and a two-step RT-PCR system (Jena qTOWER 2.0, DE).

2.8. Western Blotting. The experimental procedures were conducted following the methodology outlined in the previous study by Yi et al. [34]. Initially, INS-1 cells were collected, lysed with radio-immunoprecipitation assay buffer (Solarbio, Beijing, China) containing 1% phenylmethanesulfonyl fluoride (Solarbio, Beijing, China) and a phosphatase inhibitor cocktail (Solarbio, Beijing, China), and then incubated on ice for a duration of 30 min. The total cellular protein was extracted after centrifugation at 10,000*g* for 10 min. After collecting INS-1 cells, the nuclear and cytoplasmic proteins were extracted using nuclear and cytoplasmic protein extraction kits (Boster, Wuhan, China). Finally, the concentration of proteins in the supernatant was determined using bicinchoninic acid (BCA) protein assay kit (Solarbio, Beijing, China). The protein samples were subjected to separation via sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride (PVDF) membranes (Merck Millipore, Ireland). The PVDF membranes were blocked with 5% skimmed milk (Solarbio, Beijing, China) for 1 h at room temperature. Then, the membranes were incubated with different primary antibodies overnight at 4°C. The antibodies used in the study were as follows: rabbit (Rb) anti-Nrf2, 1 : 1000; Rb anti-Keap1, 1 : 1000; Rb anti-HO-1, 1 : 1000; Rb anti-GAPDH, 1 : 1000; Rb anti-histone deacetylase 1 (HDAC1), 1 : 1000; and Rb anti-β-actin, 1 : 1000 (all from ABclonal, China). They were then incubated with the secondary antibody (1 : 5000; Wuhan Hundred Biotechnology Co., Ltd., Wuhan, China) for 1 h at room temperature. Immunoreactive bands were detected using a contact nondestructive quantitative imager (e-Blot, Shanghai, China).

2.9. Statistical Analysis. The data were expressed as mean ± standard deviation of three replicate trials. The experimental data were statistically analyzed using IBM Statistical Package for the Social Sciences for Windows, version 26.0 (IBM Corp., NY, USA). The differences between multiple groups were analyzed using analysis of variance, and the Student *t*-test was used to analyze the differences between the two groups. p value < 0.05 indicated a statistically significant difference.

3. Results

3.1. The Effect of H_2O_2 Concentration on Viability of INS-1 Cells. In this study, the cellular model of oxidative damage was mainly used to evaluate the antioxidant capacity of the compounds. Typically, H_2O_2 serves as an oxygen radical generator for cells due to its ability to penetrate the cell membrane and enter the cell interior, subsequently generating free radicals causing cell or tissue damage [35]. Hence, H_2O_2 was selected as the agent to instigate oxidative damage within cells. We observed a decrease in cell viability corresponding to increased H_2O_2 concentrations (Figure 1).

Table 1: Sequence of primers used for real-time quantitative PCR.

Gene	Primer sequences (5′-3′)
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	F: 5′-GAAGGTCGGTGTGAACGGAT-3′ R: 5′-CCCATTTGATGTTAGCGGGAT-3′
Nrf2	F: 5′-CCTCATGTTTGCCTTCTTTGC-3′ R: 5′-GGCGGGTACATTTCTCCATC-3′
Keap1	F: 5′-CACTTCGGGGAGGAGGAGTT-3′ R: 5′-GGCAGTCGTATTTGACCCAGT-3′
Heme oxygenase-1 (HO-1)	F: 5′-TTTGAGCACCAGACCTACTGCTGAGG-3′ R: 5′-TGAGGTCCTGTACTATATATACATC-3′

Furthermore, 60 μmol/L H_2O_2 treatment of cells for 24 h resulted in a significant decrease in cell viability to 0.709 ± 0.0221 ($p < 0.05$). A further increase to 100 μmol/L H_2O_2 resulted in a substantial decline in cell viability to 0.085 ± 0.008, and the excessive reduction in cell viability may compromise the reliability and interpretability of experimental results. To induce oxidative damage while maintaining cellular viability and drawing insights from relevant literature [32], the oxidative damage model in INS-1 cells was established with 60 μmol/L H_2O_2 for 24 h. This model was then employed as the foundation for subsequent experiments.

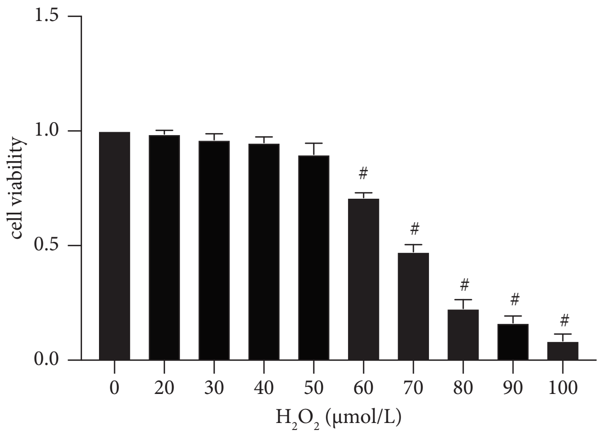

Figure 1: Cell viability of INS-1 cells in response to various concentrations of H_2O_2. Results are expressed as mean ± standard deviation; number of parallel experiments $n = 3$. 0 indicates normal group, and the other numbers in the figure indicate the dose of H_2O_2. # indicates significant difference compared to the normal group (0 μmol/L H_2O_2) ($p < 0.05$).

3.2. Effects of Small-Molecule Peptides from Lactiplantibacillus plantarum SCS2 on the Viability of INS-1 Cells. The results in Figure 2 demonstrated that an increase in the concentrations of small-molecule peptides corresponded to a decrease in cellular activity and an elevation in cytotoxicity. A significant diminution in cellular activity was observed when the concentration of small-molecule peptides with molecular weights of P1, P2, and P3 reached 0.2 mg/mL ($p < 0.05$), registering cellular activities of 0.725 ± 0.026, 0.670 ± 0.052, and 0.573 ± 0.027, respectively. This result indicated that small-molecule peptides had cytotoxic effects at concentrations greater than or equal to 0.2 mg/mL. Hence, to avoid cytotoxic effects and ensure the safe application of small-molecule peptides in future, our subsequent research opted for employing small-molecule peptides at concentrations less than 0.2 mg/mL.

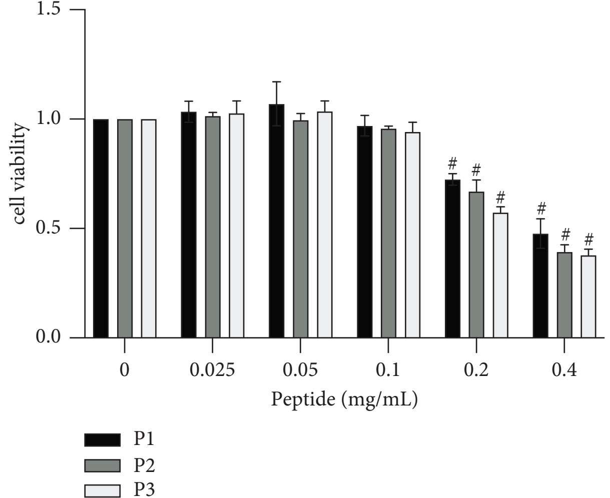

Figure 2: Effects of different concentrations of P1, P2, and P3 (0, 0.025, 0.05, 0.1, 0.2, and 0.4 mg/mL) on the viability of INS-1 cells. Results are expressed as mean ± standard deviation; number of parallel experiments $n = 3$. 0 indicates normal group, and the numbers in the figure indicate small-molecule peptides doses of 0.025 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL, and 0.4 mg/mL, respectively. # indicates significant difference compared with the normal group (0 mg/mL peptide) ($p < 0.05$).

