Ultrasonics Sonochemistry 99 (2023) 106587

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

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

Ultrasound-assisted fermentation of ginkgo kernel juice by *Lactiplantibacillus plantarum*: Microbial response and juice composition development

Jinling Chen [a,b], Qiqi Wang [a,b], Yuting Wu [a,b], Yue Wu [c], Yue Sun [a,b], Yunfei Ding [a,b], Zhen Wei [a,b], Sivakumar Manickam [d], Saikun Pan [a,b], Jie Yang [a,b,*], Yang Tao [e,*]

[a] Jiangsu Key Laboratory of Marine Bioresources and Environment/Jiangsu Key Laboratory of Marine Biotechnology, Jiangsu Ocean University, Lianyungang 222005, China
[b] Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Jiangsu Ocean University, Lianyungang 222005, China
[c] Sonochemistry Group, School of Chemistry, The University of Melbourne, Parkville, Victoria 3010, Australia
[d] Department of Petroleum and Chemical Engineering, Faculty of Engineering, Universiti Teknologi Brunei, Bandar Seri Begawan BE1410, Brunei Darussalam
[e] College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

ARTICLE INFO

Keywords:
Ginkgo kernel juice
Ultrasound-assisted fermentation
Lactiplantibacillus plantarum
Phenolic substances
Antioxidant activity

ABSTRACT

This study is aimed to explore the feasibility of ultrasound on enhancing the fermentation properties of ginkgo kernel juice by *Lactiplantibacillus plantarum* Y2. Specifically, ultrasound at 20 kHz and different intensities (mild ultrasound intensity-84.42 W/L, moderate ultrasound intensity-115.50 W/L, high ultrasound intensity-173.88 W/L) with a pulse mode were applied to facilitate the fermentation process. The number of viable cells of *Lactiplantibacillus plantarum* Y2 increased by 5.06, 5.05 and 2.19% in the sonicated groups at 173.88, 115.50 and 84.42 W/L, compared with the non-sonicated juice after 24-h fermentation. Furthermore, mild intensity ultrasonication improved the permeability of the cell membrane, which is beneficial for the metabolism of phenolics, amino acids and organic acids. Ultrasonication increased *in-vitro* antioxidant activity of fermented ginkgo kernel juice by promoting the metabolism of phenolic acids, such as ferulic acid, chlorogenic and caffeic acids. At the end of fermentation, the sonicated group at 84.42 W/L has the maximum consumptions of total sugars and proteins (increased by 12.52 and 18.73%). Moreover, the reduction rate of the poison material 4′-*O*-methylpyridoxine (MPN) in ginkgo kernel juice increased by more than 16.40% with ultrasound treatment at 173.88 W/L after the fermentation for 48 h. Overall, ultrasound can improve the metabolizations of *Lactobacillus plantarum* and reduce the toxic substances, which promoted the nutritional value and flavors of ginkgo kernel juice.

1. Introduction

Ginkgo biloba kernels are known to contain various biologically active substances, including terpenoids, flavonoids, polyphenols, and organic acids [1]. They have been traditionally used for both food and medicine and are recognized for their therapeutic and medicinal properties, such as enhancing brain function, reducing cholesterol levels, and acting as an antioxidant [2]. Despite their potential for a wide range of applications, the ginkgo kernel product is not marketable in many countries due to the presence of the toxic substance 4′-*O*-methylpyridoxine (MPN), an analogue of vitamin B6, also the ginkgoid acid and hydrogen cyanide [3]. MPN has been found in canned ginkgo boiled products at levels ranging from 0 to 25 μg/mL [4]. Excessive consumption of ginkgo kernels can result in toxic effects on the cranial nerves, gastrointestinal, and respiratory system [5]. Although there have been considerable researches on ginkgo biloba, relatively little research was conducted on ginkgo kernels. Various ginkgo products such as ginkgo tea, ginkgo wine, and ginkgo beverages are readily available in the market, but limited research has been conducted related to lactic acid bacteria (LAB) fermented ginkgo kernel juice drinks [6]. To address this issue, numerous efforts have been made to improve the fermentation of ginkgo kernel juice, reduce its toxicity, and explore its development and application.

Across various fermentation methodologies, the process of probiotic

* Corresponding authors at: Jiangsu Key Laboratory of Marine Bioresources and Environment/Jiangsu Key Laboratory of Marine Biotechnology, Jiangsu Ocean University, Lianyungang 222005, China; College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China.
E-mail addresses: yangjie@jou.edu.cn (J. Yang), yang.tao@njau.edu.cn (Y. Tao).

https://doi.org/10.1016/j.ultsonch.2023.106587
Received 11 July 2023; Received in revised form 30 August 2023; Accepted 3 September 2023

fermentation exhibits the capacity to enhance the sensory attributes of products through the bioconversion of specific organic acids and phenolic compounds [7]. Some secondary metabolites (produced by LAB fermentation) are very beneficial to the human body, improving the activity of antioxidant enzymes in cells and producing more active substances [8]. To obtain a comprehensive understanding, it is necessary to conduct a comparative analysis encompassing microbial growth, carbohydrate and protein profiles, organic acids, volatile compounds, and phenolic compounds. In our previous study, it was demonstrated that the antioxidant and antibacterial activities of fermentation samples with *L. plantarum* could be observably improved, while the content of MPN was decreased [8]. However, there is still much space for improvement in terms of the fermentation capacity of *L. plantarum*, nutrient utilization, degradation of toxic substances, and flavor enhancement. Considering the positive impacts of mild ultrasound on fermented food, it can be considered a potential technology to help microorganisms adapt to the reaction environment during the fermentation of ginkgo products.

Generally, ultrasound is a physical technique that has various biological, sonochemical effect and cavitation effects, ranging from damage to efficiency, related to the intensity used [9]. For instance, ultrasound treatment has a positive influence on improving the fermentation efficiency of *L. plantarum*, promoting cell growth and metabolism, and enhancing the fermentation process and maturation, leading to improve some physicochemical and sensory properties of fermented foods (i,e., texture, color, flavor, etc.) [10]. When ultrasound is transmitted through a liquid medium, which generates a cavitation effect. It will reduce the cavitation bubbles subsequently expand and collapse, including strong mechanical, thermal, and chemical influences that can be liberated into the surrounding fluid. Other than that, hydraulic cavitation has also been shown to have this function [11,12]. This mechanical shock generated by the propagation of the implosion of cavitation bubbles leads to enhance various mass transfer processes, which increases the rate of cellular uptake and adsorption reaction without adversely affecting cell morphology [12]. There is a study that used different intensities of ultrasound (5, 10, 15 and 20% of 37 kHz) to treat *Lacticaseibacillus casei* LUHS210 fermented cereal drinks to prepare by-products. The changes in porosity, specific volume and so on may be related to this cavitation [13]. Some ultrasound conditions are set up to assist or detect the fermentation process [14]. For instance, in the study by Ojha et al. [7], it was found that ultrasound could be the measurement to monitor the progress of fermentation or to promote fermentation. High-frequency ultrasound (>2 MHz) can be used as a tool to measure chemical composition changes during fermentation. Low-frequency ultrasound (20–50 kHz) can influence the fermentation process by improving mass transfer and cell permeability, thereby increasing process efficiency and production rate. Also, Yu et al. [10], concluded from a large number of literature review that short-term ultrasound within 30 min at a relatively low power (≤100 W), the proliferation of microorganisms in fermented food species was observed, which promoted enzyme activity and metabolic performance. In addition, high-power ultrasound (≥100 W) promotes reactions such as oxidation and hydrolysis in fermented foods to promote the maturation of fermented foods. In the study of Shokri, et al. [15], they used 23 kHz ultrasound and 3 main waves of 10 μm amplitude for 5 min were employed to improve the fermentation process. During the fermentation process, they hypothesized that *L. brevis* was suitable for the production of GABA-rich fermentation products after low-intensity ultrasound (20% amplitudes), some positive microbial responses from short *L. brevis* were observed including the increasing cell count and SGR value, while at higher intensity amplitude (30% amplitudes). Therefore, screening for suitable ultrasonic conditions can significantly contribute to the growth of bacteria and their active substance content during microbial fermentation. Wang et al. [16] employed the moderate-intensity sonication (58.3 and 93.6 W/L) in three growth stages of *L. plantarum* during the 48-h fermentation of apple juice. The influences of ultrasonic power densities on the microbial growth and the metabolization of bioactive substances (including amino acids and phenolics), and antioxidant capacity were investigated in the fermented apple juice by *L. plantarum*. *L. plantarum* responded significantly to sonication in the lag and log phases, and microbial growth was promoted and the biotransformation of malic acid was enhanced. Biosurfactant production by *L. plantarum* has also been shown to increase under ultrasound, reinforcing the adsorption of toxic substances [17]. However, according to literature reviews, few studies have focused on the ultrasound-assisted fermentation of ginkgo kernel juice by *L. plantarum*. Therefore, it is necessary to clarify the influence of ultrasound in the fermented ginkgo kernel juice and select the suitable fermentation conditions.

In this study, *L. plantarum* Y2 was separated from the seed coat of ginkgo biloba fruit was selected as the fermentation culture to investigate the effect of ultrasonic parameters (e.g., ultrasonic intensity and ultrasonic time) on the metabolic response and antioxidant properties during the 48-h fermentation of ginkgo kernel juice. Alterations in the growth of *L. plantarum* Y2, the metabolite contents (sugars, phenolics, amino acids and organic acids) as well as antioxidant capacity in vitro were identified over the entire duration of the fermentation. These results can make up the research gap of ultrasound-assisted fermentation of plant-based juice using *L. plantarum*.

2. Materials and methods

2.1. Preparation of strain and ginkgo kernel juice

L. plantarum Y2 was previously isolated from the surface of Ginkgo biloba kernels through screening and then stored at −20 °C [8]. To obtain primary inoculum, Ginkgo biloba kernels were inoculated in MRS medium at a ratio of 1:1.5 (g: mL). Then it was incubated at 37 °C for 24 h. Individual colonies were obtained from the fermentation broth by plating on MRS plates and culturing at 37 °C for 24 h. The resulting colonies were then transferred to MRS medium, preserved in 40% glycerol, and stored in a −70 °C refrigerator.

Ginkgo biloba (Heartless Ginkgo) samples were harvested from the campus of Jiangsu Ocean University in September 2022. The Ginkgo biloba kernels were separated, washed and boiled for 10 min. Next, they were stripped of their shell and seed coat, and washed with water before being pulped according to the ratio of Ginkgo biloba to distilled water (4:1 g/mL). The starch solubility needed to exceed 81.45%. α-Amylase (20 U/g) and saccharifying enzyme (30 U/g) were added as required, and the mixture was liquefied and saccharified in a temperature-controlled water bath at 50 °C for 2 h. After digestion, the acquired specimen underwent an initial filtration procedure utilizing a 100-mesh filter, followed by its exposure to a water bath set at a temperature of 90 °C for a duration of 20 min. This treatment regimen was employed with the intention of eradicating non-budding pathogenic microorganisms present within the sample [18].

