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Water extract from *Ligusticum chuanxiong* delays the aging of *Saccharomyces cerevisiae* via improving antioxidant activity

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ABSTRACT

Ligusticum chuanxiong is a common traditional edible-medicinal herb that has various pharmacological activities. However, its effects on *Saccharomyces cerevisiae* (*S. cerevisiae*) remains unknown. In this study, we found that water extract of *Ligusticum chuanxiong* (abbreviated as WEL) exhibited excellent free radical scavenging ability *in-vitro*. Moreover, WEL treatment could delay the aging of *S. cerevisiae*, an important food microorganism sensitive to reactive oxygen species (ROS) stress. Biochemical analyses revealed that WEL significantly increased the activity of antioxidant enzymes in *S. cerevisiae*, including superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR), as well as their gene expression. As a result, ROS level was significantly decreased and accompanied with the decline of malondialdehyde (MDA), which represented a state of low oxidative stress. The reduction of oxidative stress could elevate *S. cerevisiae*'s ethanol fermentation efficiency. Taken together, WEL plays a protective role against *S. cerevisiae* aging via improving antioxidant activity.

1. Introduction

Saccharomyces cerevisiae (*S. cerevisiae*), a preeminent industrial microorganism, is widely used in many fermentation products, including beverages, biofuel alcohols, baked foods, biocontrol agents, enzymes, and probiotics [1]. However, there are many factors can affect *S. cerevisiae*'s fermentation efficiency, such as aging, genetics characterization decline and environmental stress. The aging of *S. cerevisiae* is characterized by slow metabolism, large amount of secondary metabolites secretion and cell surface wrinkle and adhesion elevation, which seriously impairs product quality and yield by premature terminating fermentation process [2]. Thus, it is important to explore the methods to delay *S. cerevisiae* aging and improve fermentation efficiency.

The free radical theory of aging postulates that yeast aging is triggered by stress stimulation, which leads to macromolecular

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damage, metabolic changes and cell cycle extension [3–5]. Reactive oxygen species (ROS) is a kind of by-product of electron transfer process in mitochondrial respiratory chain that can cause cell damage directly through oxidation of bio-macromolecules or disruption of intracellular homeostasis [6]. In response to oxidative stress, yeasts possess an efficient and sophisticated antioxidant system to counteract and regulate intracellular ROS level to maintain physiological homeostasis [7]. ROS, including superoxide anion radical, hydrogen peroxide and organic peroxide, can be detoxified into water by copper/zinc superoxide dismutase (SOD1), Mn superoxide dismutase (SOD2), catalase (CAT) or glutathione reductase (GR) in *S. cerevisiae* [7]. However, when ROS level exceeding cell's scavenging capacity, several damaging effects would be appeared, especially mitochondrial dysfunction leading to ATP production decline and even cell death [6,8]. Accordingly, it has been supposed that antioxidant may have a role to delay *S. cerevisiae* aging by reducing intracellular ROS level [9–11].

In recent years, the antioxidant activity of Chinese herbs has aroused wide attention from researchers. Several Chinese herbs, such as *Curcuma longa*, *Nelumbo nucifera Gaertn* and *Herba hyperici* were reported to scavenge free radicals and delay aging in a variety of organisms [12,13]. Our group previously screened dozens of Chinese herbs in their advantages in antioxidant effects and found that the water extract of *Ligusticum chuanxiong* (WEL) showed excellent free radical scavenging capacity *in-vitro* (data not published). *Ligusticum chuanxiong* (LC) is a medicinal herb that possesses versatile pharmacological functions, including antiatherosclerosis, antimigraine, antiaging, and anticancer properties, which is widely applied in traditional Chinese medicine for treating headaches, blood extravasation, and arthritis [14]. A recent study showed that the absolute alcohol extract of LC rhizome and leaves exhibited well antioxidant actions, including 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-Diphenyl-2-picrylhydrazyl radical 2, 2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) radicals scavenging as well as ferric cyanide reducing power [15]. Tetramethylpyrazine (TMP), an active component of LC, can reduce the generation of excitatory toxic free radicals induced by red alginate in rat hippocampus, which thereby stabilizes mitochondrial function and protects brain from oxidative injury [16]. Moreover, LC significantly extends the average and maximum life span of *Caenorhabditis elegans* (*C. elegans*) through regulating the expression of aging and longevity related genes [17,18].

Although the antioxidant activity of LC has been confirmed in many organisms, little is known about its impact on intracellular ROS content and cell aging in *S. cerevisiae*. In the present study, WEL's antioxidant and anti-aging actions in *S. cerevisiae* as well as the underlying presumable mechanisms were investigated. Furthermore, the effect of WEL on ethanol fermentation efficiency of *S. cerevisiae* was also evaluated. The present study provides a candidate method for delaying *S. cerevisiae* aging to improve fermentation efficiency.

2. Materials and methods

2.1. Plant material and extraction

Dried LC rhizomes were purchased from Guizhong Pharmacy (Liuzhou, China), and the drying method was noted in the instruction manual in which fresh LC rhizomes were washed with water and cut into 2-mm slices then dried in 50 °C oven for 2 h. For bioactive substances extraction, 10 g dried LC rhizomes were crushed and mixed with 50 mL double-distilled water (ddH_2O), and shaked at 37 °C, 200 rpm for 2 h. The mixture was concentrated to 5 mL by boiling after crushing with ultrasonic wave (40 kHz, 400 W) for 30 min. Then, the concentrated solution was centrifuged at 10,000 rpm for 5 min, and the supernatant was collected and filtered through a 0.22-μm filter for sterilization. Finally, ddH_2O was added into the supernatant to reach a final volume of 10 mL, and the concentration of WEL was defined as 10 mL extract obtained from 10 g dried LC rhizomes (1 g/mL). The chemical composition of WEL was identified by high performance liquid chromatography (HPLC) that performed at Institute of Quality Standard and Monitoring Technology for Agro-products of Guangdong Academy of Agricultural Sciences (Guangzhou, China), and the components were showed in Table S1.

