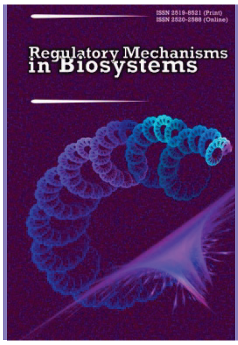

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Detoxification of hydrocyanide by fermentation of apricot seeds with probiotic *Lactobacillus* strains isolated from breast milk

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Many plants produce substances known as cyanogenic glycosides, which, when hydrolyzed, can release hydrogen cyanide. These include apricot (*Prunus armeniaca* L.) seeds, the subject of the study presented here. Amygdalin when metabolized, turns into hydrocyanide, which can cause cyanide poisoning if ingested in large quantities. The current study used *Lactobacillus* spp. that were isolated from breast milk. High concentrations of hydrocyanic acid, which apricot seeds contain, are dangerous during fermentation by *Lactobacillus* spp. The aim of the present study is to recognize the most effective methods for detoxifying apricot seeds and fermented amygdalin extract, while conserving or enhancing the physical properties, chemical structure, minerals and amino acids. Activation of bacterial isolates was followed by fermentation. Next a cellular cytotoxicity test for extract was conducted. After fermentation, we evaluated the cytotoxic effect on cancer and normal cell *in vitro*. After fermentation, a detoxification of apricot seeds was shown resulting from the presence of compound and improvement of vitamins formed due to fermentation. The use of *Lactobacillus* bacteria isolated from breast milk resulted in the production of a new fermented milk product containing aqueous extract of apricot seeds free from life-threatening toxicity. Fermentation produced a new probiotic with less toxic hydrocyanide, fatty acid, and amino acid, showing cytotoxic effects on cancer cells and minimal toxicity to normal cells.

Keywords: *Prunus armeniaca*; amygdalin; probiotic; *Lactobacillus*; cancer cell line.

Introduction

Many plants produce substances known as cyanogenic glycosides, which, when hydrolyzed, can release hydrogen cyanide. This ability, known as cyanogenesis, has been documented for periods in plants such as apricots, peaches, almonds, and other important food plants (Tahir et al., 2024). Large numbers of people are daily exposed to low concentrations of cyanogenic compounds in many foods. This exposure may imply a risk to human health (Park et al., 2024).

Apricot seeds are rich in proteins, lipids, and chemicals called cyanogenic glycosides – which, when hydrolyzed, can generate poisonous hydrogen cyanide (HCN). Apricot seeds are well-known for their nutritious richness. Despite the seeds' possible health advantages, their overconsumption puts human health at serious risk due to their high cyanide level. Probiotic-based fermentation presents a potentially effective way to detoxify these substances. Amygdalin-containing seeds, like apricot seeds, can ferment and release hydrogen cyanide (HCN), a hazardous substance. When metabolized, amygdalin turns into HCN, which can cause cyanide poisoning if ingested in large quantities (Akhone et al., 2022).

Beneficial bacterial strains called *Lactobacillus* spp. are frequently found in fermented foods in the human gut. These microbes have the ability to ferment and metabolize a wide range of substances, possibly including cyanogenic glycosides like amygdalin. Because the *Lactobacillus* spp. strains isolated from breast milk are tailored to human biology, they may provide better metabolic pathways to neutralize HCN, which may lead to greater detoxifying capabilities. Thus, treating or removing hydrogen cyanide from such products is essential to ensure their safety for human consumption. Commonly present in human breast milk, *Lactobacillus* species have been shown to possess probiotic qualities and the ability to hydrolyze cyanogenic glycosides. The purpose of this work is to reduce cyanide levels by fermenting apricot seed extracts using *Lactobacillus* bacteria that were isolated from breast milk (Rowland et al., 2018).

High concentrations of hydrocyanic acid which apricot seeds contain are dangerous to human health. It has been demonstrated that conventional detoxification techniques, including soaking in water or chemical treatments, can lower these levels. However they may have some negative effects on the seeds' nutritional value (Dempsey & Corr, 2022).