A 1% (v/v) inoculum per 100 mL of sterilized ginkgo kernel juice was inoculated into a 250 mL sealed conical flask, with an initial live count of approximately 6.54 Log CFU/mL. The fermentation was conducted in an incubator at 37 °C for 48 h, and samples were obtained at 0, 4, 12, 24, 36 and 48 h. Subsequently, bacterial cells were eliminated through centrifugation at 10,000×*g* and 4 °C for 10 min. The resulting supernatant was then collected for subsequent chemical analysis.

2.2. Ultrasound-assisted fermentation of ginkgo kernel juice

Ultrasound treatment was conducted using a 20 kHz ultrasound processor (GZ98-III, Wuxi Gangzheng Technology Co., China). The ultrasonic probe was put at a constant position and 1 to 2 cm underneath the surface of the ginkgo kernel juice for a certain time. The sonicated samples were put in an ice-filled container to keep the temperature at 30 °C during the period of sonication. The impacts of sonication at 84.42, 115.50 and 173.88 W/L on the physiological properties of

microbial strain and composition of ginkgo kernel juice was studied, a temperature of 30 °C. Ultrasonication was conducted in a pulsed mode of 5 s on, 5 s off, total time lasted for 6 min. At the fermentation for 0, 4, 12, 24, 36 and 48 h, around 2 mL of unfermented and fermented juice was taken and the supernatant was carefully collected after centrifugation (10,000×*g*, 10 min, 4 °C) for the further physicochemical analysis.

2.3. Analytical methods

2.3.1. Viable cell counts and pH value

The determination of viable cell counts was carried out using the standard plate method [19]. The fermentation broth was appropriately diluted and then spread on MRS culture medium. The pH changes of the non-sonicated and sonicated ginkgo kernel juice during fermentation were monitored using a pH meter (PHS-3C, Shanghai INESA Scientific Instruments Co., Ltd., China).

2.3.2. Cell morphology of L. plantarum Y2

Morphological characterization was performed using a HITACHI transmission electron microscope, model HT7800. The resolution in scanning transmission mode was 0.20 nm and the magnification ranged from 50 to 1,000× for low resolution and 200–200,000× for high resolution. The pixel size was 1024 × 1024 and the frame rate was 160 fps. The *L. plantarum* Y2 fermented for 4 and 12 h were treated with three ultrasonic intensities (84.42, 115.50 and 173.88 W/L) and observed under electron microscope.

2.3.3. Contents of total proteins

The change in total protein concentration during the fermentation of ginkgo kernel juice was analyzed according to the Komas Brilliant Blue assay [20], with minor modifications. Briefly, a 0.1 g fermentation broth was used for protein extraction along with 3 mL of extraction buffer (200 mM Tris, 10 mM EDTA, pH 5.5). The supernatant obtained was then assayed for protein content at an ultraviolet absorbance value of 595 nm using Komas Brilliant Blue G-250 (Suzhou Biotech, China). Thereafter, soluble protein contents in non-sonicated and sonicated ginkgo kernel juice were determined by utilizing the standard curve generated from bovine serum albumin (BSA) (Suzhou, China) as a standard and total protein content in samples was expressed as mg/mL.

2.3.4. Contents of total sugars

The total sugar contents of unfermented and fermented ginkgo kernel juice were determined by the phenol sulphate method [21]. Briefly, sugars in unfermented and fermented ginkgo kernel juice were hydrolyzed into monosaccharides by concentrated H_2SO_4, such dehydrated components can combine with phenol to form products with yellow or orange color. The absorbance of the products was recorded by UV–vis spectrometry at 470 nm. Glucose was used to establish the standard curve and the results were expressed as the mg/mL of glucose equivalents.

2.3.5. Total phenolic content

The total phenolic content of unfermented and fermented ginkgo kernel juice was determined by the Folin-Ciocalteu method with slight modifications [22]. Specifically, the diluted unfermented or fermented ginkgo kernel juice was mixed with diluted Folin-Ciocalteu reagent (10% concentration) in proportion to 1:5 (v:v). After reacting for 3 min, 2 mL of Na_2CO_3 (7.5% w/v) was added to. The resulting mixture was then stored at 20 °C and avoided the light for 45 min and the absorbance was measured at 765 nm. The calibration curve was established using gallic acid (GA, purity >99.9%) as a standard and the result was expressed as mg GAE/L.

2.3.6. Total flavonoids content

The total flavonoid content was determined by referring to the method of Kwaw with minor modifications [23]. A mixture of 1 mL of the sample, 4 mL of distilled water, and 0.3 mL of 5% (w/v) $NaNO_2$ was prepared by vortexed and left to stand for 4 min. Subsequently, 1 mL of 10% $AlCl_3$ (w/v) was added and mixed, and allowed to stand for 4 min. Then, 2 mL of NaOH (0.1 mM) and 2.4 mL of distilled water were added to bring the final volume to 1 mL. The mixture was put at 20 °C for 10 min with occasional shaking, and the absorbance of fermented juice was determined at 510 nm. A standard curve was established using rutin as a standard the results were expressed in μg/mL of rutin equivalents.

2.3.7. Contents of individual phenolic compounds

HPLC analysis was performed to identify and quantify the individual phenolic compounds in unfermented and fermented ginkgo kernel juice [24]. The device used was the Agilent 1260 HPLC system coupled with a chromatographic column (Inertsil ODS-35, 4.6 × 250 mm, 5 μm). Mobile phases A and B was consisted of aqueous 1% (v:v) acetic acid and methanolic 1% (v:v) acetic acid. The flow rate, injection volume and column temperature were 0.6 mL/min, 20 μL and 25 °C, respectively. The gradient was set as: 0–10 min, 10–26% B; 25 min, 40% B; 45 min, 65% B; 55 min, 95% B; 58 min, 10% B; 65 min, 10% B. The phenolic acids and flavonoids were measured at 280 nm and 350 nm, respectively. HPLC grade phenolic standards (gallic, hippuric, protocatechuic, chlorogenic, *p*-hydroxybenzoic, *p*-vinyl guaiacol, rhizobin, rhizolic, cinnamaldehyde, caffeic, eugenic, *p*-coumaric, ferulic, homovanillic, catechin and proanthocyanidinB_2) (purity > 99.9%) were applied to establish the calibration curves.

2.3.8. Contents of organic acid

The organic acid profiles in unfermented and fermented ginkgo kernel juice were analyzed using a Shimadzu LC-2010A system (Shimadzu, Tokyo, Japan) equipped with an Agilent TC-C18 column (4.6 × 25 mm, 5 μm) [25]. Isocratic elution was performed using 0.08 M KH_2PO_4 solution (pH 2.5) and the measurement was carried out at 210 nm. The column temperature, flow rate and injection volume were set to 30 °C, 0.7 mL/min and 20 μL, respectively. HPLC grade organic acids (oxalic acid, quininic acid, lactic acid, pyruvic acid, mango acid, malic acid, citric acid, fumaric acid and succinic acid) (purity > 99.9%) were selected as standards to establish the calibration curves.

2.3.9. Contents of free amino acid

The amino acid compositions in unfermented and fermented ginkgo kernel juice were analyzed based on a Shimadzu LC-2010A system (Shimadzu, Tokyo, Japan) equipped with an Agilent 2622PH column (4.6 × 60 mm I.D.) [26]. The flow rate was carefully adjusted to 0.4 mL/min, while the column temperature was precisely maintained at 57 °C throughout the experiment. The reaction temperature was set to a controlled 135 °C, and the detection wavelength was specifically selected at 570 nm. For each analysis, a precise injection volume of 20 μL was used. HPLC-grade free amino acid standards (purity > 99.9%) were bought as standards to establish the calibration curves.

2.3.10. Volatile profile

Analysis of volatile components were performed using gas chromatography-mass spectrometry (GC-IMS). The air from the vial was expelled by filling the headspace vial with nitrogen [27,28]. The thermo RSH autosampler was used to hold 5 mL of the sample and the extraction head was aged at 250 °C for 30 min after being placed into the gas chromatography inlet. The bake oven was set at 80 °C, the water bath was kept for 20 min, and the absorbent extraction was lasted for 30 min. After the extractor head was inserted into the inlet port for 5 min of resolution, the extraction process was completed automatically using the SPME module of the inlet, and the data was collected with the start-up instrument.

Separation and collection of volatile components were conducted using a triple quadrupole gas chromatograph (Trace 1310/TSQ 9000, Thermo Scientific) [28]. The gas chromatographic separation was performed using a TG-5MS capillary column, and the carrier gas used was

He (99.999%). The ionization mode was El, with the electron energy set at 70 eV, and the scan mass range was 33–450 amu. The column temperature was initially set to 50 °C for 5 min, followed by a gradual increase to 200 °C at a rate of 5 °C per minute for 2 min. Finally, the temperature was further increased to 250 °C at a rate of 10 °C per minute for 2 min. HPLC-grade volatile components standards (catechin, hippuric acid, homovanillic acid, *p*-coumaric acid, phloretin, *p*-vinyl guaiacol, proanthocyanidinB_2, chlorogenic acid, *p*-hydroxybenzoic acid, Syringic acid, hypochlorous acid, ferulic acid, cinnamic aldehyde, gallic acid, protocatechuic acid, caffeic acid, rutin, quercetin) were selected as standards to establish the calibration curves. The results were expressed in relative content (%).

2.3.11. Content of 4′-O-methylpyridoxine (MPN)

The content of MPN in fermented ginkgo kernel juice was determined following the method of Yoshimura et al. [29], the mobile phase and gradient elution were modified. The Agilent 1260 HPLC instrument equipped with a fluorescence detector and ZORBAX SB-C18 column (250 × 4.6 mm, 5 μm) was used to analyze MPN in ginkgo kernel juice. The mobile phases A consisted of 5 mM K_3PO_4 and 5 mM $C_5H_{13}NaO_3S$ (pH 2.5). 100% CH_3CN was used as mobile phase B and gradient settings of 0–10 min, 4–10% B; 15 min, 15% B; 30 min, 4% B. The column temperature and flow rate were set at 30 °C and 1.0 mL/min, respectively. The injection volume and the detection wavelength were 20 μL and 291 nm, respectively.

