Journal of Functional Foods 76 (2021) 104320

Contents lists available at ScienceDirect

Journal of Functional Foods

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

Anti-inflammatory effects of three selenium-enriched brown rice protein hydrolysates in LPS-induced RAW264.7 macrophages via NF-κB/MAPKs signaling pathways

Mingju Feng [a,1], Xiaoya Wang [a,1], Hua Xiong [a], Tingting Qiu [a], Hua Zhang [b], Fanghua Guo [a], Li Jiang [b], Yong Sun [a,*]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, Jiangxi, China
[b] Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, Jiangxi, China

ARTICLE INFO

Keywords:
Selenium brown rice
Protein hydrolysates
Anti-inflammatory property
NF-κB
MAPKs

ABSTRACT

In the present study, three selenium-enriched brown rice protein hydrolysates were prepared by trypsin, their anti-inflammatory mechanism *in vitro* were investigated. Results obtained from LPS-induced RAW264.7 cell model showed that the 1.0–3.5 kDa peptide fractions exhibited the most effective anti-inflammatory property through inhibiting the production of nitric oxide (NO), prostaglandin E_2 (PGE_2) and pro-inflammation cytokines including interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α). The mRNA and protein expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) were also suppressed by 1.0–3.5 kDa peptide fractions. Additionally, the phosphorylation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPKs) signal proteins were blocked after 1.0–3.5 kDa peptide fraction treatments. Above all, the observed anti-inflammatory effects of selenium-enriched brown rice protein hydrolysates were closely related to their Se content. These results illustrated that the 1.0–3.5 kDa peptide fractions could be a novel functional food ingredient for inflammation-related diseases treatment.

1. Introduction

Selenium is one of the crucial trace elements with various important biological functions such as antioxidant, immunity and antifatigue activities not only for human but also for animals (Rayman, 2000). According to the surveys, about 72% of the regions is deficient selenium or low selenium in China, which caused many diseases for instance Keshan disease, Kashin-Beck disease (Ge & Yang, 1993; Oropeza-Moe, Wisløff, & Bernhoft, 2015). Therefore, Se-enriched products have been widely concerned, including those from Se-enriched peanuts (Zhao et al., 2016), rice (Fang et al., 2019), green tea, *Cardamine violifolia*, and other plants (Zhu et al., 2019). In addition, Se occurs in different forms including inorganic and organic. Organic Se, especially those rich in selenoprotein, selenium polysaccharide and selenium nucleic acid, has getting more attention due to the better toxicological safety, bioavailability and physiological activity than inorganic Se (Zhu et al., 2019).

Rice is one of the most important staple foods for Chinese residents, selenium enriched rice is generally the main source of dietary Se (Chen, Xu, Hu, & Pan, 2002). Selenium-enriched brown rice has higher nutritional value than polished rice, the selenium in selenium-enriched brown rice is mainly combined with protein. Furthermore, compared with nonenzymatically hydrolyzed rice protein, the activity of peptides produced from rice protein by enzymatic hydrolysis is significantly higher. Researches indicated that Se-containing proteins hydrolysates exhibited varied biological activities such as antioxidant (Liu, Zhao, Chen, & Fang, 2015), immunomodulatory (Fang et al., 2017, 2019) and relief of toxicity of heavy metals (Xu et al., 2016). Recently, evidence has emerged that oxidative stress plays a crucial role in the development and perpetuation of inflammation (Lugrin, Rosenblatt-Velin, Parapanov, & Liaudet, 2014). Yet, study about the effect and mechanism of Se-containing proteins hydrolysates on inflammation particularly from brown rice remains minor.

Inflammation is a defense response of living tissue with the vascular system to harmful stimuli (Ruslan, 2008), which considered to be a critical risk reason for the pathogenesis of chronic diseases. Macrophages activated by lipopolysaccharide (LPS) could produce pro-

* Corresponding author.
E-mail address: yongsun@ncu.edu.cn (Y. Sun).
[1] These authors contribute equally to this manuscript.

https://doi.org/10.1016/j.jff.2020.104320
Received 10 August 2020; Received in revised form 22 November 2020; Accepted 1 December 2020
Available online 18 December 2020

inflammatory cytokines, chemokines, nitric oxide (NO) and prostaglandin E_2 (PGE_2) (Udenigwe, Je, Cho, & Yada, 2013). Excessively accumulation of pro-inflammatory cytokines and mediators could trigger dysfunction and certain chronic diseases such as rheumatoid arthritis, psoriasis and inflammatory bowel disease (Valledor, Comalada, Santamaría-Babi, Lloberas, & Celada, 2010). Therefore, regulating the activation of macrophages is a complementary strategy for controlling the inflammatory process (Jiang et al., 2017). NF-κB and MAPKs, two classical inflammation related pathways, have been widely investigated in RAW264.7 macrophage. Various protein hydrolysates form different sources have been shown to have anti-inflammation activity through regulating NF-κB and MAPKs (Wen et al., 2016). Thus, study about the anti-inflammatory activity and underlying mechanism of Se-containing rice proteins hydro necessary lysates is necessary and meaningful.

The aim of the present study was to investigate the anti-inflammatory mechanism of three types Se-enriched brown rice protein hydrolysate fractions in LPS-stimulated RAW264.7 macrophages. The production of pro-inflammatory cytokines, related mRNA and protein expression levels were determined by ELISA, RT-qPCR and Western Blot. The correlation between anti-inflammatory property of selenium-enriched brown rice protein hydrolysates and Se content was evaluated by Pearson correlation analysis. This study may provide scientific basis for the application of selenium-enriched brown rice protein hydrolysate in inflammation-related diseases.

2. Materials and method

2.1. Materials and reagents

Three Se-enriched brown rice were supplied by Hunan Taoyuan Rich Selenium Agricultural Products Base (Hunan, China), named Shuangjiangkou Selenium-rich brown rice (SSR), Taoyuan selenium-rich brown rice (TSR), Mayang selenium-rich brown rice (MSR), respectively. Cytochrome C (l.25 kDa), aprotinin (6.50 kDa), bacillus (1.45 kDa), ethine-ethine-tyrosine-arginine (0.45 kDa), ethionine-ethine-ethine (0.19 kDa) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Phosphate buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM) was acquired from Beijing Solarbio Science & Technology Corporation Limited (Beijing, China). Fetal bovine serum (FBS), lipopolysaccharides (LPS, from Escherichia coli O55: B5) were purchased from Sigma (St. Louis, MO, USA). ABTS, FRAP and NO reagent kit were purchased from Beyotime Inst itute of Biotechnology (Shanghai, China). The PGE, IL-6, IL-1β and TNF-α ELISA kits were obtained from MEIMIAN. Primary antibodies against p-JNK, JNK, p-ERK, ERK, p-p38, p38, p- IκBα, IκBα, p-p65, p65, iNOS, COX-2, β-actin were purchased from Cell Signaling Technology (MA, USA). GAPDH was obtained from Servicebio Biological Technology Corporation Limited (Wuhan, China). All other chemicals and reagents used were of analytical grade.

2.2. Preparation and enzymatic hydrolysis of Se-enriched brown rice protein

An optimized alkali extraction method was employed as previously described (Fang, Zhang, Brittany, Joseph, & Hu, 2010; Liu, Chen, Zhao, Gu, & Yang, 2011) with some modifications. Briefly, the flour of Se-enriched brown rice was defatted with hexane (w/v, 1:3), homogenized, then mixed with 0.05 mol/L NaOH solution in a weight/volume ratio of 1:20 and stirred continuously for 3–4 h at room temperature. The supernatant was collected, repeated extraction three times. Se-enriched brown rice protein was precipitated from the supernatant by adding HCl, the supernatant was discarded, resultant precipitate was dried by a freezer dryer and storage at −20 °C for further analyze. The selenium rich brown rice protein was hydrolyzed with Trypsin in optimal pH and temperature as recommended by the manufacturer Liu et al. (2015). The protein was dissolved in deionized water, the solution was hydrolyzed by the Trypsin at a concentration of 0.2% (w/w, enzyme/substrate) in a shaking water bath at 55 °C for 1 h. After that, the reaction was terminated by heating at 90 °C for 15 min to inactivate enzyme. Supernatants were centrifugated at 4000 g for 15 min, freeze-dried and stored at −20 °C, named as Shuangjiangkou selenium rich brown rice protein hydrolysates (SSPHs); Taoyuan selenium rich brown rice protein hydrolysates (TSPHs); Mayang Selenium rich brown rice protein hydrolysates (MSPHs). A molecular weight cut-off (MWCO, 3.5 and 1.0 kDa) on a Quixstand bench-top system (LNG-MUF-112B, Shanghai, China) was used to fractionated the hydrolysates. Three peptide fractions were obtained and designated as: MW < 1.0 kDa (1), MW:1.0–3.5 kDa (2), MW > 3.5 kDa (3). Selenium rich brown rice protein hydrolysates were resolved in PBS (1 mg/PBS 1 mL) and diluted into 25, 50, 100 μg/mL, further used for the cell experiments.

