Food Bioscience 62 (2024) 105105

Contents lists available at ScienceDirect

Food Bioscience

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

Biological transformation of herbal extracts through Lactic Acid Bacteria fermentation: A study on Milk thistle and liquorice root extract

Alireza Amini , Zeinabossadat Ebrahimzadeh Mousavi *, Seyed Mohammad Mousavi

Bioprocessing and Biodetection Lab (BBL), Department of Food Science, Technology, and Engineering, University of Tehran, Karaj, Iran

ARTICLE INFO

Keywords:
Milk thistle
Liquorice
Fermentation
Lactic cid bacteria
Probiotic

ABSTRACT

Herbal extracts have recently gained attention in pharmaceuticals, supplements, and cosmetics. This study explored the impact of Lactic Acid Bacteria (LAB) fermentation on the functional properties of milk thistle and licorice root extracts to develop health-enhancing beverages. Biological transformation using microorganisms or enzymes was employed to improve the stability and efficacy of these extracts. After 36 h of fermentation, bacterial growth in both extracts surged approximately two-fold. The licorice root extract showed a particularly significant increase in total phenolic content and antioxidant activity, attributed to bacterial enzymes breaking down complex, high-molecular-weight phenolic compounds into simpler, more potent free phenols. Despite a reduction in total flavonoid content across all samples, this did not indicate a decline in biological activity. High-Performance Liquid Chromatography (HPLC) analysis revealed a notable increase in silymarin concentration in the milk thistle extract, rising from 0.59 mg mL^{-1} to 2.5 mg mL^{-1} after fermentation with *Lactiplantibacillus plantarum*. Glycyrrhizic acid in the licorice root extract slightly decreased following fermentation with *L. plantarum*, while no significant changes were observed in extracts fermented with *L. acidophilus*. Cell viability studies during cold storage showed that the bacterial count in the licorice root extract remained within acceptable limits (10^6 CFU mL^{-1}) for four weeks, ensuring ongoing health benefits. However, in the milk thistle-fermented extract, no viable cells were detected by the third week. These findings demonstrate the potential of LAB-fermented extracts for developing functional beverages with enhanced health benefits and therapeutic properties.

1. Introduction

In recent years, there's been a notable move towards herbal treatments in pharmaceuticals, food supplements, and cosmetics. This shift highlights a growing recognition of nature's healing potential in these industries (Bernela, Seth, Kaur, Sharma, & Pati, 2023; Kumar Srivastava, 2018). Herbal medicine and natural plant materials comprise diverse biologically active compounds and antioxidants, displaying effects like anti-aging, anti-cancer, anti-inflammatory properties, and immune system enhancement. However, these substances can be unstable and occasionally cause irritation upon extraction, posing toxicity risks to the human body (Shahrajabian & Sun, 2023). Consequently, recent efforts have focused on stabilizing natural extracts, mitigating their toxicity, or transforming them into more stable derivatives to heighten their efficacy. One approach involves employing biological transformation techniques utilizing microorganisms or enzymes. Fermentation stands out as a prominent example among these methods.

Milk thistle with the scientific name *Silybium marianum* is an annual or biennial plant that is native to the Mediterranean region and is grown and cultivated all over the world. The active compound of this plant is Silymarin. Silymarin is a mixture of flavonolignans contained in the coat of Milk thistle seed including Silybin, Isosilybin, Silychristin and Silydianin (Liu et al., 2023). Silymarin exhibits the ability to stop liver cell degeneration, enhance liver cleansing, improve detoxification processes, and facilitate the restoration of impaired liver cells (Liu et al., 2023). Its primary mechanisms involve the scavenging of free radicals, particularly combating lipid peroxidation, and elevating glutathione (G-SH) levels in the liver, thereby exerting a protective effect on liver health (Elwekeel, Elfishawy, & Abouzid, 2013). In addition, Silymarin has been reported as an excellent antioxidant, free radicals scavenger and lipid peroxidation inhibitor and inflammation suppressor (Bhattacharya, 2011). Conventional research has concentrated on the extraction and purification of Silymarin with organic substances, which was inapplicable to the food industry (Liu et al., 2023).

* Corresponding author. Tel.: ++98-26-32248804, Fax: +98-26-32249453.
E-mail addresses: aminialireza395@gmail.com (A. Amini), zeinab.mosavi@ut.ac.ir (Z.E. Mousavi), mousavi@ut.ac.ir (S.M. Mousavi).

https://doi.org/10.1016/j.fbio.2024.105105
Received 1 July 2024; Received in revised form 9 September 2024; Accepted 12 September 2024
Available online 20 September 2024

Liquorice, with the scientific name of *Glycyrrhiza glabra*, belongs to the genus *Glycyrrhiza* of the Papilionaceae family. Liquorice is a perennial plant native to the Mediterranean region and central and south-western Asia (Dastagir & Rizvi, 2016; Husain, Bala, Khan, & Khan, 2021). The liquorice plant is widely used in food, confectionery, pharmaceuticals and tobacco products as a flavoring agent worldwide recognized as safe (GRAS) (Anwar et al., 2023; Jiang, Zhang, True, Zhou, & Xiong, 2013; Quirós-Sauceda, Ovando-Martínez, Velderrain--Rodríguez, González-Aguilar, & Ayala-Zavala, 2016). Glycyrrhizin, the primary active compound found in liquorice root, has a long historical ap-plication in traditional medicine for relieving conditions like bronchitis, gastritis, and jaundice. Glycyrrhizinic acid, the main bioactive triterpene glycoside in the Chinese herb *Gancao*, exhibits diverse biological effects, including anti-inflammatory, antioxidant, antiviral, immune-modulating, and liver-protective properties (Yuan, Wang, Chen, Shan, & Di, 2020). However, due to its high relative molecular mass and polarity, the absorption of glycyrrhizinic acid following oral administration is challenging (Yuan et al., 2020). For its efficacy to be realized, glycyrrhizinic acid necessitates enzymatic deglycosylation by diverse gut microbiota, converting it into glycyrrhetinic acid (GA), which can then be absorbed into the bloodstream (Yuan et al., 2020).

Around the world, especially in Asia, different plants are fermented for medicinal purposes. However, little is known about the fermentation products of medicinal herbal plants (Köberl et al., 2019). Lactic Acid Bacteria are widely used as fermentation strains. To date, around 20 strains have been effectively applied to plant-based fermentation. Among the most common are *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus* (Yang et al., 2024). These fermentative microorganisms alter plant composition by releasing chemically bound compounds, breaking down plant macromolecules, and synthesizing new metabolites. As a result, fermented plants are enriched with phytochemicals and exhibit enhanced bioactivity and bioavailability (Dogan & Tornuk, 2019). Various studies have investigated the impact of fermentation on the bioactive ingredients in herbal medicines (Hussain et al., 2016; Wen, Yan, & Chen, 2013). These studies assessed the effect of lactic acid fermentation on the bioavailability of isoflavones, saponins, phytosterols, and phenols. The primary conclusion drawn from these research endeavors is that fermentation could effectively augment the pharmacological and therapeutic effectiveness of herbal formulations in food and cosmetics by facilitating the release of functional ingredients into the relevant extracts.

Based on the literature review, limited research has been conducted on exploring the bioconversion of active components present in liquorice and Milk thistle extracts. Therefore, in this study, we aimed to investigate the growth and effects of probiotic *L. plantarum* and *L. acidophilus* in liquorice root and Milk thistle extracts. In addition, we have also evaluated the effect of fermentation antioxidant activity and phenolic and flavonoid compounds Ultimately, our goal is to use these fermented extracts as the basis for a health-promoting beverage formulation.

2. Materials and methods

2.1. Preparation of the extracts

The liquorice root concentrate was supplied by Shirin Darou Co. (Shiraz, Iran), and the milk thistle liquid extract was obtained from Giyah Kala (Sabzevar, Iran). The liquorice root concentrate was diluted from Brix 70°–10°, and the milk thistle extract from Brix 20°–5°, using distilled water. The Pearson square method and a refractometer (Bellingham, England) were used to achieve the desired Brix levels, and the extracts were pasteurized for 15 min at 80 °C. The extracts were then cooled to room temperature by placing them in an ice water bath and subsequently kept refrigerated until use.

