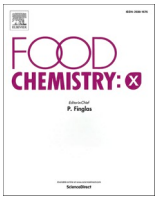
Food Chemistry: X 19 (2023) 100837

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Proteomics reveals energy limitation and amino acid consumption as antibacterial mechanism of linalool against *Shigella sonnei* and its application in fresh beef preservation

Rongrong He [a], Haiming Chen [a], Hao Wu [a], Jicai Liu [a], Weijun Chen [a,b], Ming Zhang [a], Wenxue Chen [a,*], Qiuping Zhong [a,*]

[a] HNU-HSF Collaborative Innovation Laboratory, College of Food Sciences & Engineering, Hainan University, 58 People Road, Haikou 570228, China
[b] Chunguang Agro-product Processing Institute, Wenchang 571333, China

ARTICLE INFO

Keywords:
Shigella sonnei
Linalool
Antibacterial mechanism
Proteomic
Energy limitation
Amino acid consumption

ABSTRACT

Meat is often contaminated by food-borne pathogens, resulting in significant economic losses. Linalool from plant essential oils (EOs) has been reported to have excellent antibacterial properties. Therefore, this study aims to elucidate the mechanism of linalool against *Shigella sonnei* (*S. sonnei*) based on proteomic and physiological indicators. The results indicated that linalool severely perturbed the expression levels of intracellular proteins, of which 208 were up-regulated and 49 were down-regulated. Moreover, linalool exerted its inhibitory effect mainly through the induction of amino acid limitation and insufficient energy levels based on the pathways involved in differential expressed proteins (DEPs). After 8 h, alkaline phosphatase (AKP) leakage increased 20.96 and 21.52-fold in the MIC and 2MIC groups while protein leakage increased 2.17 and 2.50-fold, respectively, which revealed the potential of linalool on cell structure damage combined with nucleic acid leakage. In addition, the ATP content decreased to 36.92% and 18.84% in the MIC and 2MIC groups, respectively when processed for 8 h. In particular, linalool could effectively control the quality change of fresh beef by measuring pH, total volatile basic nitrogen (TVB-N), total viable counts (TVC) while not affecting its sensory acceptability based on the result of sensory evaluation. This research provides theoretical insights for the development of linalool as a new natural antibacterial agent.

1. Introduction

Meat including beef is often an ideal carrier for pathogenic bacteria due to its high protein, fat and moisture content (Lin, Mao, Sun, Rajivgandhi, & Cui, 2019). *Shigella*, is commonly detected in multiple foods including ground beef (Ahmed & Shimamoto, 2015; Kang, Liu, Liu, Wu, & Li, 2018), which not only threatens human health, but also causes great economic losses. *Shigella*, a gram-negative bacteria, consists of four species including *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*, which ranked third after *Salmonella* and *Campylobacter* in the bacteria associated with food-related infections (Zhang et al., 2011). As one of the *Shigella*, *Shigella sonnei* (*S. sonnei*) is expanding unprecedentedly in industrialized regions including Asia, Latin America and the Middle East due to the resistance exhibited to multiple antibiotics (Gu et al., 2012; Thompson, Duy, & Baker, 2015). Despite improvements in drinking water and food hygiene, Shigellosis caused by *S. sonnei* was still internationally prevalent, and its multi-drug resistant strains were rapidly expanding (Tickell et al., 2017; Ud-Din et al., 2013). They can transmit exogenous genes related to mobile genetic components including transposons, integrons, R-plasmids and genome islands related to bacterial chromosomes (Ud-Din et al., 2013). Once the intestinal tract is infected with *S. sonnei*, abdominal cramping, diarrhea, nausea, vomiting, fever, and severe fluid loss may occur (Chai, Lee, Kim, & Oh, 2016). Therefore, the risk of *S. sonnei* infection combined with negative perceptions of synthetic preservatives, has forced researchers to seek effective and safe natural antibacterial agents.

In recent years, essential oils (EOs) from natural ingredients have attracted the attention of researchers. Reports confirmed that EOs including oregano and clove have excellent antibacterial effects against a variety of pathogenic bacteria (Cui, Zhang, Li, & Lin, 2019; Hu, Li, Dai, Cui, & Lin, 2019). However, inconsistencies in composition due to geographic origin and harvest time may limit the application of EOs in

* Corresponding authors.
E-mail addresses: hnchwx@vip.163.com (W. Chen), hainufood88@163.com (Q. Zhong).

https://doi.org/10.1016/j.fochx.2023.100837
Received 29 November 2022; Received in revised form 7 July 2023; Accepted 11 August 2023
Available online 12 August 2023

the food industry (Burt, 2004). One of the effective ways to overcome this problem is to isolate specific components with antibacterial potential from EOs. Linalool is widely found in the EOs including green huajiao and basil (Diao, Hu, Feng, Li, & Xu, 2013; Hollander & Yaron, 2021; Pereira, Severino, Santos, Silva, & Souto, 2018), proving to have excellent inhibitory effect on multiple pathogenic bacteria. Our previous studies also found that linalool has outstanding inhibitory potential against spoilage and pathogenic bacteria including *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Listeria monocytogenes*, mainly by disrupting cell structure and impairing respiratory metabolism (Guo et al., 2021a; He et al., 2022a; Liu et al., 2020b). Although the mechanisms of linalool inhibition on *S. sonnei* have been initially reported based on the membrane damage and oxidative stress (He et al., 2023), the mechanisms involved at the molecular level have not been systematically investigated. Furthermore, it was reasonable to speculate that components from EOs would disrupt the expression of bacterial intracellular protein profiles according to previous report (Du, Yin, Yang, He, & Sun, 2021). However, the response mechanism of *S. sonnei* to linalool has not been clearly clarified based on physiological indicators combined with proteomic technology.

Proteomics was widely used as a highly sensitive method to study the action mechanisms of different drugs (Yang et al., 2016). Therefore, proteomic technology was used to analyze the mechanism of *S. sonnei* response to linalool in this study and its preservation potential for fresh beef. In addition, alkaline phosphatase (AKP), protein and nucleic acid leakage were also measured to validate the insights drawn in proteomics. Particularly, intracellular ATP content and SDS-PAGE were also performed as components of the mechanism of linalool against *S. sonnei*. This investigation aims to reveal the molecular mechanism of *S. sonnei* inhibition by linalool using proteomics techniques, encouraging the proposal of linalool as a natural antibacterial agent in the food industry.

2. Materials and methods

2.1. Bacterial strains and chemicals

S. sonnei ATCC 25931 was purchased from Guangdong Microbial Culture Preservation Center (Guangzhou, China), and cultured at 37 °C with nutrient broth medium (Solarbio Science & Technology Co., Ltd, China). Linalool (purity > 99%) was provided by Hainan Camphora Biotech Co., Ltd. (Hainan, China). AKP A059-1, Bicinchoninic acid (BCA) A045-4 and ATP A095-1-1 assay kits were obtained from Nanjing JianCheng Bioengineering Institute (Nanjing, China).

2.2. Determination of cell wall integrity

Referring to our previous report, the minimum inhibitory concentration (MIC) of linalool against *S. sonnei* used in this study was 1.5 mL/L (He et al., 2023). Subsequently, the leakage of AKP was used as an indicator to evaluate the cell wall integrity and simply, linalool at final concentrations of MIC and 2MIC was added as treatments when the bacterial suspension was grown to log phase. The supernatant was obtained for the determination of AKP content by centrifugation (6000 *g*, 4 °C, 10 min). In particular, the absorption value was measured at a wavelength of 520 nm using a microplate reader (SP-Max3500FL, Shanghai Flash Spectrum Biotechnology Co., Ltd., Shanghai, China).

2.3. Leakageof extracellular proteins and nucleic acids

The bacterial suspension was treated with linalool (MIC and 2MIC) after incubation to log phase and subsequently, the supernatant was collected by centrifugation (6000 *g*, 4 °C, 10 min) when treated for 0, 2, 4, 6, 8 h, respectively. Nucleic acid leakage was defined as absorbance at 260 nm and the content of protein was quantified using BCA protein assay kit.

2.4. SDS-PAGE

Intracellular proteins were analyzed using Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) following the procedures (Sitohy, Mahgoub, & Osman, 2012). Cells in log phase were harvested by centrifugation (6000 *g*, 4 °C, 10 min) and then resuspended after washing with PBS (phosphate buffer solution, 0.01 M, pH 7.2). Subsequently, linalool (MIC and 2MIC) was added to the bacterial suspension for co-culture and the samples were centrifuged again to get the cell pellet at 4 h. The prepared bacteria cells were processed with ultrasound (power 300 W, 15 min, interval 5 s) to release intracellular proteins. The samples (10 μL) were loaded on SDS-PAGE electrophoresis (Bio-Rad Mini-PROTEAN® Tetra, Bio–Rad Laboratories, Hercules, USA) at 80 V for 30 min followed by 120 V for 60 min. Subsequently, the gel was stained using coomassie blue R-250 stain solution and decolorized in water/methanol/acetic acid (5:4:1, v/v). Finally, the eluted protein bands were visualized by photographing.