2.2. Antioxidant activity test

The antioxidant properties of WEL were detected using *in-vitro* chemical methods. Vitamin C (VC, 2 g/L) was used as the positive control. DPPH radical scavenging assay was performed according to the method described by Tristantini et al. [19]. Briefly, the test samples were mixed with an equal volume of DPPH (0.2 mM) and incubated for 30 min at room temperature, and the absorbance was measured at 517 nm in a microplate reader (Infinite F Nano+, Tecan, Switzerland). DPPH radical scavenging rate $(\%) = \frac{A3+A2-A1}{A2} \times 100$, where A_1, A_2 and A_3 are the absorbance of sample, blank control and absolute ethyl alcohol control, respectively.

Superoxide radical scavenging assay was performed according to the method described by Xu et al. [20] with slight modification. The samples (100 μL) were added to the reaction solution containing 450 μL of 50 mM Tris-HCl (pH 8.2) and 40 μL of 25 mM pyrogallol. The mixture was incubated at room temperature for 5 min, then 100 μL of 8 M HCl was added to terminate the reaction, and the absorbance was measured at 299 nm. Superoxide radical scavenging rate $(\%) = \frac{A2-A1}{A2} \times 100$, where A_1 and A_2 are the absorbance of sample and blank control, respectively.

Hydroxyl (•OH) radical scavenging assay was performed according to the method described by Gao et al. [21]. Firstly, a reaction solution containing 100 μL of 0.75 mM phenanthroline, 200 μL of 0.15 M PBS (pH 7.4), 100 μL of 0.75 mM $FeSO_4$ and 100 μL of H_2O_2 (0.01%, v/v) was prepared. Then the samples (100 μL) were added, and the mixtures were incubated for 60 min at 37 °C. The absorbance was measured at 536 nm, and the ability to scavenge •OH radical was calculated as *Scavenging rate* $(\%) = \frac{A1-A0}{A2-A0} \times 100$, where A_0, A_1 and A_2 are the absorbance of blank control, sample and H_2O_2, respectively.

WEL's reducing power was determined by the method described by Gao et al. [22]. The reaction mixture contained 250 μL of 0.2 M

phosphate buffer (pH 6.8), 100 μL of $K_3Fe(CN)_6$ (1%, w/v), and 200 μL of samples. After incubation at 50 °C for 20 min, 120 μL of $FeCl_3$ (0.1%, w/v) was added to stop the reaction. The absorbance was measured at 700 nm, and the reducing power was calculated as Reducing power (%) $= \frac{A2}{A1} \times 100$, where A_1 and A_2 are the absorbance of VC and sample, respectively.

2.3. S. cerevisiae culture and treatment

S. cerevisiae strain GGSF16 used in this study was previously isolated from a distillery by our team and preserved in Fermentation Engineering Institute (Guangxi University of Science and Technology, China). Its genetic characteristic and peculiarity were described previously [23,24]. *S. cerevisiae* were cultured in yeast extract peptone dextrose (YPD) broth (20 g/L glucose, 20 g/L peptone, and 10 g/L yeast extract) at 30 °C for 24 h and then diluted to 1:10 in fresh YPD broth with different concentrations of WEL (0, 0.1, 0.5, 1.0 g/L) for incubation in a shaker with rotary speed at 150 rpm. *S. cerevisiae* were collected by centrifugation at 10,000 rpm for 2 min after incubated with different times depending on subsequent experiments.

2.4. S. cerevisiae viability assay

Propidium iodide (PI) was used to detect *S. cerevisiae* viability by its permeability of damaged membrane [25]. *S. cerevisiae* were collected after WEL treated for 4 and 14 days and washed three times with phosphate buffered saline (PBS) solution. Then, *S. cerevisiae* were stained with PI (1 μg/mL) for 1 min on ice at dark. The fluorescence of PI was detected in a flow cytometer (NovoCyte Flow Cytometer, ACEA Biosciences, Inc. USA) with excitation at 488 nm and emission at 572 nm. PI negative cells represent live cells, whereas PI positive cells represent dead cells.

In addition, *S. cerevisiae* viability after WEL treated for 4 days was also detected by spotting test [26,27]. Briefly, *S. cerevisiae* were re-suspended in sterile PBS and diluted to 10^6, 10^5, 10^4, and 10^3 cells/mL. An aliquot of 3 μL of cell suspension was inoculated onto solid YPD medium and cultured at 30 °C for 24 h. The colony growth of *S. cerevisiae* was inspected and imaged.

2.5. Real-time quantitative PCR

After treated with WEL for 12 h and 24 h, total RNAs of *S. cerevisiae* were extracted using RNAprep Pure Cell/Bacteria Kit according to the manufacturer's instructions (TIANGEN, Beijing, China). Total RNAs were quantified by UV-spectrophotometer (Nanodrop 2000c, Thermo, USA) and qualified by 1% agarose gel electrophoresis. 1 μg of total RNAs were reverse-transcribed to cDNAs using 5 × FastKing-RT SuperMix (TIANGEN, China). Real-time quantitative PCR (qPCR) mixtures were prepared using SYBR Green qPCR Mix (GDSBio, Guangzhou, China) and run on the LightCycler 96 Sequence Detection System (Roche, Switzerland). *Actin* gene was used as the internal reference, and the expression of each gene was normalized to *actin* using comparative Ct method [28]. All primers used in this study were listed in Table 1.

2.6. Measurement of enzyme activity

After treated with WEL for 24 h, *S. cerevisiae* were collected and washed with PBS for three times to remove YPD medium. *S. cerevisiae* were re-suspended in a suitable homogenization buffer depending on the enzymes detected, then moderate 400-μm silica beads were added and homogenized for 6 min by grinding at 4 °C [29]. The supernatants were collected after centrifuging for 2 min at 4 °C, 12,000 rpm and used for enzymes activities determination. The activity of GR, SOD and CAT enzymes were measured using respective kits (Beyotime Bio., Shanghai, China) according to the manufacturer's instructions. The protein concentration of *S. cerevisiae* lysates was detected by BCA Protein Concentration Assay Kit (Beyotime, China). GR, SOD and CAT enzymes activities were expressed as U/μg protein. Details of the procedures were described by previous methods [30–32].