The chemicals in apricot seeds, such as cyanogenic glycosides like amygdalin, which when digested, can produce lethal hydrogen cyanide, can be fermented by lactobacilli. Lactobacilli have the ability to ferment these substances and change them into non-toxic byproducts, lessening the negative effects. Because lactobacilli are able to ferment cyanogenic glycosides, there is a chance that a smaller quantity of harmful hydrogen cyanide will be produced during digestion (Barakat et al., 2022; Nausad et al., 2024). The fermenting process produces healthy oils with antioxidant qualities while also improving the nutritional profile of apricot seeds and eliminating toxic substances (Nausad et al., 2024).

The use of strains of lactic acid bacteria from safe sources, which is breast milk. The goal of this work is to investigate how *Lactobacillus* species that have been isolated from human breast milk can reduce hydrocyanide chemicals found in fermented apricot seed extract in order to facilitate detoxification (D'Alessandro et al., 2022). It is hypothesized that the cyanide level of apricot seeds can be greatly reduced by utilizing the enzymatic activity of these strains during the fermentation process, potentially providing probiotic advantages and making the seeds safer to consume.

The current study aims to recognize the most effective methods for detoxifying seeds of apricot and fermented amygdalin extract, while conserving or enhancing the physical properties, chemical structure, minerals and amino acids. Moreover, the current study will assess whether the newly created probiotic after fermentation is safe for normal cells.

Materials and methods

The fruits of apricots (*Prunus armeniaca* L.) were obtained from the local Iraqi markets. The seeds were separated from the fruits obtained and then the seeds were washed and dried in the air for two weeks, the seeds were broken manually and the cotyledon, which was characterized by its bitter taste, was collected. 50 g of seeds, and part of it was ground by a grinder. About 25 g of apricot seed powder was added to 250 mL of absolute ethanol and another 25 g was added to 250 mL of distilled water and kept in a rotary shaker for 24 hours and filtered with Whatman No. 1 filter paper and re-filtered through 0.45 µm micro filter, then the extract was concentrated in the rotary evaporator at 50 °C and stored at 4 °C (Quader et al., 2018).

To initiate the process of preparing and activating the *Lactobacillus* strains from breast milk, we first diluted 10 g of milk with 100 mL of distilled water. Next, we transferred the diluted milk into 15 mL sterile test tubes, so that 9 mL of the solution was contained in each tube. We made sure the contents of the tubes were adequately disinfected by heating them for 5 to 8 minutes. After sterilization was finished, we let the tubes cool. Using micropipettes, we carefully transferred 1 mL of the bacterial isolates into each tube while maintaining sterility. After that, these tubes were incubated for 24 hours at 37 °C in an anaerobic environment using a jar with a gas bag. Other than *Lactobacillus*, bacterial species were cultured in an aerobic environment (Rezaei et al., 2023).

Human erythrocytes were used to test the cytotoxicity of alcoholic and aqueous apricot seed extract. Different concentrations of extract were used, 10, 50, 100 and 200 Pm, where 2 mL of blood was withdrawn and added to 20 mL of saline buffer phosphate and then distributed in test tubes. About 100 µL of each concentration of apricot extract was added to each tube of human blood solution and a negative control coefficient containing only physiological salt solution was used. All test tubes were placed at room temperature and the formation of hemolysis turbidity was monitored every 15, 30 and 60 minutes (Abu-Mejdad & Al-Hilfy, 2013).