2.3.12. Antioxidant activity of ginkgo kernel juice in vitro

The antioxidant activities of non-sonicated and sonicated ginkgo kernel juice were determined by free radical scavenging activity ($ABTS^{\cdot+}$) and iron-reducing antioxidant power (FRAP), as described in the previous research [30]. The calibration curves were using Trolox and $FeSO_4$ as standards, and the results were expressed as mM Trolox and mM Fe^{2+}.

2.4. Statistical analysis

All experiments and analyses were performed in triplicate. Statistical analysis was conducted using SPSS Statistics 20 (IBM Corp., NY, USA), with significance determined at $p < 0.05$. Principal component analysis was conducted using Origin 2018 (Origin Lab Corp., UK). The experimental data are presented as mean ± standard deviation.

3. Results and discussion

3.1. Effect of ultrasound-assisted fermentation on viable cell count of L. plantarum Y2 and pH value

The degree and ability of microbial growth and metabolism are represented by cellular activity. The promoting effect of different ultrasound intensities on the cells of strain Y2 was observed during the fermentation process, as evidenced by changes in the viable bacteria count of strain Y2 (Fig. 1A). It was found that the number of viable bacteria of *L. plantarum* Y2 in the fermentation broth was directly proportional to the fermentation time and gradually decreased after 24 h, due to the reduced metabolic capacity of the system in the late stages of fermentation and the threatening effect of the lower pH on the reproduction of *L. plantarum* [31]. The number of viable bacteria in all sonicated groups in the logarithmic and stable phases was higher than that in the non-sonicated group, as shown in Fig. 1A. In particular, after fermentation for 24 h, the bacterial counts in the non-sonicated group and sonicated groups at 173.88, 115.50 and 84.42 W/L were 11.15, 12.41, 12.51 and 12.95 Log CFU/mL, respectively. These results indicate that different intensities of ultrasound affect the metabolic reproduction rate of *L. plantarum* Y2 to different degrees. Throughout the whole fermentation period, ultrasound treatment at 173.88 W/L consistently had the highest values of viable bacteria, implying that the ultrasonic intensity in this group was the most impactful in promoting the growth of *L. plantarum* Y2. Generally, the permeability of cell membranes can be improved by the cavitation effect produced by mild ultrasound treatment, which can accelerate the growth and reproduction of cells [32]. The leaching of nutrients from the juice, facilitated by ultrasound treatment, may have been rapidly absorbed and used by *L. plantarum* Y2 to promote its growth and reproduction.

A trend of continuous decline in the pH value of both non-sonicated group and sonicated groups at 84.42, 115.50 and 173.88 W/L was observed, in line with Fig. 1B. The pH of the ultrasonic fermented broth was lower than that of the conventional fermentation broth, which was dropped significantly in the first 12 h of fermentation, the pH decreased from 6.11 ± 0.01 at the beginning to 3.81 ± 0.04, 3.73 ± 0.05, 3.75 ± 0.02 and 3.7 ± 0.04 in the unfermented group, 84.42, 115.50 and 173.88 W/L sonicated groups respectively. In the study of Liao et al. [33], ultrasound increased the consumption of lactose to produce glucose and galactose, thus the pH value has dropped. In addition, according to Shokri and Gholamhosseinpour et al. [34,35], ultrasound can accelerate the acidification of *L. plantarum* subspecies by increasing the activity level of extracellular *β*-galactosidase, thus promoting the hydrolysis of lactose. It was observed that the pH decreased gently between 12 and 48 h of fermentation and steadily between 3.49 ± 0.02 and 3.51

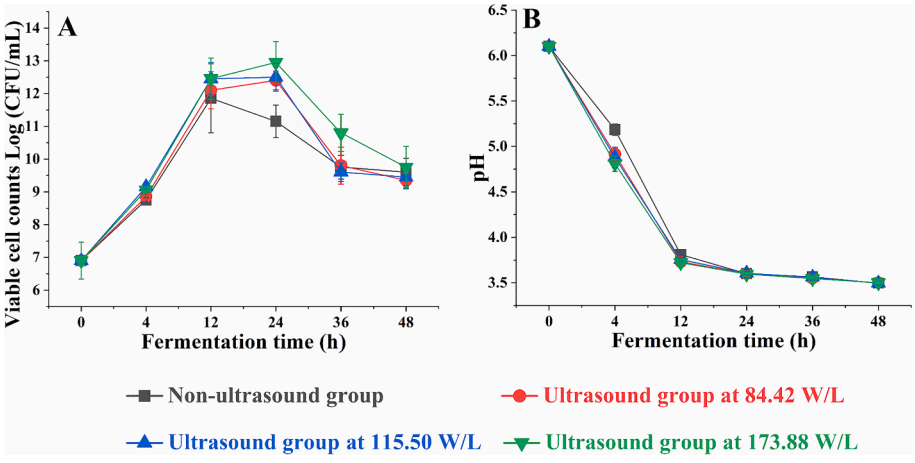

Fig. 1. The Viable cell count (A) and changes of pH during the fermentation of ginkgo kernel juice (B) during the fermentation of ginkgo kernel juice for 48 h.

± 0.01 at the 48-h fermentation. Considering that the fermentation of *L. plantarum* Y2 is entering its decay phase and the acid production capacity has reduced.

3.2. Effect of ultrasound-assisted fermentation on the microbial morphology

The effects of ultrasonic intensity and fermentation time on *L. plantarum* Y2 cell morphology are illustrated in Fig. 2, where the degree of cell rupture is directly proportional to the ultrasonic intensities. A slight rupture of the cell membrane was observed in the ultrasound-assisted fermentation at 173.88 W/L compared to the non-sonicated group in the fermentation for the first 4 and 12 h (the length of cell lysates is 300–400 nm), probably resulting in the leaching of some intracellular nutrients into the extracellular environment. However, in the ultrasound groups with 84.42 and 115.50 W/L (the length of cell lysates are 50–100 nm and 150–200 nm, respectively), it's slighter than the 173.88 W/L ultrasound group. Therefore, it is deduced that the ultrasound intensity and duration did not cause obvious destruction to the cells, resulting only in a slight rupture. The cells were able to gradually recover during the fermentation processes [36]. It was reported that ultrasound has been shown to enhance the cell membrane permeability on the surface of *L. plantarum* and remove metabolic by-

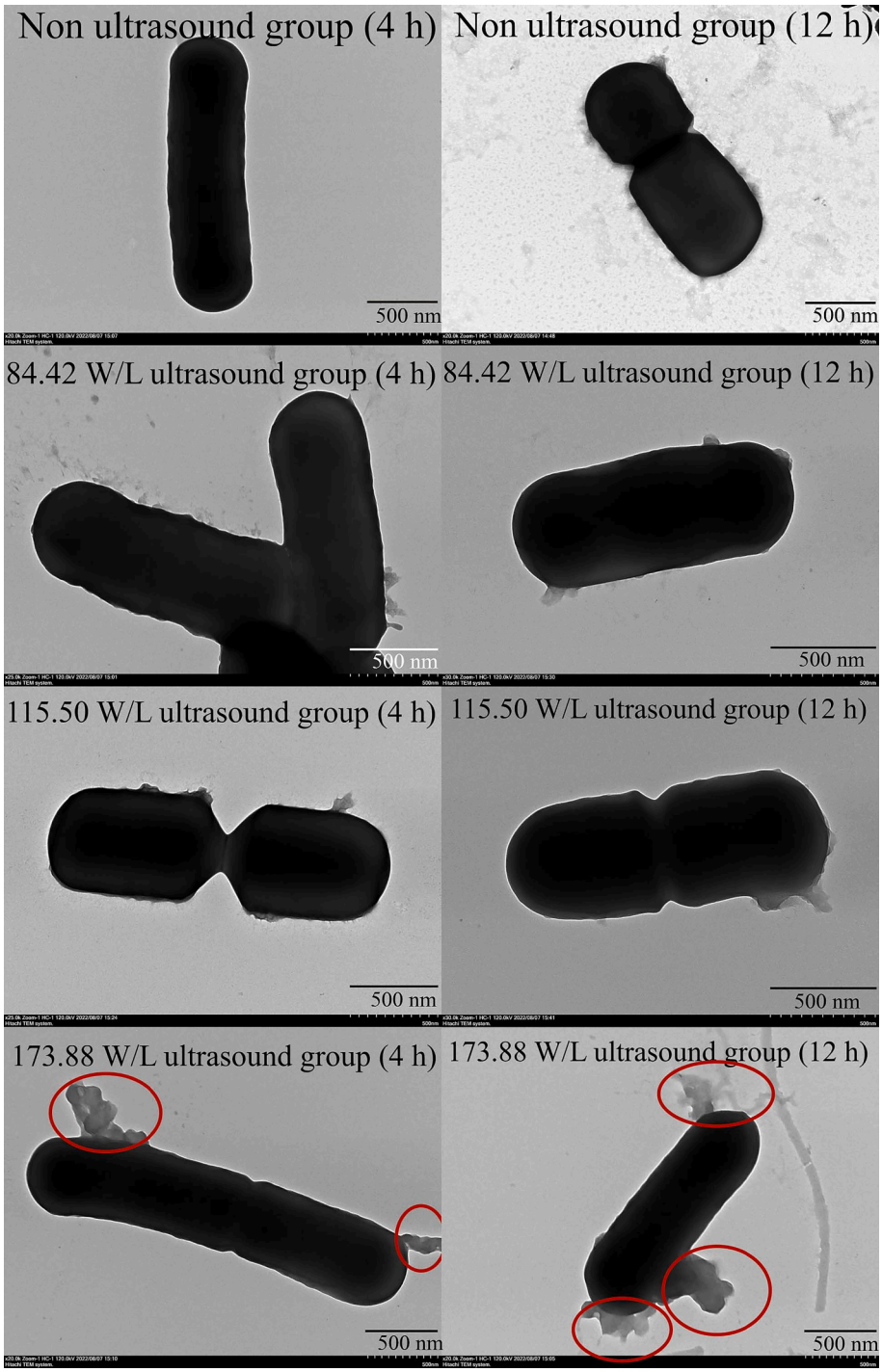

Fig. 2. The microstructure of *L. plantarum* Y2 cell at different sonication conditions and durations observed by Transmission electron microscopic (TEM).

products, thereby facilitating the mass transfer of *L. plantarum* cells [37]. It was speculated in the study of Lu. [38] et al. that the cavitation generated by ultrasound treatment improves the efficiency of nutrient-microorganism interactions and enhances the leaching rate of intracellular substances, then accelerating microorganism growth. However, it has also been shown that excessive ultrasound treatment may cause physical damage and change of microbial cell membranes, leading to leakage of cell contents and cell death [39].