3.3. Effects of Small-Molecule Peptides from Lactiplantibacillus plantarum SCS2 on the Oxidative Damage of INS-1 Cells. As depicted in the results of Figure 3, a marked reduction was observed in cell viability in the model group compared with the control group ($p < 0.05$). Small-molecule peptides with molecular weights of P1, P2, and P3 increased cell activity, and the highest cell viability was achieved when the concentration of small-molecule peptides was 0.05 mg/mL. Furthermore, P1 delivered superior protection against H_2O_2-induced oxidative damage in INS-1 cells at all tested concentrations compared with P2 and P3. We concluded that the small-molecule peptides of *L. plantarum* SCS2 had a protective effect against H_2O_2-induced oxidative damage in INS-1 cells when the small-molecule peptide concentration was 0.05 mg/mL. Therefore, subsequent experimental procedures were planned to use a small-molecule peptide concentration of 0.05 mg/mL.

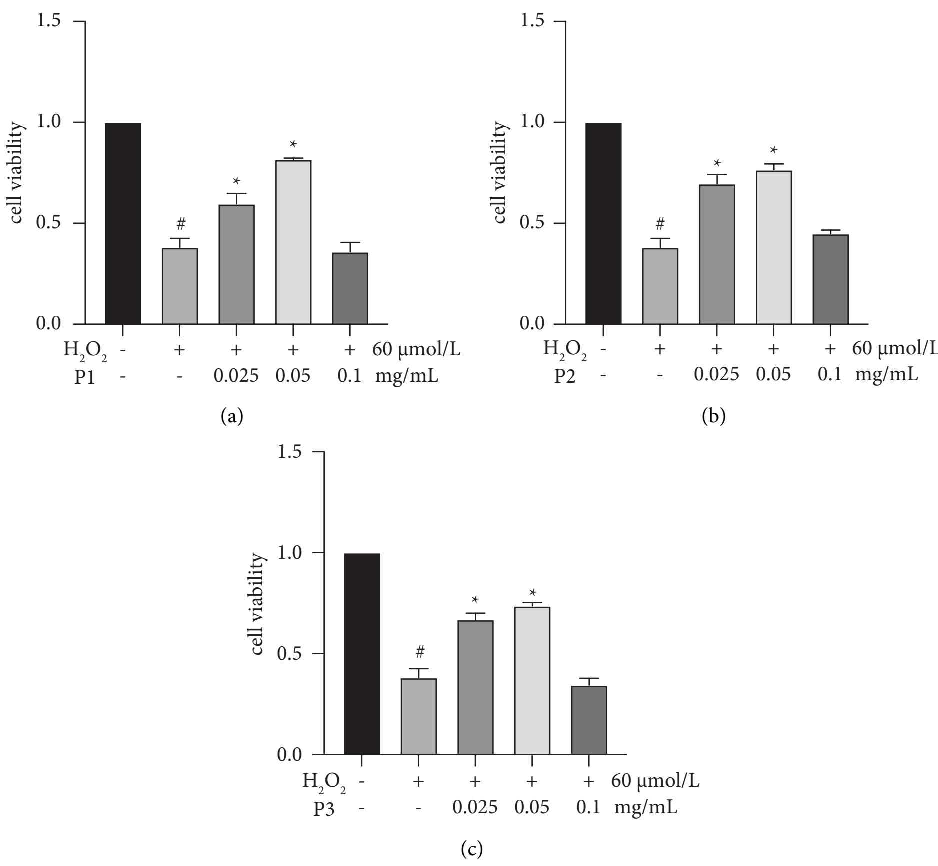

Figure 3: (a–c) Effects of different concentrations of P1, P2, and P3 (0, 0.025, 0.05, and 0.1 mg/mL) on the cell viability of INS-1 with H_2O_2-induced oxidative damage. Results are expressed as mean ± standard deviation; number of parallel experiments $n = 3$. *indicates significant difference compared with the model group ($p < 0.05$). # indicates significant difference compared with the normal group ($p < 0.05$).

3.4. Effects of Small-Molecule Peptides from Lactiplantibacillus plantarum SCS2 on the Levels of ROS and MDA. Based on the results of Figure 4, it could be seen that the model group displayed significantly elevated levels of ROS and MDA compared with the control group ($p < 0.05$), indicating that oxidative damage occurred in the cells after H_2O_2 treatment, alongside a considerable generation of ROS and MDA. However, pretreatment with small-molecule peptides resulted in a reduction of intracellular ROS and MDA concentrations across all three groups compared with the model group, with the lowest ROS and MDA levels observed in the P1 group relative to the P2 and P3 groups. In summary, the evidence suggested that the small-molecule peptides could protect INS-1 cells from H_2O_2-induced damage by inhibiting the cellular production of ROS and MDA, and P1 might have stronger antioxidant effects than the other two small-molecule peptides.

3.5. Effects of Small-Molecule Peptides from Lactiplantibacillus plantarum SCS2 on the Levels of Antioxidant Enzymes. From the results in Figure 5, it could be found that the activities of SOD, CAT, and GPx significantly reduced in the model group compared with the normal group ($p < 0.05$). Relative to the model group, the cells pretreated with small-molecule peptides demonstrated notably enhanced activities of SOD, CAT, and GPx, with the highest levels observed in the P1 group among the three small-molecule peptide categories ($p < 0.05$). When excessive ROS are generated, the cells have antioxidant defense systems to counteract the resulting damage [36]. SOD, CAT, and GPx, as crucial antioxidant enzymes, facilitate the reduction of oxidative damage within cells. P1 could markedly upregulate the activities of important antioxidant enzymes in oxidatively damaged cells, reducing oxidative damage in INS-1 cells ($p < 0.05$). In addition, combined with the preceding results on the effect of three small-molecule peptides of *L. plantarum* SCS2 on cellular oxidative damage, we concluded that P1 exhibited superior antioxidant effects compared with P2 and P3. The rest of this manuscript discusses our exploration of how P1 ameliorates oxidative damage in INS-1 cells by mediating the Keap1-Nrf2 signaling pathway.