Several reports related to the quantitative analysis of fermented amygdalin have indicated the existence of several methods, and these methods possess many disadvantages such as low sensitivity, long retention time and requirements for large quantities of organic solvents. Compared to the method used in our current study, which included analysis using liquid chromatography – liquid mass spectroscopy (LC-MS-MS) Bio analysis due to good specificity, sensitivity and short analytical time Tools and conditions LC-MS-MS. A liquid chromatography system of the Agilent 1200 series was used, which was linked to the quaternary mass spectrometry API4000 in addition to electrolyte ionization (ESI). Chromatographic separation was achieved in a Gemini analytical column C18 50 × 2.0 mm, 5 microns; Phenomenex), Torrance, CA) at a temperature of 40 °C. The mobile phase consists of methanol and water (85:15; v/v) with a flow rate of 0.25 mL/min at an operating time of 3.0 min. Injection volume was 5 µL Calibration and Quality Control Sample Counter for (LC-MS-MS) (Sulfianti et al., 2023).

Hydrocyanic acid in apricot seed cotyledon powder according to Association of Official Analytical Chemists after fermentation of the extract for cotyledons, the sample was incubated at 10 g in a conical flask (800 mL) with 200 mL of distilled water for 6 hours, after which a steam distillation of apricot seed cotyledon infusion was performed to collect 150 mL of distilled water in a flask containing 20 mL of sodium hydroxide solution with a concentration of 2.5% and then the distillation was diluted to a volume of 250 mL. 100 mL of previously prepared distilled dilute was taken and 8 mL of ammonium hydroxide concentration 6 was added to it, and then 2 mL of potassium iodine solution 5% was added with good shaking and titration of the resulting solution by silver nitrate solution known as standard 2%. Next we calculated milligrams of hydrocyanic acid according to Xu et al. (2017).

After taking 1.0 g from a sample of oil of apricot seed that was placed in a in a tightly sealed tube, the dissolved oil was then added, followed by the addition of 10 mL of methanol solution suppressing 1% pure concentrated sulfuric acid, then heated in an oven at 90 °C for 90 minutes. After cooling to room temperature, distilled water was added, and the extraction process was repeated three times. Extraction after collecting and filtering through anhydrous sodium sulfate. After that, the filtrate was concentrated using nitrogen gas and stored in a refrigerator until the analyses were performed. The prepared fatty acid methyl esters were separated using a DB-S fused silica capillary column (60 cm in length, 0.32 mm inner diameter). The temperature program started at 150 °C and increased at a rate of 3 °C/min until reaching 240 °C. The injection unit was maintained at 230 °C, while the detector was set at 250 °C. The identification of the separated fatty acids was achieved by comparing their retention times (Rt) with those of known fatty acid standards (Pawar & Nema, 2023).

An atomic absorption spectrophotometer (Perkin Elmer) was used to determine the mineral elements (copper, calcium, iron, magnesium, manganese, and zinc). A flame photometer was used to measure the levels of sodium and potassium. The ammonium molybdate colorimetric method was utilized to ascertain the phosphorus level. The blue color's intensity was quantified at a wavelength of 625 nm, adhering to the Association of Official Analytical Chemists recommendations (Isaac & Kerber, 1971; Ullah et al., 2022).

After taking a sample containing 25 mg of apricot seed will considered with 10 mL of hydrochloric acid, extended with a reducing agent as mercaptoethanol 0.01%, then subjected to hydrolysis by heating in an oven at 110 °C for 24 hours. After cooling to room temperature, the filtrate was then diluted to a final volume of 25 mL. The second beaker containing solid potassium hydroxide (KOH) and another containing concentrated sulfuric acid (H_2SO_4) were used to ensure complete drying of the sample under vacuum conditions (Boisen et al., 2000).

Cancer cell lines were maintained in RPMI-1640 supplemented with fetal bovine, penicillin, and streptomycin, and used in breast cancer AMJ13 and normal cell HBL100 incubation. The cytotoxic effect of plant compounds was assessed using an (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay (MTT) cell viability assay in 96-well plates. Cell lines were treated with different concentrations for 24 hours, and after 72 hours, cell viability was assessed by adding MTT solution.