3.3. Effect of ultrasound-assisted fermentation on total sugar and protein contents

The total sugar content in all the fermented ginkgo kernel juice exhibited a declining trend during the whole fermentation process, as illustrated in Fig. 3A. This was particularly evident in the fermentation for 48 h, the total sugar content of non-sonicated and sonicated ginkgo kernel juice (84.42, 115.50 and 173.88 W/L) decreased significantly from 126.83 ± 2.74 mg/mL to 75.21 ± 3.62, 59.33 ± 2.91, 63.52 ± 1.76 and 65.71 ± 3.27 mg/mL, respectively. During fermentation, the total sugar content of all three sonicated groups was lower than the non-sonicated group. After fermentation, the total sugar consumption for the un-sonicated group was 40.5%, and ultrasonication at 84.42 and 173.88 W/L promoted total sugar consumption by microbial strains (48.2% ~ 53.5%). This result suggests that the mild ultrasound intensity was more helpful to increase the degree of sugar consumption by *L. plantarum*, providing more energies for microbial growth [8].

The in-process ultrasonication during probiotic fermentation by *L. plantarum* Y2 had certain effect on total protein content in ginkgo kernel juice. Total protein content in fermented ginkgo kernel juice exhibited a continuous decreasing trend. According to Fig. 3B, the protein content in the original ginkgo kernel juice was 9.61 ± 0.26 mg/mL, in the end of fermentation, the three sonicated samples at 84.42, 115.50 and 173.88 W/L contained the total protein contents of 3.23 ± 0.47, 3.32 ± 0.05 and 3.55 ± 0.08 mg/mL, which was lower than the un-sonicated samples (5.03 ± 0.56 mg/mL). Also, there was a difference in the microbial metabolism of proteins at different ultrasound intensities. Compared with ultrasonication at high intensity (173.88 W/L), ultrasonication at moderate intensity (115.50 W/L) further promoted the decrease of soluble proteins in the ginkgo kernel juice, particularly in the first 4-h fermentation. These results may be due to the reason that ultrasound promoted the utilization of soluble proteins by *L. plantarum* during the lag phase [40]. As a result, this could have a positive impact on the extent of protein consumption by *L. plantarum* Y2 in the mild ultrasound intensity. As similar as the study of Shokri et al. [34], in which moderate intensity ultrasound also promoted the utilization of protein by *Lactococcus lactis*.

3.4. Effect of ultrasound-assisted fermentation on the contents of total phenolics and flavonoids

Phenolics, including flavonoids, are secondary metabolites produced by plants which affect the sensory and nutritional properties of foods [30]. The total phenolic content in both non-ultrasound treated and ultrasound treated ginkgo kernel juices experienced an increase trend in the first 12-h fermentation, and a decrease trend in the later 12-h fermentation, and a slight increase until the 48-h fermentation (Fig. 3C). After fermentation, there were no discernible variations in the total phenolic contents among all the treatments, the in-process ultrasonication exerted certain influence on total phenolic content of ginkgo kernel juice. For example, after fermentation for the first 12 h, the total phenolic content in the ginkgo kernel juice without ultrasonication was 523.06 ± 5.09 mg/L, whereas the samples sonicated at 84.42, 115.50 and 173.88 W/L possessed the total phenolic contents of 496.93 ± 8.91, 513.15 ± 8.91 and 494.23 ± 5.09 mg/L, respectively. The attenuation of phenolics by ultrasound could be contributed to the enhancement of hydrolysis of some phenolics by the extracellular enzymes produced by *L. plantarum* [41]. The drop in the fermentation between 12 and 24 h may be due to the decarboxylation of some phenolics. For example,

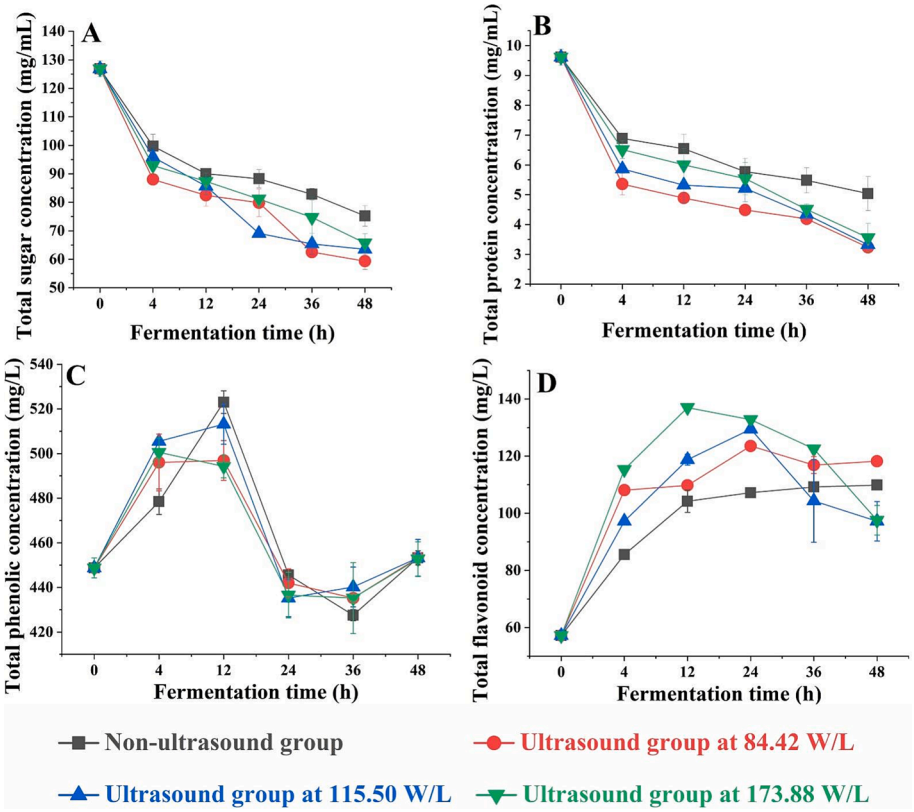

Fig. 3. The changes in the contents of total phenolics (A), flavonoids (B), proteins (C) and sugars (D) during the fermentation of ginkgo kernel juice for 48 h.

these extracellular enzymes produced by the microbial strains, i.e. general decarboxylase, could convert *p*-coumaric and caffeic acids to *p*-vinyl guaiacol, which is further transformed to volatile phenolic compounds such as ethyl derivatives [42]. In the end of fermentation, total phenolic contents in all the ginkgo kernel juices were decreased from 448.73 ± 4.45 to 453.24 ± 8.28 mg/L, and there was no distinction in the total phenolic content among samples with and without sonication.

Changes in total flavonoid content of unfermented and fermented ginkgo kernel juices with and without ultrasound assistance is presented in Fig. 3D. The content of total flavonoids in all groups showed an obviously increase trend during the first 12-h fermentation, roughly, from 57.22 to 104.22 ~ 137.01 mg/L. After fermentation for 12 h, the ultrasound-treated samples at 173.88 W/L possessed the highest total flavonoid content, being 137.01 ± 5.31 mg/L, compared with 104.22 ± 3.92 mg/L in the non-sonicated sample. This is consistent with the increase in phenolics described above, the ultrasound also promotes the conversion of some flavonoids [42]. Then, the higher power ultrasound group (115.50 and 173.88 W/L) showed a decreasing trend from 12 to 48 h of fermentation, however, the mild power ultrasound group (84.42 W/L) and the non-sonicated group were showed a slow increasing trend. The final values were ranging from 97.22 to 118.22 mg/L. The sonicated group at 84.42 W/L was the most beneficial to the increment of total flavonoids, with the content of 118.22 ± 3.15 mg/L at the end of fermentation (48 h). This could be that the mild intensity (84.42 W/L) ultrasound promotes the growth of microorganisms, which in turn secrete some extracellular enzymes such as *β*-glucosidase, *p*-coumaric acid decarboxylase and therefore promote the conversion of phenolic substances [41]. Similar as the results obtained by Elnour et al. [43,51],

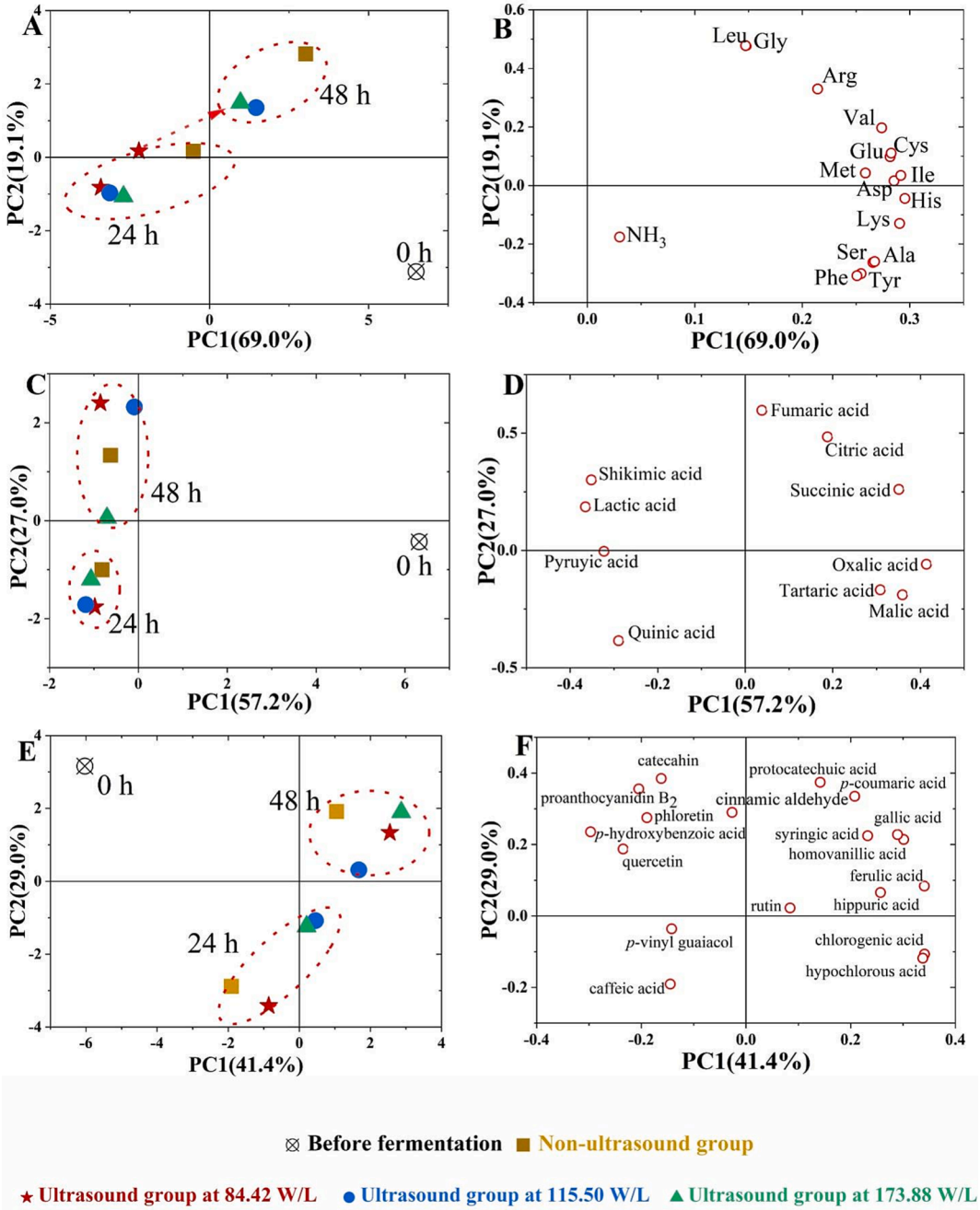

Fig. 4. Principal component analysis (PCA) plots for organic acids free, amino acids and phenolic acids during ultrasound assisted fermentation of ginkgo kernel juice. A and B: free amino acid scores and loading plots for samples fermented by ultrasound at 24 and 48 h; C and D: organic acid scores and loading plots for samples fermented by ultrasound at 24 and 48 h; E and F: phenolic acid scores and loading plots for samples fermented by ultrasound at 24 and 48 h.

the ultrasonic technique enhanced the contents of total flavonoids.