3.6. Effects of P1 on the Expression of Keap1-Nrf2 Pathway-Related Genes. Our study explored the change in mRNA expression levels for the key constituents of the Keap1-Nrf2 signaling pathway, such as Nrf2, Keap1, and HO-1. The results in Figures 6(a)–6(c) showed that a marked decrease in the mRNA expression of Nrf2 and HO-1 and an increased expression of Keap1 mRNA were observed in the model group relative to the control group ($p < 0.05$). Strikingly, P1

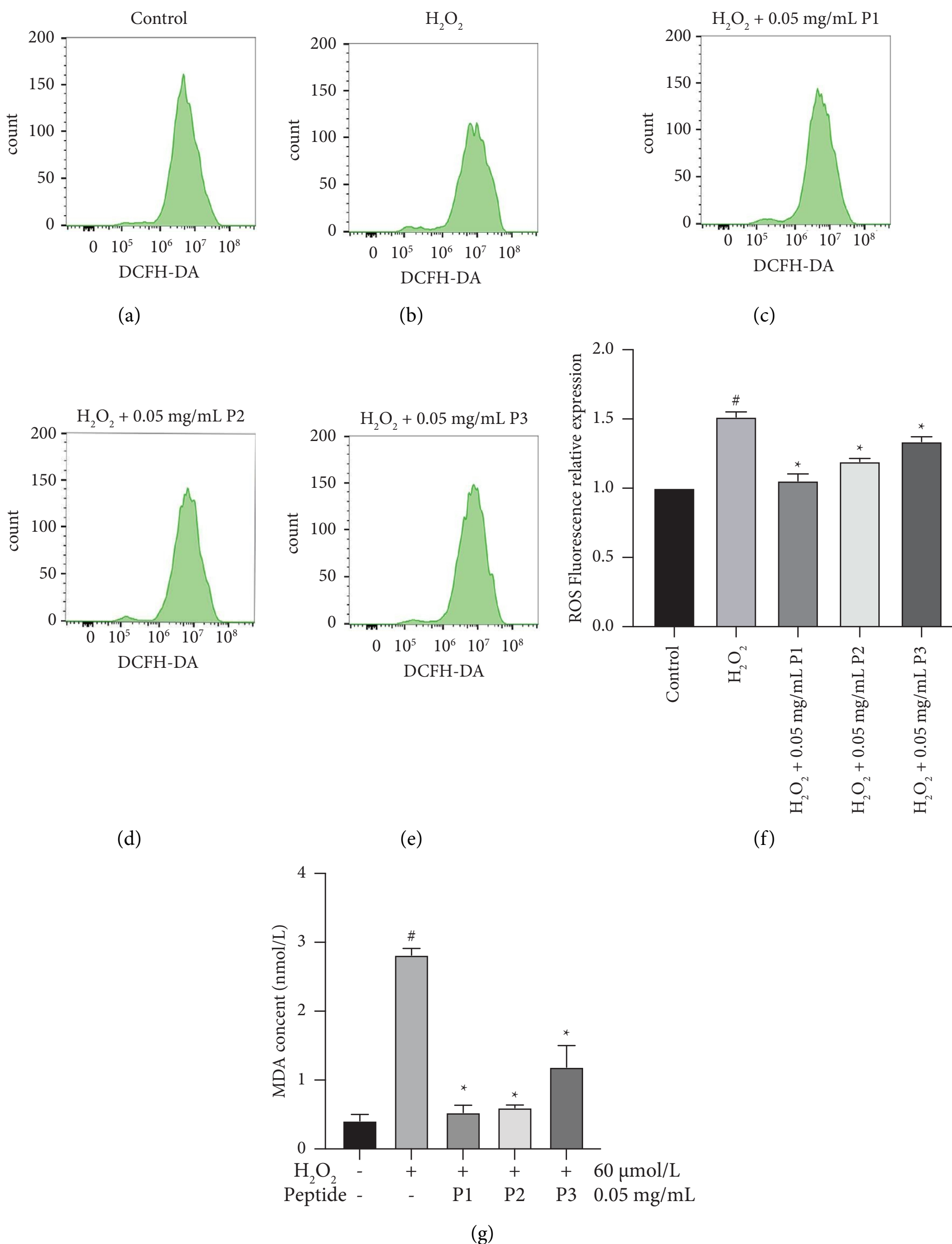

Figure 4: (a–f) Effects of P1, P2, and P3 on ROS levels in H_2O_2-induced oxidative damage in INS-1 cells. (g) Effects of P1, P2, and P3 on MDA levels in H_2O_2-induced oxidative damage in INS-1 cells. Results are expressed as mean ± standard deviation; number of parallel experiments $n = 3$. *indicates significant difference compared with the model group ($p < 0.05$). # indicates significant difference compared with the normal group ($p < 0.05$).

promoted the expression of antioxidant genes Nrf2 and HO-1 and decreased the expression of Keap1 mRNA compared with that in the model group ($p < 0.05$). These findings indicated that the molecular mechanism of P1 alleviating oxidative damage in INS-1 cells might be through the Keap1-Nrf2 signaling pathway.

3.7. Effects of P1 on the Expression of Proteins Related to Keap1-Nrf2 Signaling Pathway. The results in Figures 7(a)–7(d) indicated that compared with the control group, the expression of Nrf2 protein and its downstream antioxidant enzyme HO-1 protein significantly decreased and Keap1 protein expression significantly increased in the model group ($p < 0.05$). Contrastingly, the pretreatment with P1 markedly increased the expression levels of Nrf2 protein and its downstream antioxidant enzyme HO-1 protein while simultaneously curtailing the expression of Keap1 protein in the oxidatively compromised cells relative to the model group ($p < 0.05$). This suggested that P1 could potentially exert antioxidant effects through modulating the Keap1-Nrf2 signaling pathway.