2.5. Measurement of intracellular ATP concentrations

Linalool (MIC and 2MIC) was added to the bacterial suspension when the bacteria grew to log phase. Cell precipitates were collected by centrifugation and resuspended in saline for sonication as described in 2.4. Next, ATP in the samples was measured to assess the level of intracellular energy perturbation under linalool stress with the instructions of ATP content assay kit.

2.6. Proteomic analysis

2.6.1. Sample preparation

The change in protein profile of *S. sonnei* treated with linalool was analyzed to uncover the mechanism of linalool as description of Liao et al. (2019). Generally, the final concentration of linalool at MIC was selected as the treatment when *S. sonnei* was cultured to log phase. Subsequently, cells were harvested by centrifugation (3000 *g*, 4 °C, 10 min) and washed 3 times with PBS when co-cultured for 4 h. Cell pellets were quenched with liquid nitrogen immediately for proteomic analysis.

2.6.2. Protein extraction

Lysate (4% SDS, 100 mM Tris-HCl, pH 7.6) was added to the cell pellet and then pellets were disrupted using MP Fastprep-24 homogenizer (6.0 M/S, 30 s, 2 times). Boiling water bath was performed for 10 min after sonication and subsequently, the supernatant was collected by centrifugation (14000 *g*, 15 min). Afterwards, the protein content was determined with BCA protein assay kit when the supernatant was filtered using 0.22 μm filters. Protein (20 μg) was mixed with loading buffer and then treated with boiling water bath for 5 min for SDS-PAGE analysis.

2.6.3. FASP (Filter aided sample Preparation) enzymatic hydrolysis and TMT labeling

Referring to previous report (Sarengaowa et al., 2019), protein solution (150–200 μg) were reduced with dithiothreitol (DTT, 100 mM) for 5 min in boiling water. Protein samples were centrifuged under the condition (12500 *g*, 15 min) after mixing with UA buffer (8 M Urea, 150 mM Tris-HCl, pH 8.5). Subsequently, the protein was alkylated using IAA butter (Iodoacetamide, 100 uL) at 600 rpm for 1 min and centrifuged at 12,500 *g* for 15 min before left standing for 30 min at 25 °C. In particular, this process was performed twice. After TEAB was mixed with the sample for centrifugation (12500 *g*, 15 min), the solution was incubated under agitation (600 rpm, 1 min) when mixing with TEAB (0.1 M, 40 μL) buffer containing trypsin (4 μg). Peptides (100 μg) were labeled with TMT kit (Thermo Scientific, USA) as directed by the instructions due to the high accuracy compared to label-free proteomic.

2.6.4. LC–MS/MS analysis

The peptide solution was fractionated with Thermo Scientific Easy nLC equipped column (Thermo Fisher Scientific, Acclaim PepMap RSLC 50um X 15 cm, nano viper, P/N164943). A fivestep linear gradient of A/B mixture (A: ddH_2O with 0.1% formic acid, B: ACN with 0.1% formic acid) was applied to elute the peptides. The sample was loaded onto the analytical column for separation by the autosampler and the flow rate was 300 nL/min when the column was equilibrated with 100% A. The elution gradient was as follows: 0–5 min, the linear gradient of B was 3%-5%; 5–75 min, the linear gradient of B was 5%-28%; 75–80 min, the linear gradient of B increased from 28% to 38%; 80–84 min, the linear gradient of B rose to 99%; 84–90 min, B was maintained at 99%. Samples were analyzed using Q Exactive plus (Thermo Fisher Scientific, Shanghai, China)-coupled tandem mass spectra after chromatographic separation. The detection method was positive ion, and the scanning range of precursor ion was 350–1800 *m/z*. MS1 resolution was 70,000 and AGC target was 3e6 with Level 1 Maximum IT of 50 ms. The acquisition parameters of the secondary spectrum were as follows: MS2 Activation Type was HCD; Isolation window was 2.0 *m/z*; MS2 resolution was 17,500 or 35000; AGC target was 2e5; Minimum AGC target was 2e3; Secondary Maximum IT was 50 ms. The collected charge was between 2 and 6, the ion dynamic exclusion time was 30 s, with the Normalized Collision Energy of 30 eV.

2.6.5. Proteomics data analysis

Pearson correlation, PCA and heat map analyses were performed using the R language in this study. Difference level >1.2 or <0.83 folds was defined as differentially expressed protein (DEPs) combined with p value < 0.05 based on previous study (Sarengaowa et al., 2019). Then, Blast2GO was carried out for functional annotation and classification with DEPs and the Kyoto Encyclopedia of Genes and Genomes (KEGG) was employed using KOALA (KEGG Orthology And Links Annotation) software to display the functions from the metabolic pathways.

2.7. Application in fresh beef

In the current study, the preservation potential of linalool was also investigated by selecting fresh beef as a food model. Specifically, fresh beef was cut into pieces (2 cm × 2 cm, 10 *g*) after sterilization with UV irradiation, and all samples were inoculated with *S. sonnei* (10^6 CFU/mL, 10 μL) by spot painting. The samples were left for 30 min to ensure that the bacteria were adsorbed and then divided into 4 groups for processing (linalool at MIC and 2MIC concentrations, 1% ethanol and sterile water). Subsequently, the prepared fresh beef samples were stored in sterile packaging bags at 4 °C and the quality was evaluated by quantitative indicators including pH, total volatile basic nitrogen (TVB-N), the total viable counts (TVC) and sensory evaluation.

2.7.1. pH

The blender was used to homogenize the beef samples when mixed with sterile saline at a ratio of 1:50 (v/v). After soaking for 30 min, the samples were filtered to obtain a filtrate for pH determination with a pH meter, previously calibrated with pH 4.00 and pH 7.00 buffers.

2.7.2. Total volatile basic nitrogen (TVB-N)

TVB-N values of beef samples during storage were also quantified in this study. Briefly, the samples were dispersed in deionized water (75 mL) and then filtered using a stirrer to obtain the supernatant after thorough mixing. Subsequently, TVB-N analyses were performed using the automatic Kjeldahl analyzer (K9840, Shandong Haineng Scientific Instrument Co., Ltd., China). Finally, hydrochloric acid (0.1 mol/L) was used to titrate distillation products and the results were expressed as mg of TVB-N/100 g of beef.

2.7.3. Total viable counts (TVC)

The TVC was determined by plate counting method according to the Chinese National Standard (GB/T 4789.2–2016). The churned beef samples were mixed with sterile saline and diluted at a ratio of 1:9 (v/v). The diluted samples were uniformly coated on the cooled medium and incubated for 48 h before observation. The result of microbial count was recorded as Log CFU/g of beef sample.

2.7.4. Sensory evaluation

The effect of linalool on the sensory properties of beef samples was evaluated through a panel of 12 semi-trained students in food science and engineering. In particular, the sensory evaluation of this study consisted of 5 parts including “color”, “odor”, “texture”, and “overall-acceptability”, which were evaluated using a 5-point descriptive range with reference to previous report (Shokri, Parastouei, Taghdir, & Abbaszadeh, 2020). The higher the score of the sample, the better the quality of the related index, where 5 means the best and 1 means the worst. Samples were randomly assigned to group members for evaluation at 1, 3, 5, 7, and 9 days during the evaluation process. In addition, samples with scores < 3 were specified as unacceptable.

2.8. Statistical analysis

Graphs were performed using Origin 2018 (Origin Lab Co., Northampton, MA, USA) and results were expressed as mean ± standard deviation (SD) with three replicates in each experiment. The significance of data was conducted by one-way analysis of variance (ANOVA), followed by Duncan’s multiple range tests using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). In particular, significance analysis was performed between MIC of linalool and blank control in the proteomics data while in MIC of linalool, 2MIC of linalool, 1% ethanol and blank control among other indicators. A p-value < 0.05 was considered as a significant difference.

3. Results

3.1. Cell wall integrity analysis

In this study, the content of AKP was maintained at a very low level and hardly changed significantly in the absence of linalool (Fig. 1A). However, the AKP activity showed a significant increasing trend (p < 0.05) and reached a maximum at 6 h under linalool stress, implying that the addition of linalool caused severe damage to the cell wall thereby leading to the leakage of the AKP. Similar report also supported that linalool can damage the cell wall structure of *Shewanella putrefaciens* (Guo et al., 2021b). A potential explanation might be attributed to the interaction between the hydrophobic group of linalool and the hydrophobic peptidoglycan (Vadillo-Rodriguez, Cavagnola, Perez-Giraldo, & Fernandez-Calderon, 2021).