Results

The cytotoxicity test findings for the apricot seed aqueous extract show a distinct tendency of increasing hemolysis at higher concentrations and longer exposure durations. After 15 minutes, there was no appreciable hemolysis at a low concentration of 10 ppm; only minor effects were observed at subsequent times. On the other hand, considerable hemolysis started to happen at modest doses of 50 and 100 ppm, suggesting that the extract starts to harm red blood cell membranes. Critical hemolysis was seen by the time the concentration hit 200 ppm, indicating significant toxicity (Table 1).

The extract contained substances such cyanogenic glycosides, which may be the cause of this toxicity. Higher levels appear to carry serious health hazards, even while lower quantities seem safe. We endeavoured to comprehend the effects of the extract and investigate possible protective agents that could lessen its cytotoxic effects.

Table 1
Cytotoxicity test utilizing the apricot seeds aqueous extract

Concentration, ppm	After 15 minutes	After 30 minutes	After 60 minutes
10	no hemolysis (0%)	mild hemolysis (2%)	slight hemolysis (5%)
50	slight hemolysis (5%)	moderate hemolysis (15%)	moderate hemolysis (25%)
100	moderate hemolysis (15%)	significant hemolysis (35%)	severe hemolysis (50%)
200	significant hemolysis (40%)	severe hemolysis (65%)	critical hemolysis (90%)

Following fermentation, the concentration of hydrocyanic acid fell from 7.3 to 3.3, suggesting a possible decrease in toxicity. Crude Protein: rose dramatically to 70.91 from 53.62 indicating improved nutritional value after fermentation. Crude Fat: showed a decrease in fat content, going from 2.66 to 1.22. Total Ash: showed a slight shift in the mineral composition, falling from 2.83 to 2.46. Crude Fiber: showed a slight decrease in fiber content, going from 34.96 to 33.20. While lowering anti-nutritional elements like hydrocyanic acid, the fermentation procedure appeared to increase protein content (Table 2).

Table 2
Nutritional and anti-nutritional changes before and after fermentation

Components	Before fermentation	After fermentation	Change, %
Hydrocyanic acid, mg/100 g	7.30	3.30	–54.79
Crude protein, %	53.62	70.91	–32.23
Crude fat, %	2.66	1.22	–54.14
Total ash, %	2.83	2.46	–13.09
Crude fiber, %	34.96	33.20	–5.03

Considerable alterations were seen when fatty acid composition was compared before and after fermentation. A decrease in stearic acid from 2.6 to 1.2 and a decrease in oleic acid from 56.3 to 45.6 show that fermentation promotes the breakdown of saturated fats. This pattern was further reinforced by the decrease in both palmitoleic and palmitic acids. Linoleic acid, on the other hand, showed resilience to the effects of fermentation by staying steady at about 24. A small decrease in arachidic and linolenic acids was observed. Overall, there appears to be a shift toward a healthy fat profile as unsaturated acids climbed from 91.9 to 92.3 while saturated acids declined from 6.09 to 5.65. These modifications suggest that the fermented product has increased nutritional value, which calls for more research into the underlying metabolic processes (Table 3).

Table 3
Fatty acid content of apricot seed extract before and after fermentation

Fatty acid	Before fermentation, %	After fermentation, %
Oleic	56.3	45.6
Stearic	2.6	1.2
Palmitoleic	0.4	0.2
Palmitic	3.5	2.3
Linoleic	24.2	24.2
Arachidic	0.09	0.08
Linolenic	0.14	0.12
Unsaturated acids	91.9	92.3
Saturated acids	6.1	5.7

The content of apricot seed meal of mineral elements before and after fermentation. It is noted that the seed flour after removing the toxin (fermentation) contains relatively higher concentrations of some elements compared to before removing the toxin (fermentation), and it becomes clear that the most abundant elements (the highest concentration) were in the seed (Table 4).