3.5. Effect of ultrasound-assisted fermentation on contents of free amino acids

The original data on the change of free amino acids under fermentation with and without sonication are listed in Supplementary Table S1. These free amino acids may be originated from the above changes in ginkgo kernel juice protein contents, which could be degraded into peptides and free amino acids [40]. The loading and score plots of PCA analysis of free amino acids data are illustrated in Fig. 4A and 4B.

The first two principal components (PCs) accounted for 88.1% of the total variance in free amino acids, suggesting that these two PCs adequately captured the variations in free amino acids throughout the fermentation process. At the same time, the distribution of fermented ginkgo kernel juice samples at various stages was observed in different regions, indicating significant changes in the free amino acid profile during the fermentation process. Before fermentation, the ginkgo juice samples had the positive PC1 value, and after 24-h fermentation, all the samples had negative PC1 values, In the end of fermentation, PC1 values for all the samples increased. Combined with Fig. 4B, the contents of all detected free amino acids (Asp, Ser, Glu, etc.) first decreased and then increased during 48-h fermentation of ginkgo kernel juice. The decrease in free amino acid levels can be attributed to the catabolism of amino acids, which is one of the mechanisms by which microorganisms adapt to the fermentation environment [44]. For example, the Glu content in the ginkgo kernel juice was 57.11 ± 3.03 mg/L before fermentation, and then changed to 47.31 ± 2.06 and 54.10 ± 3.38 mg/L after fermentation for 24 and 48 h, respectively. This may be due to the action of *L. plantarum* decarboxylase, which could convert Glu to γ-aminobutyric acid [45]. Similarly, the content of Arg in all fermented samples first experience a decreased trend to 18.65 ~ 20.78 mg/L within 24-h fermentation following a continuously increase to 18.80 ~ 24.34 mg/L during the late 24-h fermentation period. The consumption of amino acids can benefit the microbial growth, generate energies by decarboxylation [46]. In comparison, the cellular autolysis and the release of proteins from the died *L. plantarum* may be hydrolyzed into small peptides and amino acids [47], which can cause the increase of amino acid in the later 24-h fermentation.

From Supplementary Table S1, the contents of Leu, Gly, Arg, Val, Glu, Cys, Met, Ile in three sonicated ginkgo kernel juice were lower than that of the non-sonicated one after fermentation for 24 and 48 h. In addition, the lowest concentration of these amino acids in the three sonicated groups was observed when the fermentation reached to 24 h. For example, after fermentation with *L. plantarum* for 24 h, the contents of Leu and Gly in the non-sonicated group of ginkgo kernel juice was 4.07 ± 0.16 and 1.60 ± 0.02 mg/L, respectively. In comparison, their contents decreased to 2.80 ± 0.87 and 1.12 ± 0.04 mg/L in the sonicated sample at 84.42 W/L. This may be explained by that ultrasound properly promoted the microbial utilization of these amino acids [40]. Liu et al. [48] reported that Leu can be metabolized into 3-methylbutyraldehyde and then reacts with some sulfur compounds to produce aromatic substances in the presence of transaminases and decarboxylases. Accordingly, ultrasound may facilitate the aforementioned reaction to affect the metabolization of amino acids. In particular, mild ultrasound (84.42 W/L) was able to promote the conversion of free amino acids appropriately, while moderate and higher intensity ultrasound (115.50 and 173.88 W/L) was likely to be counterproductive.

3.6. Effect of ultrasound-assisted fermentation on contents of organic acids

PCA score and loading plots were combined to analyze the influence of the organic acids profile of fermented ginkgo kernel juice (Fig. 4C and 4D). The original data of organic acid profile is summarized in Supplementary Table S2. It can be found that 84.2% of the total variance associated with organic acids were explained by the first two principal components (PCs), indicating that these two PCs were sufficient to explain the changes in organic acid profile during fermentation. Before fermentation, the ginkgo juice samples had the positive PC1 value, and after 24-h and 48-h fermentation, all the samples had negative PC1 values. The loading diagram presented in Fig. 4D indicates that malic acid, tartaric acid, citric acid, oxalic acid, fumaric acid and succinic acid had positive PC1 values, whereas lactic acid, shikimic acid, pyruvic acid, and quininic acid had negative PC1 values. Based on both score and loading plots, it can be confirmed that the contents of malic acid, tartaric acid, citric acid, oxalic acid, fumaric acid and succinic acid descended with fermentation, and the contents of fumaric acid, malic acid and citric acid got increased. Before fermentation, the contents of malic acid and citric acid in the ginkgo kernel juice samples were 2443.18 ± 29.39 and 420.28 ± 2.87 mg/L, respectively. After fermentation for 48 h, the abovementioned two organic acid contents in the non-sonicated group samples were 1676.03 ± 17.46 and 336.21 ± 2.93 mg/L. The decreased malic acid and increased citric acid may be related to the malolactic fermentation that *L. plantarum* converted malic acid into lactic acid and carbon dioxide [49].

As can be seen in Supplementary Table S2, the three sonicated groups contributed to the significant increases in quininic acid, pyruvic acid, shikimic acid, fumaric acid and lactic acid content. Compared with the sonication at 173.88 W/L, sonication at 84.42 and 115.50 W/L have an obviously influence on the metabolizations of organic acids. For instance, shikimic acid in the ginkgo kernel juice was 4.81 ± 0.78 mg/L before the fermentation. The maximum shikimic acid content (61.69 ± 2.10 mg/L) was obtained from the fermented ginkgo kernel juice with ultrasound treatment at 84.42 W/L, followed by sonicated groups at 115.50 W/L (56.38 ± 4.51 mg/L), 173.88 W/L (55.23 ± 3.36 mg/L) and non-sonicated group (46.22 ± 4.96 mg/L). The increased shikimic acid content may be contributed by the biotransformation of phosphoenolpyruvate acid [50], which may be facilitated by the physical effects of ultrasound. During the first 24-h fermentation, quininic acid content has the greatest increase compared with other detected organic acids. Compared to non-sonicated group (3395.96 ± 6.62 mg/L), the concentrations of quininic acid in the sonication samples at 84.42, 115.50 and 173.88 W/L were increased by 14.29% (3962.29 ± 27.04 mg/L), 14.24% (3960.07 ± 24.83 mg/L) and 22.74% (4395.96 ± 6.62 mg/L), respectively. Quininic acid, lactic acid, and shikimic acids are beneficial to the balance between sugars and acids [51]. Accordingly, ultrasound at 84.42 W/L or 115.50 W/L may promote the nutritional and commercial values of ginkgo kernel juice by increasing these organic acid contents.

3.7. Effect of ultrasound-assisted fermentation on contents of phenolic acids

Bioconversions can be affected by ultrasound, impacting the quality and nutritional value of the fermented product. Throughout the fermentation, the PC1 scores of samples after ultrasound-assisted fermentation for 24 h were lower than those of ultrasound-assisted fermentation for 48 h (Fig. 4E). The detailed data was listed in Supplementary Table S3. The results showed that 70.4% of the total variance associated with phenolic acids was explained by the two principal components (PCs), demonstrating that these two PCs were sufficient to explain the changes in organic acid profiles during fermentation. Before fermentation, the ginkgo juice samples had the negative PC1 value, and after fermentation for 24 h, the samples with ultrasound intensities of 115.50 and 173.88 W/L had positive PC1 values, the un-sonicated and moderate-sonicated groups at 84.42 W/L had negative values for PC1, after fermentation for 48 h, all the samples had negative PC1 values. Based on loading plot (Fig. 4F), protocatechuic acid, *p*-coumaric acid, clove acid, hippuric acid, gallic acid, phloretin, chlorogenic acid, homovanillic acid and ferulic acid are on the positive side of PC1, whereas catechin, proanthocyanidin B_2, *p*-hydroxybenzoic acid, cassia

bark aldehyde, *p*-vinyl guaiacol, and caffeic acid are on the negative side of PC1. Based on both score and loading plots, it can be confirmed that the contents of the former increased with fermentation, and the latter got attenuated.

Before fermentation, the catechin, *p*-coumaric acid, *p*-vinyl guaiacol, *p*-hydroxybenzoic acid, ferulic acid, chlorogenic acid and caffeic acid content of ginkgo kernel juice were 422.20 ± 9.35 mg/L, 3.16 ± 0.11 mg/L, 0.65 ± 0.00 mg/L, 82.13 ± 2.96 mg/L, 0.90 ± 0.02 mg/L, 2.02 ± 0.11 mg/L and 0.24 ± 0.03 mg/L (Supplementary Table S3). During the 48-h fermentation process, the metabolism of phenolic acids was significantly promoted when ultrasound was applied. In particular, these phenolic acid levels ranged from 376.35 ~ 378.29 mg/L, 2.27 ~ 3.27 mg/L, 0.001 ~ 0.93 mg/L, 2.5 ~ 2.75 mg/L, 4.18 ~ 5.60 mg/L, 5.24 ~ 5.58 mg/L and 0.19 ~ 0.20 mg/L after ultrasound-assisted fermentation at 84.42, 115.50 and 173.88 W/L for 24 h, respectively. For example, compared with non-sonicated sample, the catechin content in the sonicated at 84.42 W/L had the lowest value (376.35 ± 3.51 mg/L) after fermentation for 24 h. This is contrary to the research by Wang et al. [16] who showed an increase trend in catechin content after sonicated at 58.3 and 93.6 W/L. It is possible that the higher intensity ultrasound (115.50 and 173.88 W/L) in our studies facilitates the conversion of the protocatechuic acid to a decarboxylation and inhibits the formation of catechins [52]. The content of caffeic acid in the non-sonicated group showed a trend of increasing and then decreasing during the fermentation process, with the highest caffeic acid content at fermentation for 24 h (0.51 ± 0.01 mg/L). In the three sonication groups, caffeic acid content presented a continuously decreasing trend to 0.19 ± 0.00 mg/L ~ 0.20 ± 0.00 mg/L. The decline of the content of caffeic acid in three sonicated groups may be contributed by its degradation caused by the high temperature of fermented broth and the thermal effects of ultrasound [53]. Differently, ferulic acid content at three sonicated group was increased by 4 – 5 folds after fermentation for 24 h. It has been proposed that extracellular enzymes are generated by *L. plantarum* fermentation to break the ester bond of ferulic acid attached to arabinoxylan and arabinose residues, thus promoting the secretion of ferulic acid [54]. Therefore, ultrasound may facilitate the release of extracellular enzymes to improve the metabolization of ferulic acid.