2.7. Measurement of ROS content

2′,7′-Dichlorofluorescin diacetate (DCFH-DA) and Dihydroethidium (DHE) were used to detect the level of ROS and superoxide anion free radicals in *S. cerevisiae*, respectively. DCFH-DA is a fluorescence probe that can enter into cells and be hydrolyzed to DCFH

Table 1
Sequences of Primers for qPCR.

Genes	Forward Primers (5′-3′)	Reverse Primers (5′-3′)	Reference
Catt	AGACCAGACGGCCCTATCTT	ACGGTGGAAAAACGAACAAG	Gene ID: 852979
Cata	CCACTGAACTTCTTCGCACA	GAAAACAAACGGGCCTGTAA	Gene ID: 851843
Sod1	AACGTGGGTTCCACATTCAT	CACCATTTTCGTCCGTCTTT	Gene ID: 853568
Sod2	GGGTGGTTTGTCATTGCTCT	CCTTGGCCAGAAGATCTGAG	Gene ID: 856399
Nma111	GATGGGTATGCTGTGGCTTT	TTCAGCAGCCATTGAACTTG	Gene ID: 855600
Aif1	GCGGTGGCTTTGTTAATTGT	CGGCGAGGTGTCTAAAGAAG	Gene ID: 855811
Mca1	GACGAAGAAGATGGGATGGA	GGCCAACATCCTTCCAAATA	Gene ID: 854372
Sch9	TTCGGAAGACAGAGCGAAAT	CCGTTGGCATCGAGTAGAAT	Gene ID: 856612
Actin	CGAATTGAGAGTTGCCCCAG	CGGCTTGGATGGAAACGTAG	Gene ID: 850504

by esterase. Intracellular ROS can oxidize nonfluorescent DCFH to fluorescent DCF. Thus, DCF fluorescence intensity reflects the level of intracellular ROS [33]. Dihydroethidium (DHE) is also a fluorescence probe that can be dehydrogenated by intracellular superoxide anions to generate ethidium, which can produce red fluorescence after binding with DNA or RNA. Thus, ethidium fluorescence intensity reflects the level of intracellular superoxide anions [34].

The procedures of DCFH-DA and DHE (Beyotime Bio., Shanghai, China) to detect ROS and superoxide anions were performed according to the previously described [35,36]. Briefly, *S. cerevisiae* were collected and washed three times with PBS after WEL treated for 24 h as described in section 2.3. Then, *S. cerevisiae* were re-suspended in 10 μM DCFH-DA solution or 4 μM DHE solution (Beyotime Bio., Shanghai, China), and incubated in dark at 30 °C for 20 min, with overturning tubes every 5 min to promote probes entry into *S. cerevisiae*. After incubation, *S. cerevisiae* were washed thrice with PBS to remove the extracellular DCFH-DA or DHE, then re-suspended in PBS. Subsequently, the fluorescence of DCF and ethidium were detected by flow cytometry with excitation at 488 nm and emission at 530 nm for DCF, excitation at 488 nm and emission at 572 nm for ethidium. Intracellular levels of ROS and superoxide anions were represented by the mean fluorescence intensity (MFI) of DCF and ethidium, respectively.

2.8. Measurement of malondialdehyde (MDA) content

MDA is a natural product of lipid oxidation, an oxidation reaction occurred under oxidative stress [37]. Thus, detection intracellular MDA content can reflect the level of lipid oxidation, which indicates the state of oxidative stress. MDA content in *S. cerevisiae* was detected using Lipid Peroxidation MDA Assay Kit (Beyotime, China) according to the manufacturer's instructions. Briefly, *S. cerevisiae* were collected and washed three times with PBS as described in section 2.3 after WEL treated for 48 h. Then, *S. cerevisiae* were homogenized in radio immunoprecipitation assay (RIPA) lysis buffer containing 1% protease inhibitor cocktail (Beyotime, China) by grinding with beads, and the supernatants were collected after centrifuging for 10 min at 4 °C and 12,000 rpm. 100 μL samples and standards provided in the kit were mixed with 200 μL 1 × working buffer and boiled for 15 min. After cooling to room temperature, the absorbance of MDA in the samples and standards was measured at 532 nm in 96-well plate in the microplate reader. MDA concentration was calculated according to the standard curve and expressed as μmol MDA per mg protein.

2.9. Scanning electron microscope

S. cerevisiae were collected and washed three times with PBS as described in section 2.3 after WEL treated for 27 days. Then, *S. cerevisiae* were fixed in 2.5% glutaraldehyde at 4 °C for overnight as described by Hulínská et al. [38]. Fixed *S. cerevisiae* were dehydrated through a series of increasing concentrations of ethanol (20%, 40%, 60%, 80% and 100%). After washed three times with 100% acetone, *S. cerevisiae* were cooled to −70 °C and dried into powder by a vacuum freeze-dryer (Christ Alpha 2LD plus, Germany). The morphological changes of *S. cerevisiae* were observed and imaged by scanning electron microscope (Phenom XL G2, Netherlands).