2.3. Determination of total Se contents

The Se contents of SPHs were determined according to previous studies (Chen et al., 2002; Liu, Cao, Bai, Wen, & Gu, 2009) with slightly modifications. Samples (0.5 g) were digested with a mixture of ultra-pure HNO_3 and ultra-pure H_2O_2 (5 mL, 4:1, v/v) in a microwave digestion system (MARS6, CEM, Matthews, NC, USA). After that, the digestion solutions were incubated at 120 °C until the mixture became colorless and clear. Finally, solutions were diluted with double-distilled water to a final volume of 25 mL. The content of Se in eventual solutions was analyzed by dual-hydride generation atomic fluorescent spectrometry (AFS-8230, Beijing Jitian Instrument, China).

2.4. Identified the amino acid sequence of TSPHs-2 fraction

The amino acid sequences of TSPHs-2 fraction were identified by Scide Triple TOF® 5600 + LC-MS/MS. The Data Dependent Acquisition (DDA) mode acquires the MS1.MS2 spectrum data. Obtained MS/MS data were analyzed by Protein Pilot, the sequences were searched in the protein database to match with parent proteins.

2.5. Cell culture

RAW264.7 cells were cultured in DMEM supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin and 10% fetal bovine serum (FBS) at 37 °C in humidified 5% CO_2 atmosphere. The medium was changed every other day. For subcultures, cells were passaged at approximately 80% confluence, harvested and seeded at a dilution of 1:4. Logarithmic growth phase cells were used in the following experiments.

2.6. Cell viability

Cell viability was determined by MTT assay (Li, Gao, Du, Cheng, & Mao, 2018; Ren et al., 2018). Briefly, RAW 264.7 cells were seeded at a density of 2×10^4 cells/well in 96-well plates. After overnight adherence, cells were incubated with various concentrations of peptide fractions for 24 h. Then, MTT solution (10 μL, 5.0 mg/mL) was added to each well and incubated at 37 °C. After 4 h, the medium was discarded and DMSO was added to dissolve the formazan dye. The absorbance of each well at 570 nm was determined using a microplate reader (Bio-Rad, Hercules, CA, USA). Cell viability was expressed as a percentage of the optical density of each treatment group relative to control group.

2.7. NO, PGE_2, TNF-α, IL-6 and IL-1β determination

RAW264.7 cells were seeded into 24-well plates (3×10^5 cells/well). After 12 h, cells were incubated with peptide fractions for 12 h, and then stimulated with or without LPS (1.0 μg/mL) for 24 h, supernatants were collected. The production of NO was determined with the Griess reagent. The levels of IL-1β, IL-6, TNF-α and PGE_2 in cell culture supernatant

were determined with ELISA kits according to the manufacturer's instructions, respectively.

2.8. Reverse transcription polymerase chain reaction (RT-PCR)

RAW264.7 cells were plated in 24-well (1×10^6 cells/well) and incubated 12 h. Cells were treated with different concentrations of peptide fractions (25, 50, 100 μg/mL) for 12 h prior to the stimulation with LPS (1 μg/mL) for 24 h. For determining the relative gene expression of TNF-α, IL-1β, IL-6, iNOS and COX-2, total RNA was extracted using RNAeasy™ Animal RNA Isolation Kit with Spin Column (Beyotime Biotechnology, China). The concentration and purity of RNA were determined by measuring the absorbance at 260 nm and 280 nm using a microplate reader. Subsequently, the first-stranded cDNA was synthesized using PrimeScript™ RT reagent Kit (TaKaRa, Japan). At the real-time PCR step, the cDNA was used for quantitative real-time PCR using TB Green® Premix Ex Taq™ (TaKaRa, Japan). The reaction mixture was prepared for PCR at 95 °C for 30 s, then subjected to 40 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 30 s and extension at 65 °C for 5 s, with an additional 5 min extension at 95 °C in the Real-time System (Bio-Rad, CFX Connet, CA,USA). The primer sequences for each gene were summarized in Table 1. The relative changes of gene expression were calculated using method of $2^{-\triangle\triangle CT}$.

2.9. Western blot analyses

RAW264.7 cells were plated in 6-well (2×10^6 cells/well), after incubated overnight, attached cells were pretreated with peptide fractions (25, 50, 100 μg/mL) for 12 h prior to the stimulation with LPS (1 μg/mL) for 1 h, then cells were collected and total proteins were extracted by Cell lysis buffer for Western and IP (Beyotime Biotechnology, Shanghai, China). BCA protein assay kit was used to determine the concentration of protein. An equal amount of protein for each sample was subjected to 10% SDS-PAGE electrophoresis and transferred to PVDF membranes. After blocking with 5% skim milk, the PVDF membrane was incubated with primary antibodies at 4 °C overnight, followed by incubation with the peroxidase conjugated secondary antibody for 1 h at room temperature. Band intensities were measured using Image J software. Quantitative data were normalized by internal control (GAPDH) further expressed as relative protein expression.

2.10. Statistical analyses

All assays in the current study have been repeated at least in triplicate and data were expressed as means ± standard deviations (SD). Significant differences ($P < 0.05$) between the groups were evaluated using Student *t*-test. All of the statistical analyses were conducted using SPSS software.

3. Results

3.1. Effects of SPHs on cell viability and production of NO

The cell viability treated with selenium-rich brown rice protein hydrolysates fractions (0, 25, 50, 100, 150, 200, 250 μg/mL) for 24 h were measured by MTT assay. As shown in Fig. 1A, Results suggested that different peptides fractions exhibited no cytotoxicity within the concentration range of 0–250 μg/mL. To determine the relationship between concentrations and anti-inflammatory effect of peptide fractions, three different concentrations (25, 50 and 100 μg/mL) were selected for further study.

As shown in Fig. 1B, LPS treatment evidently improved the content of NO approximately 4.56-fold compared with the control group. However, SSPHs, TSPHs, MSPHs treatments remarkedly decreased the production of NO ($P < 0.01$). In addition, compared with high molecular weight (Mw > 3.5 kDa) SSPHs, TSPHs, MSPHs with molecular weight < 1.0 kDa and 1.0–3.5 kDa fraction exhibited more effective NO inhibition activity, particularly at 100 μg/mL relative to the LPS group (58.33%, 72.11%, 42.38%, respectively). In order to further investigate the potential anti-inflammatory mechanism of SSPHs, TSPHs, MSPHs peptide fractions with molecular weight at 1.0–3.5 kDa were selected for the following experiments.

3.2. SPHs inhibited the production of PGE_2, TNF-α, IL-1β, and IL-6 in LPS-induced RAW 264.7 cells

As shown in Fig. 2, compared with the control group, the levels of PGE_2, TNF-a, IL-1β, and IL-6 were significantly increased (1.96, 1.96, 2.52 and 2.04-folds) after LPS treatment, while pretreatment with 1.0–3.5 kDa peptide fraction decreased ($P < 0.05$) the levels of these mediators. Compared with the LPS group, the inhibitory activities of

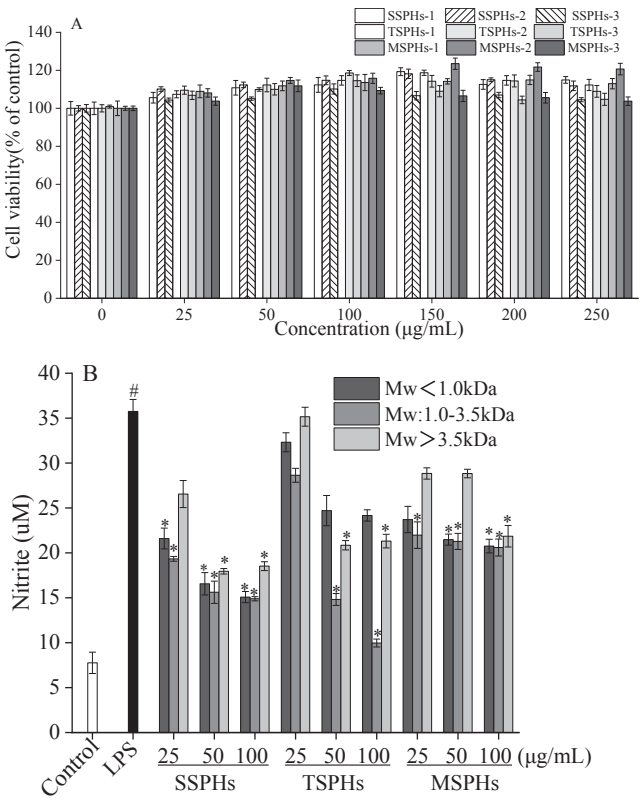

Fig. 1. Effects of SSPHs, TSPHs, MSPHs with different molecular weights on cell viability (A) and NO production in LPS-induced RAW264.7 macrophages (B). SSPHs: Shuangjiangkou selenium rich brown rice protein hydrolysates; TSPHs: Taoyuan selenium rich brown rice protein hydrolysates; MSPHs: Mayang Selenium rich brown rice protein hydrolysates; number 1, 2, 3 represent that hydrolysates with molecular weights < 1.0 kDa, 1.0–3.5 kDa, >3.5 kDa, respectively. The results are expressed as the mean ± SD. # indicated $P < 0.01$ compared with control group; * indicated $P < 0.01$ compared with LPS group.