Table 4
Element content (mg/100 g) of apricot seed extract
before and after fermentation

Element	Before fermentation	After fermentation
Potassium	513.90	520.30
Magnesium	138.76	200.34
Sodium	64.78	67.11
Calcium	70.08	150.98
Manganese	0.98	15.50
Iron	6.34	5.66
Zinc	8.33	10.33
Phosphorus	655	700
Copper	1.23	1.33

The content of amino acids in apricot seeds is altered in a number of ways during fermentation. Leucine, isoleucine, threonine, and methionine are among the amino acids that exhibit minor increases, indicating an improvement in the overall quality of the protein. The seeds' potential as an antioxidant is increased when cysteine levels are greatly raised. Lysine and valine, on the other hand, decrease little; lysine drops by 10.57, which could marginally lower the necessary amino acid value of the seeds. All things considered, fermenting enhances the nutritious value of apricot seeds (Table 4). A large number of non-essential amino acids in apricot seeds exhibit slight alterations after detoxification (fermentation). While other amino acids such as glutamic acid, serine, and tyrosine only exhibit minor increases, aspartic acid and histidine show considerable increases. Proline does not change, although arginine and lysine both slightly drop. The fermentation process affects the amino acid concentration in a modest but generally beneficial manner overall, especially when it comes to increasing certain essential amino acids including histidine (Table 5).

Table 5
Content (mg/100 g) of apricot seed containing essential amino acids before and after detoxification (fermentation)

Amino acid	Before fermentation	After fermentation
Valine	4.60	4.48
Leucine	5.84	5.93
Isoleucine	6.96	7.06
Threonine	2.70	2.91
Lysine	3.50	3.13
Phenylalanine	5.02	5.32
Methionine	1.75	1.83
Cystine	0.13	0.20

The effect of apricot seed before and after formation have cytotoxic effect on breast cancer (Table 6, 7).

Table 6
Content (mg/100 g) of apricot seed containing
non-essential amino acids before and after detoxification (fermentation)

Amino acid	Before fermentation	After fermentation
Aspartic	11.08	12.18
Glutamic	24.32	24.42
Glycine	4.82	4.66
Alanine	5.17	5.12
Histidine	3.20	4.38
Arginine	7.44	7.18
Serine	4.29	4.33
Proline	4.59	4.59
Tyrosine	3.00	3.04

Table 7
Effect of viability of AMJ3 percentage
before and after fermentation (mean ± standard deviation)

Concentration, μM	Viability of AMJ3, %	
	before fermentation	after fermentation
5	100.0 ± 0.1^{d}	100.0 ± 0.9^{d}
10	90.3 ± 3.1^{c}	95.2 ± 7.1^{c}
25	81.4 ± 2.0^{bc}	84.7 ± 8.1^{bc}
35	69.8 ± 18.8^{bc}	79.7 ± 2.5^{bc}
45	54.9 ± 3.2^{b}	70.4 ± 16.1^{bc}
65	40.9 ± 6.7^{a}	55.4 ± 2.1^{b}
75	31.4 ± 2.5^{a}	50.3 ± 3.1^{b}

Note: different letters indicate samples that differ significantly from each other based on the results of comparison using the Tukey test ($P < 0.05$).

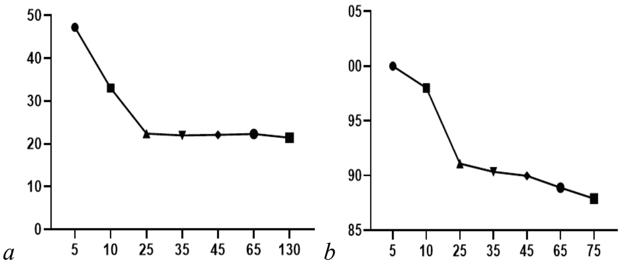

Fig. 1. Effect on normal cell HBL100 before and after fermentation: the abscissa axis is the concentration (μm), the ordinate axis is the viability (%) of HBL100 cells before fermentation (*a*) and after fermentation (*b*)