3.2. SDS-PAGE

The SDS-PAGE electrophoresis analysis was performed in this study and the protein bands of samples and Markers were visualized in Fig. 1B, where Markers ranged from 10 kDa to 180 kDa. The protein bands were clear without linalool. The bands were blurred and even some bands were barely discernible when exposed to linalool. The current result suggested that the loss of soluble protein was extremely significant combined with the result of intracellular protein content (Fig. S1A). These blurred protein bands revealed the inhibitory effect of linalool on protein synthesis of *S. sonnei* and leakage of proteins, which were thought to interfere with physiological functions due to proteins responsible for information transmission and cell structure (Kohanski, Dwyer, & Collins, 2010). Our previous study found that linalool also reduced intracellular protein content of *Shewanella putrefaciens* (Guo et al., 2021b) and speculated that it may be due to the following reasons: protein leakage caused by loss of cellular integrity; excessive protein consumption in response to stimuli or blocked protein synthesis when

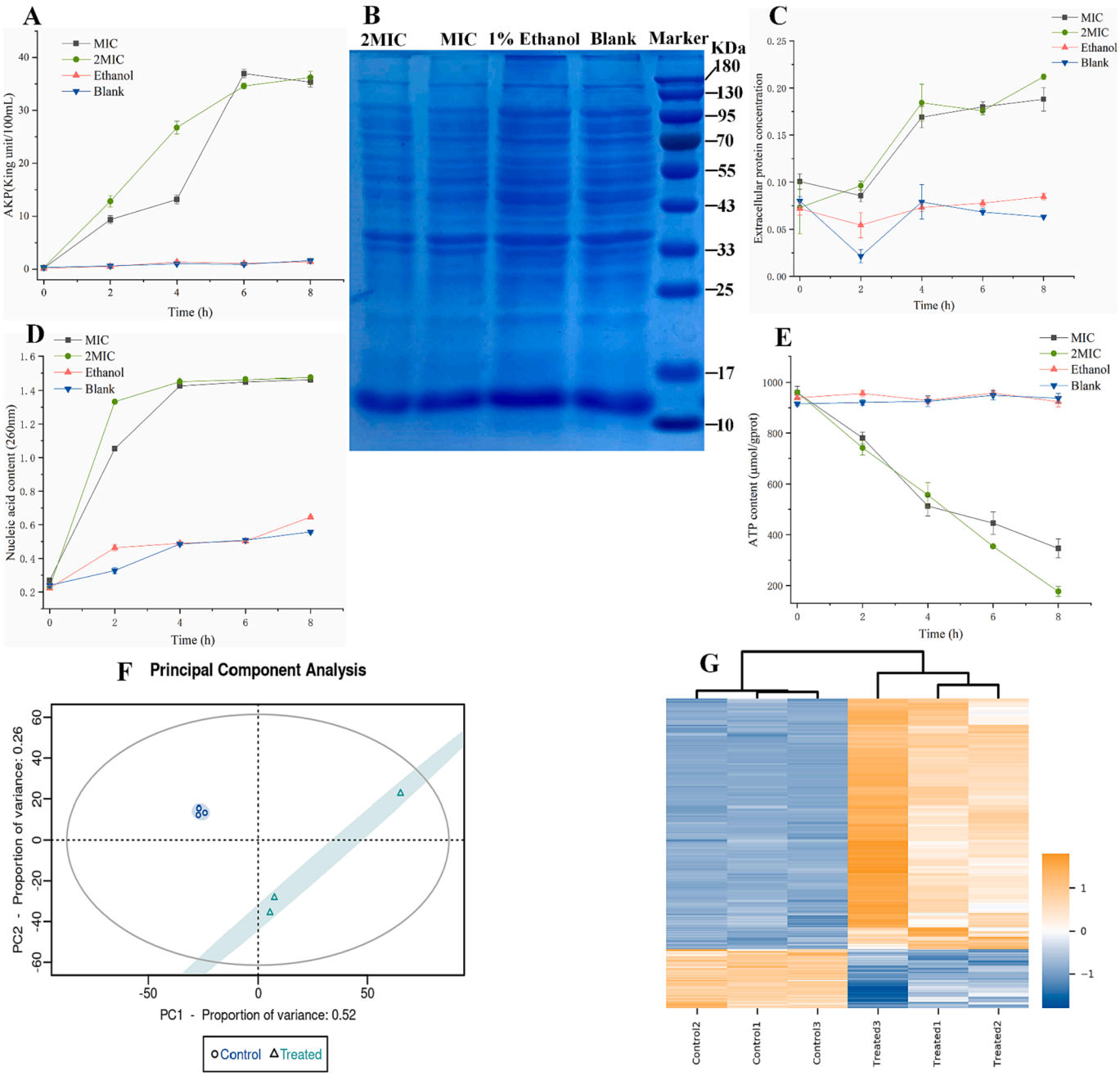


Fig. 1. The leakage of AKP in *S. sonnei* with or without linalool (A); SDS–PAGE profiles of *S. sonnei* under linalool stress (B); Extracellular protein (C) and nucleic acid (D) contents in *S. sonnei* when exposed to linalool; Intracellular ATP content in *S. sonnei* when treated with linalool (E). PCA (F) and Heat map (G) analysis of the protein profiles in *S. sonnei* between control and linalool treatment.

exposed to linalool.

3.3. Cell membrane integrity assessment

The content of extracellular macromolecules including proteins and nucleic acid was also measured as an indicator to monitor the integrity of the cell membrane in the current work. As shown in Fig. 1C and 1D, both protein and nucleic acid contents were increased with the prolongation of linalool acting time ($p < 0.05$). The amount of nucleic acid leakage in the MIC group and the 2MIC group was 2.61 and 2.63 times that of the control group, respectively, while the amount of protein leakage was 3.02 and 3.33 times. Taken together, the current evidence revealed that membrane damage may make an important contribution to the inhibition of *S. sonnei* by linalool.

3.4. Intracellular ATP content analysis

ATP is widely used to assess a variety of cellular functions including material transport across cell membranes, which may be a potential target for understanding how natural antibacterial agents work (Bajpai, Sharma, & Baek, 2013). As illustrated in Fig. 1E, intracellular ATP content was rapidly dissipated when linalool was selected as the treatment ($p < 0.05$). ATP content decreased by 63.08% and 81.16% in the MIC and 2MIC groups, respectively, revealing the phenomenon of energy shortage in the cell. It implied an intracellular energy deficit, which was attributed to the excessive consumption of ATP caused by excessive apoptosis (Zamaraeva et al., 2005). Another important factor in the decrease of intracellular ATP content might be the leakage of intracellular ATP due to the loss of cell membrane integrity.

3.5. Global analysis of proteomic responses

Microbial responses to linalool can be analyzed with advanced information provided by proteomics (Hu et al., 2017). A quantitative proteomic approach based on TMT label was employed to obtain the global protein profile of *S. sonnei* after exposure to linalool. Pearson's correlation coefficient and principal component analysis (PCA) were performed to assess the reproducibility of protein quantification (Fig. S1B and 1F). These data supported that there were significant changes in the protein profiles of the linalool-treated and control group ($p < 0.05$), which was consistent with the normalized heatmap results (Fig. 1G). Subsequently, the DEPs were plotted in Fig. S1C in the form of volcano map. A total of 257 DEPs were identified, of which 208 were overexpressed and 49 were downregulated (Fig. S1D). In order to more intuitively display the differences in protein expression between different groups, the contents of the top 9 proteins with significant expression were displayed in the form of scatter plots (Fig. S2).

3.6. Gene ontology (GO) analysis

The obtained DEPs were further categorized according to GO annotations (Fig. 2A), which were classified based on cellular components (CC), biological process (BP) and molecular functions (MF). In BP, most of DEPs mainly involved in cellular process, metabolism process and biological regulation. The CC analysis indicated that these proteins were mainly located in cell part, protein-containing complex and membrane part. Binding, catalytic activity and structural molecular activity contained a large number of DEPs known from MF. Significant enrichment analysis of GO functional annotations was conducted to observe whether DEPs has significant enrichment trends in certain functional types. The top 10 enriched classification results of the three categories were displayed in the form of bubble charts (Fig. S3A). Among the three categories, the most enriched were transport (in BP), out membrane-bounded periplasmic space (in CC) and structural constituent of ribosomes (in MF).

3.7. KEGG pathway analysis

KEGG analysis was performed to discover the metabolic or signaling pathways that DEPs involved in. Fig. 2B showed that the top 20 KEGG pathways were sorted according to the number of DEPs, and the pathways with more DEPs mainly included metabolic pathways, ribosomes, ABC transport, microbial metabolism in diverse environments and biosynthesis of secondary metabolites. These data supported that intracellular metabolic processes were disrupted in the presence of linalool based on the highest number of DEPs involved. To identify significantly affected metabolic and signal transduction pathways, KEGG pathway enrichment analysis was also carried out through fisher's exact test (Fig. S3B). Pathways that were highly enriched and possibly related to potential antibacterial mechanisms mainly involved ribosomes, cellular chemotaxis, phosphotransferase system (PTS) and ABC transporters.

3.8. Domain annotation and enrichment analysis

It is important to study the domain of proteins for understanding the biological functions of proteins and their evolution. The domain enrichment results were summarized in Fig. 3A and Fig. 3B after statistical analysis. Pathways with higher enrichment involved Solute-binding protein family 3, conserved site, Solute-binding protein family 3/*N*-terminal domain of MltF, and Cold-shock conserved site, containing 5, 6, and 4 DEPs, respectively. Moreover, except for the Globin-like superfamily, the proteins involved in the other domains all showed a tendency to be overexpressed ($p < 0.05$).

