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The Role of Fermentation Process to Break Down Pesticide Residues in Milk

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Abstract: Pesticides are widely used in the control of weeds, harmful insects, and microbial pollution. Although they are useful in production and agriculture, excessive and inappropriate use causes some health problems, including cancers, neurological diseases, and endocrine problems. Therefore, recent research on how to remove the residues of these pesticides using microorganisms, including lactic acid bacteria or probiotics has been conduct. This study relied upon the fermentation process of milk to breakdown pesticides residue. The milk fermentation process depends on the activity of Lactic Acid Bateria (LAB), as these bacteria produce lactic acid. In this study, the role of this bacterium was observed in reducing the percentage of Glyphosat pesticide. This observation was made by measuring its percentage in High-performance liquid chromatography technology (HPLC). The initial concentration of glyphost in milk (a sample that had not been processed) was calculated to be 58.2 mg/ml. After 18 hours of fermentation, the concentration of Glyphost was significantly lower than it had been and had reached 14.4 mg/ml the results showed that there were differences in the mean levels of Glyphosat residues in milk after and before the fermentation process.

Keywords: Lactic Acid, Microbial Contamination, Milk Fermentation, Pesticides and Probiotic.

Research Paper

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INTRODUCTION

Milk, with its nearly perfect balance of nutrients, is often hailed as nature's healthiest food discovery. Babies, kids, seniors, and the sick all rely heavily on milk and other dairy products as part of their regular diet. There are multiple opportunities for chemical contamination throughout the milk and dairy product supply chain. The feed and other parts of the animal's diet, including the water, can all contribute to this contamination (Schopf *et al.*, 2023). Yogurt is a fermented milk drink that typically contains acidophilus bacillus, bifidobacteria, and Bulgarian bacillus (lactobacillus). Substances such as lactic acid, alcohol, carbon dioxide, antibiotics, and vitamins are formed due to the vital activity of the microflora in the yogurt. All of these compounds, when present in low enough concentrations, have beneficial effects on human health, including normalizing gastrointestinal tract activity, blocking the growth of pathogenic microflora, and boosting immunity (Fontcuberta, *et al.*, 2006).

Species of lactic acid bacteria (LAB) have been widely used in food fermentation around the world. Milk fermentation is dependent on the activity of LAB, which enables the transformation of milk into high-quality fermented milk products Multiple LAB are in common usage, and their function can be broken down into starters and non-starters, which can also include adjunct cultures. The acid production is the primary function of starting cultures in production, and they help with ripening as well. However, non-starter cultures contribute far more throughout the ripening phase than they do during the growing stage (yantyati *et al.*, 2014).

Streptococcus thermophilus and Lactobacillus bulgaricus are two of the most popular LAB cultures used in yoghurt production. Together, they synergistically produce volatile metabolites that give yoghurt its characteristic flavor. They benefited each other by increasing production of lactic acid and aromatic compounds, which was achieved by releasing amino acids and organic acids from the milk. Lactic acid is the primary contributor to the tangy, tangy flavor of yogurt, but other fragrance compounds also play a role (Routray and Mishra 2011). Herbicides include glyphosate. Broadleaf plants and grasses can be eradicated by applying it to the leaves of the plants. Some crops' maturation and development can be controlled by applying glyphosate in the form of its sodium salt. The first use of glyphosate in the United States was approved in 1974. In the United States, glyphosate is among the most popular herbicides. It is used for weed control in industrial areas as well as in agriculture, forestry, lawns, and gardens. Some glyphosate-based treatments can be used to manage aquatic vegetation (Henderson *et al.*, 2010). Numerous studies have been conducted on qualitative and quantitative aspects of the significant impact of microorganisms on pesticide breakdown (Kiruthika *et al.*, 2025). According to the findings of Bo and Zhao (2010), the growth of lactic acid bacteria in bovine milk has the potential to lower the amounts of residues caused by certain organophosphorous pesticides, which in turn may minimize the potential for danger posed by the product. According to the findings of Bo *et al.*, 2011, lactic acid starters improved the organophosphorous breakdown in varying degrees in whole milk. Pseudomonas and Serratia species that were isolated from soil were shown to be beneficial in degrading the DMT leftovers, as was discovered by Grant *et al.,* (2002).