2.2. Bacterial strains

Lactiplantibacillus plantarum DSMZ 20174 and *Lactobacillus acidophilus* DSMZ 20079 were purchased in lyophilized form from DSMZ GmbH Co., Germany. They were reactivated by cultivation in De Man, Rogosa, and Sharpe (MRS) broth (Merck, Germany) under anaerobic conditions at 37 °C for 48 h. Subsequently, the broth cultures were transferred onto MRS agar plates and incubated under the same conditions. The stock culture was preserved in MRS with 20% glycerol at −20 °C.

2.3. Fermentation of the extracts

A culture of probiotic lactic acid strains was prepared by inoculating a few cell colonies from agar plates into MRS broth, and then incubating them at 37 °C for 12–18 h. After this incubation period, 10% of the volume was aseptically withdrawn and centrifuged at 3200×*g* for 6 min at 25 °C. The resulting pellet was washed twice with physiological saline solution and centrifugation was repeated. Subsequently, the bacteria were introduced into the pasteurized extracts. Notably, the initial cell count, approximately 10^7 to 10^8 per milliliter of extract, was determined using the McFarland scale. The bacterial cultures were diluted with sterile distilled water, and their optical density was assessed using a spectrophotometer at 625 nm. This optical density was then compared to that of a 0.5 McFarland standard, which corresponds to a concentration of 1.5×10^8 CFU mL^{-1}. To promote proliferation of lactic acid strains, 2 g L^{-1} of yeast extract was supplemented in the Milk thistle extract before fermentation (Perjéssy, Hegyi, Nagy-Gasztonyi, & Zalán, 2022). The inoculated extracts were then incubated at 37 °C for 72 h, with periodic sampling for both growth estimation and chemical analysis. Viable cell counts were determined using the standard plate counting method on MRS agar medium, expressed as colony-forming units per milliliter of the sample (CFU mL^{-1}).

2.4. pH meausurement

A digital pH meter was used to measure the pH of the samples. The samples were first filtered using a 0.2 μm sterile syringe filter (Millipore, Germany).

2.5. Determination of antioxidant activity using DPPH inhibition assay

The 1,1-diphenyl-2-picryl hydroxyl radical (DPPH, Sigma, USA) is a hydrophobic radical known for its peak absorbance at 517 nm. In the DPPH assay, hydroxyl groups in antioxidant compounds donate H^+ to the DPPH radical, leading to a reduction in radical concentration, subsequently lowering the absorbance at 517 nm. The recorded absorbance served as an indicator of the extracts free radical scavenging capacity. The methodology is derived from Brand-Williams, Cuvelier (Brand-Williams, Cuvelier, & Berset, 1995). with minor adaptations. A 0.1 mM methanolic DPPH solution was freshly prepared daily, stored in an aluminum foil-covered flask, and kept in the dark at 4 °C until utilized.

For analysis, 3 ml of undiluted juice was vigorously mixed with 1 ml of methanolic DPPH and shaken thoroughly. The resulting mixture was then incubated at room temperature for 30 min, and the absorbance was measured at 517 nm using a spectrophotometer (CE2502, Cecil Instruments, UK). The percentage of DPPH radical quenching was calculated based on the observed decrease in radical absorbance. The radical scavenging activity was quantified as the inhibition percentage using the following formula (Mousavi, Mousavi, Razavi, Emam-Djomeh, & Kiani, 2011):

$$\%\text{Radical scavenging activity} = \frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \times 100 \quad (1)$$

where Abs control is the absorbance of the control (1 methanolic DPPH

solution + 3 ml methanol), and Abs sample is the absorbance of the extract at 517 nm.

2.6. Determination of total phenolic contents

A 1 mL volume of the diluted sample with 95% ethanol (1:2 v/v) or standard solution of gallic acid (20, 40, 60, 80 and 100 μg mL^{-1}) was combined with 1 mL of Folin–Ciocalteu's reagent and 1 ml of sodium carbonate solution (7.5% w/v). The resulting mixture was incubated at room temperature for 30 min. Following this, 2 mL of distilled water were introduced, and the mixtures were vigorously vortexed. The absorbance of the final mixture was measured at 750 nm. The total phenolic content of each sample was determined as the average of three replicates, expressed in gallic acid equivalents, denoted as μg mL^{-1} based on the calibration curve prepared using the same standard (Castro-López et al., 2016).

2.7. Total flavonoid content determination

The total flavonoid content was determined following the method outlined by previous studies (Ivanov, Dyankova, & Pavlova, 2021). In summary, 500 μL of each extract was mixed with 1.50 mL of 95% ethanol, 0.10 mL of 10% aluminum chloride solution, 0.10 mL of 1 M sodium acetate solution, and 2.80 mL of distilled water. The mixture was then incubated for 40 min in the dark at room temperature, and the absorbance was measured at 415 nm. To quantify the concentration of flavonoids, a calibration curve was constructed using quercetin as the standard reference.

2.8. HPLC measurement of active ingredient

2.8.1. Quantitative measurement of silymarin content in milk thistle extract

The determination of Silymarin content was conducted using an HPLC system (Knauer, Germany) equipped with a Knauer-UV K2501:288 nm detector and a separation column (Eclipse-XBD-C18, dimensions: 12.5 cm × 4.6 cm × 5 μm) set at room temperature. The mobile phases A and B consisted of phosphoric acid, methanol, and water, with compositions of 0.5:35:65 V/V/V and 0.5:50:50 V/V/V, respectively, at a flow rate of 0.8 mL min^{-1}.

The procedure involved dissolving 60 mg of the plant extract in a 100 ml flask with the addition of 50 ml of Ethanol. The extract was further dissolved in Methanol using an ultrasonic bath. The resulting solution was brought to a final volume of 100 ml with the same solvent and filtered through a 0.45 μm filter before being collected in HPLC vials. These vials were then sealed and stored at 5 °C in a refrigerator prior to injection into the HPLC system. The concentration of Silymarin in the prepared solution was quantified by measuring the area under the Silymarin peak in the sample and correlating it with the calibration curve. To construct the calibration curve, 10 mg of standard silymarin (Sigma, USA) powder was weighed and dissolved in 50% ethanol to a final volume of 100 mL. Calibration solutions with concentrations of 10, 20, and 100 ppm were then prepared from this stock solution. After filtering through a 0.45-μm syringe filter, the solutions were ready for injection.

2.8.2. Quantitative measurement of glycyrrhizic acid in the extract

Glycyrrhizic acid analysis was performed using an HPLC system (Knauer, Germany) equipped with a Knauer-UV K2501:254 nm detector. A separation column (Eclipse-XBD-C18, dimensions: 5 cm × 4.6 cm × 5 μm) was set at room temperature. The mobile phase composed of Glacial acetic acid, Acetonitrile, and water (in the ratio of 6:30:64 V/V/V), flowed at a rate of 1.5 mL min^{-1}. For liquid product or extract analysis, 1 g of extract was mixed with 100 ml of 1% w/v ammonia solution and centrifuged. Then, 2 mL of the supernatant liquid was combined with 8 mL of ammonia solution. After filtration through a 0.45-μm syringe filter, the solution was collected in HPLC vials and subsequently injected into the HPLC system. To construct the calibration curve, 20 mg of glycyrrhizic acid (Sigma, USA) was dissolved in 100 mL of an ammonia solution (8 g L^{-1}). Solutions with concentrations of 2, 10, 20, and 50 ppm were prepared from this stock and filtered through a 0.45-μm syringe filter for injection.

2.9. Statistical analysis

All experiments were performed in triplicate and were subjected to one-way analysis of variance (ANOVA) using Minitab statistical software version 21 (Minitab Inc., USA). Mean comparisons were made using Duncan's multiple range test with a significance level of $p < 0.05$, if needed.