Table 1
Gene primers of inflammatory factor.

Gene	Forward	Reverse
COX-2	5′-TGCTGTACAAGCAGTGGCA-3′	5′-GCAGCCATTTCCTTCTCTCC-3′
iNOS	5′-CCTCACGCTTGGGTCTTGTT-3′	5′-GCACAAGGGGTTTTCTTCACG-3′
IL-1β	5′-TTGAAGTTGACGGACCCCAA-3′	5′-ATACTGCCTGCCTGAAGCTC-3′
IL-6	5′-GAGACTTCCATCCAGTTGCCT-3′	5′-TGGGAGTGGTATCCTCTGTGA-3′
TNF-α	5′-TGTCTACTCCTCAGAGCCCC-3′	5′-TGAGTCCTTGATGGTGGTGC-3′
β-actin	5′-ATCACTATTGGCAACGAGCG-3′	5′-TCAGCAATGCCTGGGTACAT-3′

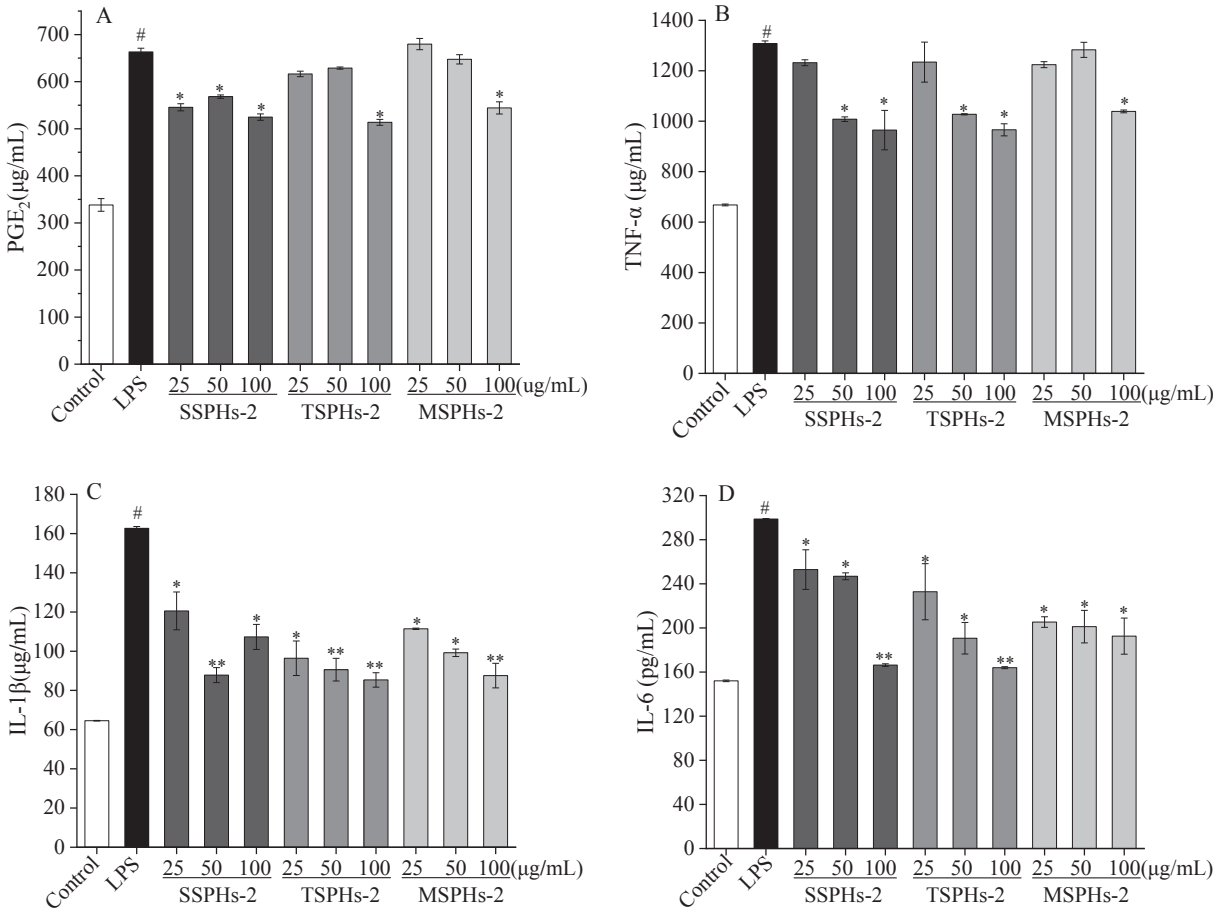

Fig. 2. Effects of SSPHs-2, TSPHs-2, MSPHs-2 on the production of PGE_2 (A), TNF-α (B), IL-1β (C), and IL-6 (D) in LPS-induced RAW264.7 macrophages. The results are expressed as the mean ± SD; # indicated $P < 0.01$ compared with the control group; * indicated $P < 0.05$, ** indicated $P < 0.01$ compared to LPS group.

SSPHs-2, TSPHs-2, MSPHs-2 at 100 μg/mL were 20.87%, 22.55% and 17.94% for PGE_2 (Fig. 2A) and 26.26%, 26.16%, and 20.6% for TNF-α (Fig. 2B). Moreover, SSPHs-2, TSPHs-2, MSPHs-2 (100 μg/mL) exhibited the most obviously inhibitory effect on the production of IL-1β about 46.03%, 47.54% and 46.19%, respectively (Fig. 2C); the inhibition rate for IL-6 at 100 μg/mL were 44.33%, 45.10% and 35.54%, respectively (Fig. 2D).

3.3. SPHs inhibited the expression of pro-inflammatory genes in LPS-induced RAW 264.7 cells

The effect of Se-enriched brown rice protein hydrolysates with molecular weights 1.0–3.5 kDa on the expression of TNF-α, IL-1β, and IL-6 mRNA were investigated by RT-qPCR. As shown in Fig. 3, LPS treatment sharply ($P < 0.05$) increased the levels of TNF-α, IL-1β and IL-6 mRNA compared with the control group. However, the improvement was reversed in a concentration-dependent manner by SSPHs-2, TSPHs-2, MSPHs-2. Particularly, high dose SPHs exhibited the most obvious inhibitory trend such as 29.96%, 52.63%, 48.33% for TNF-α (Fig. 3A); 82.76%, 77.99%, 49.77% for IL-6 (Fig. 3B) and 41.33%, 22.21%, 37.91% for IL-1β (Fig. 3C), respectively. The alterations of TNF-α, IL-1β and IL-6 mRNA were consistent with corresponding cytokines (Fig. 2), which confirmed that the anti-inflammatory effect of SSPHs-2, TSPHs-2, MSPHs-2 occurred at the transcriptional levels.