3.8. Effect of ultrasound-assisted fermentation on the volatile flavor profiles

Volatile substances have different influences on the flavor of ginkgo kernel juice. In this study, volatile substances in ginkgo kernel juice before and after fermentation were identified by GC–MS (Fig. 5). In the mixture, volatile families were detected, including aldehydes, ketones, alcohols, esters, hydrocarbons, phenolics, acids and others. Before fermentation, the number of 40 volatile substances were identified, with the largest numbers of aldehydes (8) and hydrocarbons (7). At the end of fermentation, 29, 23, 33 and 28 volatile compounds were detected in the un-sonicated and three sonicated groups at 84.42 W/L, 115.50 W/L, 173.88 W/L, respectively. Among these, the aldehydes, ketones, alcohols, esters and hydrocarbons were decreased in type after ultrasound, while the types of acids and phenolics increased after sonication.

Supplementary Table S4 shows the changes in volatile composition and relative content of unfermented and fermented ginkgo kernel juice with and without ultrasound treatments. Total contents of volatile substances increased over 328.0%, 341.0%, 313.0% and 325.0% in sonicated groups at 84.42, 115.50 and 173.88 W/L and non-sonicated group after 48-h fermentation, respectively. In addition, ultrasound enhanced the production of organic alcohols and carboxylic acids while weakened the generation of organic acids and aromatic aldehydes during the fermentation of ginkgo kernel juice using *L. plantarum* Y2. For instance, the relative contents of ethanol, 1-hexanol, acetone, acetaldehyde, nonanal, benzaldehyde, alpha-corocalene, pentadecane, acetic acid, n-decanoic acid, and edulan II were all increased after ultrasound-assisted fermentation compared to the non-sonicated group. By contrast, the relative contents of acetoin, triethyl phosphate, alpha-caracolene, tridecane, *trans*-calamenene, butyric acid and 3-methyl-butanoic acid were reduced after sonication at 84.42, 115.50 and 173.88 W/L compared to the non-sonicated group. Some metabolic transformations were promoted during the growth of *L. plantarum* and aldehydes were readily broken down by oxidation to alcohols and acids [54,55]. As a result, the increased alcohol contents further improve the metabolization of esters, leading to a decrease in aldehydes during fermentation. Observations indicate that the ultrasound-assisted fermented samples exhibited an elevated concentration of higher alcohols and esters in comparison to the naturally fermented counterparts, characterized by distinctive olfactory profiles of oiliness and fragrance. Notably, the predominant aromatic attributes of the ultrasound-assisted samples were predominantly aromatic and fruity in nature [55]. Thus, the flavor profile of post-fermented ginkgo kernel juice subsequent to ultrasonic treatment is primarily influenced by the specific composition and quantity of these two compounds. Remarkably, the sensory characteristics following ultrasonic treatment surpassed those of the control group, underscoring the enhanced gustatory quality achieved through this process.

3.9. Effect of ultrasound-assisted fermentation on 4′-O-methylpyridoxine (MPN) content

MPN has a significant impact on the safety of ginkgo kernel juice, the international limit standard of 5 mg/L [3]. The changes in MPN during 48-h fermentation are shown in Fig. 6. Before the fermentation, the MPN content of the ginkgo kernel juice was 0.44 ± 0.01 mg/L. After

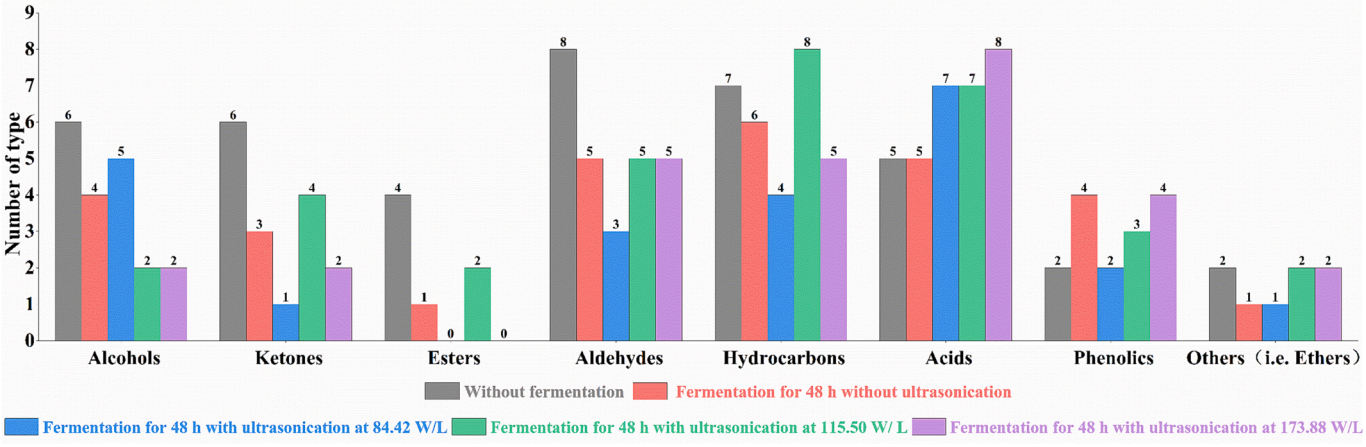

Fig. 5. Changes in relative content and number of species of volatile flavor compounds in ginkgo kernel juice fermented by *L. plantarum* Y2 for 48 h.

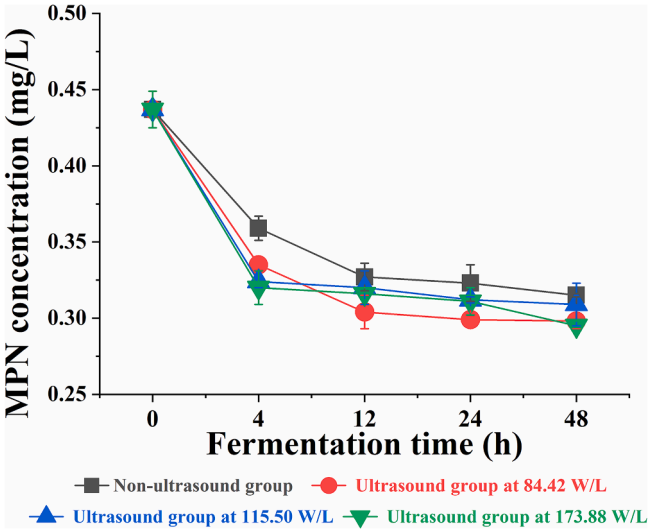

Fig. 6. The changes of the content of MPN during the fermentation 48 h for ginkgo kernel juice.

fermentation, the overall MPN content showed a decreasing trend in all sonicated and non-sonicated ginkgo kernel juice. The MPN content of fermented samples in all three sonicated groups were lower than the non-sonicated group. For example, after 4-h fermentation, the maximum MPN content was obtained from the non-sonicated group (0.36 ± 0.01 mg/L), followed by sonicated groups at 84.42 W/L (0.34 ± 0.01 mg/L), 115.50 W/L (0.32 ± 0.02 mg/L) and 173.88 W/L (0.32 ± 0.02 mg/L), respectively. It has been proposed that the production of large amounts of biosurfactants (polysaccharides and peptidoglycans) on the cell wall of *L. plantarum* to adsorb MPN during the fermentation process [56]. And after fermentation, the pH value will decrease, the peptidoglycan layer after protein denaturation will reduce crosslinking, increase pore size and provide more adsorption sites [56]. The possible mechanism of ultrasound-assisted fermentation is shown in Fig. 7. This may explain the lower MPN content observed in the sonicated fermented ginkgo kernel juice, after 48-h fermentation, the MPN content below the

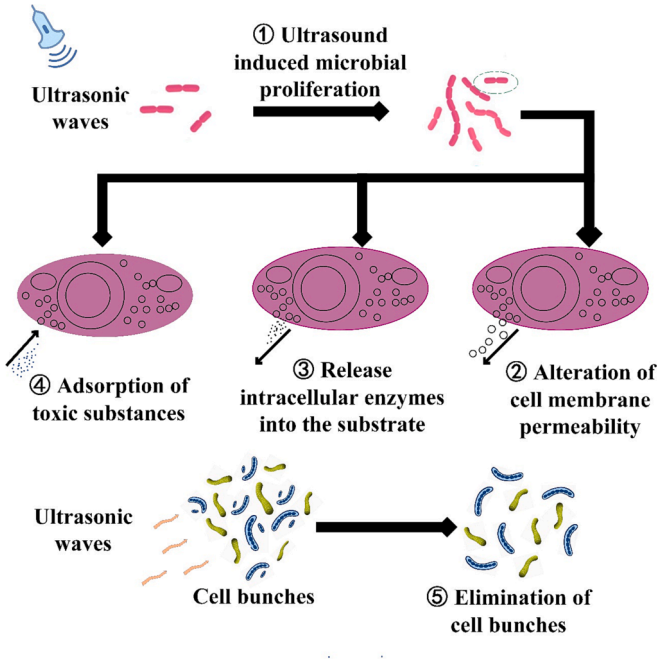

Fig. 7. The possible physical mechanism of ultrasound-assist fermentation.

international standards.