Discussion

Many plants produce substances known as cyanogenic glycosides which, when hydrolyzed, can release hydrogen cyanide. This ability, known as cyanogenesis is related to the toxicology test. Red blood cell hemolysis and concentration and exposure time are clearly correlated with the cytotoxicity of apricot seed aqueous extract. Hemolysis is minimal at lower concentrations (10 ppm), but occurs significantly at higher values (50 ppm and above), suggesting possible membrane injury. At 200 ppm, critical hemolysis is seen, indicating significant toxicity, most likely as a result of the extract's cyanogenic glycoside content. Cyanogenic glycosides, which have the ability to damage red blood cell membranes, are the cause of the extract's toxicity (Jang et al., 2018). It could be required to use protective processes to reduce these cytotoxic effects because lower amounts seem safer, as stated by Ferreira et al. (2011). The extract has medicinal potential, but higher doses pose serious health threats, thus more research into protective compounds is needed. The fermentation process performs an essential function in improving the nutritional content of particular food products while decreasing damaging anti-nutritional components. The current study investigated the pre- and post-fermentation effects of fermentation on essential nutritional and anti-nutritional components, particularly hydrocyanic acid, crude protein, crude fat, total ash, and crude fiber. The fermentation process significantly changes the nutritional and anti-nutritional profiles of various substrates, enhancing their value for consumption. Particularly, hydrocyanic acid levels decrease, indicating reduced toxicity, while crude protein content rises, indicating improved nutritional quality. The nutritional value provided by this food is enhanced through fermentation, resulting in a significant increase in crude protein between 53.6% to 70.9%, which agrees with the results obtained by Mukhtar (2022) and Zhao et al. (2024). There was a decrease in crude fat from 2.66% to 1.22%, which could be advantageous for some dietary requirements, as suggested by Rambu et al. (2024). While natural fiber demonstrated a slight reduction, indicating greater digestibility, total ash concentration demonstrated a 13% diminution, suggesting changes in mineral arrangement (Knez et al., 2023). The concentration of hydrocyanic acid fell from 7.3 to 3.3 mg/100 g, which characterizes a 54.8% decrease. Changes in the fatty acid, mineral, and amino acid profiles of apricot seeds indicate a discernible effect of fermentation on the nutritional makeup of the seeds. These changes point to a move toward higher nutritional quality, especially in terms of the amount of protein, the concentration of minerals, and the makeup of fatty acids that may influence the seeds' antioxidant qualities (Knez et al., 2023; Kang et al., 2024). A great deal of research has been done on the modification of dietary fiber from *Agrocybe cylindracea*, especially when contrasting the treatment with sodium hydroxide with other techniques such high-temperature treatment, cellulase modification, and Lactobacillus fermentation, which are important for synthesizing a new probiotic for treatment of breast cancer and has less effect on the normal cell. There are valuable differences between the impact of apricot seed extract on malignant and normal cells. Studies reveal that the extract is less cytotoxic to normal cells, like HBL100, but has a higher cytotoxic effect on breast cancer cells. Similar to findings with the grape seed extracts, which demonstrated significant proliferation suppression in MCF-7 cells, apricot seed extract exhibits concentration-dependent cytotoxicity against breast cancer cell lines (Sharma et al., 2004). The extract operates well before fermentation, which is consistent with research showing that polyphenolic chemicals can cause cancer cells to undergo apoptosis via mitochondrial pathways (Abbaspour, 2024). On the contrary, the extract shows less cytotoxicity when applied to normal cells such as HBL100, indicating a selective activity that protects healthy tissues (Agarwal et al., 2000). Because it targets malignant cells while minimizing damage to healthy cells, this selective cytotoxicity is essential for therapeutic applications (Abbaspour, 2024).

Conclusion

Fermentation substantially produced a new probiotic, which probiotic had less toxic of hydrocyanide with little change in fatty acid and amino acid after fermination. It was shown to have cytotoxic effect on cancer cells with little or less toxic effect on normal cells, which makes it a good option for additional study and possible therapeutic uses.

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