3.9. Analysis of metabolic pathways

The data obtained revealed that changes in protein profiles might be one of the important targets for linalool to exert its bacteriostatic effect. Subsequently, the screened DEPs were introduced into KEGG for metabolic network analysis to gain an insight into the action of linalool against *S. sonnei*. The result showed that highlighted pathways were related to amino acid and protein metabolism, DNA replication and

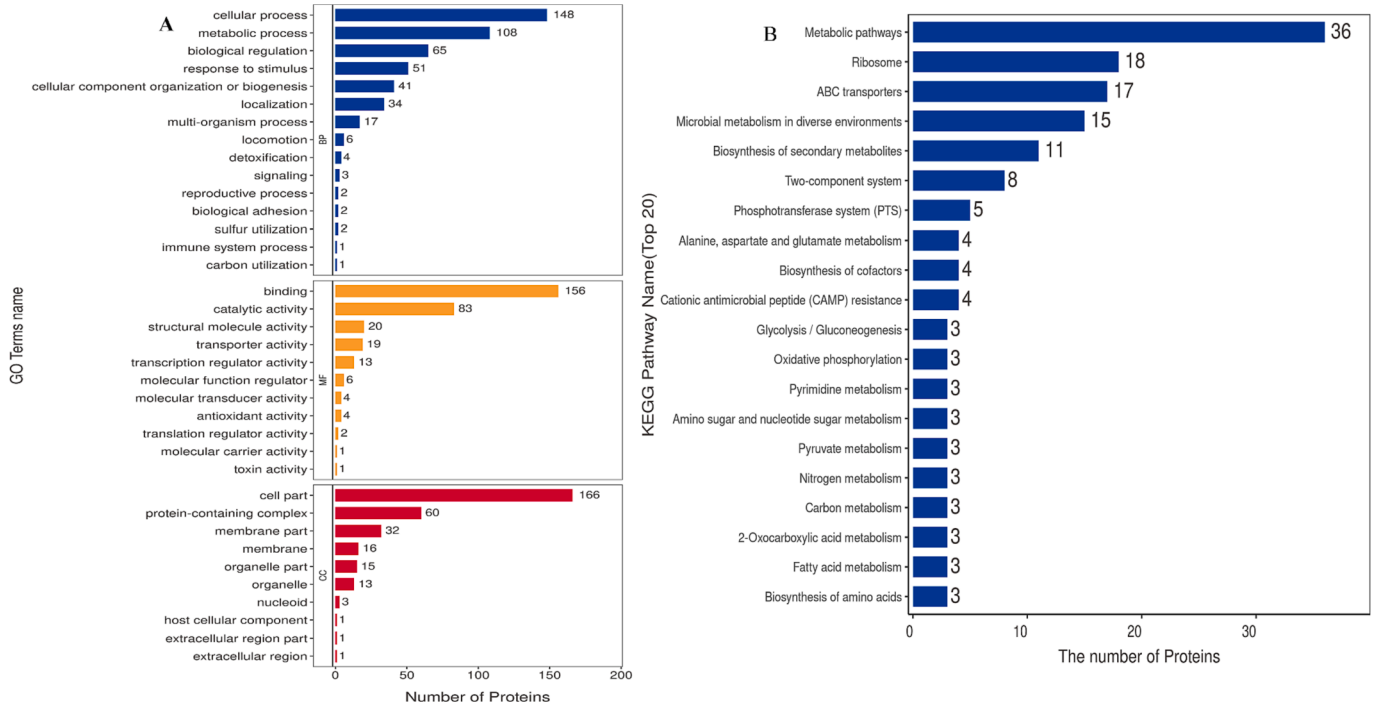


Fig. 2. Gene Ontology (GO) functional annotation of differentially expressed proteins (DEPs) (A). KEGG pathway annotation according to DEPs (C).

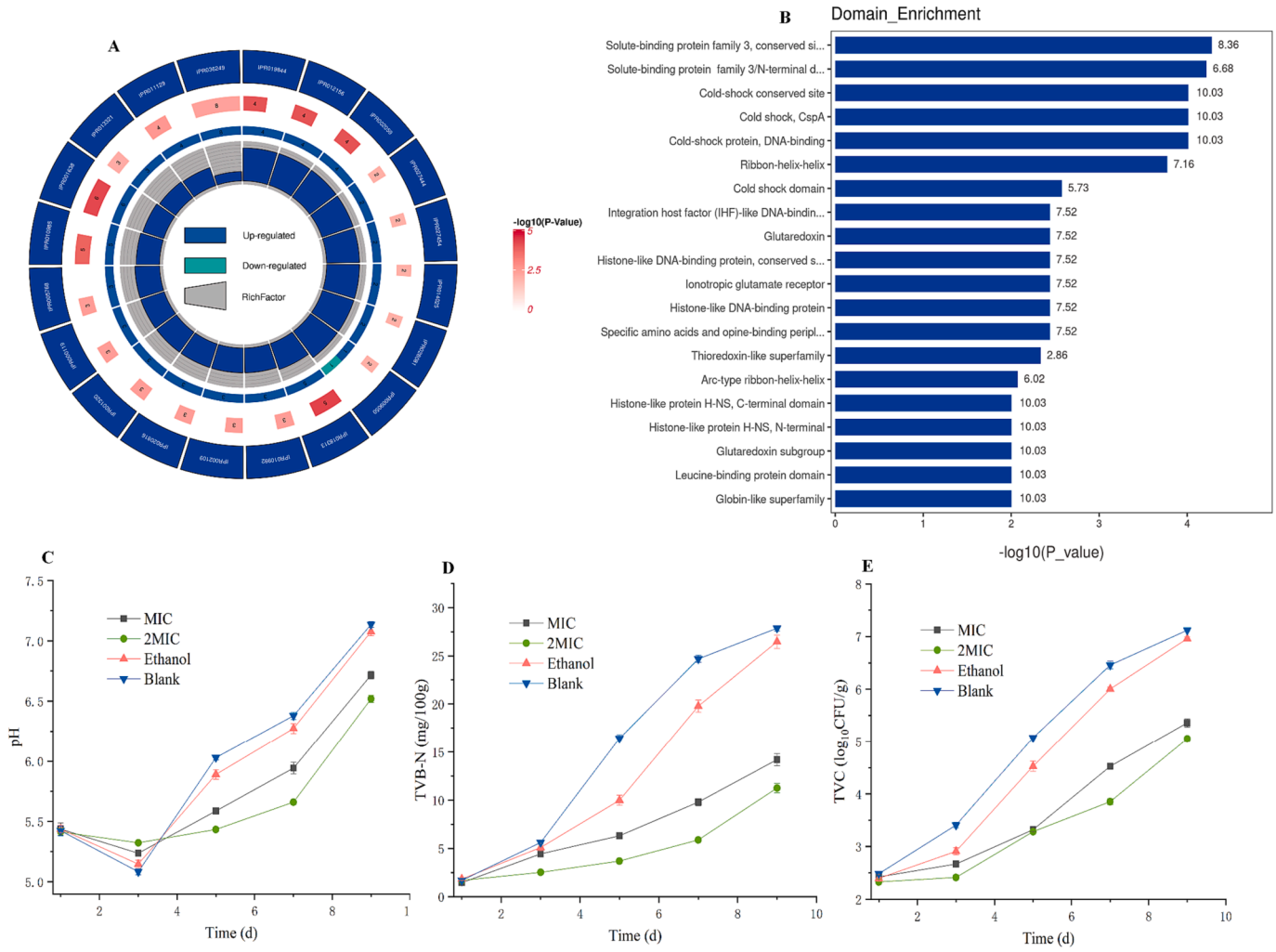


Fig. 3. Enrichment analysis of domains in the form of a circle plot (A); Enrichment analysis of domains in histogram format (B). Application of linalool in fresh beef preservation, pH (C); TVB-N (D) and TVC (E).

repair, energy metabolism and environmental stimulus response. Other important pathways including PTS, amino sugar and nucleotide sugar metabolism, and peptidoglycan biosynthesis were also enriched.