3. Results and discussion

3.1. Microbial growth and pH changes

The assessment of microbial growth in Milk thistle and liquorice root extract was evaluated using microbial population changes during fermentation under aerobic culture conditions at 37 °C for 72 h. As shown in Fig. 1(a and b), the inoculated LAB (*L. plantarum* and *L. acidophilus*) into both extracts remained initially in the lag phase for about 12 h and afterward, the bacteria entered the logarithmic phase of growth in which the population of bacteria increased significantly. The cell number of *L. acidophilus* increased from the initial cell number of about 5.7×10^7 CFU mL^{-1} to its maximum value of 3.52×10^9 CFU mL^{-1} at hour 36 of Milk thistle fermentation. Meanwhile, during the 36 h of fermentation, the initial cell population of *L. plantarum* (3.8×10^7 CFU mL^{-1}) exhibited a logarithmic increase, reaching approximately 3.73×10^9 CFU mL^{-1}, marking a two-fold surge. Subsequently, the population remained in a stationary phase for approximately 20 h. Following this stage, the cell count declined as it entered the subsequent death phase. At the end of the fermentation process using Milk thistle extract, the count of *L. acidophilus* was reduced 3.1×10^6 CFU mL^{-1}, while *L. plantarum* attained a count of 4.4×10^8 CFU mL^{-1} (Fig. 1a). This decline may be attributed to the accumulation of acidic substances and a depletion in carbon and nitrogen sources (Mahmoudi, EbrahimzadehMousavi, & Khodaiyan, 2021). Microorganisms partially utilize the sugars in the extract to produce organic acids during fermentation, which causes a decrease in pH (de Oliveira, de Medeiros, de Assis, & Sousa Júnior, 2024). Lactic Acid Bacteria (LAB) generally need nutrient-rich and complex media for cell growth. Thus, this observed behavior could be attributed to either a deficiency in specific nutrients or the exhaustion of available nutrients that probiotics consumed in the early stages of fermentation (de Oliveira et al., 2024)

Regarding the liquorice extract, over the subsequent 48 h, the cell population in *L. acidophilus* and *L. plantarum* escalated to 8.1×10^8 CFU mL^{-1} and 6.9×10^8 CFU mL^{-1}, respectively. Shortly after this surge, the population declined, entering the death phase. However, this decline is more pronounced in Milk thistle extract (Fig. 1b).

Fig. 2 (a and b) display the pH variations observed in Milk thistle and liquorice root extracts before and after fermentation. The initial pH value in both extracts were above 5. However, with the introduction of the two lactic acid cultures and fermentation process resulted in a drastic decrease in the pH, reducing it to below 4.5 after 72 h. In the case of the Milk thistle extract, the pH readings after fermentation with *L. acidophilus* and *L. plantarum* concluded at 4.45 and 4.46, respectively.

This decline in pH can be attributed to the generation of acidic metabolites, the depletion of environmental buffering capacity, and the consumption of carbon sources by bacterial activity during their rapid logarithmic growth phase (Mousavi & Mousavi, 2019). Toward the latter hours of fermentation, a deceleration in pH reduction occurred, likely due to excessive acid accumulation during the initial 36 h when bacterial growth was at its peak. As acidity increased, bacterial growth slowed, subsequently curbing acid production and minimizing pH

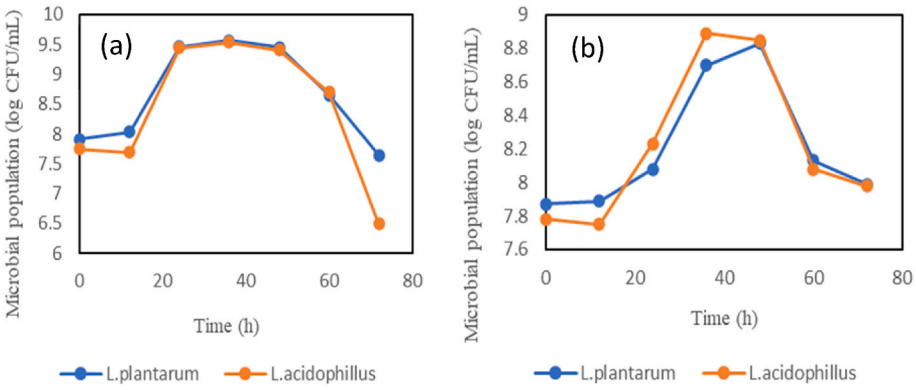

Fig. 1. Growth kinetic of *L. plantarum* (Lp) and *L. acidophillus* (La) in Milk Thistle extract (A) and licorice root extract (B) during fermentation.

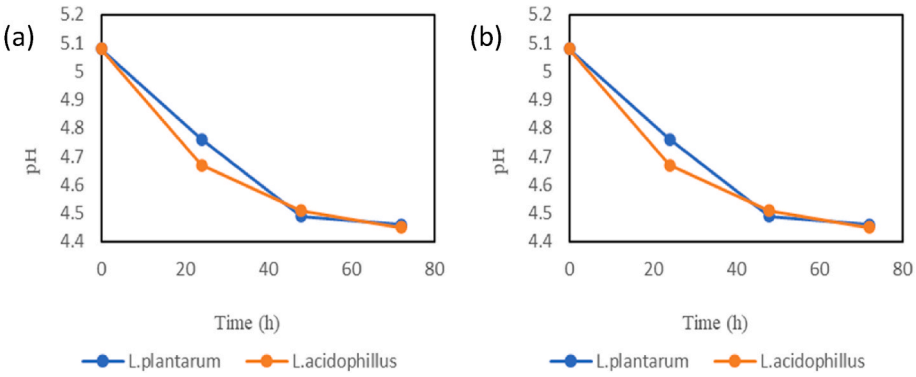

Fig. 2. Changes in the pH of Milk thistle extract (A) and licorice root extract (B) during fermentation by *L. plantarum* (Lp) and *L. acidophillus* (La).

alterations (Nagpal, Kumar, & Kumar, 2012). These findings align with previous studies by Mahmoudi et al. (2021) and Budiari et al. (2019)

3.2. Antioxidant activity

Table 1 showcases the alterations in antioxidant activity observed before and after fermentation in Liquorice and Milk thistle extracts. Surprisingly, in the case of fermented Milk thistle extracts, no significant change ($p < 0.05$) was detected in the antioxidant activity of the samples. The initial antioxidant activity in the Milk Thistle extract registered at 91.44% ± 0.87%, and post-fermentation, increased this value to 92.68% and 93% with *L. acidophilus* and *L. plantarum*, respectively. Indeed, the antioxidant activity of the Milk Thistle extract was already high before fermentation, and the fermentation process effectively maintained this high antioxidant activity in the samples Another study reported a DPPH radical inhibition percentage of 94.94% for Milk thistle hydroalcoholic extract, affirming its remarkable antioxidant potency (Akkaya & Yilmaz, 2012).

Table 1
Antioxidant activity, total phenolic and total flavonoid content of Milk thistle and lico-rice root extracts before and after fermentation.

Sample	Antioxidant Activity (%)	Total phenolic content (mg L^{-1})	Total flavonoid content (mg L^{-1})
Milk thistle extract		data	
Un fermented	91.44 ± 0.87[aa]	259.14 ± 1.77[a]	243.40 ± 19.50[a]
Fermented by L. *plantarum*	93.00 ± 0.38[a]	268.32 ± 7.33[b]	211.29 ± 5.07[b]
Fermented by L. *acidophilus*	92.68 ± 0.86[a]	265.79 ± 5.24[b]	202.34 ± 2.19[b]
Liquorice root extract			
Un fermented	88.35 ± 0.96[b]	268.47 ± 1.31[c]	229.76 ± 3.37[a]
Fermented by L. *plantarum*	93.16 ± 1.03[a]	309.44 ± 2.12[a]	218.62 ± 0.94[b]
Fermented by L. *acidophilus*	92.03 ± 0.15[a]	296.98 ± 3.86[b]	219.85 ± 1.61[b]

[a] Different letters indicate statistically significant differences with $p < 0.05$.

Table 1 demonstrates a noteworthy increase ($p < 0.05$) in the antioxidant activity of liquorice samples after fermentation. The initial antioxidant activity of liquorice root was quantified at 88.35% ± 0.96%. Post-fermentation, this activity notably increased to 92.03% ± 0.15% with *L. acidophilus* and further to 93.16% ± 1.03% with *L. plantarum*. Evidence shows that some LAB can increase the activity of antioxidative enzymes to protect cells from damage caused by oxidative stress (Feng & Wang, 2020). The enhanced free radical scavenging rate of fermented liquorice root extract can be attributed to the significant increase in total flavonoids and phenols. Additionally, microbial metabolism may boost compounds with proton-donor properties, thereby improving their free radical scavenging capability (Du et al., 2024). Hur et al. (2014) similarly concluded that fermentation has the potential to enhance both the phenolic content and antioxidant activity of various plants, with the specific outcomes contingent upon the initiating microorganisms. Tian et al. (2022). showed that *L. plantarum* exhibited excellent antioxidant capacity through superoxide an-ion (O2-) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity method. Also, in recent studies, recent studies have indicated that *L. acidophilus* can increase the antioxidant activity by increasing the phenolic content of plant materials and fruits (Wen, Ma, & Liu, 2023).