3.4. SPHs inhibited mRNA and protein expressions of iNOS/COX-2 in LPS-induced RAW 264.7 cells

As shown in Fig. 4A and B, the mRNA and protein levels of iNOS/COX-2 were markedly ($P < 0.01$) upregulated by LPS treatment compared with the control group. Whereas, peptide fractions (1.0–3.5 kDa) treatment markedly inhibited the improvement. Compared with the LPS group, the inhibitory rate of SSPHs-2, TSPHs-2, MSPHs-2 (100 μg/mL) on the mRNA relative expression were 70.98%, 77.95%, 51.04% for iNOS and 77.83%, 35.70%, 46.16% for COX-2, respectively (Fig. 4A). Similar trends were found at protein levels detected by western blotting assay (Fig. 4B). 1.0–3.5 kDa peptide fractions treatment resulted in remarkably decrease on the protein expressions of iNOS/COX-2 compared with the LPS group. Compared with the LPS group, the inhibitory rate of SSPHs-2, TSPHs-2, MSPHs-2 (100 μg/mL) on the protein level were 63.76%, 51.28%, 28.89% for iNOS and 78.75%, 81.96%, 22.89% for COX-2, respectively (Fig. 5B). Reductions of iNOS/COX-2 mRNA and protein expression were consistent with the results of NO (Fig. 1B) and PGE_2 (Fig. 2A), which indicated that the 10–3.5 kDa peptide fractions reduced the production of NO and PGE_2 by down-regulating iNOS/COX-2 mRNA and protein expression.

3.5. Effects of SPHs on NF-κB and MAPKs signaling pathways in LPS-induced RAW264.7 cells

To gain the insights into the molecular mechanisms underlying the inhibitory effect of 1.0–3.5 kDa peptide fractions on pro-inflammatory cytokines and mediators, NF-κB and MAPKs signaling pathways were further investigated. As shown in Fig. 5A, LPS treatment strongly ($P < 0.01$) induced the phosphorylation of IκBα (3.38-fold) and p65 (8.08-fold) compared with the control group. However, SSPHs-2, TSPHs-2, MSPHs-2 fractions (100 μg/mL) treatment obviously ($P < 0.01$) inhibited the phosphorylation of IκBα by 30.8%, 73.7%, 42.1% and p65 by

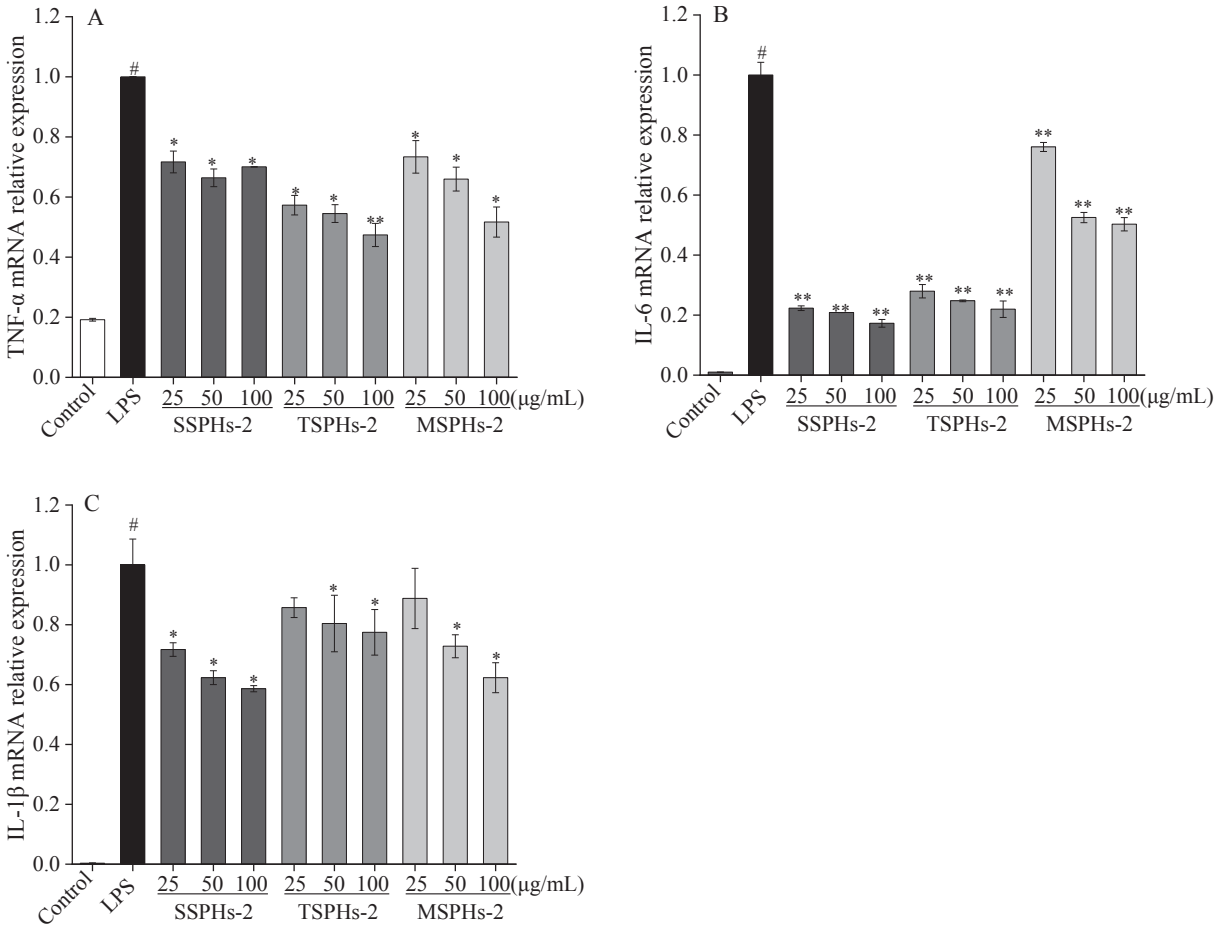

Fig.3. Effects of SSPHs-2, TSPHs-2, MSPHs-2 fraction on the mRNA expression of TNF-α (A), IL-1β (B), and IL-6 (C) in LPS-induced RAW264.7 macrophages. The results are expressed as the mean ± SD; # indicated P < 0.01 compared with the control group; * indicated $P < 0.05$, ** indicated $P < 0.01$ compared to LPS group.

28.1%, 47.4%, 50.9%, respectively, relative to LPS alone group. In addition, the phosphorylation levels of the ERK, JNK, and p38 in macrophages were increased about 6.07, 6.89 and 9.52-fold in the LPS treated group compared with the control group. Similarly, the phosphorylation levels were inhibited by 1.0–3.5 kDa peptide fractions, especially at the concentration of 100 μg/mL. Compared with the LPS group, SSPHs-2, TSPHs-2, MSPHs-2 fractions (100 μg/mL) prevented the phosphorylation about 44.72%, 68.84%, 55.11% for ERK, 75.73%, 51.90%, 58.44% for JNK; 58.27%, 63.36%, 51.04% for p38. The results demonstrated that the activation of MAPK signaling pathway was blocked by 1.0–3.5 kDa peptide fractions.

3.6. Correlation between Se concentration and anti-inflammatory activity

To further investigate whether selenium is involved in the anti-inflammatory capacity of SPHs, the selenium content was determined by hydride atomic fluorescence spectrometry. As shown in Table 2, Se content in SSPHs-2, TSPHs-2, MSPHs-2 was 590.32 ± 1.97, 913.44 ± 11.76, 1785.18 ± 7.63 μg/kg, respectively. The relationship between Se content and the inflammatory effect of SPHs was determined by correlation analysis. The results in Table 3 indicated that the inhibitory effect of 1.0–3.5 kDa peptide fractions (100 μg/mL) on the secretion of proinflammatory mediators (NO, PGE_2, TNF-a, IL-1β, and IL-6) had a positive correlation with the Se concentration; the value of the coefficient R was approximately 0.847–1.000. Therefore, the Se concentration was maybe one of the factors that affected the anti-inflammatory activity of 1.0–3.5 kDa peptide fractions.

3.7. Identified the amino acid sequence of TSPHs-2 fraction by LC-MS/MS.

TSPHs-2 fraction which exhibit the highest anti-inflammatory activity was selected for the amino acid sequence analysis by LC-MS/MS. As shown in Fig. 6, the sequences Ala-Leu- Leu- Leu-Gln-Ala-Val-Gln-Ser-Gln-Tyr-Glu-Glu-Lys (ALLLQAVQS QYEEK) were identified by searching the database, and the sequences contained mostly hydrophobic amino acids, such as Glutamine residue(Q), Leucine(L), Alanine (A), Valine(V), and aromatic amino acid of Tyrosine, which were characteristic anti-inflammatory activity amino acids according to the previous study (Ma, Liu, Shi, & Yu, 2016; Marcela, Blanca, Jose, Rosalva, & Cristina, 2018). The results indicated that selenium-enriched brown rice protein peptide (1.0–3.5 kDa) is rich in amino acid residues related with anti-inflammatory activity.