3.9.1. Amino acid and protein metabolism

Bacterial cells consume about 50% of their energy to produce protein, while 20–40% of protein synthesis is directed to ribosomes and other translation factors (Russell & Cook, 1995). Therefore, the ribosome, as a site for protein synthesis, is critical for bacterial survival. However, the ribosome-associated proteins were all overexpressed except for ribosome large subunit L19 based on the results in Fig. 4. These evidences suggested that protein starvation might have occurred in the cell, causing the upregulation of ribosomal proteins when exposed to linalool, which was also supported by results for SDS-PAGE and intracellular soluble proteins. Similar study considered that natural bacteriostatic agents might achieve bacteriostatic properties by overconsumption of amino acids (Liu et al., 2020a), which acted as a negative feedback regulation signal to stimulate ribosome expression. In the biosynthesis of various amino acids, the protein involved in valine, leucine and isoleucine biosynthesis (LeuD) was overexpressed. However, the expression of protein responsible for arginine biosynthesis (aspc), which was also involved in the biosynthesis of phenylalanine, tyrosine and tryptophan, was inhibited. A potential explanation for the unbalanced expression of proteins involved in the synthesis of these amino acids might be the depletion and accumulation of specific amino acids within the cell. Previous study has also shown that under stress conditions, bacterial cells usually activated some amino acid biosynthesis pathways to survive (Horinouchi et al., 2010). Unlike previous report that amino acid restriction downregulated genes controlling amino acid metabolism (Liu et al., 2020a), we found that some amino acid metabolisms were down-regulated, while some were up-regulated. The pathways of amino acid metabolism up-regulation included D-amino acid metabolism (alr and dadA), degradation of valine, leucine and isoleucine (fadA). Meanwhile, arginine and proline metabolism (aspC and adiA), tyrosine metabolism (aspC) were inhibited. Some amino acid metabolisms were expressed unbalanced, such as the down-regulation of aspC, and the overexpression of pyrB and pyrI, which involved in alanine, aspartate, and glutamate metabolism. In cysteine and methionine metabolism, aspC was inhibited while mrsC was significantly expressed. Proteins involved in phenylalanine metabolism were also expressed unbalanced, with aspC repressed and dadA activated. Overall, the addition of linalool might have caused amino acid restriction to activate most of the proteins participating in ribosomal in an attempt to alleviate this stress. Upregulation of specific amino acid synthesis pathways (valine, leucine and isoleucine biosynthesis) and inhibition of certain amino acid metabolism (tyrosine metabolism, arginine and proline metabolism) may also serve this purpose (Liu et al., 2020). However, more research was still needed to elucidate the mechanism of amino acid disorder in the next step.

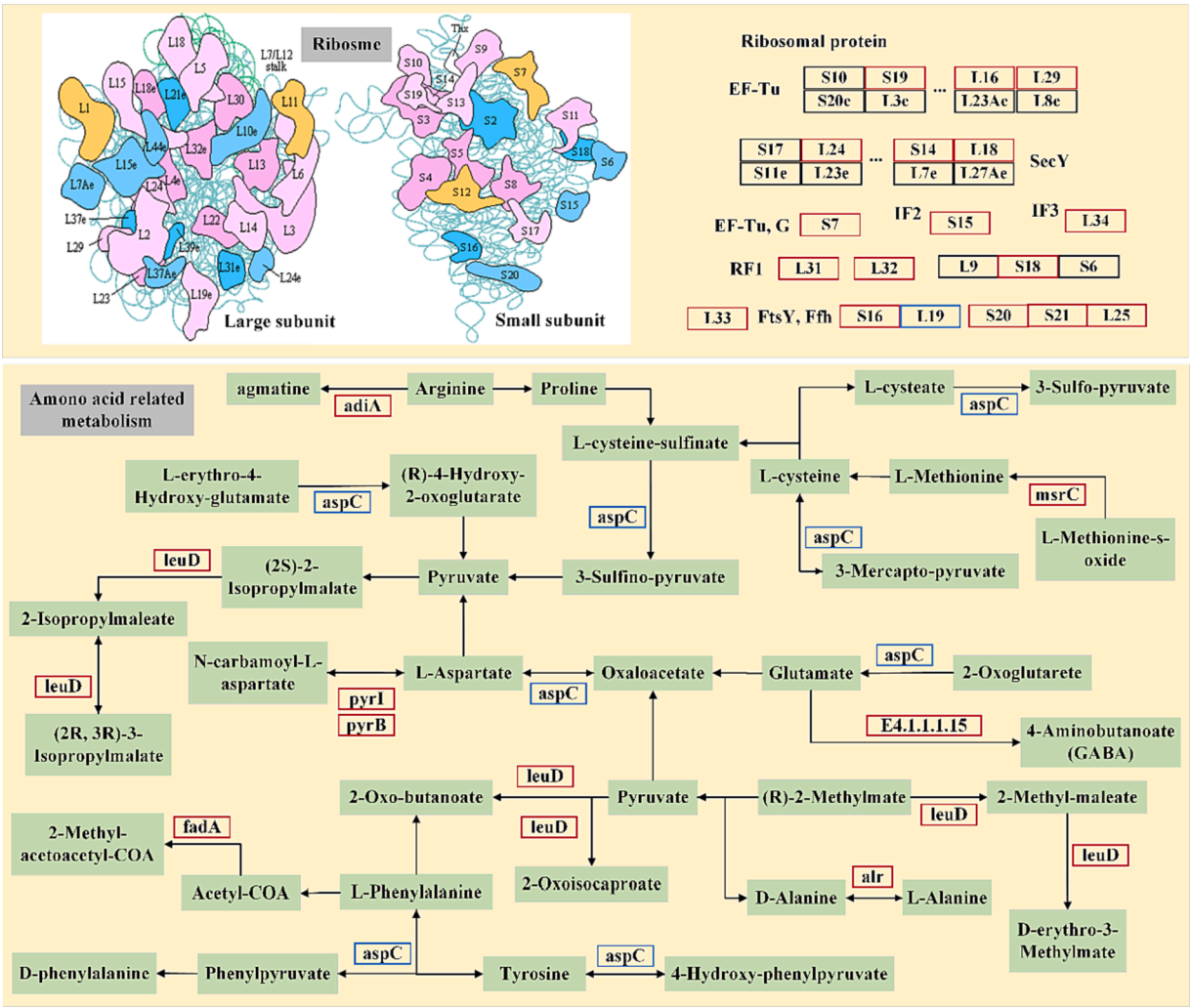


Fig. 4. Pathway analysis of DEPs related to amino acid and protein metabolism. The colors used in the ribosomal pathway (top left) include blue, pink and yellow to distinguish between different proteins The red box reprents up-regulated proteins and blue indicates down-regulated proteins in the mapped metabolic network pathways. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.9.2. *Energy metabolism*

Multiple pathways related to energy metabolism were perturbed in the presence of linalool (Fig. 5), which was thought to be a direct damage of linalool to the energy system or a derivative effect of amino acid restriction (Guo et al., 2021b). We also previously demonstrated that linalool emulsion exerted antibacterial effect by inhibiting respiratory metabolism mainly through TCA (He et al., 2022b). In this study, it was found that TCA was also enriched due to the DEPs in this pathway named DLAT (down-regulated) and acnB (up-regulated) detected. The proteins including uxaA, actP and yihX were detected to be overexpressed, and DLAT participated in the synthesis of Acetyl-COA was inhibited. Downstream of acetyl-COA, the proteins (fadA and accB) involved in synthetic Malonyl-COA and isocitrate were up-regulated, which Acetyl-COA deficiency might appear. In this case, the proteins involved in fatty acid degradation (fadA) and fatty acid metabolism (fadA and fabD) were activated, which was likely to be an emergency energy supply phenomenon caused by insufficient energy. This phenomenon was consistent with previous study that fatty acids can be effective in providing energy under lactic acid stress (Kang et al., 2021). Furthermore, the proteins involved in fatty acid biosynthesis (accB and fabD) were up-regulated as well as unsaturated fatty acid biosynthesis protein (tesA). A potential explanation for the activation of fatty acid synthesis was a salvage mechanism for cell membrane damage since linalool has been shown to have a damaging effect on the membrane (Guo et al., 2021a). A series of electron transport reactions generate ATP through the electron transport chain to power cells during oxidative phosphorylation (Nolfi-Donegan, Braganza, & Shiva, 2020). The proteins CyoA (up-regulated), CydA and NuoA (both down-regulated) involved in this pathway were identified to show differential expression compared to the control group. There was evidence that components from EOs can compete with substrates for the binding sites of enzyme molecules to influence their affinity (Zhou, Li, Siva, Cui, & Lin, 2021). Therefore, the inhibition of CydA and NuoA (in NADH dehydrogenase) by linalool was attributed to the above mechanism. Subsequently, the downregulation of CydA and NuoA caused the blockage of electron transport and thus may activate the expression of protein CyoA in the downstream transport chain (cytochrome *c* oxidase). In the electron transport chain, NuoA is involved in the oxidation of NADH to transfer electrons and CydA is responsible for the transfer of electrons to cytochrome *c*. Inhibition of the expression of both will lead to blocked electron transport and ultimately affect the production of ATP, corresponding to the above-mentioned viewpoint that the ATP content was reduced. Furthermore, imbalances in the proteins expression of galactose metabolism, starch and sucrose metabolism, and carbon metabolism proteins all suggested perturbations in energy system of *S. sonnei*, which might contribute to its antibacterial effect.