The fermentation process facilitates the breakdown of complex polyphenols into simpler and more biologically active compounds. As a result, free phenolic compounds exhibit a height-ened inhibitory effect (Martins et al., 2011). Also, during similar research by Eweys, Zhao, and

Darwesh (2022) they stated that the fermentation of *Cinnamomum cassia* by *L. plantarum* led to an increase in antioxidant activity, and they reported the heightened antioxidant efficacy observed in fermented Cinnamon is linked to elevated levels of distinct phenolic compounds and flavonoids. Another similar result in fermented fireweed leaves was reported by Lasinskas et al. (2023).

3.3. Total phenolic and total flavonoid

The data presented in Table 1 show the total phenolic contents of both unfermented and fermented extracts. Surprisingly, there was a significant change ($p < 0.05$) observed in the total phenol content of Milk thistle extract post-fermentation. The initial total phenol content of Milk thistle extract was measured at 259.14 ± 1.77 mg of gallic acid equivalents per liter of extract. Following fermentation with *L. plantarum*, it increased to 268.32 ± 7.33 mg of gallic acid equivalents per liter of extract. Similarly, fermentation of this extract with *L. acidophilus* resulted in a phenolic content of 265.79 ± 5.24 mg of gallic acid equivalents per liter of extract.

In contrast to the total phenolic changes observed in the Milk thistle extract after fermentation, the liquorice root extract exhibited a more significant increase in total phenolic content. The initial value was measured at 268.47 ± 1.31 mg of gallic acid equivalents per liter of extract, which then surged to 309.44 ± 2.12 mg L^{-1} in the liquorice extract fermented by *L. plantarum* and rose to 296.98 ± 3.86 mg of gallic acid equivalents per liter of the fermented sample with *L. acidophilus*. Phenolic compounds are generally found in conjugated forms with one or more sugar moieties, as glycosides, linked through OH group (O-glycosides) or through carbon–carbon bonds (C-glycosides) (Eseberri et al., 2022). Numerous studies highlight that LAB in fermentation produce enzymes such as β-glucosidase. These enzymes play a crucial role in breaking down phenolic glycosides into aglycones, which exhibit superior radical scavenging abilities (Hashemi & Jafarpour, 2020). Research on black bean fermentation has shown the production of β-galactosidase by certain microorganisms. This enzyme effectively breaks down complex, high mo-lecular weight phenolic compounds into simpler free phenols, consequently boosting the antioxidant potency of the fermented product (Lee, Hung, & Chou, 2008). In addition, recent research on Avocado plant leaf fermentation shows that LAB with enzymatic activity such as glycosidases or decarboxylases are capable of releasing phenolic compounds initially attached to the plant cell wall and therefore improve their bioavailability and also the total phenolic content significantly increased in avocado leaf extract after fermentation with *L. plantarum* (De Montijo-Prieto et al., 2023). In another similar study in 2023, it was announced that different strains of LAB can lead to an increase in total phenolic compounds in red ginger and African Black Nighshade (Mulyani et al., 2023; Irakoze, Wafula, & Owaga, 2023). A robust and positive correlation was identified between the total phenolic content (TPC) and the outcomes of the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. This correlation strongly suggests a notable connection between TPC and antioxidant activity, likely attributable to the phenolic compounds' ability to donate protons, consequently reducing DPPH radicals into DPPH2 (Mwamatope, Tembo, Chikowe, Kampira, & Nyirenda, 2020).

The total flavonoid content of the Milk Thistle and liquorice root extract before and after fermentation was determined and is reported as shown in Table 1 The initial amounts of total flavonoid content for the Milk thistle extract and liquorice extracts were 243.4 ± 19.5 and 229.76 ± 3.37 mg of Quercetin per liter of the extract, respectively. After fermentation with *L. plantarum*, these initial values in Milk thistle extract and liquorice extract decreased to 211.29 ± 5.07 and 218.62 ± 0.94 mg L^{-1}, respectively. Following fermentation with *L. acidophilus*, the contents in the mentioned extracts further decreased to 202.34 ± 2.19 and 219.85 mg L^{-1}, respectively. In summary, the decline in total flavonoids after fermentation does not necessarily indicate a loss of biological activity. This reduction is more likely due to a significant decrease in conjugated flavonoids, and it may be attributed to the metabolic transformations that occur during the fermentation process, leading to the formation of other metabolites (He, Zhang, Wang, Wang, & Luo, 2022). Microbial metabolism of phenolic compounds generates a wide range of new metabolites through various bioconversion pathways, including glycosylation, deglycosylation, ring cleavage, methylation, glucuronidation, and sulfate conjugation, depending on the microbial strains and substrates involved (Huynh, Van Camp, Smagghe, & Raes, 2014). The release of phenolic compounds means these compounds are obtained in a soluble free form in the fermentation medium, enhancing the value of the resulting extracts and food products (Huynh et al., 2014). Additionally, the microbial hydrolysis of polyphenols can enhance their bioavailability in food materials, resulting in the production of bioactive components that are more easily digested, absorbed, and utilized by the human body (Yang et al., 2023). Researchers have determined that the composition of the food matrix greatly influences alterations during fermentation. The decrease in the concentration of flavonoids and phenolic compounds is attributed to the fermentation process, primarily due to the degradation of polyphenolic compounds by LAB (Li et al., 2018). In a similar investigation, the total flavonoid contents in orange peels notably decreased by approximately 50% after fermentation with *L. plantarum* (Deba-Rementeria, Paz, Estrada, & Vázquez-Araújo, 2023; He et al., 2022). In simpler terms, fluctuations in temperature and pH levels can contribute to a reduction in flavonoid quantities, with higher temperatures hastening their degradation (Wijayanti & Setiawan, 2017). Diverse outcomes have been documented in the fermentation of various plant materials using LAB. For example, fermenting the Jaruk Tigaron plant and apple juice resulted in observable increases in overall flavonoid content post-fermentation. This underscores the potential of LAB-driven fermentation to augment flavonoid concentrations in these materials (Nazarni, Purnama, Umar, & Eni, 2016; Ankolekar et al., 2012), In addition, during the fermentation of peppers, there was an increase in flavonoid levels, while in the case of carrot fermentation, an overall decline in flavonoid content was observed (Ahmad, Jalaluddin, & Panda, 2014). In addition, in research performed by He et al. flavonoids can undergo a conversion into liberated states during the fermentation of soybean and tea. However, a reduction in the overall quantity may not necessarily signify a decrease in biological effectiveness.

3.4. Analysis of silymarin content in milk thistle extract

The Silymarin concentration in Milk thistle extract, was assessed before and after fermentation using HPLC and the relevant data are presented in Fig. 3. As mentioned earlier in this paper, Silymarin comprises a mixture of flavonoids. In this study, we have provided the total content measurement of this mixture. Initially, the unfermented Milk thistle extract contained 0.59 mg mL^{-1} of Silymarin. Following fermentation with *L. plantarum*, this concentration increased significantly to 2.5 mg mL^{-1}. Additionally, after fermentation with *L. acidophilus*, the Silymarin concentration reached 0.91 mg mL^{-1}. The percentage increase in Silymarin content in the sample subjected to *L. plantarum* fermentation was notably remarkable at 323.72%, surpassing the initial value by over four times. Furthermore, the Milk thistle extract subjected to *L. acidophilus* fermentation exhibited a 54.23% increase, which was more than 1.5 times the initial value. The findings revealed a significant difference in the increase of Silymarin content between the sample fermented with *L. plantarum* and the one fermented with *L. acidophilus*. It has been documented that *L. plantarum* exhibits robust glucosidase activity, suggesting that the rise in the quantity of active compounds following fermentation can be attributed to the influence of the glucosidase enzyme (Dueñas, Fernández, Hernandez, Estrella, & Muñoz, 2005). The glucosidase enzyme catalyzes the hydrolysis of β-glucosidic bonds in several phenolic compounds, which primarily exist as conjugates with one or more sugar residues linked to hydroxyl groups (Hussain et al., 2016). Enzymatically breaking down