The process of fermenting milk has traditionally relied on the action of LAB, which play an essential part in the transformation of milk from its raw material state into fermented milk products. As starter cultures in the milk fermentation business, a wide variety of LAB strains cultivated specifically for commercial purpose are utilized. The starter cultures of LAB were obtained from the sequencing activities, and they went through an isolating, selecting, and confirming phase. During the process of making fermented milk products in an industrial setting, researchers have identified and implemented a number of behaviors and features that are unique to each individual strain of LAB that is employed. LAB's capacity to acidify milk (Mäyrä-Mäkinen and M. Bigret, 2004).and their ability to provide flavor and texture by transforming milk protein as a result of their prolytic activities are two of the most important features they possess (Griffiths and Tellez 2013, Kongo 2013). Yogurt and other fermented milk products, such as cheese and yoghurt, have a flavor that is mildly acidic and has a delightful freshness to them.

The purpose of this project is to investigate whether or not the procedure of fermentation utilizing lactic acid bacteria (LAB) is effective for the detoxification of pesticides.

Materials and Methods

Milk samples (250 mL) are collect from local markets to prepare the culture we inoculated heat treated-milk (90 to 95°C for 5 min) with 3% (wt/wt).

Lactobacillus strains (Bacteria collected from food sample at scientific research comment ion in bacteria laboratory) anaerobically at 37°C for 18 h, and maintained it at 4°C until milk fermentation (within 48 h). Milk fermentation took place at 43°C for 6 h and post-ripening at 4°C for 18 h before sensory, evaluation and texture analysis. During acidification; pH decreased below 4.6. Milk-gelatin occurs at pH 5.2 to 5.4, when lactose converts to lactic acid in milk medium. The pH value of the yogurt was determined by inserting the electrode directly in to the yogurt-samples.

Preparation of Glyphosate Standards

Stock-solution (ss){1mg/ml} were prepared from Glyphosate standard in methanol Ab% and kept in freezer at -20c◦. The stock standard solution was used for up to 3month. Suitable concentration of working standards-solution (WSS) were prepared from stock solution (SS) by dilution with methanol Ab%, immediately prior to sample preparation.

Determination of Glyphosate Residues in Samples

The-separation occurred on liquid chromatoghraphy equipped with binary delivery-pump. The eluted peaks were monitored by UV-Vis 10 Avp-SPD spectrophotometer.

Extraction and Separation Procedure for Glyphosate Quantification in Samples

1. Shake 50 ml with 5ml MeoH.
2. 0.5gm sodium oxalate for 1min in 250ml separation funnel.
3. Add 20 ml diethyl ether and shake again for 1min and repeat with 25 ml petroleum either.
4. After separating the phase (centrifuging for 5 min at 1500 rpm).
5. Transfer the organic phase into another separating funnel.
6. Add 50ml (1+ v/v diethylether and petroleum. ether wash solvent over sodium sulfanhyd.
7. Evaporate by usingrotary-evaporate at 35c◦.

HPLC Conditions

Chromatographic column: NH2 (5μm) 200 mm x 4.6 mm, mobile phase: 85% water solution KH_2PO_4 with 1,5% NaOH 3M; 15% Acetonitrile, for 16 min, debit: 0,8 ml/min, Fluorescence detection Ex. 265 nm, Em. 310 nm.

RESULTS AND DISCUSSION

The HPLC method was used in the laboratory investigations of residual Glyphosat, and the results showed that there were differences in the mean levels of Glyphosat residues in milk after and before the fermentation process. The results of the Glyphosate that was still present in the milk are shown in the figure (3-1). After 18 hours of the fermentation process, the effect of indigenous lactic acid bacteria and streptococcus thermophilus milk on the degradation of Glyphost is shown in Figures (3-2). The initial concentration of glyphost in milk (a sample that had not been processed) was calculated to be 58.2 mg/ml in table 1. After 18 hours of fermentation, the concentration of Glyphost was significantly lower than it had been and had reached 14.4 mg/ml. Similar findings were reported by Navab *et al*, who reported the laboratory effects of Pseudomonas spp. isolated from soil on DDT, DDF, DDD, and HCH. The degradation of insecticides during fermentation refer to several mechanisms, non-biological and biological process. The chemical and mechanical breakdown of insecticides such as hydrolysis refer to non-biological process will biological degradation mentioned the breakdown by microorganisms. (Kiruthika *et al*., 2025; Sarlak *et al.,* 2021). Bhatt *et al*., (2021) found bacteria in soil degrade pesticides by enzyme reaction that split chemical bound by oxidation and hydrolysis. Soil bacterial extremely effective degraders of pesticides compound achieved through hydrolytic, oxidative, reductive and ring cleavage mechanism, this bacteria achieve rapid and substantial reducer with recorded effectiveness of 85-98% within 5-30 days. (Gynedra *et al*., 2025). Julia *et al.,* (2026). Found that the use of lactic acid bacteria shortened the half-life of organophosphate pesticides in food. This degradation may result from abiotic or biotic mechanisms, or a combination of both.