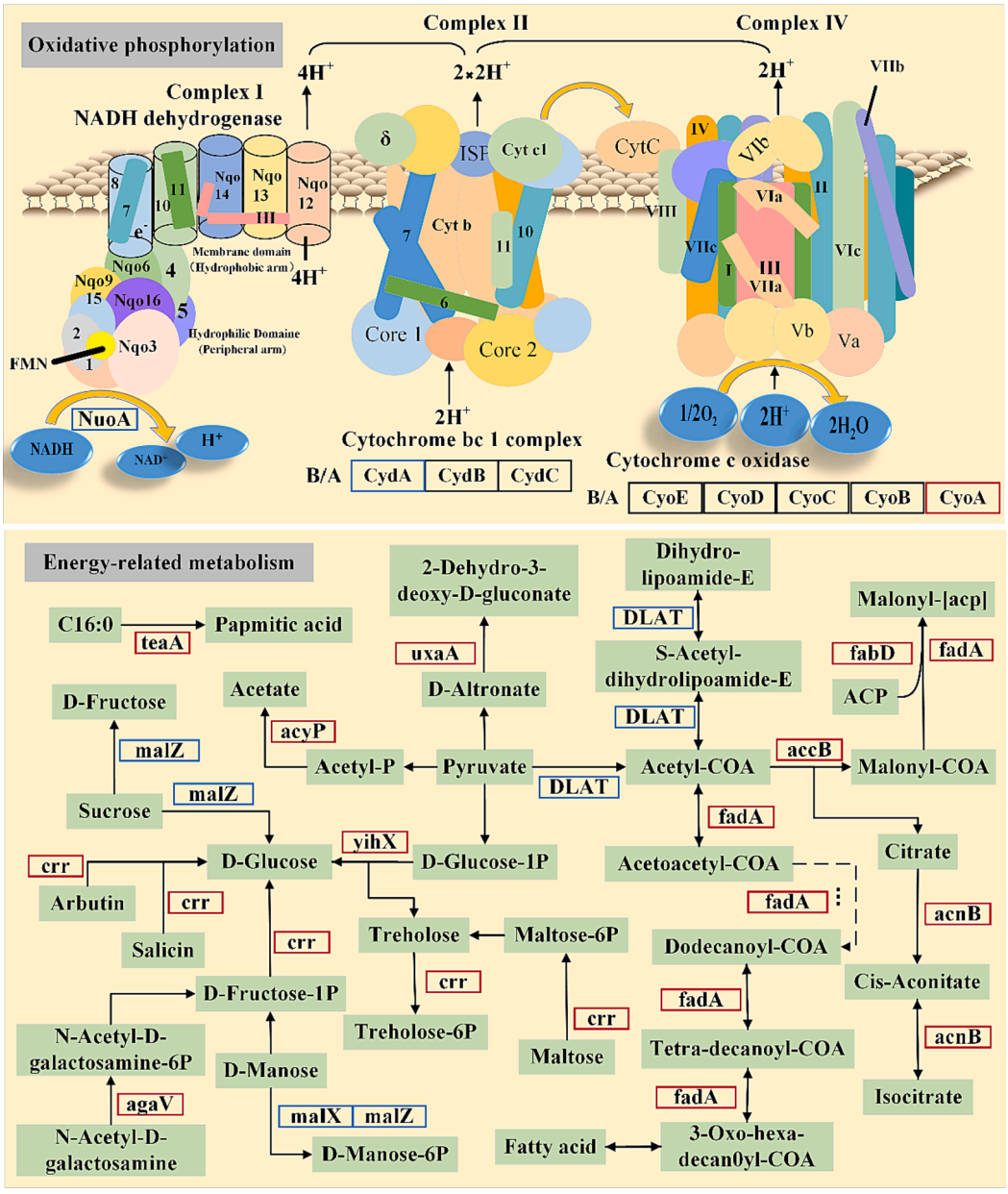


Fig. 5. Pathway analysis of DEPs related to energy metabolism.

3.9.0.3. DNA replication and repair

DNA replication and repair systems are critical for bacteria to survive in response to stimuli especially under stress (Wang et al., 2020). Pathways related to DNA replication and repair were summarized and visualized in Fig. S4. The DNA replication process requires the coordinated action of multiple enzymes including helicase, DNA polymerase III holoenzyme and DNA ligase (Yi & Lu, 2019). It was found that the protein θ (holE) involved in DNA polymerase III was significantly expressed, suggesting that the DNA replication pathway was activated. The underlying hypothesis was that the lack of intracellular nucleic acid stimulated the DNA replication pathway, consistent with the above result of detecting nucleic acid leakage. Activation of pyrimidine metabolism also supported this point, in which pyrI and pyrB were detected showing a trend of up-regulation. However, there was an imbalanced expression of proteins participated in purine metabolism due to inconsistent expression trends observed for adK (up-regulated) and deoD (down-regulated). Preliminary research claimed that purines can play crucial repair and survival functions when *E. coli* was under acid stress (Shayanfar, Broumand, & Pillai, 2018). In particular, deoD was also involved in the interconversion between various substances, and all this revealed that linalool caused disturbance of intracellular genetic material. Various stimuli and pressures from the external environment often damage the structure and function of DNA, causing biological mutations and even death. This is where mismatch repair becomes significantly more important by correcting replication errors, recombination intermediates and some forms of DNA chemical damage (Sarker, Cooper, & Hazra, 2021). Mismatch repair and homologous recombination were among the important repair mechanisms and identified to be both activated due to overexpression of hole (DpoIII) and PriC. Collectively, these evidences supported that *S. sonnei* employed up-regulation of DNA repair (mismatch repair and homologous recombination) to counteract DNA lesions caused by linalool.

Environmental stimulus response and other important pathways including phosphotransferase (PTS), peptidoglycan, amino sugar and nucleotide sugar metabolism were also enriched in this study (Fig. S5

and S6) and relevant description was presented in the Supporting information.

3.10. Application in fresh beef

Common preservation methods such as heat treatment may cause significant damage to the nutritional and sensory quality of foods (Ozen & Singh, 2020). In addition, non-thermal technologies, including cold plasma, are often limited by gas type, voltage and frequency (Bezerra et al., 2023). Therefore, the present work used beef as a food model to explore the preservation potential of linalool by coating. The preservation potential of linalool on fresh beef was analyzed by measuring the indicators including pH, TVB-N, TVC and sensory evaluation. The pH value continued to rise throughout the storage period although it decreased on the third day (Fig. 3C). Furthermore, the rate of change in the linalool-treated group was consistently less than that in the control group, which revealed that the presence of linalool delayed pH changes in fresh beef. pH, as an important indicator affecting protein stability, the cause of its content reduction was lactic acid, phosphoric acid an amino acids from glycolysis, ATP and protein decomposition (Jia et al., 2021). Afterwards, various amines produced by microbial activity and endogenous enzymatic activity forced the pH to trend upward (Wang, Hu, Gao, Ye, & Wang, 2017). The result for TVB-N was presented in Fig. 3D and it was found that the content of TVB-N showed an increasing trend throughout the storage period. It can be speculated that the unfavorable living environment was generated in the presence of linalool and then inhibited the activities of microorganisms and endogenous enzymes, which reduced the production rate of amines. Similarly, the rate of increase was also smaller compared to the absence of linalool ($p < 0.05$). As an important indicator for assessing meat and meat products, TVB-N is derived from the degradation of proteins and other nitrogenous compounds by enzymes and microorganisms in meat. Therefore, in order to elucidate the reasons behind the variation in TVB-N content, the content of TVC in beef samples was also determined in the current study. The results revealed that TVC also showed an increasing trend in all fresh beef and a low rate of increase was monitored in the linalool treated group (Fig. 3E). After 9 days, TVC was reduced by 1.76 and 2.07 log CFU in MIC and 2MIC groups, respectively. In particular, the sensory evaluation results of fresh beef after linalool treatment were summarized in Table 1. All attributes scored higher in colour, odour, texture and overallacceptability initially and then began to decline with storage time. In addition, the scores of the linalool-treated group were consistently higher than those of the control group. In this study, samples with scores > 3 were specified as acceptable. Thus, it can be seen that the treated group samples were still acceptable after 9 days in terms of sensory properties while the untreated ones has decayed at day 7. All in all, linalool did not affect the acceptability of the fresh beef samples, which suggested that linalool has the potential to be developed as a natural antibacterial agent.

Overall, linalool exerted outstanding antibacterial ability against *S. sonnei* mainly by damaging cellular structure, altering intracellular protein profiles and inducing energy restriction accompanied by depletion of specific amino acids and activation of stress responses.

4. Conclusions

This work confirmed that intracellular protein levels were significantly changed after linalool treatment according to the sequencing results of proteomics. The KEGG analysis showed that the enriched pathways mainly included amino acid and protein metabolism, energy metabolism, DNA replication and repair, and environmental stimulus response accompanied by perturbation of flagellar assembly and PTS. Furthermore, the decrease in intracellular ATP content also illustrated the emergence of energy limitation when exposed to linalool. The leakage of AKP, protein and nucleic acid also indicated the damage of cell structure under linalool stress. In conclusion, the inhibitory mechanism of linalool on *S. sonnei* can be described as: damage to cellular structure, excessive consumption of specific amino acids and energy limitation accompanied by activation of stimulatory responses. Encouragingly, linalool can effectively control the quality deterioration of fresh beef without affecting sensory acceptance. These results provided new insights into the mechanism by which linalool inhibited *S. sonnei* and explored its potential as a natural antibacterial.

CRediT authorship contribution statement

Rongrong He: Software, Writing – original draft. **Haiming Chen:** Validation. **Hao Wu:** Data curation. **Jicai Liu:** Investigation. **Weijun Chen:** Data curation, Resources. **Ming Zhang:** Project administration, Resources. **Wenxue Chen:** Writing – review & editing. **Qiuping Zhong:** Methodology.