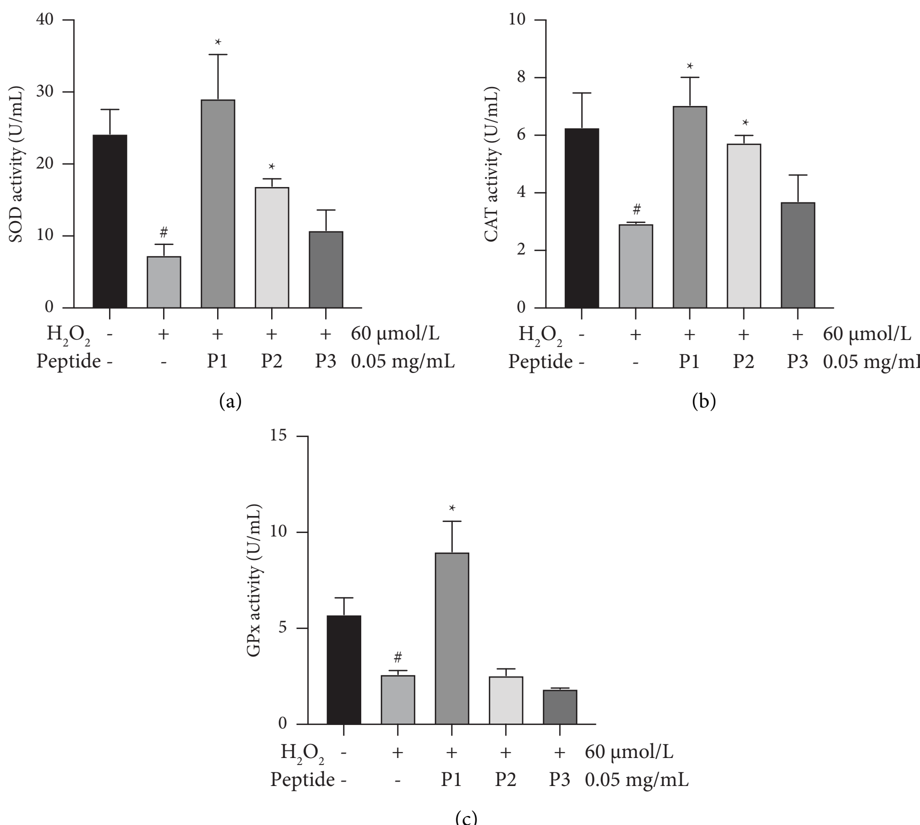

Figure 5: (a) Effects of P1, P2, and P3 on the SOD activity of H_2O_2-induced oxidative damage in INS-1 cells. (b) Effects of P1, P2, and P3 on the CAT activity of H_2O_2-induced oxidative damage in INS-1 cells. (c) Effects of P1, P2, and P3 on the GPx activity of H2O2-induced oxidative damage in INS-1 cells. Results are expressed as mean ± standard deviation; number of parallel experiments $n = 3$. *indicates significant difference compared with the model group ($p < 0.05$). # indicates significant difference compared with the normal group ($p < 0.05$).

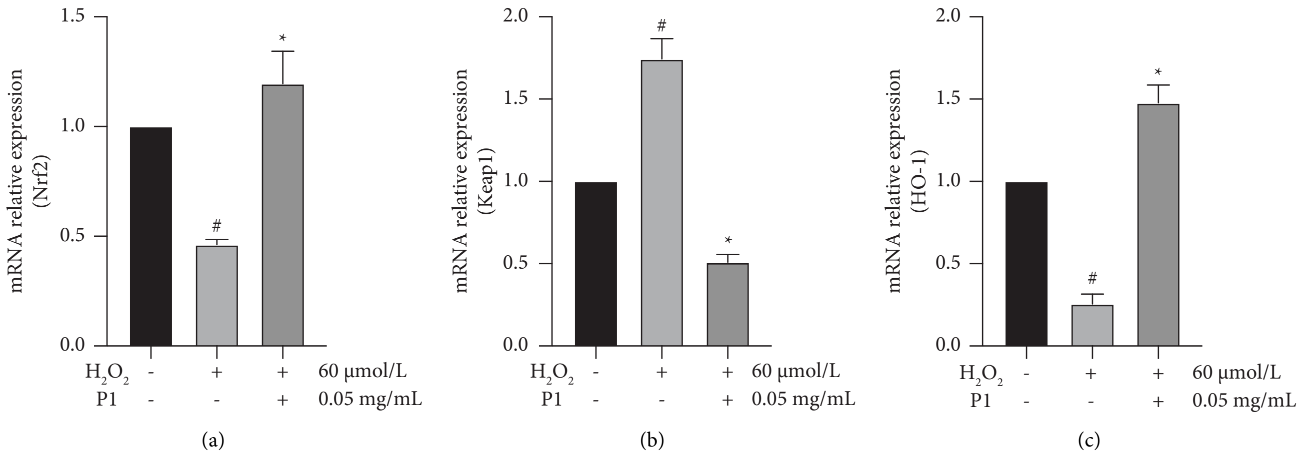

Figure 6: (a–c) Effects of P1 on H_2O_2-induced mRNA expression of Nrf2, Keap1, and HO-1 in INS-1 cells. Results are expressed as mean ± standard deviation; number of parallel experiments $n = 3$. *indicates significant difference compared with the model group ($p < 0.05$). # indicates significant difference compared with the normal group ($p < 0.05$).

3.8. Effects of P1 on the Expression of Nrf2 Proteins in the Nucleus and Cytoplasm. We employed nuclear internal control protein antibodies to probe the cytoplasmic proteins in the initial stage of the experiment and vice versa. As demonstrated by the results in Figure 8(a), we could ascertain the successful extraction of both nuclear and cytoplasmic proteins. As depicted in Figures 8(b)–8(d), a significant decrease in Nrf2 expression in the nucleus and a significant increase in the cytoplasm were observed after H_2O_2 treatment compared with that in the normal group

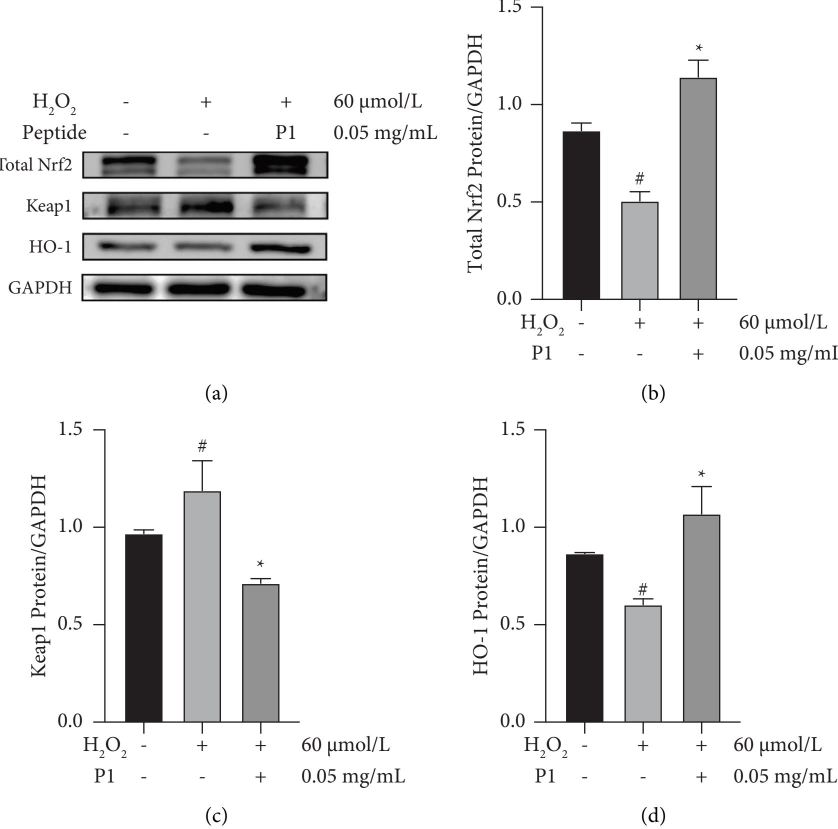

Figure 7: (a–d) Effects of P1 on the protein expression of total Nrf2, Keap1, and HO-1 in H_2O_2-induced INS-1 cells. Results are expressed as mean ± standard deviation; number of parallel experiments $n = 3$. *indicates significant difference compared with the model group ($p < 0.05$). # indicates significant difference compared with the normal group ($p < 0.05$).