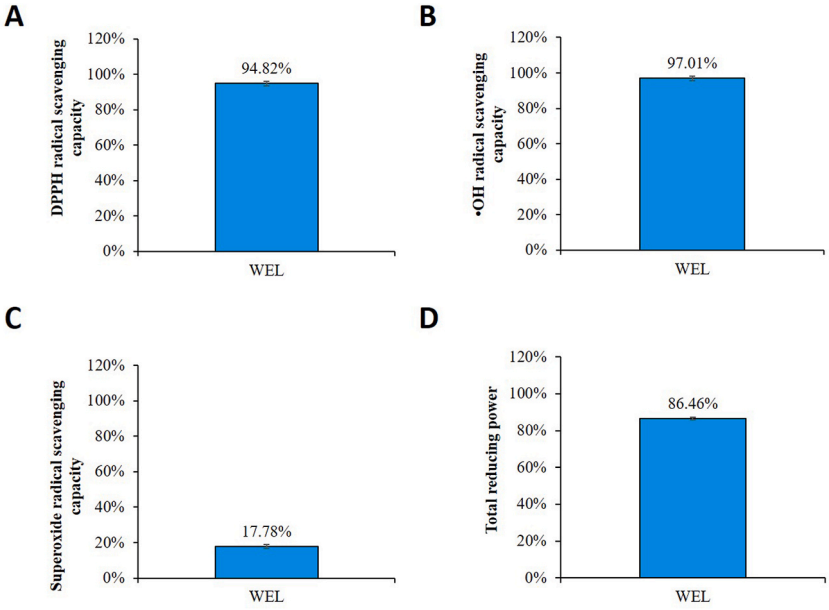

Fig. 1. The *in-vitro* antioxidant effects of WEL. The relative scavenging capacity of WEL to DPPH radical ($P = 0.03$) (A), •OH radical ($P = 0.33$) (B) and superoxide anion ($P < 0.001$) (C) and WEL's total reducing power ($P = 0.001$) (D) normalized to. vitamin C (VC), a well-known antioxidant served as positive control, n = 3.

2.10. Detection of ethanol fermentation efficiency

For ethanol fermentation efficiency detection, *S. cerevisiae* GGSF16 were inoculated to YPD fermentation broth containing 250 g/L sucrose without (Ctrl) or with 1.0 g/L WEL. After fermentation at 30 °C, 150 rpm for 3, 6, 9, 12, 24, 36, 48 and 60 h, 1 mL fermented broth supernatant was collected and used for ethanol concentration detection by gas chromatograph (SCION 8300, Netherlands) as described by Souza et al. [39]. Ethanol production rate was calculated as $rate\left(\frac{g}{L}/h\right) = \frac{m}{t}$, m: ethanol production during sampling time, t: sampling time.

2.11. Statistical analysis

Each experiment in this study was repeated at least three times. Data were expressed as mean ± sem and analyzed by one-way analysis of variance (ANOVA) followed with Duncan's multiple range test or independent-samples T test using IBM SPSS Statistics 25 software. P value < 0.05 was considered statistically significant and indicated by different letters or asterisks in the histogram.

3. Results

3.1. The in-vitro antioxidant effects of WEL

WEL's antioxidant effects were determined by *in-vitro* scavenging DPPH, •OH and superoxide anion radicals as well as total reducing power analysis (Fig. 1). Results showed that WEL's DPPH radical (94.82%) and •OH radical (97.01%) scavenging capacities were comparable to VC, a well-known antioxidant served as positive control (Fig. 1A-B), although superoxide anion radical (17.78%) scavenging capacity lower than VC (Fig. 1C). Total reducing power of WEL was 86.46% compared to VC (Fig. 1D). These results suggested that WEL possessed excellent antioxidant activities.

3.2. WEL delays the aging of S. cerevisiae

PI staining was used to discriminate live *S. cerevisiae* and dead *S. cerevisiae*. Flow cytometry results showed that the mortality of *S. cerevisiae* was gradually decreased with the increase of WEL concentrations, and 1.0 g/L WEL significantly decreased the mortality from 4.93 ± 1.24% to 1.51 ± 0.29% at 4 days treatment and 54.29 ± 4.52% to 27.99 ± 3.82% at 14 days treatment (Fig. 2A-B). Spotting test also showed that WEL increased spot numbers in a dose-dependent manner (Fig. 2C), suggesting an anti-aging effect of WEL on *S. cerevisiae*. Moreover, scanning electron microscope revealed that aged *S. cerevisiae* were characterized by collapse, shrinkage and sticking together after culturing for 27 days, while the morphology of *S. cerevisiae* treated with WEL displayed plump, complete and smooth surface (Fig. 2D), which suggests that WEL can delay the aging of *S. cerevisiae*.

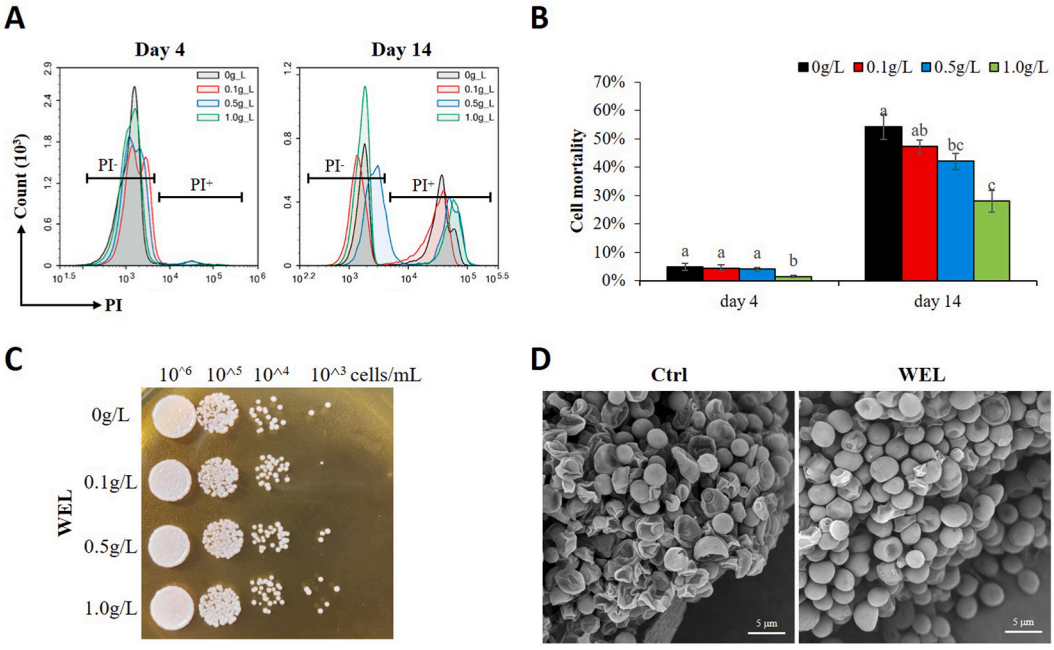

Fig. 2. WEL delays the aging of *S. cerevisiae*. The effects of different concentrations WEL on *S. cerevisiae* mortality detected by flow cytometry using PI staining (A, B), n = 3–4. Spotting test (C) and morphology (D) of *S. cerevisiae* after different concentrations of WEL treatment.