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.

Table 1
Effect of linalool on sensory attributes of fresh beef during storage.

Sensory parameter	Treatments	Storage times (days)				
		1	3	5	7	9
Color	MIC	4.86 ± 0.02^{a}	4.48 ± 0.04^{b}	4.16 ± 0.02^{b}	3.72 ± 0.03^{b}	3.16 ± 0.02^{b}
	2MIC	4.87 ± 0.03^{a}	4.66 ± 0.03^{a}	4.32 ± 0.04^{a}	4.11 ± 0.03^{a}	3.29 ± 0.04^{a}
	1% Ethanol	4.86 ± 0.02^{a}	4.26 ± 0.02^{c}	3.50 ± 0.03^{c}	2.91 ± 0.03^{c}	NT
	Blank	4.85 ± 0.02^{a}	4.08 ± 0.03^{d}	3.39 ± 0.02^{d}	2.82 ± 0.03^{d}	NT
Odor	MIC	4.86 ± 0.02^{a}	4.56 ± 0.03^{b}	4.15 ± 0.03^{b}	3.84 ± 0.03^{b}	3.44 ± 0.02^{b}
	2MIC	4.86 ± 0.02^{a}	4.67 ± 0.02^{a}	4.40 ± 0.04^{a}	4.18 ± 0.02^{a}	3.78 ± 0.03^{a}
	1% Ethanol	4.85 ± 0.02^{a}	4.16 ± 0.01^{c}	3.53 ± 0.01^{c}	2.72 ± 0.02^{c}	NT
	Blank	4.86 ± 0.03^{a}	4.10 ± 0.04^{d}	3.29 ± 0.03^{d}	2.48 ± 0.03^{d}	NT
Texture	MIC	4.85 ± 0.02^{a}	4.55 ± 0.03^{b}	4.14 ± 0.02^{b}	3.86 ± 0.01^{b}	3.26 ± 0.02^{b}
	2MIC	4.86 ± 0.02^{a}	4.65 ± 0.02^{a}	4.26 ± 0.03^{a}	4.08 ± 0.02^{a}	3.58 ± 0.03^{a}
	1% Ethanol	4.86 ± 0.02^{a}	4.26 ± 0.02^{c}	3.61 ± 0.04^{c}	2.87 ± 0.03^{c}	NT
	Blank	4.86 ± 0.02^{a}	4.12 ± 0.04^{d}	3.41 ± 0.04^{d}	2.44 ± 0.02^{d}	NT
Overall-acceptability	MIC	4.91 ± 0.02^{a}	4.51 ± 0.02^{b}	4.15 ± 0.02^{b}	3.51 ± 0.02^{b}	3.15 ± 0.02^{d}
	2MIC	4.91 ± 0.03^{a}	4.68 ± 0.02^{a}	4.26 ± 0.02^{a}	3.73 ± 0.03^{a}	3.31 ± 0.04^{a}
	1% Ethanol	4.90 ± 0.02^{a}	4.17 ± 0.03^{c}	3.54 ± 0.03^{c}	2.84 ± 0.02^{c}	NT
	Blank	4.91 ± 0.03^{a}	4.07 ± 0.02^{d}	3.30 ± 0.04^{d}	2.66 ± 0.02^{d}	NT

Notes: all values were showed as means ± standard deviation. Means with lowercase alphabet in the same column indicated significant difference ($p < 0.05$). NT: no test, it meant that the meat samples were rotten.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the Natural Science Foundation of China (32160591) and Hainan Provincial Natural Science Foundation of China (320RC515, 2019RC120).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100837.

References

Ahmed, A. M., & Shimamoto, T. (2015). Molecular characterization of multidrug-resistant Shigella spp. of food origin. *International Journal of Food Microbiology, 194*, 78–82. https://doi.org/10.1016/j.ijfoodmicro.2014.11.013

Bajpai, V. K., Sharma, A., & Baek, K. H. (2013). Antibacterial mode of action of Cudrania tricuspidata fruit essential oil, affecting membrane permeability and surface characteristics of food-borne pathogens. *Food Control, 32*(2), 582–590. https://doi.org/10.1016/j.foodcont.2013.01.032

Bezerra, J. D., Lamarao, C. V., Sanches, E. A., Rodrigues, S., Fernandes, F. A. N., Ramos, G. L. P. A., … Campelo, P. H. (2023). Cold plasma as a pre-treatment for processing improvement in food: A review. *Food Research International, 167*. ARTN 11266310.1016/j.foodres.2023.112663.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods–a review. *International Journal of Food Microbiology, 94*(3), 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Chai, C., Lee, S., Kim, J., & Oh, S. W. (2016). Synergistic Antimicrobial Effects of Organic Acids in Combination with Carvacrol Against Shigella Sonnei. *Journal of Food Safety, 36*(3), 360–366. https://doi.org/10.1111/jfs.12251

Cui, H. Y., Zhang, C. H., Li, C. Z., & Lin, L. (2019). Antibacterial mechanism of oregano essential oil. *Industrial Crops and Products, 139*. ARTN 11149810.1016/j.indcrop.2019.111498.

Diao, W. R., Hu, Q. P., Feng, S. S., Li, W. Q., & Xu, J. G. (2013). Chemical Composition and Antibacterial Activity of the Essential Oil from Green Huajiao (Zanthoxylum schinifolium) against Selected Foodborne Pathogens. *Journal of Agricultural and Food Chemistry, 61*(25), 6044–6049. https://doi.org/10.1021/jf4007856

Du, G. F., Yin, X. F., Yang, D. H., He, Q. Y., & Sun, X. S. (2021). Proteomic Investigation of the Antibacterial Mechanism of trans-Cinnamaldehyde against Escherichia coli. *Journal of Proteome Research, 20*(5), 2319–2328. https://doi.org/10.1021/acs.jproteome.0c00847

Gu, B., Cao, Y., Pan, S. Y., Zhuang, L., Yu, R. B., Peng, Z. H., … Tong, M. Q. (2012). Comparison of the prevalence and changing resistance to nalidixic acid and ciprofloxacin of Shigella between Europe-America and Asia-Africa from 1998 to 2009. *International Journal of Antimicrobial Agents, 40*(1), 9–17. https://doi.org/10.1016/j.ijantimicag.2012.02.005

Guo, F., Chen, Q., Liang, Q., Zhang, M., Chen, W., Chen, H., … Chen, W. (2021). Antimicrobial Activity and Proposed Action Mechanism of Linalool Against Pseudomonas fluorescens. *Frontiers in Microbiology, 12*, Article 562094. https://doi.org/10.3389/fmicb.2021.562094

Guo, F., Liang, Q., Zhang, M., Chen, W., Chen, H., Yun, Y., … Chen, W. (2021). Antibacterial Activity and Mechanism of Linalool against Shewanella putrefaciens. *Molecules, 26*(1). https://doi.org/10.3390/molecules26010245

He, R., Wu, H., Liu, J., Chen, W., Chen, W., Chen, H., … Gu, F. (2023). Mechanism of membrane damage to Shigella sonnei by linalool from plant essential oils: A driver of oxidative stress. *Industrial Crops and Products, 193*, Article 116255.

He, R. R., Chen, W. J., Chen, H. M., Zhong, Q. P., Zhang, H. L., Zhang, M., & Chen, W. X. (2022). Antibacterial mechanism of linalool against L. monocytogenes, a metabolomic study. *Food Control, 132*. ARTN 10853310.1016/j.foodcont.2021.108533.

He, R. R., Zhang, Z. K., Xu, L. L., Chen, W. J., Zhang, M., Zhong, Q. P., … Chen, W. X. (2022). Antibacterial mechanism of linalool emulsion against Pseudomonas aeruginosa and its application to cold fresh beef. *World Journal of Microbiology & Biotechnology, 38*(4). ARTN 5610.1007/s11274-022-03233-4.

Hollander, A., & Yaron, S. (2021). Pore-forming treatments induce aggregation of Salmonella Senftenberg through protein leakage. *Food Microbiology, 96*, Article 103721. https://doi.org/10.1016/j.fm.2020.103721

Horinouchi, T., Tamaoka, K., Furusawa, C., Ono, N., Suzuki, S., Hirasawa, T., … Shimizu, H. (2010). Transcriptome analysis of parallel-evolved Escherichia coli strains under ethanol stress. *BMC Genomics, 11*. Artn 57910.1186/1471-2164-11-579.