($p < 0.05$). Interestingly, the level of Nrf2 nuclear translocation significantly increased in oxidatively damaged cells after P1 intervention compared with that in the model group. The Keap1-Nrf2 pathway is a classical signaling pathway that regulates oxidative stress in the body [37], and the translocation of Nrf2 across the membrane into the nucleus serves as a critical marker of the Keap1-Nrf2 pathway activation. Therefore, the results of this study showed that P1 could significantly reduce the expression of Keap1 protein and promote the translocation of Nrf2 to the nucleus, thereby activating downstream antioxidant enzymes and ultimately leading to the alleviation of cellular oxidative damage.

4. Discussion

ROS react with the double bonds of polyunsaturated fatty acids in the event of cellular oxidative damage, resulting in the formation of lipid hydroperoxides. MDA is a primary product of these lipid hydroperoxides [38]. Consequently, ROS and MDA serve as critical markers in detecting oxidative stress. It is imperative to efficiently and promptly eliminate excess ROS through the endogenous antioxidant defense systems to mitigate the extent of oxidative damage [39]. SOD, CAT, and GPx play indispensable roles as the initial line of defense against oxidative stress [40]. However, the body's endogenous antioxidant systems may be inadequate to clear the surplus ROS when the degree of cellular damage surpasses the cell's capacity for adaptive regulation. In such cases, exogenous antioxidants can intervene by stimulating specific intracellular signaling pathways, thereby effectively reducing ROS levels [41]. In this study, the antioxidant capacity of small-molecule peptides from *L. plantarum* SCS2 was explored by examining the antioxidant enzyme activities of cells and the changes in ROS and MDA levels. The results of the study showed that ROS and MDA levels significantly increased and antioxidant enzyme activities significantly decreased in INS-1 cells subjected to H_2O_2-stimulated cells. The small-molecule peptides of *L. plantarum* SCS2 significantly reduced ROS and MDA levels and increased antioxidant enzyme activity in oxidatively damaged cells, with the strongest antioxidant capacity of P1 from *L. plantarum* SCS2. These results aligned with the findings of another study that concluded that *L. plantarum*HFY09-fermented soymilk improved the activities of SOD, CAT, and GPx and reduced the MDA level in serum, liver, and brain tissues of mice with D-galactose-induced oxidative aging [42]. Unlike the present study in

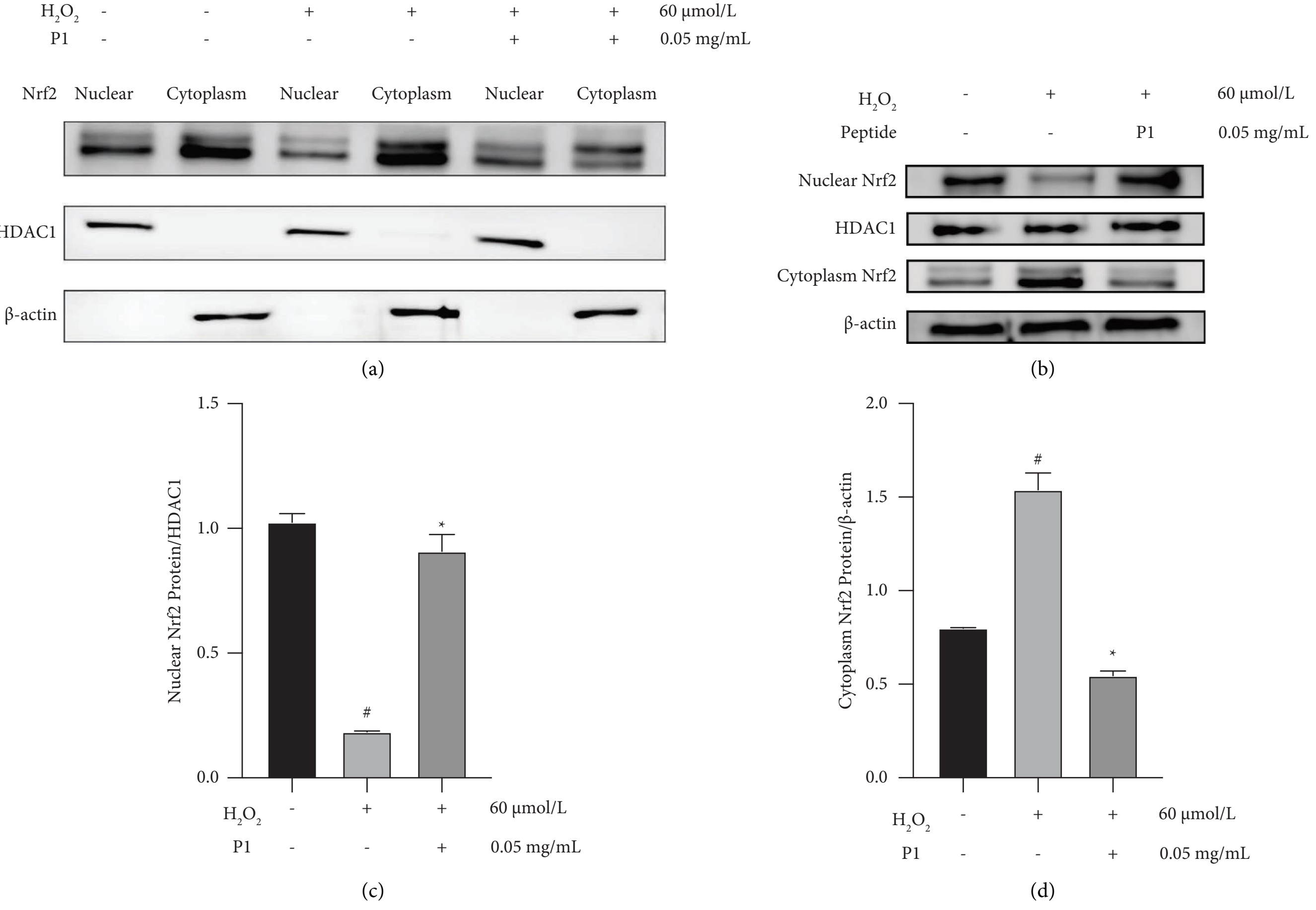

Figure 8: (a) Validation results of nuclear and cytoplasmic protein extraction experiment. (b–d) Effects of P1 on the protein expression of Nrf2 in the nucleus and cytoplasm of H_2O_2-induced INS-1 cells. Results are expressed as mean ± standard deviation; number of parallel experiments $n = 3$. *indicates significant difference compared with the model group ($p < 0.05$). # indicates significant difference compared with the normal group ($p < 0.05$).

which small-molecule peptides in *L. plantaru*m SCS2 cells reduced oxidative damage to cells, the main components of *L. plantarum*HFY09-fermented soymilk having antioxidant effects were low-molecular-weight active peptides and soy isoflavones, which together reduced oxidative stress in mice.