3.3. WEL affects the expression of aging-related genes in S. cerevisiae

To further investigate the effect of WEL on *S. cerevisiae*'s aging, aging-related genes expressions were detected by qPCR. *Nma111*, *aif1*, *Sch eme 9* and *mca1* are markers of aging and cell death in yeast. Results showed that WEL significantly decreased the expression of *nma111* at 12 h and 24 h (Fig. 3A). Different concentrations of WEL did not affect *aif1* expression at 12 h, but low dosage of WEL (0.1 g/L) decreased and high dosage of WEL (1.0 g/L) increased *aif1* expression at 24 h (Fig. 3B). The expression of *Sch eme 9* was not affected by WEL at 12 h, but increased at 24 h in a dose-dependent manner (Fig. 3C). *Mca1*'s expression was only significantly changed at 12 h under 0.1 g/L WEL treatment (Fig. 3D). Taken together, WEL can affect the expression of aging-related genes in *S. cerevisiae* at different extents.

3.4. WEL elevates the activity of anti-oxidative enzymes in S. cerevisiae

Based on the excellent antioxidant effects of WEL *in-vitro*, we speculated that WEL delays *S. cerevisiae* aging by modulating anti-oxidant system. The activities of SOD, CAT and GR anti-oxidative enzymes in *S. cerevisiae* were detected, and the results showed that SOD, CAT and GR activities were gradually elevated with the increase of WEL concentrations (Fig. 4A-C). The elevation of anti-oxidative enzymes activities in *S. cerevisiae* mainly accounted for the increased expression of anti-oxidative enzymes genes, since 1.0 g/L WEL significantly increased the expression of *sod1*, *sod2*, *catt* and *cata* mRNAs both at 12 h and 24 h (Fig. 5A-D). Taken together, these results indicate that WEL can enhance the ability of *S. cerevisiae* to against intracellular oxidative stress.

3.5. WEL reduces ROS levels in S. cerevisiae

In order to clarify whether WEL elevated anti-oxidative enzymes activities affected ROS levels in *S. cerevisiae*, the intracellular ROS and superoxide anion contents were respectively detected by DCFH and DHE fluorescence probes through flow cytometry. Flow cytometry results showed that WEL caused both of DCFH and DHE fluorescence curves shifted to the left (Fig. 6A and C), and the mean fluorescence intensities of DCFH and DHE were significantly decreased compared to WEL untreated samples (Fig. 6B and D), suggesting that WEL can reduce ROS contents in *S. cerevisiae*. Consistently, the level of intracellular MDA, an indicator of oxidative stress, was also gradually decreased with the increase of WEL concentrations (Fig. 6E).

3.6. WEL improves ethanol fermentation efficiency of S. cerevisiae

WEL inhibiting oxidative stress to delay *S. cerevisiae* aging prompted us to detect whether WEL can improve ethanol fermentation of

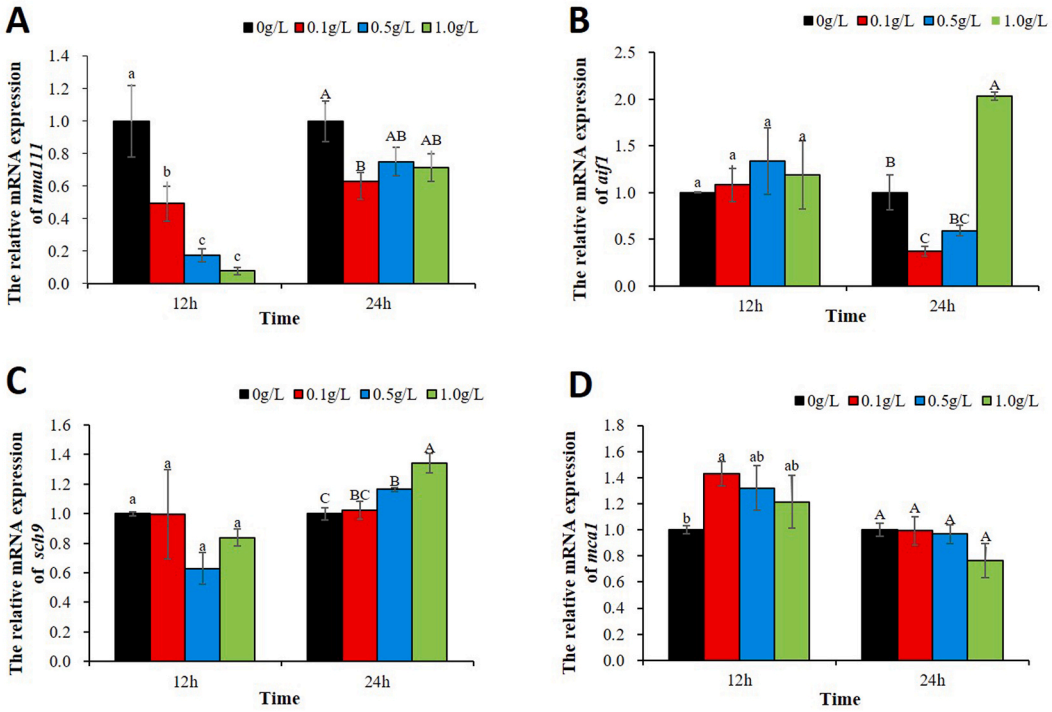

Fig. 3. Effects of WEL on aging-related genes expression in *S. cerevisiae.* The mRNA expression of *nma111* (A), *aif1* (B), Sch eme 9 (C) and *mca1* (D) of *S. cerevisiae* after different concentrations of WEL treated for 12 h and 24 h, n = 3.