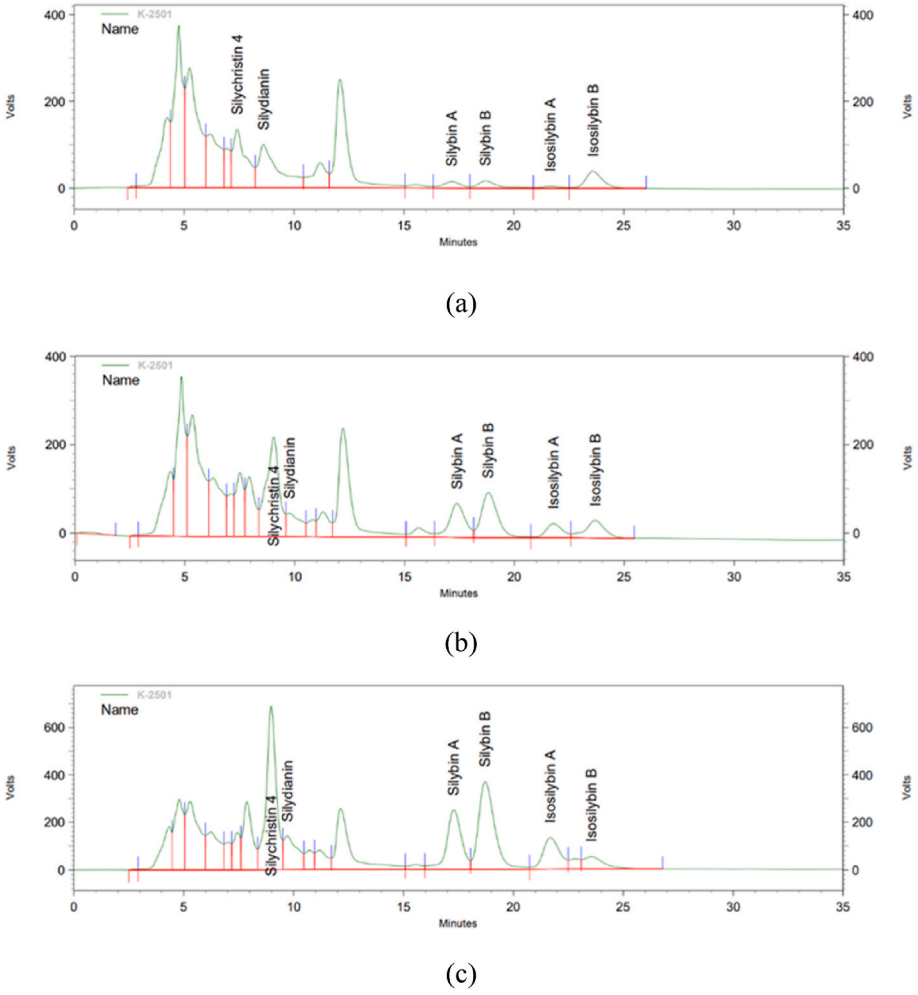

Fig. 3. HPLC results of silymarin analysis: (a) Un Fermented, (b) Fermented with L. *plantarum*, (c) Fermented with L. *acidophilus*.

phenolic glucosides is regarded as an efficient method to boost the concentration of free polyphenols and enhance the nutraceutical properties of various substrates (Hussain et al., 2016). The main hurdle affecting the efficacy of Silymarin in treatment lies in its poor bioavailability. This issue stems from several factors, including silybin's high reactivity during conjugation, limited permeability through intestinal epithelial cells, low solubility in water, and rapid elimination through bile and feces. Only a small amount (10–17%) of the overall plasma Silybin exists in its free and unbound state (Koushki, FarrokhiYekta, & Amiri-Dashatan, 2023). Generally, many natural flavonoids are in glycoside forms, which are less efficiently absorbed by the body compared to their aglycone counterparts. Apart from a fraction of flavonoid glycosides in the daily diet that are hydrolyzed into aglycones by digestive system enzymes; the majority are transformed into aglycones by the intestinal microbiota in the large intestine and interact with the body's own functions (Sandoval et al., 2020). Employing probiotic bacteria via fermentation has consistently been a method for boosting bioavailability. In this research, using this method yielded promising results in enhancing Silymarin's bioavailability and concentration.

3.5. Analysis of glycyrrhizic acid content in liquorice extract

Glycyrrhizic acid is commonly used as a sweetening agent in confectionery items such as candies and pastries. Additionally, it serves medicinal purposes in the treatment of liver and skin disorders. Recently, there has been discussion regarding its potential as a promising candidate for an antiviral drug to combat the ongoing COVID-19 pandemic, whether used alone or in combination with other medications (Li, Wan, & Yang, 2022). In this study, the analysis of samples (Fig. 4) revealed that the initial concentration of Glycyrrhizic acid in liquorice extract was 2.6 mg mL^{-1} before fermentation with the selected LAB strains. This concentration remained unchanged after fermentation with *L. acidophilus*. However, it was observed that the level of glycyrrhizic acid experienced a slight reduction to 2.34 mg mL^{-1} following fermentation with *L. plantarum*. In this study, the reduction in glycyrrhizic acid during fermentation with *L. plantarum* could be assigned to the enzymatic digestion of this compounds and formation of other compounds. As previously noted, upon consuming liquorice, microorganisms and enzymes in the gastrointestinal tract convert glycyrrhizic acid into 18β-glycyrrhetinic acid and glucuronic acid (Lu, Li, Dai, & Ouyang, 2006). The hydrolysis of glycoside molecules in glycyrrhizic acid generates a more potent aglycone known as 18-β-glycyrrhetinic acid, which exhibits increased absorption capacity (Hattori et al., 1985). Consequently, ongoing scientific endeavors aim to produce 18β-glycyrrhetinic acid from glycyrrhizic acid by utilizing microbial strains or beta-glucuronidase enzymes in laboratory experiments involving liquorice root (Ahmad et al., 2014). β-Glucuronidase, found in numerous organisms including bacteria, is a glycosidase enzyme that facilitates the breakdown of β-linked glucuronides, resulting in the formation of different derivatives and free glucuronic acid (Zou, Liu, Kaleem, & Li, 2013). From a biological standpoint, pentacyclic triterpenoids (PTs), which possess a core structure composed of five rings, have garnered significant interest due to their pharmacological properties. Specifically, 18β-glycyrrhetinic acid and its derivatives demonstrate a wide range of

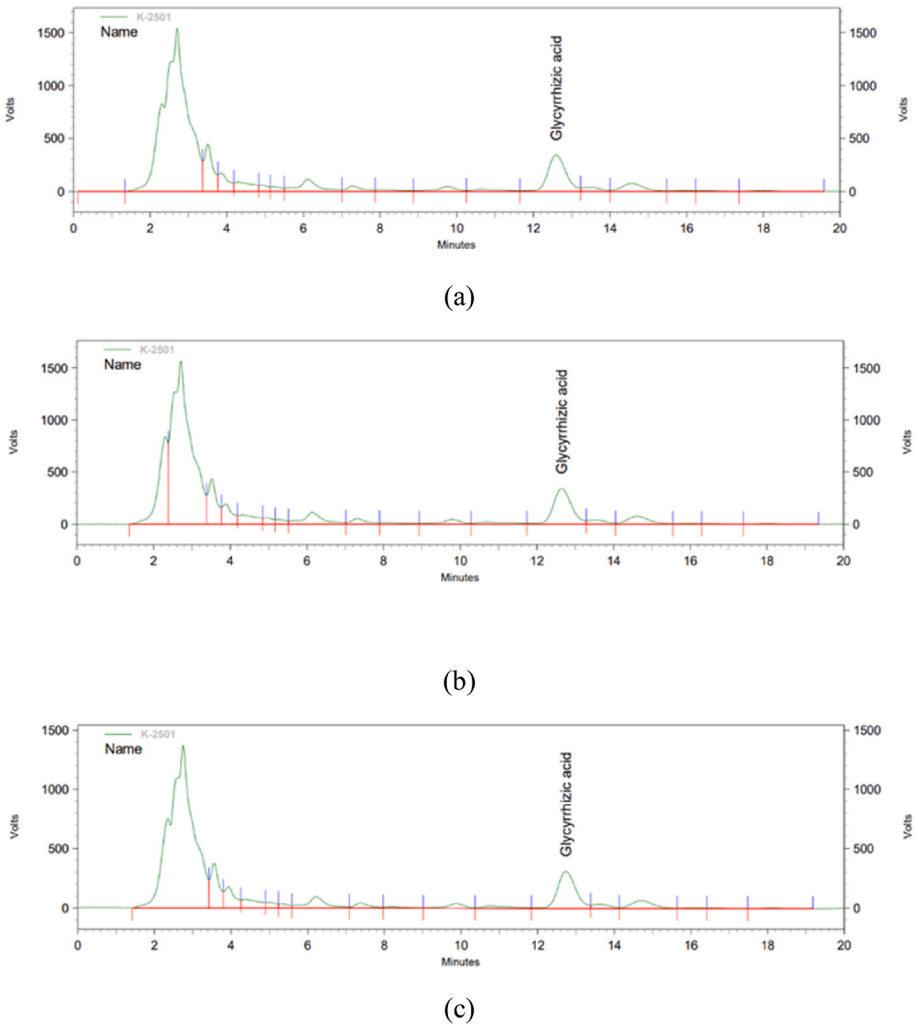

Fig. 4. HPLC results of Glycyrrhizic acid. (a) Un Fermented, (b) Fermented with L. *plantarum*, (c) Fermented with L. *acidophilus*.