Furthermore, the Keap1-Nrf2 pathway is activated during oxidative stress, and Nrf2 disassociates from Keap1, becomes activated, and translocates into the nucleus, consequently initiating the expression of phase II detoxification and antioxidant enzymes [43–45]. HO-1, predominantly regulated by Nrf2, is considered to play a pivotal role in endogenous defense against oxidative stress [46]. Therefore, the present study sought to explore the mechanisms through which small-molecule peptides mitigated oxidative stress by examining the changes in the expression levels of Keap1, Nrf2, and the antioxidant enzyme HO-1 in H_2O_2-induced oxidatively damaged cells. An increase in Keap1 expression level and a decrease in Nrf2 and HO-1 expression levels were observed after H_2O_2 induction of oxidative damage in INS-1 cells. However, the pretreatment with P1 effectively reduced the expression of Keap1 and promoted the expression of Nrf2 and HO-1 in these cells. The results of a previous study showed that the pretreatment with *L. plantarum* KSFY06 decreased Keap1 expression and increased the expression of Nrf2 and downstream antioxidant enzymes expression, indicating that *L. plantarum* KSFY06 could inhibit oxidative stress injury in mice through the Keap1-Nrf2 pathway [47]. This was consistent with the results of our study. On the contrary, Wang et al. [48] argued that *L. plantarum*Y16-fermented soymilk increased the protein expression levels of Keap1, Nrf2, and HO-1 and then enhanced the activities of SOD, CAT, and other antioxidant enzymes related to the Keap1-Nrf2 signaling pathway, thereby protecting HepG2 cells from oxidative damage caused by 2,2′-azobis (2-amidinopropane) dihydrochloride. This was inconsistent with the changes in the protein expression levels of Keap1 in our experiments. It was found that the sustained accumulation of Nrf2 might have adverse consequences such as apoptosis and tumorigenesis [49, 50]. As cells returned to normalcy, Keap1 levels increased to regulate the ongoing accumulation of intracellular Nrf2 [51]. Therefore, the increase in Keap1 protein expression in oxidatively damaged cells by *L. plantarum*Y16-fermented soymilk might be associated with this factor. Furthermore, this study revealed that P1 could promote Nrf2 nuclear translocation in H_2O_2-induced oxidative damage in

INS-1 cells. This result further demonstrated that P1 might reduce oxidative damage in cells by activating the Keap1-Nrf2 pathway. The results of a study concluded that the pretreatment with *L. plantarum* ZLP001 did not promote Nrf2 entry into the nucleus in oxidatively damaged cells [52], contradicting our results. Wang et al. [52] suggested that this discrepancy might be due to the activation of Nrf2 by *L. plantarum* ZLP001 prior to their detection time point. Hence, the cells were capable of resisting oxidative damage without requiring sustained Nrf2 accumulation in the nucleus.

Many recent studies investigated the beneficial effects of postbiotics on the host, such as anti-inflammatory, immunomodulatory, antiproliferative, and antioxidant effects [53]. The postbiotic components can be broadly classified into two categories: beneficial microbial cellular components and metabolites. The latter include extracellular polysaccharides, intracellular polypeptides, and small active molecules such as short-chain fatty acids [54]. This study focused on the small-molecule peptides in *L. plantarum* SCS2, microbial cellular components. We examined their potential to exert antioxidant influence during oxidative stress via regulating the Keap1-Nrf2 pathway. However, this study only confirmed the antioxidant effect of intracellular small-molecule peptides of *L. plantarum* SCS2 *in vitro*. Therefore, further studies are needed to verify the *in vivo* antioxidant effect and investigate the key substances within P1 of *L. plantarum* SCS2 that exerted antioxidant effects. This will facilitate the development and use of postbiotics in functional foods.

5. Conclusions

P1 from *L. plantarum* SCS2 ameliorated oxidative damage in INS-1 cells by promoting Nrf2 entry into the nucleus and upregulating antioxidant enzymes, with this effect being mediated through regulation of the Keap1-Nrf2 signaling pathway. Next, the research will focus on isolating and purifying the key components responsible for the antioxidant properties of P1 derived from *L. plantarum* SCS2. Subsequently, our goal is to investigate the potential antioxidant effects exhibited by these components *in vivo* and *in vitro*.

Abbreviations

ARE: Antioxidant response element
CAT: Catalase
CCK-8: Cell counting kit-8
cDNA: Complementary DNA
DCFH-DA: 2′,7′-dichlorofluorescin diacetate
GPx: Glutathione peroxidase
GAPDH: Glyceraldehyde 3-phosphate dehydrogenase
H_2O_2: Hydrogen peroxide
HO-1: Heme oxygenase-1
HDAC1: Histone deacetylase 1
INS-1 cells: Rat insulinoma cells
Keap1: Kelch-like ECH-associated protein 1
L. plantarum SCS2: *Lactiplantibacillus plantarum* SCS2
LAB: Lactic acid bacteria
MDA: Malondialdehyde
Nrf2: Nuclear factor erythroid 2-related factor 2
PBS: Phosphate-buffered saline
PVDF: Polyvinylidene fluoride
ROS: Reactive oxygen species
RPMI 1640: Roswell Park Memorial Institute 1640
RT-qPCR: Real-time quantitative polymerase chain reaction
Rb: Rabbit
SOD: Superoxide dismutase.

Data Availability

The data underpinning the conclusions of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xinyi Huang and Lishi Jiang contributed equally to the article.

Acknowledgments

We express our profound appreciation to Jiayi Sun, affiliated with the Innovative Institute of Chinese Medicine Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China, Dongmei Li from the Institute of Medical Technology, Chengdu University of Traditional Chinese Medicine, Chengdu, China, and the teachers from the Laboratory Center of Public Health Institute of Chengdu University of Traditional Chinese Medicine, Chengdu, China. Their assistance with the experimental equipment was indispensable to the successful execution of this research. This study was supported by Sichuan Science and Technology Program (2023NSFSC0180).

References

[1] C. Lian, B. Chu, W. Xia, Z. Wang, R. Fan, and L. Wang, "Persistent activation of Nrf2 in a p62-dependent non-canonical manner aggravates lead-induced kidney injury by promoting apoptosis and inhibiting autophagy," *Journal of Advanced Research*, vol. 46, pp. 87–100, 2023.

[2] H. Sies, C. Berndt, and D. P. Jones, "Oxidative stress," *Annual Review of Biochemistry*, vol. 86, no. 1, pp. 715–748, 2017.

[3] M. Halim and A. Halim, "The effects of inflammation, aging and oxidative stress on the pathogenesis of diabetes mellitus (type 2 diabetes)," *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, vol. 13, no. 2, pp. 1165–1172, 2019.

[4] J. Hancock, R. Desikan, and S. Neill, “Role of reactive oxygen species in cell signalling pathways,” *Biochemical Society Transactions*, vol. 29, no. 2, pp. 345–349, 2001.

[5] D. Lee, M. Song, and E. H. Kim, “Role of oxidative stress and nrf2/KEAP1 signaling in colorectal cancer: mechanisms and therapeutic perspectives with phytochemicals,” *Antioxidants*, vol. 10, no. 5, p. 743, 2021.

[6] M. Zhang, C. An, Y. Gao, R. K. Leak, J. Chen, and F. Zhang, “Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection,” *Progress in Neurobiology*, vol. 100, pp. 30–47, 2013.