3.10. Effect of ultrasound-assisted fermentation on antioxidant capacity

Fig. 8A and 8B illustrate the impact of ultrasound processing during the logarithmic phase on the antioxidant capacity of fermented ginkgo kernel juice. The scavenging capacity of $ABTS^{+}$ radicals and the ability to reduce ferric to ferrous ions in ginkgo kernel juice exhibited consistent enhancement throughout the entire fermentation period [30]. Before the fermentation, the $ABTS^{\cdot+}$ radical scavenging capacity in the ginkgo kernel juice was 21.65 ± 1.34 mM Trolox. The highest $ABTS^{\cdot+}$ radical scavenging capacity was observed in the sonication group at 173.88 W/L, which was 11.3%, 16.0% and 23.3% higher than the non-sonicated and sonicated groups at 84.42 and 115.50 W/L after 12-h fermentation, respectively. The increased antioxidant capacities in sonicated samples may be related to the enhancement of phenolic acid metabolism, such as chlorogenic acid and butyric acid. Differently, the reducing ferric ion antioxidant capacity in the ginkgo kernel juice was 0.53 ± 0.21 mM Fe^{2+} before the fermentation (Fig. 8B). The reducing ferric ion antioxidant capacity of all sonicated groups were lower than that of the non-sonicated group after 48-h fermentation. According to the report, ultrasound can help the degradation of unstable phenolics such as caffeic acid, resulting in a reduction of antioxidant activity [57]. Is clear that phenolic acids like catechin, *p*-coumaric acid and *p*-vinyl guaiacol were converted and increased to different degrees under different intensities of sonication. This is positively correlated with the rise in both antioxidant activities of fermented ginkgo kernel juice.

4. Conclusions

In general, ultrasound successfully promoted the fermentation of ginkgo kernel juice with *L. plantarum* Y2. Compared to the non-sonicated group, ultrasound-assisted fermentation groups exhibited higher number of viable bacteria in the logarithmic and stable phases. In addition, lower ultrasonic power density level (84.42 W/L) with mild physical effects has a better performance on the metabolization of cellular antioxidant active components such as chlorogenic and butyric acids, free forms of phenolic compounds and other by-products. After 48-h fermentation, the maximum consumptions of total sugars and total proteins in sonicated group at 84.42 W/L were 53.21% and 66.3%, respectively. Furthermore, ultrasound-assisted fermentation facilitated the hydrolysis of chlorogenic acid to caffeic acid, the solubilization of ferulic acid, and the conversion of proanthocyanidin B_2. The distribution of all sonicated and non-sonicated samples presented obviously different on the score diagram of PCA, indicating their differences on the contents of organic acids, free amino acids, and phenolics. The degradation rates of the toxic substance MPN were decreased by 30.82% in all sonicated groups compared to the non-sonicated group. Overall, ultrasound-assisted fermentation with mild or moderate intensity can be considered as an ideal and environment-friendly technology to improve the metabolization of *L. plantarum,* the physicochemical properties of ginkgo kernel juice and the degradation of toxic substances.

Funding

This work was financially supported by grants from the Natural Science Foundation of China (No. 32100037, 51971100), Jiangsu provincial university “blue project”, Science and Technology Projects of Jiangsu (No. BE2021353), Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Institutional Review Board Statement

Not applicable.

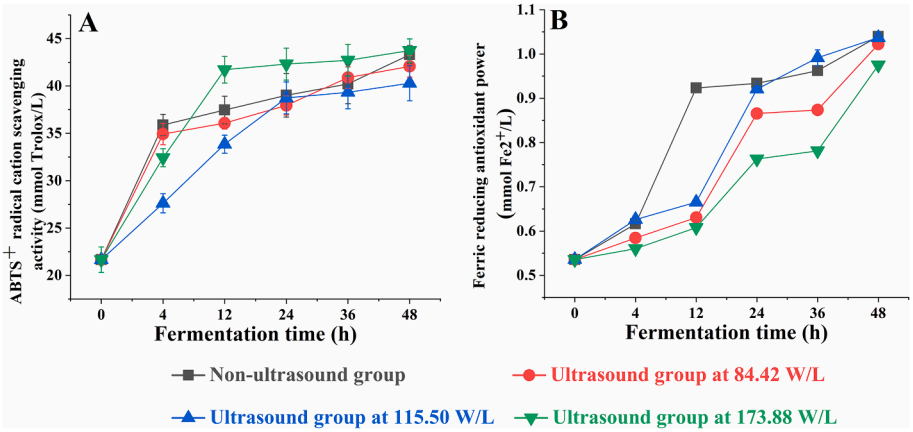

Fig. 8. Effect of ultrasonic power density levels on antioxidant capacity during the fermentation of ginkgo kernel juice. $ABTS^+$ free radical scavenging ability (A); FRAP reducing antioxidant capacity (B).

Informed Consent Statement

Not applicable.

Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors.

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.

Data availability

I have shared the link to my data/code at the Attach File step

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2023.106587.

References

[1] C. Tomino, S. Ilari, V. Solfrizzi, V. Malafoglia, G. Zilio, P. Russo, S. Proietti, F. Marcolongo, G. Scapagnini, C. Muscoli, Mild cognitive impairment and mild dementia: the role of ginkgo biloba (EGb 761®), Pharmaceuticals 14 (305) (2021) 2–19.
[2] I.D. Bateng, D.A. Soetanto, X.M. Yang, C. Zhou, F.K. Saalia, F. Li, Effect of pulsed-vacuum, hot-air, infrared, and freeze-drying on drying kinetics, energy efficiency, and physicochemical properties of ginkgo biloba L seed, J. Food Process. Eng. (2021) 376–386.
[3] B.I. Duah, X.M. Yang, Ginkgo biloba L. seed; A comprehensive review of bioactives, toxicants, and processing effects, Ind. Crop. Prod. 176 (2022), 114281.
[4] A. Arenz, M. Klein, K. Fiehe, J. Groß, C. Drewke, T. Hemscheidt, E. Leistner, Occurrence of neurotoxic 4'-*O*-methylpyridoxinein Ginkgo biloba leaves, ginkgo medications and Japanese ginkgo food, Planta. Med. 62 (06) (1996) 548–551.
[5] J.P. Zhu, H. Gong, C.E. Wu, G.J. Fan, T.T. Li, J.H. Wang, 4'-*O*-methylpyridoxine: preparation from ginkgo biloba seeds and cytotoxicity in GES-1 cells, Toxins 13 (2) (2021) 95.
[6] J. Wang, S. Ren, X.X. Fang, Data comparison of the effects of feeding Ginkgo leaf fermentation and Chinese herbal medicine on meat ducks, J. Phys. 1423 (2019), 012018.
[7] Y. Wu, S. Li, Y. Tao, D. Li, Y. Han, P.L. Show, G. Wen, J. Zhou, Fermentation of blueberry and blackberry juices using *Lactobacillus plantarum*, *Streptococcus thermophilus* and *Bifidobacterium bifidum*: Growth of probiotics, metabolism of phenolics, antioxidant capacity in vitro and sensory evaluation, Food Chem. 348 (2021), 129083.
[8] J. Yang, Y. Sun, J.L. Chen, Y. Cheng, H.R. Zhang, T.Q. Gao, F. Xu, S.K. Pan, Y. Tao, J. Lu, Fermentation of ginkgo biloba kernel juice using *L. plantarum* Y2 from the ginkgo peel: fermentation characteristics and evolution of phenolic profiles, antioxidant activities in vitro, and volatile flavor compounds, Front Nutr. 9 (2022), 1025080.
[9] X. Sun, S. Liu, S. Manickam, Y. Tao, J.Y. Yoon, X. Xuan, Intensification of biodiesel production by hydrodynamic cavitation: A critical review, Renew. Sustain. Energy Rev. 179 (2023), 113277.
[10] Z. Yu, Y. Su, Y. Zhang, P. Zhu, M. Zi, X. Zhou, Potential use of ultrasound to promote fermentation, maturation, and properties of fermented foods, Food Chem. 357 (2021), 129805.
[11] X. Sun, W. You, X. Xuan, L.i. Ji, X. Xu, G. Wang, S. Zhao, G. Boczkaj, J.Y. Yoon, S. Chen, Effect of the cavitation generation unit structure on the performance of an advanced hydrodynamic cavitation reactor for process intensifications, Chem. Eng. J. 412 (2021), 128600.
[12] X. Xuan, M. Wang, W. You, S. Manickam, Y. Tao, J.Y. Yoon, X. Sun, Hydrodynamic cavitation-assisted preparation of porous carbon from garlic peels for supercapacitors, Ultrason. Sonochem. 94 (2023), 106333.
[13] E. Bartkiene, V. Bartkevics, I. Pugajeva, A. Borisova, E. Zokaityte, V. Lele, V. Starkute, P. Zavistanaviciute, D. Klupsaite, D. Zadeike, G. Juodeikiene, The quality of wheat bread with ultrasonicated and fermented By-Products from plant drinks production, Front. Microbiol. 12 (2021), 652548.
[14] D. Novoa-Díaz, J.M. Rodríguez-Nogales, E. Fernández-Fernández, J. Vila-Crespo, J. García-Álvarez, M.A. Amer, J.A. Chávez, A. Turó, M.J. García-Hernández, J. Salazar, Ultrasonic monitoring of malolactic fermentation in red wines, Ultrason. 54 (6) (2014) 1575–1580.
[15] S. Shokri, N.S. Terefe, S.S. Shekarforoush, S. Hosseinzadeh, Ultrasound-assisted fermentation for enhancing metabolic and probiotic activities of *Lactobacillus brevis*, Chem. Eng. Process. 166 (2021), 108470.
[16] H. Wang, Y. Tao, Y. Li, S. Wu, D. Li, X. Liu, Y. Han, S. Manickam, P.L. Show, Application of ultrasonication at different microbial growth stages during apple juice fermentation by *Lactobacillus plantarum*: Investigation on the metabolic response, Ultrason. Sonochem. 73 (2021), 105486.
[17] A. Behzadnia, M.M. Nasab, B.K. Tiwari, Stimulation of biosurfactant production by *Lactobacillus plantarum* using ultrasound, Ultrason. Sonochem. 59 (C) (2019), 104724.
[18] S.Y. Binte Abu Bakar, M. Salim, A.J. Clulow, A. Hawley, J. Pelle, D.T. Geddes, K. R. Nicholas, B.J. Boyd, Impact of pasteurization on the self-assembly of human milk lipids during digestion, J. Lipid Res. 63 (5) (2022), 100183.
[19] J. Yang, Y. Sun, T.Q. Gao, Y. Wu, H. Sun, Q.Z. Zhu, C.S. Liu, C. Zhou, Y.B. Han, Y. Tao, Fermentation and storage characteristics of "Fuji" apple juice using *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus plantarum*: microbial growth, metabolism of bioactives and *in vitro* bioactivities, Front. Nutr. 9 (2022), 833906.
[20] Y. Zhao, Z. Yue, X. Zhong, J. Lei, P. Tao, B. Li, Distribution of primary and secondary metabolites among the leaf layers of headed cabbage (Brassica oleracea var. capitata), Food Chem. 312 (2020), 126028.
[21] F.A. Tamboli, H.N. More, S.S. Bhandugare, A.S. Patil, N.R. Jadhav, S.G. Killedar, Estimation of total carbohydrate content by phenol sulphuric acid method from eichhornia crassipes (Mart.) Solms, Asian J. Research Chem. 13 (5) (2020) 357–359.
[22] Z. Li, J. Teng, Y. Lyu, X. Hu, Y. Zhao, M. Wang, Enhanced antioxidant activity for apple juice fermented with *L. plantarum* ATCC14917, Molecules 24 (2019), 51.
[23] E. Kwaw, Y. Ma, W. Tchabo, M.T. Apaliya, L. Xiao, X.i. Li, M. Hu, Effect of fermentation parameters and their optimization on the phytochemical properties of lactic-acid-fermented mulberry juice, J. Food Meas. Charact. 11 (3) (2017) 1462–1473.
[24] C.E.L. Ching, L. Seunguk, M.C. Angelo, L. Liyan, B. Sungwoo, Assessment of physiological responses of bacteria to chlorine and UV disinfection using a plate count method, flow cytometry, and viability PCR, J. Appl. Microbiol. 132 (3) (2021) 1788–1801.
[25] X. Zhao, J.H. Wu, X.F. Niu, Simultaneous determination of five organic acids in beef heart persimmon juice based on HPLC method, China Brew. 41 (03) (2022) 232–237.