biological and pharmacological activities, including notable antitumor effects (Kowalska & Kalinowska-Lis, 2019). The biological activity of 18β-glycyrrhetinic acid is 20 times greater than that of glycyrrhizic acid and Scientists are currently working on producing 18β-glycyrrhetinic acid from glycyrrhizic acid using bioprocesses under *in vitro* conditions (Ahmad, Ali, & Panda, 2016). Hence, the reduction in glycyrrhizic acid content post-fermentation by *L. plantarum* likely involves the transformation of glycyrrhizic acid into compounds with higher absorption

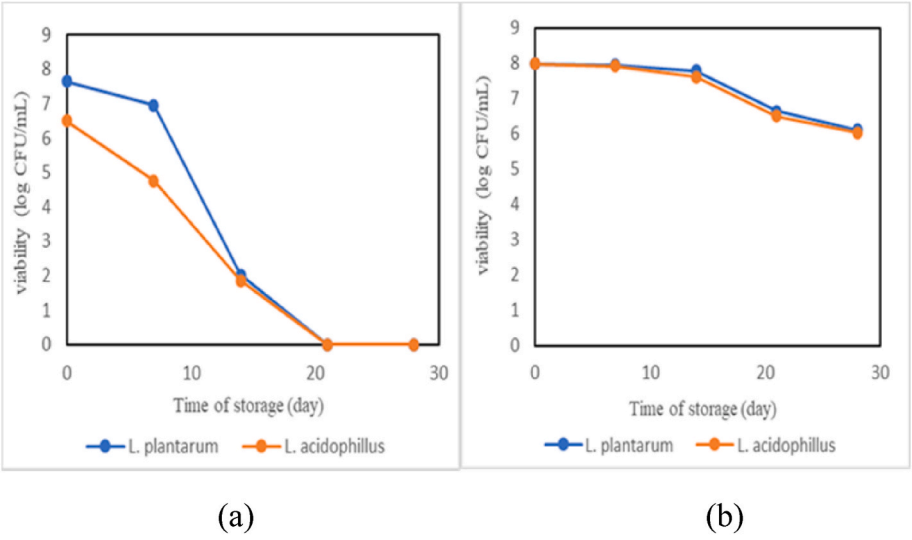

Fig. 5. Viability of probiotic bacteria in (a) Milk thistle extract and (b) licorice root.

capabilities, such as glycyrrhetinic acid.

3.6. Viability of LAB during cold storage

The viability of LAB in the extracts was monitored during storage for 28 days at 4 °C and is described in Fig. 5a and b. Following one week of refrigerated storage, a notable decrease in microbial populations was observed in the Milk thistle extract (Fig. 5a). Specifically, in the sample fermented with *L. acidophilus*, there was an approximate two-log-fold decline in microbial population, whereas in the sample fermented with *L. plantarum*, a one-log-fold reduction was noted. Later, in the subsequent week, the sample fermented with *L. acidophilus* experienced a three-log-fold decrease in microbial population, while the *L. plantarum*-fermented Milk thistle extract exhibited a remarkable four-log-fold reduction. Surprisingly, by the third week, no viable cells were detectable in the Milk thistle-fermented extracts.

According to Fig. 5b, following one week of storage, there was no notable change in the bacterial population present in the liquorice extract. However, by the third week of storage, approximately one-logarithmic reduction in the bacterial population within the liquorice-fermented extract was observed. The bacterial count in the liquorice extract remained stable and within acceptable limits throughout the fourth week. Maintaining a minimum viable cell count of 10^6 CFU mL^{-1} is recommended for delivering health benefits to consumers (Hammam & Ahmed, 2019). Sustaining this level of live cells in the liquorice extract even after four weeks of storage is a promising achievement.

The findings indicated that the probiotic bacteria within Milk thistle extracts deteriorated more rapidly. This degradation was attributed to unfavorable conditions such as low pH levels, insufficient nutrients, antimicrobial properties within the extract, and the presence of potential inhibitory compounds. In general, environments with high acidity lead to cell death through the action of hydrogen ions and mass transfer across the cell membrane. In other words, organic acids enter the microbial cell and release H^+ ions, causing significant changes in cell function and inhibiting microbial growth (Maia, Domingos, & de São José, 2023). The reduction in bacterial viability during storage could be attributed to multiple factors, including a decline in sugar levels within the samples, the accumulation of organic acids as metabolic by-products, and variations in the storage temperature (Yoon, Woodams, & Hang, 2004). It has also been reported that the increase in acidity as a result of the fermentation process can reduce the survival and viability of probiotic bacteria, and it was also specifically reported that the survival of probiotic organisms depends on factors such as the oxygen level in the product, the penetration of oxygen through the packaging, and storage time (Yáñez, Marques, Gírio, & Roseiro, 2008). Several methods have been employed to improve and sustain microbial cell viability. Among these methods, cell immobilization in various carriers, including composite matrix systems, has recently gained attention. This technique aims to protect probiotics from various types of environmental stress, such as changes in pH and heat treatments (Terpou et al., 2019). Additionally, the development of synbiotic combinations represents another approach in functional foods that helps stimulate the growth of probiotics (Terpou et al., 2019).

4. Conclusion

In recent decades, the notion of food has evolved significantly. With growing interest in functional foods, the food industry has shifted to meet consumer demands for more nutritious and safer products. Several types of fermented and commercialized foods and beverages exist. Nonetheless, the focus on fermented medicinal plants and their extracts, as well as their potential in the food industry, has not yet received the attention it deserves.

The study delved into the interaction between probiotic bacteria and licorice as well as Milk thistle extracts, uncovering significant insights. The research exhibited enhanced growth, especially in the licorice extract. Moreover, fermentation yielded a dual advantage: it not only boosted the phenolic content and antioxidant activity in licorice root extract but also effectively preserved these qualities in Milk thistle extract post-fermentation. Silymarin, the main active ingredient in Milk thistle extract with health benefits, saw a marked rise in concentration after fermentation. Notably, probiotic bacteria successfully maintained their viability in licorice extract during cold storage. These findings highlight the potential of fermented extracts as foundational elements for versatile, health-centric beverages. The fermentation process significantly enhanced the therapeutic and wellness properties of these extracts, showcasing their promise for utilization in nutraceutical and functional food development. However, to fully realize the potential of these extracts, further research is needed to investigate the *in vivo* bioavailability of the active ingredients and their overall health benefits. Gaining a deeper understanding of these factors will be essential for optimizing the effectiveness and market potential of fermented extracts in the nutraceutical industry.

Funding

This research received no external funding.

CRediT authorship contribution statement

Alireza Amini: Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation, Conceptualization. **Zeinabossadat Ebrahimzadeh Mousavi:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Seyed Mohammad Mousavi:** Writing – review & editing, Software, Resources, Project administration, Data curation, Conceptualization.

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

Data will be made available on request.

Acknowledgments

We specially thanks Zarattar Novin Pharmed Co. For their technical support throughout the project.

References

Ahmad, M., Ali, M., & Panda, B. (2016). Screening and bioconversion of glycyrrhizin of *Glycyrrhiza glabra* root extract to 18 [beta]-glycyrrhetinic acid by different microbial strains. *Drug Development and Therapeutics, 7*(1), 69.

Ahmad, M., Jalaluddin, M., & Panda, B. P. (2014). Enrichment of biologically active 18-β glycyrrhetinic acid in *Glycyrrhiza glabra* root by solid state fermentation. *Annals of Microbiology, 64*(2), 683–688.

Akkaya, H., & Yilmaz, O. (2012). Antioxidant capacity and radical scavenging activity of *Silybum marianum* and *Ceratonia siliqua*. *Ekoloji Dergisi, 21*(82).

Ankolekar, C., Johnson, K., Pinto, M., Johnson, D., Labbe, R. G., Greene, D., et al. (2012). Fermentation of whole apple juice using *Lactobacillus acidophilus* for potential dietary management of hyperglycemia, hypertension, and modulation of beneficial bacterial responses. *Journal of Food Biochemistry, 36*(6), 718–738.