[7] R. Fan, Z. Li, D. Zhang, and Z. Wang, “Involvement of Nrf2 and mitochondrial apoptotic signaling in trehalose protection against cadmium-induced kidney injury,” *Metallomics*, vol. 12, no. 12, pp. 2098–2107, 2020.

[8] W. Tu, H. Wang, S. Li, Q. Liu, and H. Sha, “The anti-inflammatory and anti-oxidant mechanisms of the Keap1/Nrf2/ARE signaling pathway in chronic diseases,” *Aging and disease*, vol. 10, no. 3, p. 637, 2019.

[9] Y. Zhou, Z. Jiang, H. Lu et al., “Recent advances of natural polyphenols activators for Keap1 Nrf2 signaling pathway,” *Chemistry and Biodiversity*, vol. 16, no. 11, 2019.

[10] Z. Guo and Z. Mo, “Keap1-Nrf2 signaling pathway in angiogenesis and vascular diseases,” *Journal of Tissue Engineering and Regenerative Medicine*, vol. 14, no. 6, pp. 869–883, 2020.

[11] Q. Han, B. Kong, Q. Chen, F. Sun, and H. Zhang, “In vitro comparison of probiotic properties of lactic acid bacteria isolated from Harbin dry sausages and selected probiotics,” *Journal of Functional Foods*, vol. 32, pp. 391–400, 2017.

[12] S. Y. Jang, J. Heo, M. R. Park et al., “Genome characteristics of *Lactobacillus fermentum strain* JDFM216 for application as probiotic bacteria,” *Journal of Microbiology and Biotechnology*, vol. 27, no. 7, pp. 1266–1271, 2017.

[13] P. Hsieh, C. Chen, Y. Kuo, and H. H. Ho, “*Lactobacillus spp.* reduces ethanol-induced liver oxidative stress and inflammation in a mouse model of alcoholic steatohepatitis,” *Experimental and Therapeutic Medicine*, vol. 21, no. 3, pp. 188–191, 2021.

[14] X. Zhang, J. Xu, M. Ma et al., “Heat-killed Lactobacillus rhamnosus ATCC 7469 improved UVB-induced photoaging via antiwrinkle and antimelanogenesis impacts,” *Photochemistry and Photobiology*, vol. 99, no. 5, pp. 1318–1331, 2023.

[15] Q. Mo, S. You, H. Fu et al., “Purification and identification of antioxidant peptides from rice fermentation of Lactobacillus plantarum and their protective effects on UVA–Induced oxidative stress in skin,” *Antioxidants*, vol. 11, no. 12, p. 2333, 2022.

[16] J. Zhao, H. Fu, Y. Zhang et al., “Protective effects of *Lactobacillus reuteri* SJ-47 exopolysaccharides on human skin fibroblasts damaged by UVA radiation,” *Bioresources and Bioprocessing*, vol. 9, no. 1, p. 127, 2022.

[17] D. Luo, M. Luo, H. Wang et al., “Protective effects of *Lactobacillus rhamnosus* peptides against DSS-induced inflammatory and oxidative damages in human colonic epithelial cells through NF-κB/Nrf2/HO-1 signaling pathway,” *International Journal of Peptide Research and Therapeutics*, vol. 28, no. 4, p. 115, 2022.

[18] C. Chatterjee, S. Gleddie, and C. Xiao, “Soybean bioactive peptides and their functional properties,” *Nutrients*, vol. 10, no. 9, p. 1211, 2018.

[19] U. C. Urbizo Reyes, J. E. Aguilar Toalá, and A. M. Liceaga, “Hairless canary seeds (Phalaris canariensis L.) as a potential source of antioxidant, antihypertensive, antidiabetic, and antiobesity biopeptides,” *Food Production, Processing and Nutrition*, vol. 3, no. 1, p. 6, 2021.

[20] A. Matemu, S. Nakamura, and S. Katayama, “Health benefits of antioxidative peptides derived from legume proteins with a high amino acid score,” *Antioxidants*, vol. 10, no. 2, p. 316, 2021.

[21] S. Zhang, Y. Zhao, Y. Wang, X. Yang, C. Chi, and B. Wang, “Gelatins and antioxidant peptides from Skipjack tuna (*Katsuwonus pelamis*) skins: purification, characterization, and cytoprotection on ultraviolet-A injured human skin fibroblasts,” *Food Bioscience*, vol. 50, 2022.

[22] Q. Qiao, Q. Luo, S. Suo, Y. Zhao, C. Chi, and B. Wang, “Preparation, characterization, and cytoprotective effects on HUVECs of fourteen novel angiotensin-I-converting enzyme inhibitory peptides from protein hydrolysate of tuna processing by-products,” *Frontiers in Nutrition*, vol. 9, 2022.

[23] Y. Wang, X. Li, J. Wang, Y. He, C. Chi, and B. Wang, “Antioxidant peptides from protein hydrolysate of skipjack tuna milt: purification, identification, and cytoprotection on H_2O_2 damaged human umbilical vein endothelial cells,” *Process Biochemistry*, vol. 113, pp. 258–269, 2022.

[24] Y. Sheng, W. Wang, M. Wu et al., “Eighteen novel bioactive peptides from monkfish (Lophius litulon) swim bladders: production, identification, antioxidant activity, and stability,” *Marine Drugs*, vol. 21, no. 3, p. 169, 2023.

[25] Z. Cai, T. Yan, S. Li et al., “Ameliorative effect of dandelion (*Taraxacum officinale*) peptides on benzo (a) pyrene induced oxidative stress and inflammation in human umbilical vein endothelial cells,” *Journal of Peptide Science: An Official Publication of the European Peptide Society*, vol. 29, no. 1, p. e3447, 2023.

[26] C. Wen, J. Zhang, Y. Feng, Y. Duan, H. Ma, and H. Zhang, “Purification and identification of novel antioxidant peptides from watermelon seed protein hydrolysates and their cytoprotective effects on H_2O_2-induced oxidative stress,” *Food Chemistry*, vol. 327, 2020.

[27] L. Wu, L. Long, J. Sun et al., “Exploring the antioxidant effect of *Lactobacillus plantarum* SCS2 on mice with type 2 diabetes,” *Journal of Food Biochemistry*, vol. 45, no. 8, 2021.

[28] X. Meng, C. Chen, J. Sun, L. Jing, L. Zuo, and L. Wu, “Alleviation of oxidative stress in pancreatic tissue of hyperglycemic mice by *Lactiplantibacillus plantarum* SCS4,” *Journal of Food Biochemistry*, vol. 46, no. 9, 2022.

[29] Q. Wang, L. Li, E. Xu, V. Wong, C. Rhodes, and P. Brubaker, “Glucagon-like peptide-1 regulates proliferation and apoptosis via activation of protein kinase B in pancreatic INS-1 beta cells,” *Diabetologia*, vol. 47, no. 3, pp. 478–487, 2004.

[30] Y. He, L. Lin, H. Zheng et al., “Potential anti-skin aging effect of a peptide AYAPE isolated from Isochrysis zhanjiangensis on UVB-induced HaCaT cells and H_2O_2-induced BJ cells,” *Journal of Photochemistry and Photobiology B: Biology*, vol. 233, 2022.