4. Discussion

Recently, Se-containing proteins from Se-enriched agri-food have attracted widely attention due to the extraordinary biological activities. Among them, Se-containing rice protein has been extensively studied due to their hypoallergenic, digestive, and absorption roles in human body and biological activities such as antioxidant (Liu et al., 2015), immunomodulatory (Fang et al., 2017, 2019) and relief of toxicity of heavy metals (Xu et al., 2016) activities. Fang et al. reported the *in vitro* antioxidant activity of enzymatic hydrolysates from selenium-enriched broken rice protein and found that the hydrolysis of Se-enriched rice protein by trypsin resulted in the highest CAA. However, study about the

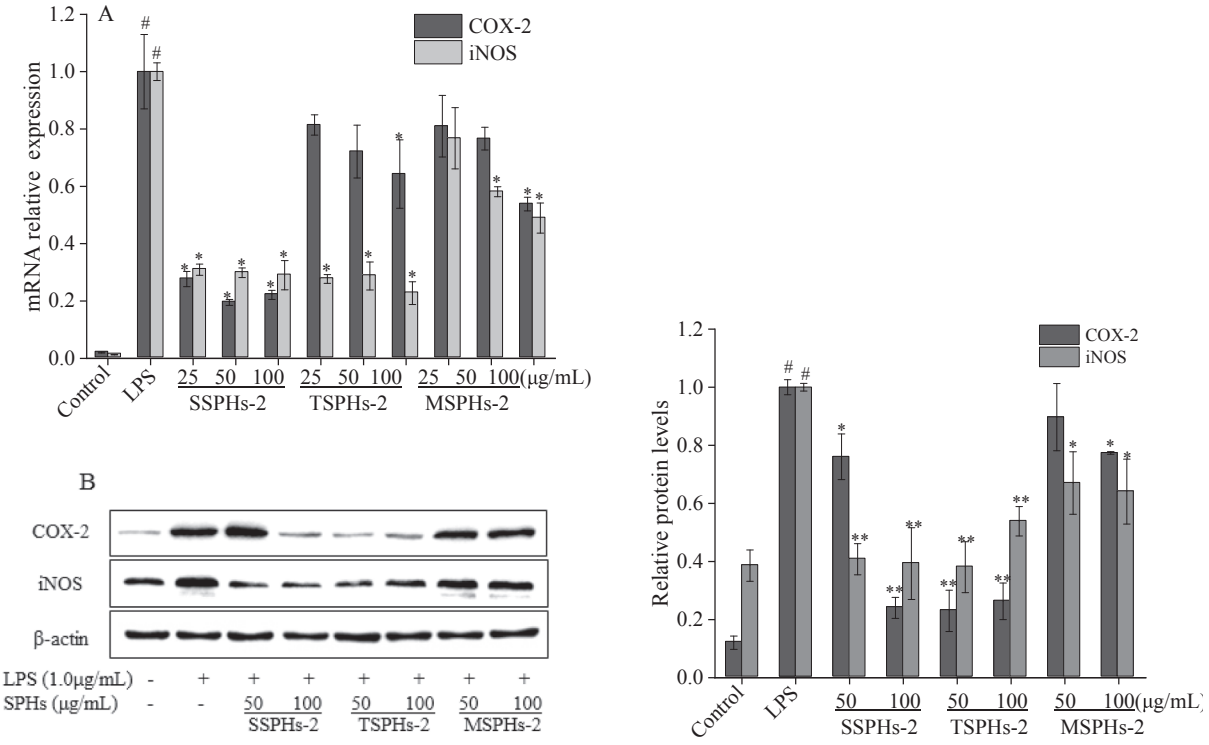

Fig. 4. Effects of SSPHs-2, TSPHs-2, MSPHs-2 fractions on the mRNA (A) and protein (B) expressions of iNOS/COX-2 in LPS-induced RAW264.7 macrophages. The results are expressed as the mean ± SD; # indicated $P < 0.05$ compared with the control group; * indicated $P < 0.05$, ** indicated $P < 0.01$ compared to LPS group.

anti-inflammatory property of Se-containing rice protein hydrolysates remains deficiency. This study determined the anti-inflammatory activity and potential mechanism of three types Se-enriched brown rice protein hydrolysate fractions in LPS-stimulated RAW264.7 macrophages. NO is an important mediator produced by iNOS in the inflammation process (Fatou, Tri, Jairo, Aluko, & Chantal, 2012). In the present study, the inhibitory effect of SSPHs, TSPHs, MSPHs with different molecular weight fractions on the production of NO were explored (Fig. 1B). The improvement of NO content induced by LPS was significantly inhibited by SPHs in different degree, fractions with molecular weight 1.0–3.5 kDa exhibited the most effective inhibitory activity, particularly at high concentration (100 μg/mL). Fang et al. (2019) found that Se-enriched rice protein acylase hydrolysates significantly reduced NO production (40.17% and 38.45%) at concentrations of 100 μg/mL and 160 μg/mL, respectively, compared with positive group. The study of Xu et al. also showed that Se-enriched rice protein hydrolysates (SPH-3, 160 μg/mL) decreased the levels of NO production (72.2%) in RAW264.7 cells induced by Pb^{2+}. In addition, LPS-stimulated macrophages lead to the release of inflammatory mediators such as PGE_2, TNF-α, IL-1β, and IL-6, all of which exhibit various biological functions, for instance regulating immune responses, inflammation and homeostasis (Zhai et al., 2016). TNF-α is a key mediator produced by activated macrophages, take part in a variety of physiological and pathological processes such as inflammation, cellular immunity and tumor immunity (Fan et al., 2015). IL-1β and IL-6 are considered as crucial pro-inflammatory cytokines which lead to kinds of inflammatory related diseases (KimKo et al., 2016). Inhibition of the excessively production of PGE_2, IL-1β, IL-6 and TNF-α was considered as an capable therapeutic approach for clinical treatment of inflammatory diseases (Kim, Ahn, & Je, 2016). The results in Fig. 2 showed that SPHs obviously decreased the production of PGE_2, IL-1β, IL-6, and TNF-α, and SSPHs-2, TSPHs-2, MSPHs-2 (100 μg/mL) exhibited the most obviously inhibitory effect. Similarly, Zhu et al. (2016) reported that Se-containing phycocyanin (Se-PC) reduced the level of IL-6, TNF-α. Ahn, Je, and Cho (2012) reported that salmon byproduct protein hydrolysates with a molecular weight of 1.0–2.0 kDa showed anti-inflammatory activity by inhibiting production of TNF-α (58.90%), IL-6 (36.42%) and IL-1β (83.50%) in LPS-induced RAW264.7 macrophages. The present study demonstrated that 1.0–3.5 kDa peptide fractions from Se-enriched brown rice at 100 μg/mL significantly inhibited the production of PGE_2, TNF-α, IL-1β, and IL-6 in LPS-induced RAW264.7 macrophages, therefore improved cellular inflammation.

Recently, studies reported that inflammatory genes were mainly expressed in activated macrophages (Zhang & Wang, 2014). The results of RT-qPCR demonstrated that the improvement of TNF-α, IL-1β, and IL-6 was reversed in a concentration-dependent manner by SSPHs-2, TSPHs-2, MSPHs-2, which is consistent with the alterations of corresponding cytokines (Fig. 2). Previous studies have demonstrated that rice protein hydrolysates fraction (RPHs-C-7–3) significantly inhibited mRNA expression of TNF-a, IL-6, and IL-1β (Li, Li, Zhou, & Cheng, 2016). Udenigwe et al. (2013) also reported that treatment of the activated cells with the almond protein hydrolysate fraction resulted in the inhibition of relative gene expressions of TNF-α, IL-1β, and IL-6. In addition, iNOS and COX-2 are two important inflammatory mediators, involved in the inflammatory process. In activated macrophages, iNOS produces a large amount of NO in the presence of arginine as a matrix; COX-2 is the key enzyme involved in the conversion of arachidonic acid to PGE_2 (Chu, Tang, Huang, Hao, & Wei, 2016). Regulating the expression of iNOS and COX-2 is considered as one of the strategies to alleviate inflammatory diseases. In the present study, the mRNA and protein expression levels of iNOS/COX-2 were markedly ($P < 0.01$) upregulated by LPS treatment, however, peptide fraction (1.0–3.5 kDa) treatments markedly reduced the improvement.