Anwar, S., Syed, Q. A., Munawar, F., Arshad, M., Ahmad, W., Rehman, M. A., et al. (2023). Inclusive overview of sweeteners trends: Nutritional safety and commercialization. *ACS Food Science & Technology, 3*(2), 245–258.

Bernela, M., Seth, M., Kaur, N., Sharma, S., & Pati, P. K. (2023). Harnessing the potential of nanobiotechnology in medicinal plants. *Industrial Crops and Products, 194*, Article 116266.

Bhattacharya, S. (2011). *Phytotherapeutic properties of milk thistle seeds: An overview*.

Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science & Technology, 28*(1), 25–30.

Budiari, S., Maryati, Y., Susilowati, A., Mulyani, H., & Lotulung, P. D. N. (2019). The effect of lactic acid fermentation in antioxidant activity and total polyphenol contents of the banana. *Musa acuminate Linn) juice.* , Article 020024. https://doi.org/10.1063/1.5134588

Castro-López, C., Sánchez-Alejo, E. J., Saucedo-Pompa, S., Rojas, R., Aranda-Ruiz, J., & Martínez-Avila, G. C. G. (2016). Fluctuations in phenolic content, ascorbic acid and total carotenoids and antioxidant activity of fruit beverages during storage. *Heliyon, 2*(9), Article e00152.

Dastagir, G., & Rizvi, M. A. (2016). *Glycyrrhiza glabra L.*(Liquorice). *Pakistan journal of pharmaceutical sciences, 29*(5).

De Montijo-Prieto, S., Razola-Díaz, M. del C., Barbieri, F., Tabanelli, G., Gardini, F., Jiménez-Valera, M., et al. (2023). Impact of Lactic Acid Bacteria fermentation on phenolic compounds and antioxidant Activity of avocado leaf extracts. *Antioxidants, 12*(2), 298. https://doi.org/10.3390/antiox12020298

de Oliveira, L. F. R., de Medeiros, N. S., de Assis, C. F., & Sousa Júnior, F. C. de (2024). Simultaneous vehiculation of probiotics and yellow mombin (*Spondias mombin L.*) seed extract to develop a new vegan multifunctional ingredient. *Lebensmittel-Wissenschaft und -Technologie, 198*, Article 116018. https://doi.org/10.1016/j.lwt.2024.116018

Deba-Rementeria, S., Paz, A., Estrada, O., & Vázquez-Araújo, L. (2023). Consumer perception and physicochemical characterization of a new product made from lactic acid fermented orange peels. *International Journal of Gastronomy and Food Science, 31*, Article 100647. https://doi.org/10.1016/j.ijgfs.2022.100647

Dogan, K., & Tornuk, F. (2019). Improvement of bioavability of medicinal herbs by fermentation with *Lactobacillus plantarum*. *Functional Foods in Health and Disease, 9*(12), 735. https://doi.org/10.31989/ffhd.v9i12.648

Du, J., Li, X., Liu, N., Wang, Y., Li, Y., Jia, Y., et al. (2024). Improving the quality of Glycyrrhiza stems and leaves through solid-state fermentation: Flavonoid content, antioxidant activity, metabolic profile, and release mechanism. *Chemical and Biological Technologies in Agriculture, 11*(1), 105.

Dueñas, M., Fernández, D., Hernandez, T., Estrella, I., & Muñoz, R. (2005). Bioactive phenolic compounds of cowpeas (Vigna sinensis L). Modifications by fermentation with natural microflora and with *Lactobacillus plantarum* ATCC 14917. *Journal of the Science of Food and Agriculture, 85*(2), 297–304.

Elwekeel, A., Elfishawy, A., & Abouzid, S. (2013). Silymarin content in *Silybum marianum* fruits at different maturity stages. *Journal of Medicinal Plants Research, 7*(23), 1665–1669. http://www.academicjournals.org/JMPR.

Eseberri, I., Trepiana, J., Léniz, A., Gómez-García, I., Carr-Ugarte, H., González, M., et al. (2022). Variability in the beneficial effects of phenolic compounds: A review. *Nutrients, 14*(9), 1925.

Eweys, A. S., Zhao, Y. S., & Darwesh, O. M. (2022). Improving the antioxidant and anticancer potential of *Cinnamomum cassia* via fermentation with *Lactobacillus plantarum*. *Biotechnology Reports, 36*, Article e00768.

Feng, T., & Wang, J. (2020). Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: A systematic review. *Gut Microbes, 12*(1), Article 1801944. https://doi.org/10.1080/19490976.2020.1801944

Hammam, A. R. A., & Ahmed, M. S. I. (2019). Technological aspects, health benefits, and sensory properties of probiotic cheese. *SN Applied Sciences, 1*(9), 1113.

Hashemi, S. M. B., & Jafarpour, D. (2020). Fermentation of bergamot juice with *Lactobacillus plantarum* strains in pure and mixed fermentations: Chemical composition, antioxidant activity and sensorial properties. *Lebensmittel-Wissenschaft und -Technologie, 131*, Article 109803. https://doi.org/10.1016/j.lwt.2020.109803

Hattori, M., Sakamoto, T., Yamagishi, T., Sakamoto, K., Konishi, K., Kobashi, K., et al. (1985). Metabolism of glycyrrhizin by human intestinal flora. II. Isolation and characterization of human intestinal bacteria capable of metabolizing glycyrrhizin and related compounds. *Chemical and Pharmaceutical Bulletin, 33*(1), 210–217.

He, Z., Zhang, H., Wang, T., Wang, R., & Luo, X. (2022). Effects of five different Lactic Acid Bacteria on bioactive components and volatile compounds of oat. *Foods, 11*(20), 3230. https://doi.org/10.3390/foods11203230

Hur, S. J., Lee, S. Y., Kim, Y.-C., Choi, I., & Kim, G.-B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry, 160*, 346–356.

Husain, I., Bala, K., Khan, I. A., & Khan, S. I. (2021). A review on phytochemicals, pharmacological activities, drug interactions, and associated toxicities of licorice (Glycyrrhiza sp.). *Food Frontiers, 2*(4), 449–485. https://doi.org/10.1002/fft2.110

Hussain, A., Bose, S., Wang, J.-H., Yadav, M. K., Mahajan, G. B., & Kim, H. (2016). Fermentation, a feasible strategy for enhancing bioactivity of herbal medicines. *Food Research International, 81*, 1–16.

Huynh, N., Van Camp, J., Smagghe, G., & Raes, K. (2014). Improved release and metabolism of flavonoids by steered fermentation processes: A review. *International Journal of Molecular Sciences, 15*(11), 19369–19388. https://doi.org/10.3390/ijms151119369

Irakoze, M. L., Wafula, E. N., & Owaga, E. E. (2023). Effect of lactic acid fermentation on phytochemical content, antioxidant capacity, sensory acceptability and microbial safety of African Black Nightshade and African Spider plant vegetables. *Bacteria, 2*(1), 48–59. https://doi.org/10.3390/bacteria2010004

Ivanov, V., Dyankova, S., & Pavlova, M. (2021). Comparative study of the antioxidant properties of silymarin and milk thistle extracts. *Trakia Journal of Sciences, 19*(1), 1–6.

Jiang, J., Zhang, X., True, A. D., Zhou, L., & Xiong, Y. L. (2013). Inhibition of lipid oxidation and rancidity in precooked Pork Patties by radical-scavenging licorice (*Glycyrrhiza glabra*) Extract. *Journal of Food Science, 78*(11), C1686–C1694. https://doi.org/10.1111/1750-3841.12273

Köberl, M., Erschen, S., Etemadi, M., White III, R. A., El-Arabi, T. F., & Berg, G. (2019). Deciphering the microbiome shift during fermentation of medicinal plants. *Scientific Reports, 9*(1), Article 13461.

Koushki, M., Farrokhi Yekta, R., & Amiri-Dashatan, N. (2023). Critical review of therapeutic potential of silymarin in cancer: A bioactive polyphenolic flavonoid. *Journal of Functional Foods, 104*, Article 105502. https://doi.org/10.1016/j.jff.2023.105502

Kowalska, A., & Kalinowska-Lis, U. (2019). 18β-Glycyrrhetinic acid: Its core biological properties and dermatological applications. *International Journal of Cosmetic Science, 41*(4), 325–331. https://doi.org/10.1111/ics.12548

Kumar Srivastava, A. (2018). Significance of medicinal plants in human life. In *Synthesis of medicinal agents from plants* (pp. 1–24). Elsevier. https://doi.org/10.1016/B978-0-08-102071-5.00001-5.