[31] J. Yang, X. Song, Y. Feng et al., “Natural ingredients-derived antioxidants attenuate H_2O_2-induced oxidative stress and have chondroprotective effects on human osteoarthritic chondrocytes via Keap1/Nrf2 pathway,” *Free Radical Biology and Medicine*, vol. 152, pp. 854–864, 2020.

[32] J. Duan, F. Zhang, M. Lu et al., “Swietenine and swietenolide from Swietenia macrophylla king improve insulin secretion and attenuate apoptosis in H_2O_2 induced INS 1 cells,” *Environmental Toxicology*, vol. 37, no. 11, pp. 2780–2792, 2022.

[33] F. Yang, X. He, T. Chen et al., “Peptides isolated from yak milk residue exert antioxidant effects through Nrf2 signal

pathway," *Oxidative Medicine and Cellular Longevity*, vol. 2021, Article ID 9426314, 17 pages, 2021.

[34] G. Yi, F. Zhao, and X. Liu, "Effect of soybean peptides against hydrogen peroxide induced oxidative stress in HepG2 cells via Nrf2 signaling," *Food and Function*, vol. 11, no. 3, pp. 2725–2737, 2020.

[35] A. Sen and J. A. Imlay, "How microbes defend themselves from incoming hydrogen peroxide," *Frontiers in Immunology*, vol. 12, 2021.

[36] P. Newsholme, V. F. Cruzat, K. N. Keane, R. Carlessi, and P. I. H. de Bittencourt Jr, "Molecular mechanisms of ROS production and oxidative stress in diabetes," *Biochemical Journal*, vol. 473, no. 24, pp. 4527–4550, 2016.

[37] W. Tu, H. Wang, S. Li, Q. Liu, and H. Sha, "The anti-inflammatory and anti-oxidant mechanisms of the keap1/nrf2/ARE signaling pathway in chronic diseases," *Aging and disease*, vol. 10, no. 3, pp. 637–651, 2019.

[38] M. Alía, S. Ramos, R. Mateos, A. B. Granado-Serrano, L. Bravo, and L. Goya, "Quercetin protects human hepatoma HepG2 against oxidative stress induced by tert-butyl hydroperoxide," *Toxicology and Applied Pharmacology*, vol. 212, no. 2, pp. 110–118, 2006.

[39] Y. Sheng, Y. Qiu, Y. Wang, C. Chi, and B. Wang, "Novel antioxidant collagen peptides of siberian sturgeon (acipenserbaerii) cartilages: the preparation, characterization, and cytoprotection of H_2O_2-damaged human umbilical vein endothelial cells (HUVECs)," *Marine Drugs*, vol. 20, no. 5, p. 325, 2022.

[40] O. Ighodaro and O. Akinloye, "First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid," *Alexandria journal of medicine*, vol. 54, no. 4, pp. 287–293, 2018.

[41] J. Kong, X. Hu, W. Cai, Y. Wang, C. Chi, and B. Wang, "Bioactive peptides from skipjack tuna cardiac arterial bulbs (II): protective function on UVB-irradiated HaCaT cells through antioxidant and anti-apoptotic mechanisms," *Marine Drugs*, vol. 21, no. 2, p. 105, 2023.

[42] X. Zhou, H. Sun, F. Tan et al., "Anti-aging effect of *Lactobacillus plantarum*HFY09-fermented soymilk on D-galactose-induced oxidative aging in mice through modulation of the Nrf2 signaling pathway," *Journal of Functional Foods*, vol. 78, 2021.

[43] Z. Zhu, Z. Shi, C. Xie, W. Gong, Z. Hu, and Y. Peng, "A novel mechanism of Gamma-aminobutyric acid (GABA) protecting human umbilical vein endothelial cells (HUVECs) against H_2O_2-induced oxidative injury," *Comparative Biochemistry and Physiology-Part C: Toxicology and Pharmacology*, vol. 217, pp. 68–75, 2019.

[44] B. Hu, H. Wei, Y. Song et al., "NF-κB and Keap1 interaction represses Nrf2-mediated antioxidant response in rabbit hemorrhagic disease virus infection," *Journal of Virology*, vol. 94, no. 10, 2020.

[45] L.-Y. Wang, R.-F. Fan, D.-B. Yang, D. Zhang, and L. Wang, "Puerarin reverses cadmium-induced lysosomal dysfunction in primary rat proximal tubular cells via inhibiting Nrf2 pathway," *Biochemical Pharmacology*, vol. 162, pp. 132–141, 2019.

[46] T. Satoh, S.-I. Okamoto, J. Cui et al., "Activation of the Keap1/Nrf2 pathway for neuroprotection by electrophillic phase II inducers," *Proceedings of the National Academy of Sciences*, vol. 103, no. 3, pp. 768–773, 2006.

[47] C. Li, J. Si, F. Tan, K. Park, and X. Zhao, "Lactobacillus plantarum KSFY06 prevents inflammatory response and oxidative stress in acute liver injury induced by D-Gal/LPS in mice," *Drug Design, Development and Therapy*, vol. 15, pp. 37–50, 2021.

[48] A. Wang, K. Hou, G. Mu, C. Ma, and Y. Tuo, "Antioxidative effect of soybean milk fermented by *Lactobacillus plantarum* Y16 on 2, 2 –azobis (2-methylpropionamidine) dihydrochloride (ABAP)-damaged HepG2 cells," *Food Bioscience*, vol. 44, 2021.

[49] M. Kobayashi and M. Yamamoto, "Nrf2-Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species," *Advances in Enzyme Regulation*, vol. 46, no. 1, pp. 113–140, 2006.

[50] I. M. Copple, C. E. Goldring, N. R. Kitteringham, and B. K. Park, "The Nrf2–Keap1 defence pathway: role in protection against drug-induced toxicity," *Toxicology*, vol. 246, no. 1, pp. 24–33, 2008.

[51] M. E. Giuliani and F. Regoli, "Identification of the Nrf2–Keap1 pathway in the European eel *Anguilla anguilla*: role for a transcriptional regulation of antioxidant genes in aquatic organisms," *Aquatic Toxicology*, vol. 150, pp. 117–123, 2014.

[52] J. Wang, W. Zhang, S. Wang, Y. Wang, X. Chu, and H. Ji, "*Lactobacillus plantarum* exhibits antioxidant and cytoprotective activities in porcine intestinal epithelial cells exposed to hydrogen peroxide," *Oxidative Medicine and Cellular Longevity*, vol. 2021, Article ID 8936907, 13 pages, 2021.

[53] L. Aghebati Maleki, P. Hasannezhad, A. Abbasi, and N. Khani, "Antibacterial, antiviral, antioxidant, and anticancer activities of postbiotics: a review of mechanisms and therapeutic perspectives," *Biointerface Research in Applied Chemistry*, vol. 12, pp. 2629–2645, 2021.

[54] P. F. Cuevas González, A. M. Liceaga, and J. E. Aguilar Toalá, "Postbiotics and paraprobiotics: from concepts to applications," *Food Research International*, vol. 136, 2020.