NF-κB was a key signaling pathway responsible for managing the transcription of pro-inflammatory cytokines and mediator, such as IL-1β, IL-6, TNF-α, NO and PGE_2 (Kim et al., 2016; Li et al., 2018). The basic NF-κB signaling pathways including receptor, receptor proximal signaling adaptors, IκB kinase complexes, IκB proteins and NF-κB dimers. When stimulated by extracellular signals, such as LPS, pro-inflammatory cytokines and reactive oxygen species, IκB kinase was

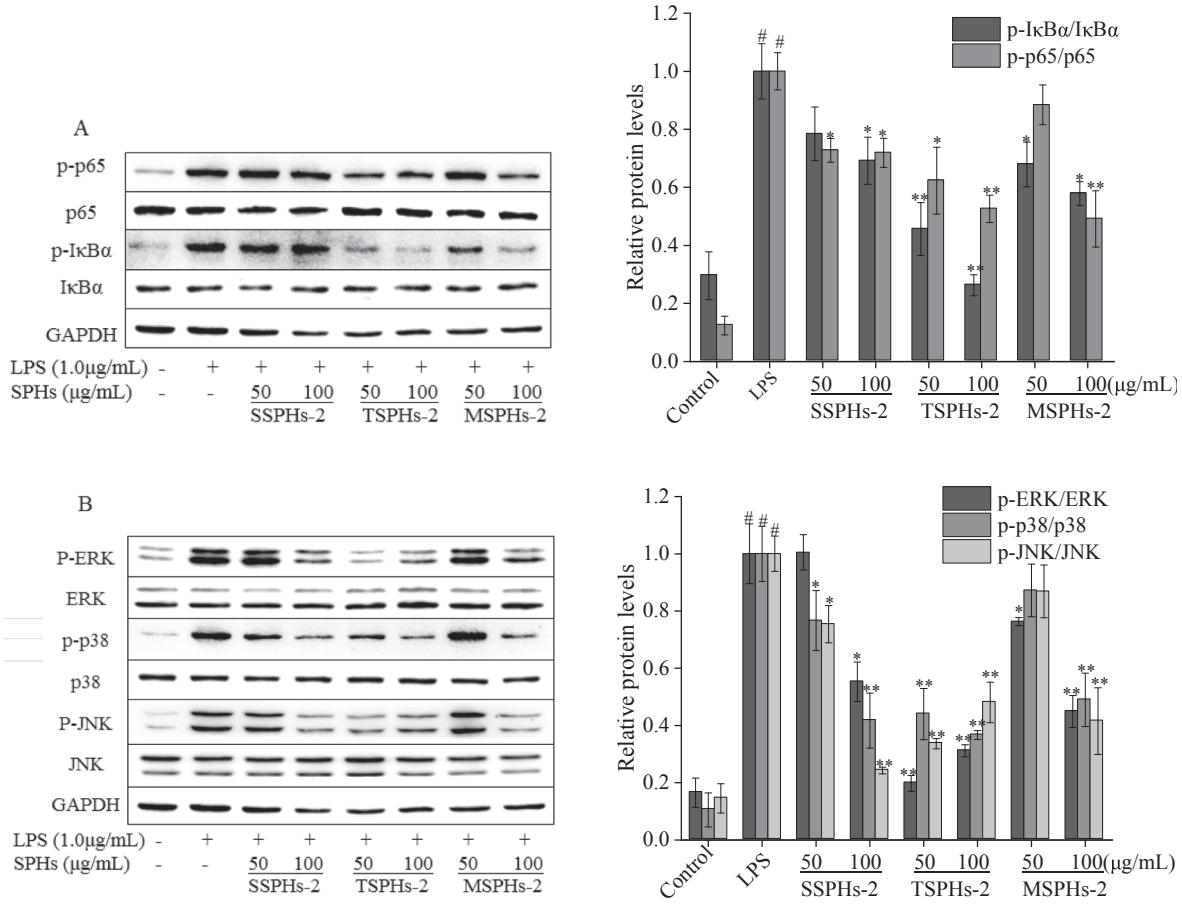

Fig. 5. Effects of SSPHs-2, TSPHs-2, MSPHs-2 fractions on NF-κB (A) and MAPKs signal pathways (B) in LPS-induced RAW264.7 macrophages. The results are expressed as the mean ± SD; # indicated $P < 0.05$ compared with the control group; * indicated $P < 0.05$, ** indicated $P < 0.01$ compared to LPS group.

Table 2
The Se contents of selenium rich brown rice protein hydrolysates.

Molecular weight distribution Mw(kDa)	Selenium content (μg/kg)		
	SSPHs	TSPHs	MSPHs
<1.0	156.16 ± 6.41[a]	358.45 ± 5.19[a]	909.48 ± 2.06[a]
1.0–3.5	590.32 ± 1.97[c]	913.44 ± 11.76[c]	1785.18 ± 7.63[c]
>3.5	451.32 ± 2.23[b]	679.38 ± 0.83[b]	1438.33 ± 10.46[b]

Table 3
Correlation analysis of Se concentration and anti-inflammatory activity of selenium rich brown rice protein hydrolysates with molecular weight 1.0–3.5 kD.

Selenium content	Anti-inflammatory activity				
	NO	PGE_2	TNF-α	IL-1β	IL-6
SSPHs-2	0.847	0.636	0.846	0.222	0.964
TSPHs-2	0.896	0.908	0.881	0.975	0.949
MSPHs-2	0.981	0.995	0.844	0.979	1.000

activated and resulted in the phosphorylation and degradation of IκBα, which exposed nuclear localization sites of NF-κB (Fan et al., 2015). Free NF-κB p65 subunit rapidly translocated into nucleus and combined with their promoter to initiate target genes expression (Zhai et al., 2016). The results in the present study showed that SSPHs-2, TSPHs-2, MSPHs-2 fractions (100 μg/mL) treatment obviously ($P < 0.01$) inhibited the phosphorylation of IκBα and p65 (Fig. 5A). These observations were in accordance with the previous findings that bioactive peptides act as negative regulators in LPS-induced activation of NF-κB pathway (Cheng, Gao, Chen, & Mao, 2015; Li et al., 2018; Oseguera-Toledo, Mejia, Dia, & Amaya-Llano, 2011). In addition to NF-κB, MAPKs signaling pathway also play an vital role in regulating the biosynthesis and secretion of inflammatory mediators (Coskun, Olsen, Seidelin, & Nielsen, 2011). With the stimulation of LPS or other inflammatory mediators, MAPKs occurred to phosphorylation and subsequently activated the downstream signaling pathways such as NF-κB (Kyriakis & Avruch, 2012; Verica & Harnett, 2013). As shown in Fig. 5B, SSPHs-2, TSPHs-2, MSPHs-2 fractions (100 μg/mL) prevented the phosphorylation of ERK, JNK, and p38 induced by LPS treatment. Similarly, hydrolysates fraction originated from Mytilus edulis and casein glycomacropeptide have been reported to inhibit LPS-induced MAPK activation in RAW 264.7 macrophages (Kim et al., 2016; Li et al., 2018). The potential of food protein-derived bioactive peptides against chronic intestinal inflammation and related mechanism has been reviewed by Zhu et al., which suggested that food protein derived bioactive peptides (BAPs) exert anti-inflammatory bioactivities through four possible mechanism pathways including NF-κB, MAPK, Janus kinase-signal transducer and activator of transcription (JAK-STAT), and peptide transporter 1 (PepT1) (Zhu et al., 2020). Among these pathways, the NF-κB and MAPK pathways are two main pathways for BAPs to inhibit inflammation. Results in the present study also showed that SPHs regulated LPS-induced RAW264.7 cells through inhibiting the phosphorylation of proteins in NF-κB and MAPK signaling pathways.