Lasinskas, M., Jariene, E., Vaitkeviciene, N., Kulaitiene, J., Adamaviciene, A., & Hallmann, E. (2023). The impact of solid-phase fermentation on flavonoids, phenolic acids, tannins and antioxidant Activity in *Chamerion angustifolium* (L.) Holub (Fireweed) leaves. *Plants, 12*(2), 277.

Lee, I.-H., Hung, Y.-H., & Chou, C.-C. (2008). Solid-state fermentation with fungi to enhance the antioxidative activity, total phenolic and anthocyanin contents of black bean. *International Journal of Food Microbiology, 121*(2), 150–156.

Li, Z., Teng, J., Lyu, Y., Hu, X., Zhao, Y., & Wang, M. (2018). Enhanced antioxidant activity for apple juice fermented with *Lactobacillus plantarum* ATCC14917. *Molecules, 24*(1), 51. https://doi.org/10.3390/molecules24010051

Li, Q., Wan, Z., & Yang, X. (2022). Glycyrrhizic acid: Self-assembly and applications in multiphase food systems. *Current Opinion in Food Science, 43*, 107–113. https://doi.org/10.1016/j.cofs.2021.11.008

Liu, Y., Wu, M., Ren, M., Bao, H., Wang, Q., Wang, N., et al. (2023). From medical herb tofunctional Food: Development of a fermented milk containing silybin and protein from Milk Thistle. *Foods, 12*(6), 1308. https://doi.org/10.3390/foods12061308

Lu, D.-Q., Li, H., Dai, Y., & Ouyang, P.-K. (2006). Biocatalytic properties of a novel crude glycyrrhizin hydrolase from the liver of the domestic duck. *Journal of Molecular Catalysis B: Enzymatic, 43*(1–4), 148–152.

Mahmoudi, B., Ebrahimzadeh Mousavi, Z., & Khodaiyan, F. (2021). A functional non-dairy beverage produced from jujube extract using probiotic Lactic Acid Bacteria. *Journal of Agricultural Science and Technology A, 23*(4), 813–824. https://jast.modares.ac.ir/article-23-38484-en.html.

Maia, M. S., Domingos, M. M., & de São José, J. F. B. (2023). Viability of probiotic microorganisms and the effect of their addition to fruit and vegetable juices. *Microorganisms, 11*(5), 1335. https://doi.org/10.3390/microorganisms11051335

Martins, S., Mussatto, S. I., Martínez-Avila, G., Montañez-Saenz, J., Aguilar, C. N., & Teixeira, J. A. (2011). Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. *Biotechnology Advances, 29*(3), 365–373.

Mousavi, Z. E., & Mousavi, M. (2019). The effect of fermentation by *Lactobacillus plantarum* on the physicochemical and functional properties of liquorice root extract. *Lebensmittel-Wissenschaft und -Technologie, 105*, 164–168.

Mousavi, Z. E., Mousavi, S. M., Razavi, S. H., Emam-Djomeh, Z., & Kiani, H. (2011). Fermentation of pomegranate juice by probiotic lactic acid bacteria. *World Journal of Microbiology and Biotechnology, 27*(1), 123–128. https://doi.org/10.1007/s11274-010-0436-1

Mulyani, H., Artanti, N., Filailla, E., Budiari, S., Maryati, Y., Melanie, H., et al. (2023). Effect of fermented red ginger (*Zingiber officinale var. rubrum*) using kombucha culture toward free radical scavenging activity. *AIP Conference Proceedings, 2902*(1).

Mwamatope, B., Tembo, D., Chikowe, I., Kampira, E., & Nyirenda, C. (2020). Total phenolic contents and antioxidant activity of *Senna singueana, Melia azedarach, Moringa oleifera* and *Lannea discolor* herbal plants. *Scientific African, 9*, Article e00481. https://doi.org/10.1016/j.sciaf.2020.e00481

Nagpal, R., Kumar, A., & Kumar, M. (2012). Fortification and fermentation of fruit juices with probiotic lactobacilli. *Annals of Microbiology, 62*, 1573–1578.

Nazarni, R., Purnama, D., Umar, S., & Eni, H. (2016). The effect of fermentation on total phenolic, flavonoid and tannin content and its relation to antibacterial activity in jaruk tigarun (*Crataeva nurvala*, Buch HAM). *International Food Research Journal, 23*(1).

Perjéssy, J., Hegyi, F., Nagy-Gasztonyi, M., & Zalán, Z. (2022). Effect of the lactic acid fermentation by probiotic strains on the sour cherry juice and its bioactive compounds. *Food Science and Technology International, 28*(5), 408–420. https://doi.org/10.1177/10820132211018044

Quirós-Sauceda, A. E., Ovando-Martínez, M., Velderrain-Rodríguez, G. R., González-Aguilar, G. A., & Ayala-Zavala, J. F. (2016). Licorice (*Glycyrrhiza glabra linn.*) oils. In *Essential oils in food preservation, flavor and safety* (pp. 523–530). Elsevier.

Sandoval, V., Sanz-Lamora, H., Arias, G., Marrero, P. F., Haro, D., & Relat, J. (2020). Metabolic impact of flavonoids consumption in obesity: From central to peripheral. *Nutrients, 12*(8), 2393. https://doi.org/10.3390/nu12082393

Shahrajabian, M. H., & Sun, W. (2023). Survey on medicinal plants and herbs in traditional Iranian medicine with anti-oxidant, anti-viral, anti-microbial, and anti-inflammation properties. *Letters in Drug Design and Discovery, 20*(11), 1707–1743.

Terpou, A., Papadaki, A., Lappa, I., Kachrimanidou, V., Bosnea, L., & Kopsahelis, N. (2019). Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients, 11*(7), 1591. https://doi.org/10.3390/nu11071591

Tian, L., Liu, R., Zhou, Z., Xu, X., Feng, S., Kushmaro, A., et al. (2022). Probiotic characteristics of *Lactiplantibacillus plantarum* N-1 and its cholesterol-lowering effect in hypercholesterolemic rats. *Probiotics and Antimicrobial Proteins, 14*(2), 337–348.

Wen, J., Ma, X., & Liu, Y. (2023). The latest research progress on application of *Lactobacillus acidophilus*. *Advances in Bioscience and Biotechnology, 14*(6), 298–307.

Wen, Y.-L., Yan, L.-P., & Chen, C.-S. (2013). Effects of fermentation treatment on antioxidant and antimicrobial activities of four common Chinese herbal medicinal residues by *Aspergillus oryzae*. *Journal of Food and Drug Analysis, 21*(2), 219–226.

Wijayanti, E. D., & Setiawan, N. C. E. (2017). The effect of lactic acid fermentation on fig (Ficus carica) fruit flavonoid. *BERKALA PENELITIAN HAYATI JOURNAL OF BIOLOGICAL RESEARCHES, 23*(1), 39–44.

Yáñez, R., Marques, S., Gírio, F. M., & Roseiro, J. C. (2008). The effect of acid stress on lactate production and growth kinetics in *Lactobacillus rhamnosus* cultures. *Process Biochemistry, 43*(4), 356–361.

Yang, F., Chen, C., Ni, D., Yang, Y., Tian, J., Li, Y., et al. (2023). Effects of fermentation on bioactivity and the composition of polyphenols contained in polyphenol-rich foods: A review. *Foods, 12*(17), 3315. https://doi.org/10.3390/foods12173315

Yang, X., Hong, J., Wang, L., Cai, C., Mo, H., Wang, J., et al. (2024). Effect of lactic acid bacteria fermentation on plant-based products. *Fermentation, 10*(1), 48. https://doi.org/10.3390/fermentation10010048

Yoon, K. Y., Woodams, E. E., & Hang, Y. D. (2004). Probiotication of tomato juice by lactic acid bacteria. *Journal of Microbiology, 42*(4), 315–318.

Yuan, T., Wang, J., Chen, L., Shan, J., & Di, L. (2020). *Lactobacillus murinus* improved the bioavailability of orally administered glycyrrhizic acid in rats. *Frontiers in Microbiology, 11*, 597.

Zou, S., Liu, G., Kaleem, I., & Li, C. (2013). Purification and characterization of a highly selective glycyrrhizin-hydrolyzing β-glucuronidase from *Penicillium purpurogenum* Li-3. *Process Biochemistry, 48*(2), 358–363. https://doi.org/10.1016/j.procbio.2012.12.008