Hu, S. F., Yu, Y. G., Wu, X. W., Xia, X. Z., Xiao, X. L., & Wu, H. (2017). Comparative proteomic analysis of Cronobacter sakazakii by iTRAQ provides insights into response to desiccation. *Food Research International, 100*, 631–639. https://doi.org/10.1016/j.foodres.2017.06.051

Hu, W., Li, C. Z., Dai, J. M., Cui, H. Y., & Lin, L. (2019). Antibacterial activity and mechanism of Litsea cubeba essential oil against methicillin-resistant Staphylococcus aureus (MRSA). *Industrial Crops and Products, 130*, 34–41. https://doi.org/10.1016/j.indcrop.2018.12.078

Jia, R., Ge, S. T., Ren, S. D., Luo, Y. F., Xiu, L., Sanabil, C., … Cai, D. (2021). Antibacterial mechanism of adzuki bean seed coat polyphenols and their potential application in preservation of fresh raw beef. *International Journal of Food Science and Technology*. https://doi.org/10.1111/ijfs.15292

Kang, J. M., Liu, L., Liu, M. H., Wu, X. X., & Li, J. K. (2018). Antibacterial activity of gallic acid against Shigella flexneri and its effect on biofilm formation by repressing mdoH gene expression. *Food Control, 94*, 147–154. https://doi.org/10.1016/j.foodcont.2018.07.011

Kang, S. M., Shi, C. L., Chang, J., Kong, F. H., Li, M. H., Guan, B. Y., … Yue, X. Q. (2021). Label free-based proteomic analysis of the food spoiler Pseudomonas fluorescens response to lactobionic acid by SWATH-MS. *Food Control, 123*. ARTN 10783410.1016/j.foodcont.2020.107834.

Kohanski, M. A., Dwyer, D. J., & Collins, J. J. (2010). How antibiotics kill bacteria: From targets to networks. *Nature Reviews Microbiology, 8*(6), 423–435.

Liao, S., Zhang, Y., Pan, X., Zhu, F., Jiang, C., Liu, Q., … Chen, L. (2019). Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant Pseudomonas aeruginosa. *International Journal of Nanomedicine, 14*, 1469–1487. https://doi.org/10.2147/IJN.S191340

Lin, L., Mao, X. F., Sun, Y. H., Rajivgandhi, G., & Cui, H. Y. (2019). Antibacterial properties of nanofibers containing chrysanthemum essential oil and their application as beef packaging. *International Journal of Food Microbiology, 292*, 21–30. https://doi.org/10.1016/j.ijfoodmicro.2018.12.007

Liu, M. M., Feng, M. X., Yang, K., Cao, Y. F., Zhang, J., Xu, J. N., … Fan, M. T. (2020). Transcriptomic and metabolomic analyses reveal antibacterial mechanism of astringent persimmon tannin against Methicillin-resistant Staphylococcus aureus isolated from pork. *Food Chemistry, 309*. ARTN 12569210.1016/j.foodchem.2019.125692.

Liu, X., Cai, J. X., Chen, H. M., Zhong, Q. P., Hou, Y. Q., Chen, W. J., & Chen, W. X. (2020). Antibacterial activity and mechanism of linalool against Pseudomonas aeruginosa. *Microbial Pathogenesis, 141*. ARTN 10398010.1016/j.micpath.2020.103980.

Nolfi-Donegan, D., Braganza, A., & Shiva, S. (2020). Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. *Redox Biology, 37*, Article 101674. https://doi.org/10.1016/j.redox.2020.101674

Ozen, E., & Singh, R. K. (2020). Atmospheric cold plasma treatment of fruit juices: A review. *Trends in Food Science & Technology, 103*, 144–151. https://doi.org/10.1016/j.tifs.2020.07.020

Pereira, I., Severino, P., Santos, A. C., Silva, A. M., & Souto, E. B. (2018). Linalool bioactive properties and potential applicability in drug delivery systems. *Colloids and Surfaces B-Biointerfaces, 171*, 566–578. https://doi.org/10.1016/j.colsurfb.2018.08.001

Russell, J. B., & Cook, G. M. (1995). Energetics of bacterial growth: Balance of anabolic and catabolic reactions. *Microbiological Reviews, 59*(1), 48–62. https://doi.org/10.1128/mr.59.1.48-62.1995

Sarengaowa, Hu, W., Feng, K., Xiu, Z., Jiang, A., & Lao, Y. (2019). Tandem mass tag-based quantitative proteomic analysis reveal the inhibition mechanism of thyme essential oil against flagellum of Listeria monocytogenes. *Food Research International, 125*, Article 108508. https://doi.org/10.1016/j.foodres.2019.108508

Sarker, A. H., Cooper, P. K., & Hazra, T. K. (2021). DNA glycosylase NEIL2 functions in multiple cellular processes. *Progress in Biophysics and Molecular Biology, 164*, 72–80. https://doi.org/10.1016/j.pbiomolbio.2021.03.003

Shayanfar, S., Broumand, A., & Pillai, S. D. (2018). Acid stress induces differential accumulation of metabolites in Escherichia coli O26:H11. *Journal of Applied Microbiology*. https://doi.org/10.1111/jam.14081

Shokri, S., Parastouei, K., Taghdir, M., & Abbaszadeh, S. (2020). Application an edible active coating based on chitosan- Ferulago angulata essential oil nanoemulsion to shelf life extension of Rainbow trout fillets stored at 4 degrees C. *International Journal of Biological Macromolecules, 153*, 846–854. https://doi.org/10.1016/j.ijbiomac.2020.03.080

Sitohy, M. Z., Mahgoub, S. A., & Osman, A. O. (2012). In vitro and in situ antimicrobial action and mechanism of glycinin and its basic subunit. *International Journal of Food Microbiology, 154*(1–2), 19–29. https://doi.org/10.1016/j.ijfoodmicro.2011.12.004

Thompson, C. N., Duy, P. T., & Baker, S. (2015). The Rising Dominance of Shigella sonnei: An Intercontinental Shift in the Etiology of Bacillary Dysentery. *Plos Neglected Tropical Diseases, 9*(6). ARTN e000370810.1371/journal.pntd.0003708.

Tickell, K. D., Brander, R. L., Atlas, H. E., Pernica, J. M., Walson, J. L., & Pavlinac, P. B. (2017). Identification and management of Shigella infection in children with diarrhoea: A systematic review and meta-analysis. *The Lancet Global Health, 5*(12), e1235–e1248. https://doi.org/10.1016/s2214-109x(17)30392-3

Ud-Din, A. M. S., Wahid, S. U. H., Latif, H. A., Shahnaij, M., Akter, M., Azmi, I. J., … Talukder, K. A. (2013). Changing Trends in the Prevalence of Shigella Species: Emergence of Multi-Drug Resistant Shigella sonnei Biotype g in Bangladesh. *PLoS One1, 8*(12). ARTN e8260110.1371/journal.pone.0082601.

Vadillo-Rodriguez, V., Cavagnola, M. A., Perez-Giraldo, C., & Fernandez-Calderon, M. C. (2021). A physico-chemical study of the interaction of ethanolic extracts of propolis with bacterial cells. *Colloids and Surfaces B-Biointerfaces, 200*. ARTN 11157110.1016/j.colsurfb.2021.111571.

Wang, J., Chi, Z., Zhao, K., Wang, H., Zhang, X., Xu, F., … Wei, Y. (2020). A transcriptome analysis of the antibacterial mechanism of flavonoids from Sedum aizoon L. against Shewanella putrefaciens. *World Journal of Microbiology and Biotechnology, 36*(7), 94. https://doi.org/10.1007/s11274-020-02871-w

Wang, Z., Hu, S. F., Gao, Y. P., Ye, C., & Wang, H. Y. (2017). Effect of collagen-lysozyme coating on fresh-salmon fillets preservation. *Lwt-Food Science and Technology, 75*, 59–64. https://doi.org/10.1016/j.lwt.2016.08.032

Yang, X. Y., Shi, T. Y., Du, G. F., Liu, W. T., Yin, X. F., Sun, X. S., … He, Q. Y. (2016). iTRAQ-Based Proteomics Revealed the Bactericidal Mechanism of Sodium New Houttuyfonate against Streptococcus pneumoniae. *Journal of Agricultural and Food Chemistry, 64*(32), 6375–6382. https://doi.org/10.1021/acs.jafc.6b02147

Yi, L., & Lu, X. (2019). New Strategy on Antimicrobial-resistance: Inhibitors of DNA Replication Enzymes. *Current Medicinal Chemistry, 26*(10), 1761–1787. https://doi.org/10.2174/0929867324666171106160326

Zamaraeva, M. V., Sabirov, R. Z., Maeno, E., Ando-Akatsuka, Y., Bessonova, S. V., & Okada, Y. (2005). Cells die with increased cytosolic ATP during apoptosis: A bioluminescence study with intracellular luciferase. *Cell Death and Differentiation, 12*(11), 1390–1397. https://doi.org/10.1038/sj.cdd.4401661

Zhang, Y. C., Zhang, L. W., Du, M., Yi, H. X., Guo, C. F., Tuo, Y. F., … Yang, L. (2011). Antimicrobial activity against Shigella sonnei and probiotic properties of wild lactobacilli from fermented food. *Microbiological Research, 167*(1), 27–31. https://doi.org/10.1016/j.micres.2011.02.006

Zhou, C., Li, C., Siva, S., Cui, H., & Lin, L. (2021). Chemical composition, antibacterial activity and study of the interaction mechanisms of the main compounds present in the Alpinia galanga rhizomes essential oil. *Industrial Crops and Products, 165*, Article 113441.