To explore the contributions of Se to the anti-inflammatory activity of 1.0–3.5 kDa peptide fraction, the correlation between the Se concentration and anti-inflammatory activity of 1.0–3.5 kDa peptide

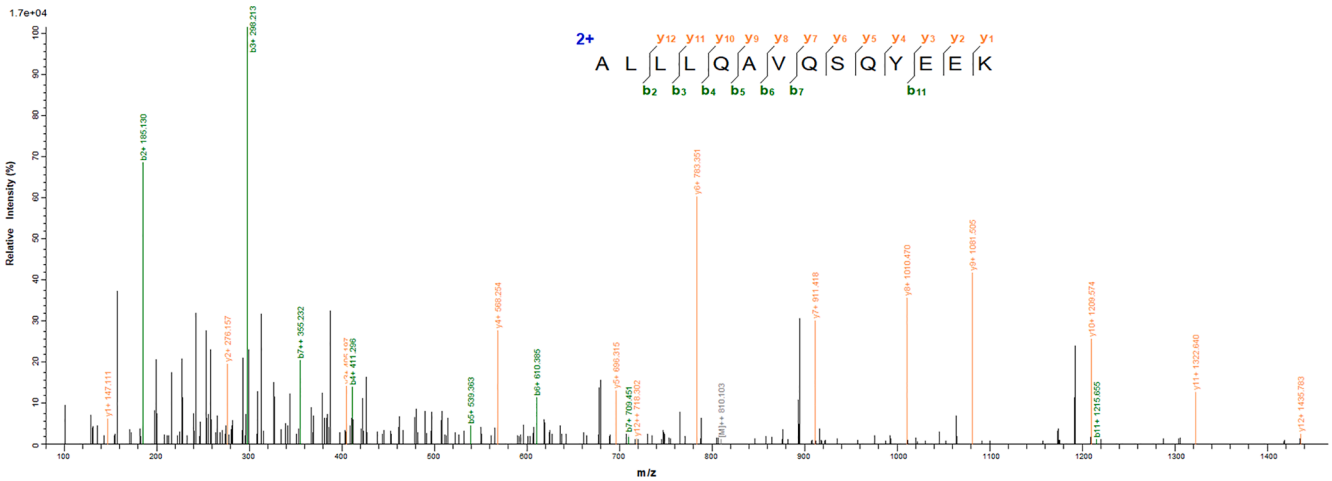

Fig. 6. Mass spectrum of TSPHs-2 fraction from Taoyuan selenium-enriched brown rice protein hydrolysate by LC-MS/MS.

fraction was analyzed by Pearson correlation analysis. The results demonstrated that the inhibitory effect of peptide fractions on the production of NO, PGE_2, TNF-a, IL-1β, and IL-6 had a positive correlation with the Se concentration. Zhu et al. confirmed that Se-containing phycocyanin (Se-PC) from Se-enriched Spirulina platensis reduced inflammation by suppressing the nuclear translocation of NF-κB and modulating the release of cytokines, including IL-6, TNF-α, MCP-1, and IL-10 (Zhu et al., 2016). A similar result showed that the Se significantly impact the biological activity of the enzymatic hydrolysates from selenium-enriched rice protein (Fang et al., 2017). The activities of Se probably act via Se-peptides or selenoamino acids in SPHs. In addition, research showed that the anti-inflammatory activity of peptides was highly dependent on their structures including amino acid composition and sequence, type of amino acid in C and N termini, charge distribution, length of peptide chain, hydrophobicity/hydrophilicity, and spatial structure, (Ma et al., 2016; Yu, Field, & Wu, 2018). Marcela et al. (2018) found that germinated soybean proteins hydrolysate peptides (QQQQQGGSQSQ, QEPQESQQ, QQQQQGG SQSQSQKG, PETMQQQQQQ) is rich in glutamine (Q) residue, which thought to be the main amino acid involved in anti-inflammatory property. In addition, peptide (Asp-Gln-Trp-Leu, DQWL) from whey protein hydrolysates showed the strongest inhibitory ability on the mRNA expression and production of IL-1β, COX-2, and TNF-α (Ma et al., 2016). These results indicated that the anti-inflammatory activity of the bioactive peptide was related with its amino acid sequence. In the present study, TSPHs-2 fraction with the sequences of Ala-Leu-Leu-Leu-Gln-Ala-Val-Gln-Ser-Gln-Tyr-Glu-Glu-Lys (ALLLQAVQS QYEEK), is the most effective anti-inflammation peptides, which were characteristic anti-inflammatory activity amino acids reported by Ma et al. (2016), Marcela et al. (2018). Above results showed that Se concentration and amino acid sequence were two vital factor that effect the anti-inflammatory of SPHs.

5. Conclusions

In the present study, the anti-inflammatory mechanism of three kinds of selenium-enriched brown rice protein hydrolysate were investigated. The results showed that the 1.0–3.5 kDa peptide fractions (SSPHs-2, TSPHs-2, MSPHs-2) exhibited the most effective anti-inflammatory activity. The levels of inflammatory mediators and corresponding mRNA and protein expression were inhibited by SSPHs-2, TSPHs-2, MSPHs-2 through regulating NF-κB/MAPKs signaling pathways. There is a positive correlation between Se content and the inflammatory property of SPHs. The most active peptide fraction was TSPHs-2 with the sequence ALLLQAVQSQYEEK. Our results showed that Se-enriched brown rice protein hydrolysates based functional foods and nutraceutical products can be a potential alternative approach to the management and prevention of inflammation or related diseases.

Ethics statement

The research did not include any human subjects and animal experiments.

CRediT authorship contribution statement

Mingju Feng: Data curation, Investigation, Methodology, Software, Writing - original draft. **Xiaoya Wang:** Data curation, Investigation, Methodology, Software, Writing - original draft. **Hua Xiong:** Supervision, Funding acquisition. **Tingting Qiu:** Data curation, Formal analysis. **Hua Zhang:** Methodology, Writing - original draft. **Fanghua Guo:** Data curation, Formal analysis. **Li Jiang:** Validation, Visualization. **Yong Sun:** Conceptualization, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

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

Acknowledgements

This study was supported by the Research Program of State Key Laboratory of Food Science and Technology, Nanchang University (Project No. SKLF-ZZA-201609 and SKLF-ZZA-201909), Key project '5511' for Science and Technology Research of Jiangxi Province (S2018ZDYFE0040), Major special projects of Jiangxi Academy of Sciences (2018-YZD1-05), National Natural Science Foundation of China (82060781) and China Postdoctoral Science Foundation (2020M671975).

References

Ahn, C. B., Je, J. Y., & Cho, Y. S. (2012). Antioxidant and anti-inflammatory peptide fraction from salmon byproduct protein hydrolysates by peptic hydrolysis. *Electronic Resource, 49*(1), 92–98.

Chen, L. C., Yang, F. M., Xu, J., Hu, Y., Hu, Q. H., Zhang, Y. L., & Pan, G. X. (2002). Determination of selenium concentration of rice in china and effect of fertilization of selenite and selenate on selenium content of rice. *Journal of Agricultural and Food Chemistry, 50*(18), 5128–5130.

Cheng, X., Gao, D. X., Chen, B., & Mao, X. Y. (2015). Endotoxin-binding peptides derived from casein glycomacropeptide inhibit lipopolysaccharide-stimulated inflammatory responses via blockade of NF-κB activation in macrophages. *Nutrients, 7*(5), 3119–3137.

Chu, H., Tang, Q., Huang, H., Hao, W., & Wei, X. (2016). Grape-seed proanthocyanidins inhibit the lipopolysaccharide-induced inflammatory mediator expression in RAW264.7 macrophages by suppressing MAPK and NF-κb signal pathways. *Environmental Toxicology & Pharmacology, 41*, 159–166.

Coskun, M., Olsen, J., Seidelin, J. B., & Nielsen, O. H. J. (2011). MAP kinases in inflammatory bowel disease. *Clinica Chimica Acta, 412*(7), 513–520.

Fan, X. L., Zhang, Y. H., Dong, H. Q., Wang, B. Y., Ji, H. Q., & Liu, X. (2015). Trilobatin attenuates the LPS-mediated inflammatory response by suppressing the NF-κB signaling pathway. *Food Chemistry, 166*, 609–615.

Fang, Y., Zhang, Y. F., Brittany, Joseph, C., & Hu, Q. H. (2010). Distribution and in vitro availability of selenium in selenium-containing storage protein from selenium-enriched rice utilizing optimized extraction. *Journal of Agricultural & Food Chemistry, 58*(17), 9731–9738.

Fang, Y., Chen, X., Luo, P. Z., Kimatu, B. M., Liu, K. L., & Hu, Q. H. (2017). The correlation between in vitro antioxidant activity and immunomodulatory activity of enzymatic hydrolysates from selenium-enriched rice protein. *Journal of Food Science, 82*(2), 517–522. https://doi.org/10.1111/1750-3841.13595.

Fang, Y., Pan, X., Zhao, E., Shi, Y., Shen, X., Wu, J., … Hu, Q. (2019). Isolation and identification of immunomodulatory selenium-containing peptides from selenium-enriched rice protein hydrolysates. *Food Chemistry, 275*, 696–702. https://doi.org/10.1016/j.foodchem.2018.09.115.

Fatou, N., Tri, V., Jairo, D., Aluko, R. E., & Chantal, M. J. E. J.o. N. (2012). Anti-oxidant, anti-inflammatory and immunomodulating properties of an enzymatic protein hydrolysate from yellow field pea seeds. *European Journal of Nutrition, 51*(1), 29–37.