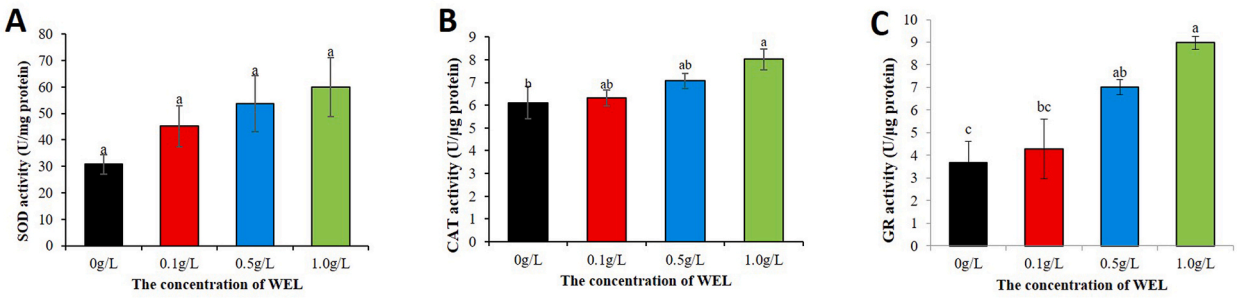

Fig. 4. Effects of WEL on antioxidative enzymes activities of *S. cerevisiae*. The activity of SOD (A), CAT (B) and GR (C) anti-oxidative enzymes of *S. cerevisiae* after different concentrations of WEL treatment, n = 3.

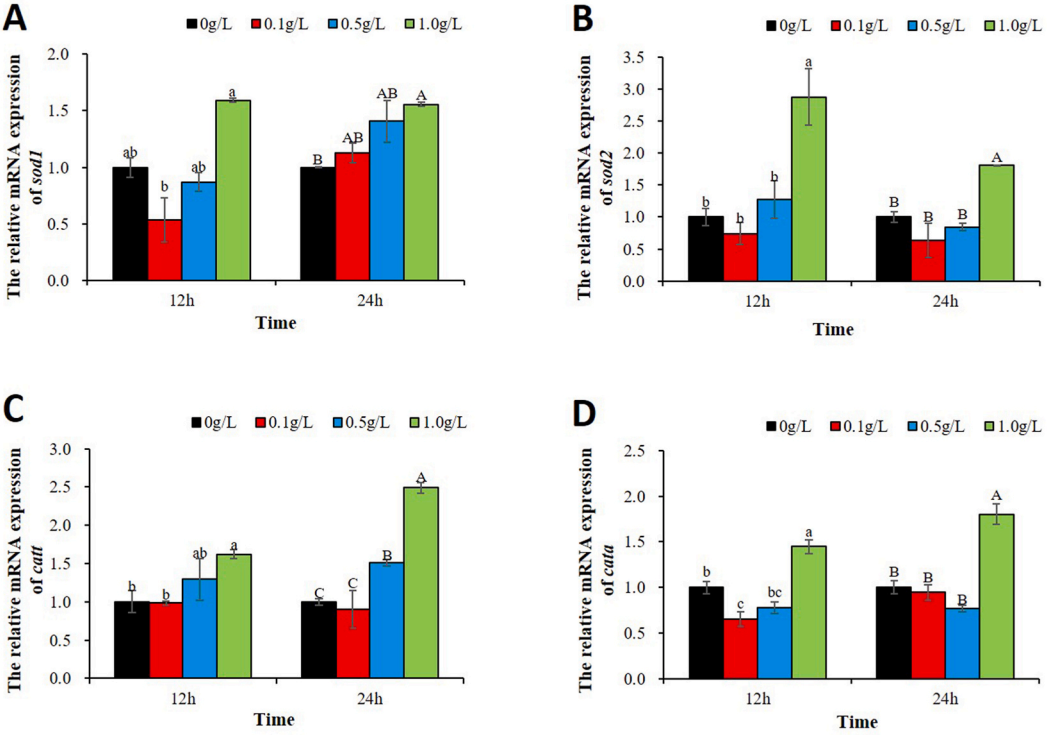

Fig. 5. Effects of WEL on the expression of anti-oxidative enzymes genes in *S. cerevisiae*. The mRNA expression of *sod1* (A), *sod2* (B), *catt* (C) and *cata* (D) in *S. cerevisiae* after different concentrations of WEL treated for 12 h and 24 h, n = 3.

S. cerevisiae. As shown in Fig. 7A, the production of ethanol was gradually increased from 3 h and reached to saturation at 24 h both in control and WEL-treated samples. Compared to control, WEL treatment could enhance the yield of ethanol fermentation (Fig. 7A), and the augment of ethanol production rate was mainly at 6 h (Fig. 7B). To sum up, WEL elevates antioxidant activity to against aging and thereby improves fermentation efficiency of *S. cerevisiae*.

4. Discussion

Numerous recent studies have focused on medicinal plants to prevent and postpone aging. A range of bioactive compounds from medicinal plants such as *Lithospermum erythrorhizon*, *Panax ginseng*, *Ginkgo biloba*, and *Rhodiola rosea* usually possess excellent ability to scavenge free radicals, which are associated with facilitate cellular senescence and lifespan extension [40]. However, the antiaging activity of *Ligusticum chuanxiong* (LC), a common traditional edible-medicinal herb, remains largely unknown. Thus, in this study, the antioxidant and antiaging activities of LC rhizome were studied in yeast model, and its potential on improving fermentation efficiency was also evaluated.