[26] E.I. Adeyeye, Amino acid composition of three species of Nigerian fish: *Clarias anguillaris, Oreochromis niloticus* and *Cynoglossus senegalensis*, Food Chem. 113 (1) (2009) 43–46.
[27] H. Yang, T. Tian, H. Gu, X. Li, G. Cai, J. Sun, D. Wu, J. Lu, Analysis of factors related to browning of Dangshan pear (Pyrus spp.) wine, Food Chem. 308 (2020), 125665.
[28] Q. Wang, R.P. Tian, Z.N. Wang, C.X. Wu, Z.J. Li, J.Y. Gong, S.S. Zhu, The relationship between soil components in different planting sites and volatile components of fragrant rice 'Gudang No. 1', Mol. Plants Breed. (2022) 1–24.
[29] T. Yoshimura, N. Udaka, J. Morita, High performance liquid chromatographic determination of ginkgo toxin and ginkgo tox in-5′-glucoside in seeds, J. Liq. Chromatogr. R. T. 29 (4) (2006) 605–616.
[30] Y.C. Wang, Y. Tao, X.Y. Zhang, Metabolic profile of ginkgo kernel juice fermented with lactic acid bacteria: a potential way to degrade ginkgolic acids and enrich terpene lactones and phenolics, Process Biochem. 76 (2019) 25–33.
[31] X. Zhang, W.L. Tian, B.J. Xie, Z. Sun, Insight into the influence of Lactic acid bacteria fermentation on the variations in flavor of chickpea milk, Foods 11 (16) (2022), 2445.
[32] A. Vildan, A.A. Sibel, Recent advances in dual effect of power ultrasound to microorganisms in dairy industry: activation or inactivation, Crit. Rev. Food Sci. 62 (4) (2020) 11–16.
[33] W. Liao, J. Shen, S. Manickam, S. Li, Y. Tao, D. Li, D. Liu, Y. Han, Investigation of blueberry juice fermentation by mixed probiotic strains: Regression modeling, machine learning optimization and comparison with fermentation by single strain in the phenolic and volatile profiles, Food Chem. 405 (2023), 134982.
[34] S. Shokri, S.S. Shekarforoush, S. Hosseinzadeh, Stimulatory effects of low intensity ultrasound on the growth kinetics and metabolic activity of *Lactococcus lactissub* sp. lactis, Process Biochem. 89 (C) (2020) 1–8.
[35] A. Gholamhosseinpour, S.M.B. Hashemi, Ultrasound pretreatment of fermented milk containing probiotic *L. plantarum* AF1: Carbohydrate metabolism and antioxidant activity, J. Food Process Eng. 42 (1) (2019), e12930.
[36] Q. He, D. Liu, M. Ashokkumar, X. Ye, T.Z. Jin, M. Guo, Antibacterial mechanism of ultrasound against escherichia coli: alterations in membrane microstructures and properties, Ultrason. Sonochem. 73 (2021), 105509.
[37] A. Behzadnia, M.M. Nasab, K.T. Brijesh, Stimulation of biosurfactant production by *L. plantarum* using ultrasound, Ultrason. Sonochem. 59 (C) (2019), 104724.
[38] H. Lu, H. Lou, T. Wei, Z. Liu, Y. Jiao, Q. Chen, Ultrasound enhanced production of mycelia and exopolysaccharide by agaricus bitorquis (Quél.) Sacc. Chaidam, Ultrason. Sonochem. 64 (2020), 105040.
[39] X. Wu, L. Yan, G. Xu, X. Wang, JuanJuan Wang, D.D. Dionysiou, High frequency ultrasonication enhances iron-catalyzed sulphate inactivation of *Escherichia coli* and *Staphylococcus aureus*, C.E.J. Adv. 8 (2021), 100170.
[40] Y.T. Lu, Q. Chen, Research progress on application of Lactic acid bacteria in livestock and poultry production, Heilongjiang Anim. Sci. Vet. Med. 51 (3) (2019) 45–46.
[41] H.C. Tseng, C.Y. Yang, Assessment of ultrasonic stress on survival and β-glucosidase activity of encapsulated *Lactiplantibacillus plantarum* BCRC 10357 in fermentation of black soymilk, Foods 11 (9) (2022), 1234.
[42] J.M. Landete, H. Rodríguez, J.A. Curiel, B.D.L. Rivas, F.L.D. Felipe, R. Muñoz, Degradation of phenolic compounds found in olive products by *L. plantarum* strains, Olives Olive Oil Health Dis. Prev. 43 (2021) 133–144.
[43] A.A.M. Elnour, M.E.S. Mirghani, N.A. Kabbashi, K.H. Musa, F. Shahabipour, N. Ashammakhi, N.H. Abdurahman, Comparative study of the characterisation and extraction techniques of polyphenolic compounds from Acacia seyal gum, F. Q. S 6 (2022) 1–10.
[44] P. Filannino, G. Cardinali, C.G. Rizzello, S. Buchin, M. De Angelis, M. Gobbetti, R. Di Cagno, M.J. Pettinari, Metabolic responses of *Lactobacillus plantarum* strains during fermentation and storage of vegetable and fruit juices, Appl. Environ. Microb. 80 (7) (2014) 2206–2215.
[45] W.L. Liu, H.M. Li, L. Liu, K.H. Ko, I. Kim, Screening of gamma-aminobutyric acid-producing Lactic acid bacteria and the characteristic of glutamate decarboxylase from *Levilactobacillus brevis* F109-MD3 isolated from kimchi, J. Appl. Microbiol. 00 (2021) 1–11.
[46] S.D. Nsogning, S. Fischer, T. Becker, Investigating on the fermentation behavior of six lactic acid bacteria strains in barley malt wort reveals limitation in key amino acids and buffer capacity, Food Microbiol. 73 (2018) 245–253.
[47] O. Martínez-Pinilla, Z. Guadalupe, Z. Herńandez, B. Ayestaŕan, Amino acids and biogenic amines in red varietal wines: the role of grape variety, malolactic fermentation and vintage, Eur. Food Res. Technol. 237 (2013) 887–895.
[48] Z.Y. Liu, Q.M. Liu, S. Wei, Q.X. Sun, Q.Y. Xia, D. Zhang, W.Z. Shi, H.W. Ji, S.C. Liu, Quality and volatile compound analysis of shrimp heads during different temperature storage, Food Chem. X (2021) 12.
[49] P.M.I. Cañas, M.R. Carrasco, E.G. Romero, A.M. Morales, J.M. Heras-Manso, G. C. Bueso, Co-Existence of inoculated yeast and Lactic acid bacteria and their impact on the aroma profile and sensory traits of tempranillo red wine, Fermentation 6 (1) (2020) 17.
[50] Q.i. Sheng, L. Yi, B. Zhong, X. Wu, L. Liu, B. Zhang, Shikimic acid biosynthesis in microorganisms: current status and future direction, Biotechnol. Adv. 62 (2023), 108073.
[51] M.G. Beiro-Valenzuela, I. Serrano-García, R.P. Monasterio, M.V. Moreno-Tovar, E. Hurtado-Fernández, J.J. González-Fernández, J.I. Hormaza, R. Pedreschi, L. García, A. Carrasco-Pancorbo, Characterization of the polar profile of bacon and fuerte avocado fruits by hydrophilic interaction liquid chromatography-mass spectrometry: distribution of non-structural carbohydrates, quinic acid, and chlorogenic acid between seed, mesocarp, and exocarp at different ripening stages, J. Agric. Food Chem. 71 (2023) 5674–5685.
[52] A.F. Sanchez-Maldonado, A. Schieber, M.G. Ganzle, Structure-function relationships of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria, J. Appl. Microbiol. 111 (2011) 1176–1184.
[53] T. Belwal, I.D. Bhatt, G. Cravotto, Effect of ultrasound on extraction and stability of polyphenols from berberis jaeschkeana C.K. schneid fruits: a comparative study, Sustain. Chem. Pharm. 27 (2022), 100649.
[54] P.C. Nobahle, A.A. Stephen, E.M. Vimbainashe, S. Tinotenda, R. Fabienne, S. Dharini, Influence of Lactic acid bacterium strains on changes in quality, functional compounds and volatile compounds of mango juice from different cultivars during fermentation, Foods. 11 (5) (2022), 682.
[55] L. Cai, W. Wang, J. Tong, L. Fang, X. He, Q.i. Xue, Y. Li, Changes of bioactive substances in Lactic acid bacteria and yeasts fermented kiwifruit extract during the fermentation, LWT- Food Sci. Tech-Brazil. 164 (2022), 113629.
[56] H. Gong, C.-e. Wu, G.-J. Fan, T.-T. Li, J.-H. Wang, T. Wang, Determination of native contents of 4′-*O*-methylpyridoxine and its glucoside in raw and heated ginkgo biloba seeds by high-performance liquid chromatography, J. Food Meas. Charact. 14 (2) (2020) 917–924.
[57] M. Li, X. Chen, J. Deng, D. Ouyang, D.a. Wang, Y. Liang, Y. Chen, Y. Sun, Effect of thermal processing on free and bound phenolic compounds and antioxidant activities of hawthorn, Food Chem. 332 (2020), 127429.