Ge, K., & Yang, G. J. (1993). The epidemiology of selenium deficiency in the etiological study of endemic diseases in China. *The American Journal of Clinical Nutrition, 57*(2 Suppl), 259S.

Jiang, F., Guan, H., Liu, D., Wu, X., Fan, M., Han, J. J. F., & Function. (2017). Flavonoids from sea buckthorn inhibit the lipopolysaccharide-induced inflammatory response in RAW264.7 macrophages through the MAPK and NF-κB pathways. *Food Function, 8*(3), 1313.

Kim, Y. S., Ahn, C. B., & Je, J. Y. (2016). Anti-inflammatory action of high molecular weight Mytilus edulis hydrolysates fraction in LPS-induced RAW264.7 macrophage via NF-κB and MAPK pathways. *Food Chemistry, 202*, 9–14.

KimKo, S. C., Ye, B. R., Kim, M. S., Kim, J., Ko, E. Y., & Jung, W. K. (2016). 5-Bromo-2-hydroxy-4-methyl-benzaldehyde inhibited LPS-induced production of pro-inflammatory mediators through the inactivation of ERK, p38, and NF-κB pathways in RAW 264.7 macrophages. *Chemico Biological Interactions, 258*, 108–114.

Kyriakis, J. M., & Avruch, J. (2012). Mammalian MAPK signal transduction pathways activated by stress and inflammation: A 10-year update. *Physiological Reviews, 92*(2), 689.

Li, W., Chen, Y. H., Zhang, L., Yu, H. X., Zhou, X., You, H. X., & Cheng, Y. J. (2016). Rice protein hydrolysates (RPHs) inhibit the LPS-stimulated inflammatory response and phagocytosis in RAW264.7 macrophages by regulating the NF-κB signaling pathway. *Rsc Advances, 6*(75), 71295–71304.

Li, T. G., Gao, D. X., Du, M., Cheng, X., & Mao, X. Y. (2018). Casein glycomacropeptide hydrolysates inhibit PGE2 production and COX2 expression in LPS-stimulated RAW 264.7 macrophage cells via Akt mediated NF-κB and MAPK pathways. *Food & Function, 9*(4), 10.1039.C1037FO01989K.

Liu, K. L., Cao, X. H., Bai, Q. Y., Wen, H. B., & Gu, Z. X. (2009). Relationships between physical properties of brown rice and degree of milling and loss of selenium. *Journal of Food Engineering, 94*(1), 69–74.

Liu, K. L., Chen, F. S., Zhao, Y., Gu, Z. X., & Yang, H. S. (2011). Selenium accumulation in protein fractions during germination of Se-enriched brown rice and molecular weights distribution of Se-containing proteins. *Food Chemistry, 127*(4), 1526–1531. https://doi.org/10.1016/j.foodchem.2011.02.010.

Liu, K. L., Zhao, Y., Chen, F. S., & Fang, Y. (2015). Purification and identification of Se-containing antioxidative peptides from enzymatic hydrolysates of Se-enriched brown rice protein. *Food Chemistry, 187*, 424–430. https://doi.org/10.1016/j.foodchem.2015.04.086.

Lugrin, J., Rosenblatt-Velin, N., Parapanov, R., & Liaudet, L. (2014). The role of oxidative stress during inflammatory processes. *Biological Chemistry, 395*(2), 203–230. https://doi.org/10.1515/hsz-2013-0241.

Ma, Y., Liu, Y., Shi, H. M., & Yu, L. L. (2016). Isolation and characterization of anti-inflammatory peptides derived from whey protein. *Journal of Dairy Science, 99*(9), 6902–6912.

Marcela, Blanca, Jose, Rosalva, & Cristina. (2018). Peptides derived from in vitro gastrointestinal digestion of germinated soybean proteins inhibit human colon cancer cells proliferation and inflammation. *Food Chemistry, 242*(MAR.1), 75–82.

Oropeza-Moe, M., Wisløff, H., & Bernhoft, A. J. J. T. E. M. B. (2015). Selenium deficiency associated porcine and human cardiomyopathies. *Breast Cancer, 31*, 148–156.

Oseguera-Toledo, Mejia, Dia, & Amaya-Llano. (2011). Common bean (Phaseolus vulgaris L) hydrolysates inhibit inflammation in LPS-induced macrophages through suppression of NF-KB pathways. *Food Chemistry, 127*(3), 1175–1185.

Rayman, M. P. J. L. (2000). The importance of selenium to human health. *Lancet, 356*(9225), 233–241.

Ren, D. Y., Wang, P., Liu, C. L., Wang, J., Liu, X. Q., Liu, J. S., & Min, W. H. (2018). Hazelnut protein-derived peptide LDAPGHR shows anti-inflammatory activity on LPS-induced RAW264.7 macrophage. *Journal of Functional Foods., 46*, 449–455.

Ruslan, M. (2008). Origin and physiological roles of inflammation. *Nature, 454*(7203), 428–435.

Udenigwe, C., Je, J. Y., Cho, Y. S., & Yada, R. Y. (2013). Almond protein hydrolysate fraction modulates the expression of proinflammatory cytokines and enzymes in activated macrophages. *Food & Function, 4*(5), 777.

Valledor, A. F., Comalada, M., Santamaría-Babi, L. F., Lloberas, J., & Celada, A. J. A. i. I. (2010). Chapter 1–Macrophage proinflammatory activation and deactivation: A question of balance. Advances in Immunology 108, 1–20.

Verica, P., & Harnett, M. M. (2013). Mitogen-activated protein kinases as therapeutic targets for rheumatoid arthritis. *Drugs, 73*(2), 101–115.

Wen, L., Chen, Y., Zhang, L., Yu, H., Xu, Z., You, H., & Cheng, Y. J. R. A. (2016). Rice protein hydrolysates (RPHs) inhibit the LPS-stimulated inflammatory response and phagocytosis in RAW264.7 macrophages by regulating the NF-κB signaling pathway. *RSC Advances, 6*(75), 71295–71304.

Xu, Z., Fang, Y., Chen, Y., Yang, W. J., Ma, N., Pei, F., & Hu, Q. H. (2016). Protective effects of Se-containing protein hydrolysates from Se-enriched rice against Pb2+-induced cytotoxicity in PC12 and RAW264.7 cells. *Food Chemistry, 202*, 396–403.

Yu, W. L., Field, C. J., & Wu, J. P. (2018). Purification and Identification of Anti-inflammatory Peptides from Spent Hen Muscle Proteins Hydrolysate. *Food Chemistry, 253*. S030881461830102X.

Zhai, X. T., Zhang, Z. Y., Jiang, C. H., Chen, J. Q., Ye, J. Q., Jia, X. B., … Song, J. (2016). Nauclea officinalis inhibits inflammation in LPS-mediated RAW 264.7 macrophages by suppressing the NF-κB signaling pathway. *Journal of Ethnopharmacology, 183*, 159–165.

Zhang, L., & Wang, C. C. (2014). Inflammatory response of macrophages in infection. *Hepatobiliary & Pancreatic Diseases International, 13*(2), 138–152.

Zhao, P., Wang, Y., Zhang, Y., Guo, T., Zhang, Z., Zhang, W. J., … Ashraf, M. A. (2016). The extraction of different proteins in selenium enriched peanuts and their antioxidant properties. *Saudi Journal of Biological Sciences, 23*(3), 353–357. https://doi.org/10.1016/j.sjbs.2015.08.007.

Zhu, C. H., Ling, Q. J., Cai, Z. C., Wang, Y., Zhang, Y., Hoffmann, P. R., … Huang, Z. (2016). Selenium-containing phycocyanin from Se-enriched spirulina platensis reduces inflammation in dextran sulfate sodium-induced colitis by inhibiting NF-kappa B activation. *Journal of Agricultural and Food Chemistry, 64*(24), 5060–5070. https://doi.org/10.1021/acs.jafc.6b01308.

Zhu, S., Du, C. D., Yu, T., Cong, X., Liu, Y. F., Chen, S. W., & Li, Y. (2019). Antioxidant activity of selenium-enriched peptides from the protein hydrolysate of cardamine violifolia. *Journal of Food Science, 84*(12), 3504–3511. https://doi.org/10.1111/1750-3841.14843.

Zhu, W., Ren, L., Zhang, L., Qiao, Q., Farooq, M. Z., & Xu, Q. (2020). The potential of food protein-derived bioactive peptides against chronic intestinal inflammation. *Mediators of Inflammation, 2020*, 6817156. https://doi.org/10.1155/2020/6817156.