LC exhibits free radical scavenging capacity *in-vitro* and antioxidant property in different organisms [14]. In this study, WEL was

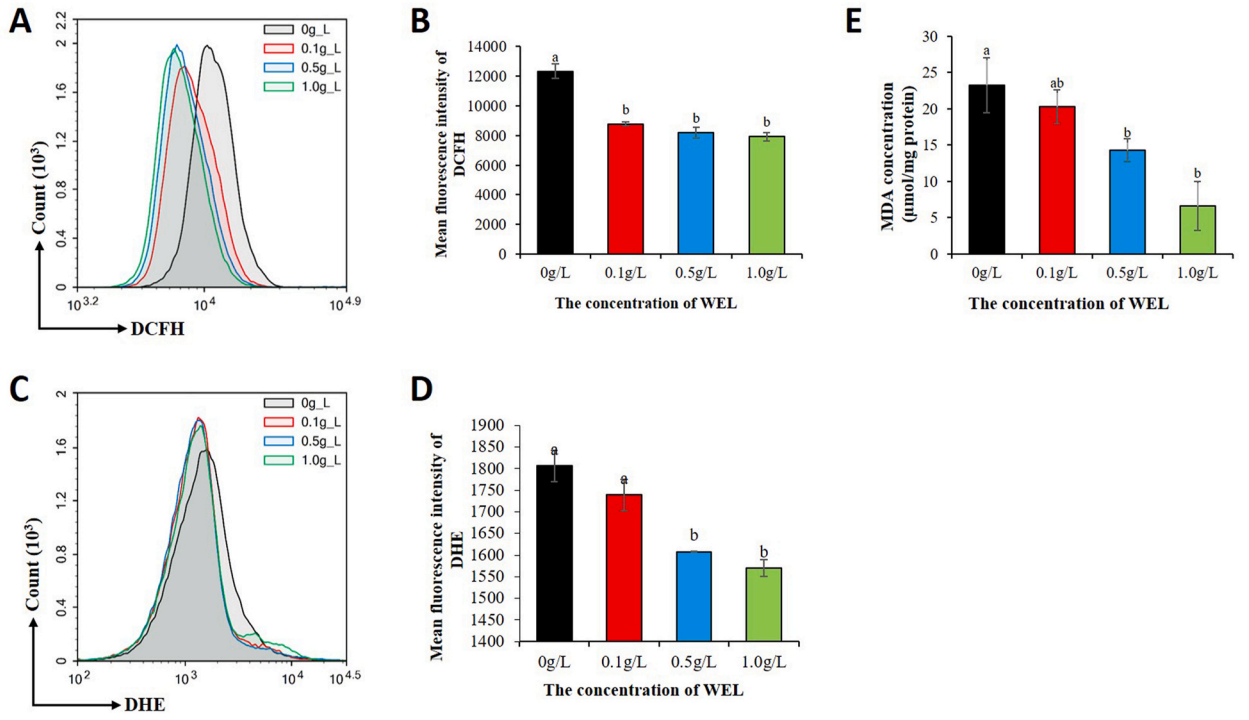

Fig. 6. Effects of WEL on oxidative stress levels in *S. cerevisiae*. The levels of ROS detected by DCFH-DA fluorescence probe (A, B), superoxide anions detected by DHE fluorescence probe (C, D) and MDA (E) in *S. cerevisiae* after different concentrations of WEL treatment, n = 3.

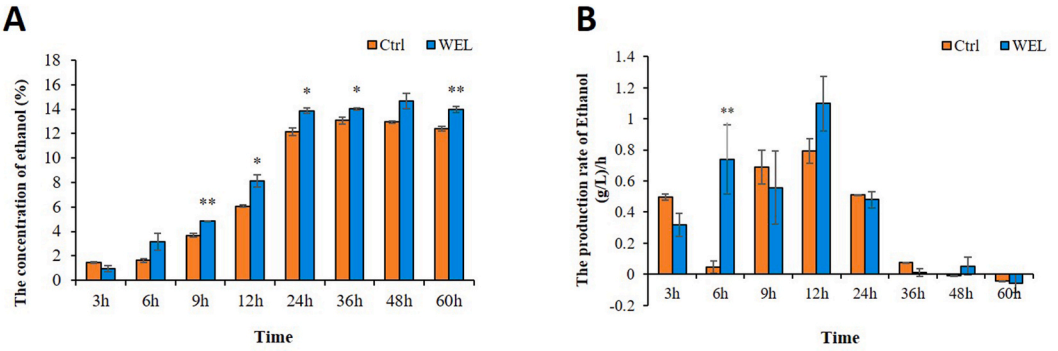

Fig. 7. Effects of WEL on ethanol fermentation of *S. cerevisiae*. The produced ethanol concentration (A) and the production rate (B) in *S. cerevisiae* at different fermentation times after WEL treatment.

proved to scavenge DPPH and •OH radicals *in-vitro* and possess strong reducing power. Consistent with our results, ethyl acetate and methanol extracts from LC showed high radical scavenging rates for DPPH, ABTS, and •OH radicals [41,42]. A recent liquid chromatography-mass spectrometry (LC-MS) analysis confirmed that LC rhizome extracts contain abundant phytochemicals, including phthalides, alkaloids, organic acids, terpenes, and polyphenols [15].The free radical scavenging capacity of LC may be attributed to it contained polyphenols, which have hydroxyl groups and can donate electrons to promote H_2O_2 conversion to H_2O [43,44]. Different solvents can recover compounds with different compositions and contents from one medicinal herb, and neutral solvents are more favorable to extract polyphenols [41]. In addition, the fermentation of microorganisms, such as *S. cerevisiae* [45] and *Lactobacillus plantarum* [46] can affect the antioxidant activity of plant-based food material through release and production of bioactive components, for example polyphenols [47,48]. The antioxidant potential of LC extracts has been considered to the synergistic or additive actions among multiple secondary metabolites [49]. Therefore, we speculated that WEL's antioxidant effects may be related to the interactions between various active compounds, while the main functional chemical components identification requires further investigation.

Aging is a multifactorial process that leads to progressive decline in the function of cells, tissues and organism. Although aging is inevitable, it is entirely possible to slow the rate. The results from this study clearly showed that WEL enriched with polyphenols

significantly delayed the aging of *S. cerevisiae*. Polyphenols with strong antioxidant activity are often identified as anti-aging agents that help to slow down cellular aging [50], which may aid to delay the aging of *S. cerevisiae*. Similar activities to delay yeast aging have been reported in other medicinal herbs, such as clove extract, roselle petal extract, and *Polyalthia longifolia* leaf extract, which may account for their significant antioxidant activity [27,51]. In addition, LC extracts were proved to significantly extend the mean and maximum lifespan of *C. elegans* [17,18]. To our knowledge, this is the first research to demonstrate the antiaging potential of LC rhizome extract on *S. cerevisiae*. During the proliferation of yeast, mitochondria constantly produce several types of harmful free radicals, such as superoxide and peroxide, which lead to the accumulation of oxidative stress and accelerate aging process [52]. Thus, antioxidant system is essential for maintaining physiological homeostasis and delaying aging. As demonstrated in this study, antioxidant-enriched WEL treatment may enhance endogenous antioxidant activities to reduce excessive amount of harmful oxygen derivatives existed in yeast cells, and thereby facilitate cell proliferation.

To understand the underlying mechanism of WEL delaying *S. cerevisiae* aging, the antioxidant system in *S. cerevisiae* was further detected. WEL could enhance the antioxidant activity of *S. cerevisiae* as evidenced by the effects of WEL on yeast antioxidant enzymes activities, oxidative stress level and genes expression. As mentioned above, cellular endogenous antioxidant defense system should eliminate and detoxify ROS in oxidative stage. However, when cellular antioxidant defense system is overwhelmed by an excess of free radicals, there has a destructive impact on cellular function that leads to cell aging. Taken the free radical scavenging capacity of WEL *in-vitro* into consideration, we speculated that medicinal herbs rich with antioxidants can assist cells in scavenging free radicals. Glutathione (GSH) produced by GR is one of non-enzymatic endogenous antioxidant in *S. cerevisiae* for free radicals scavenging. The principal role of GSH is to reduce oxidative stress, and depleting cellular GSH showed hypersensitivity and inability to respond to H_2O_2 stress in *S. cerevisiae* [53]. In addition, SOD, a classical antioxidant enzyme, is reported to relieve oxidative stress and prolong yeast lifespan [54,55]. In this study, WEL significantly reduced *S. cerevisiae*'s oxidative stress by enhancing antioxidant enzymes activities, including SOD, CAT and GR, suggesting that WEL plays an important role to delay *S. cerevisiae* aging through antioxidant effects.

WEL elevated antioxidant system in *S. cerevisiae* has been shown to play an essential role in protecting cells from oxidative stress by eliminating ROS and superoxide anion. RT-qPCR experiment for antioxidant enzymes and aging related genes was conducted to further understand the mechanism of WEL extending *S. cerevisiae* lifespan. Although these genes play essential roles in aging and antiaging processes, their exact contributions in WEL-treated *S. cerevisiae* are unknown. In the present study, WEL could increase the expression of antioxidant genes including *sod1*, *sod2*, *catt* and *cata* that are associated with aging delay, and decrease the expression of apoptosis related gene *nma111* in *S. cerevisiae.* It has been reported that antioxidant enzymes genes *sod1* and *sod2* are closely related to the regulation of yeast lifespan in which knockout of *sod1* and *sod2* caused a rapid yeast death [54]. In this study, the up-regulated *sod1* and *sod2* genes may play a vital role in free radical scavenging activity, which maintains the redox balance and postpones the aging progression of WEL-treated *S. cerevisiae*. Similar results were also reported on *sod* genes expression in *P. longifolia* leaf extract treated *S. cerevisiae* [27].

In addition to affecting the expression of antioxidant genes, WEL also regulated the expression of aging and apoptosis related genes. Nma111 and Mca1 belong to proteases that are implicated in yeast programmed cell death. Cells lacking *nma111* show stronger survivability than wild-type cells in response to heat-shock, and overexpression of *nma111* promotes apoptosis under stress conditions [56]. Moreover, S. cerevisiae apoptosis induced by toxicant is accompanied by increasing cellular oxidative stress and nma111 gene expression [57,58]. *Aif1* is a mitochondria related apoptosis-inducing factor, and deletion of *aif1* protects *S. cerevisiae* from caspofungin-induced programmed cell death [59].Thus, these genes act as markers for programmed cell death and are involved in oxidative stress. In this study, WEL significantly decreased the expression of *nma111* and *aif1* gene, which indirectly indicated that WEL might decrease the apoptosis of *S. cerevisiae*. It is reported that Scheme *9* gene encodes a key protein kinase for longevity control, and deletion of *Sch eme 9* gene in yeast showed a longevity phenotype compared to the wild type yeast [60]. Furthermore, *Scheme 9* was proved to regulate intracellular protein ubiquitination by mediating stress responses in *S. cerevisiae* [61]. Inconsistently, WEL gradually increased the expression of Scheme *9* gene with the increasing concentrations of WEL administration in this study. We hypothesized that some substances existed in WEL could specifically affect the expression of *sch* gene.

Alcohol fermentation is widely used in alcohol, wine and food industries. Additionally, fuel ethanol, a new type of clean renewable energy, also relies on the ethanol fermentation of *S. cerevisiae*. Improving fermentation efficiency is quite necessary for food and energy industries. In this study, WEL was added into fermentation broth and improved the yield of ethanol fermentation to a certain extent. As mentioned above, the improvement in fermentation efficiency was mainly due to WEL delayed *S. cerevisiae* aging by increasing antioxidant ability. To our knowledge, the role of LC improving ethanol fermentation in *S. cerevisiae* has not reported yet, which suggests the potential application of WEL in industrial fermentation.

5. Conclusions

In this study, we found that water extract from *Ligusticum chuanxiong* had excellent capacities to scavenge DPPH, •OH and superoxide anion radicals *in-vitro*. Biochemical analyses results showed that WEL significantly elevated antioxidant enzymes activities to reduce oxidative stress levels and thereby delayed the aging of *S. cerevisiae*. More importantly, WEL could improve the efficiency of ethanol fermentation in *S. cerevisiae*. Altogether, the present study provides a new direction for improving ethanol fermentation and even renewable energy development using *S. cerevisiae*.

Declarations

Author contribution statement

Yinhui Yan: Performed the experiments; Analyzed and interpreted the data.

Qianxing Zou: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. </p>

Yueqi Zhou: Huan He: Wanguo Yu: Haijun Yan: Performed the experiments.

Yi Yi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Zaoya Zhao: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

5.1. Data availability statement

Data will be made available on request.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19027.

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