Aslan and Aziz studied the effect of indigenous lactic acid bacteria in vegetable mix on the degradation of diazinon and malathion after 48 h of fermentation process, The highest population of bacteria i.e. Lactobacilli, Streptococci, Leuconostoc and Pediococci, was observed in first 24 h of fermentation. After this period the cfu/mL decreased remarkably. After 24 hours of fermentation, the pH reached 4.0, then slowly decreased, reaching 3.6 after 24 hours, as Lactic acid bacteria showed a high ability to reduce the concentration of decreasing the malathion concentration from 3.5 to 0.5 mg kg-1 after 48 h of fermentation. The manufacture of yoghurt from Buffalos' and Cows' milk resulted in a decrease levels of the studies pesticides by Abou-Donia *et al.,* (2010) where the reduction levels of HCB, Lindane, Aldrin, Heptachlor expoide, Chlordane and DDT. Similar research by Zhang *et al*., (2006) mentioned that the manufacturing of the yoghurt pasteurization and sterilization including (heat treatments) and the action of the microorganisms during storage caused a significant decrease in the levels of pesticides in yoghurt. These result confirmed our current data since the Buffalos' and cows' leben stored for at least one week inside the cotton fabric bag to get rid of their water and become condensed (Ali, 2017).

Table 3-1: Result table (Uncal-F\ glyphosate 10 ppm.

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	6.82	1325.06	114.18	100.0	100.0	0.10	
	Total	1325.06	114.18	100.0	100.0		

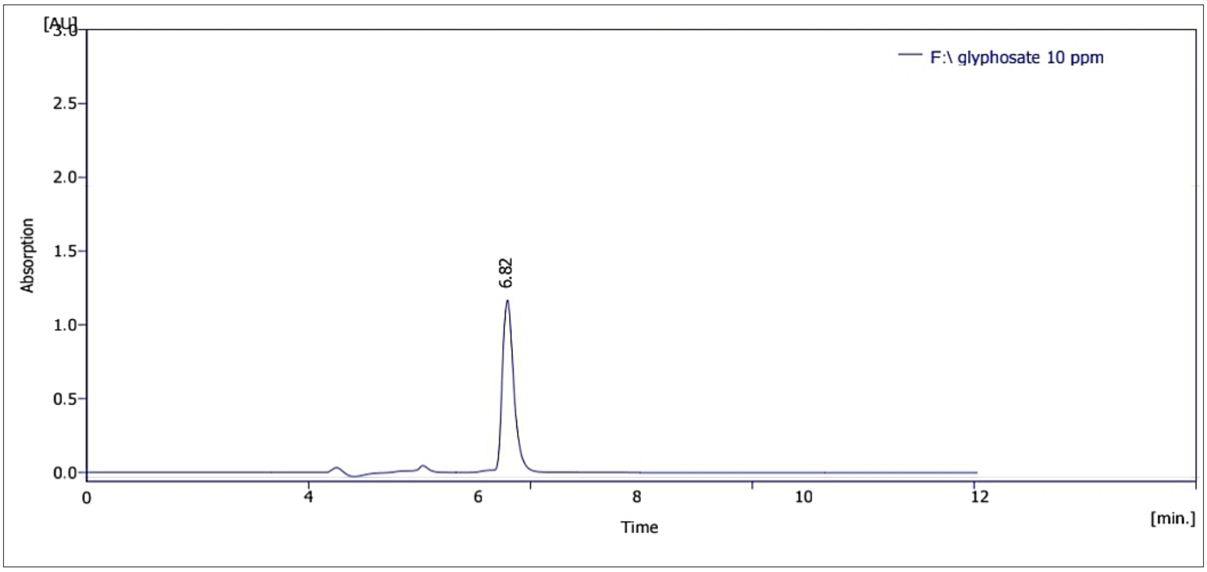

Figure 3-1: Glyphost pesticide standard curve in HPLC

Table 3-2: Result chromatography table (Uncal-F\ milk)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.77	1589.23	125.25	23.58	23.58	0.14	
2	6.98	38565.58	652.36	48.56	48.56	0.33	
3	9.11	3256.66	214.55	22.25	22.30	0.35	
	Total	43411.36	992.23	100.00	100.00		

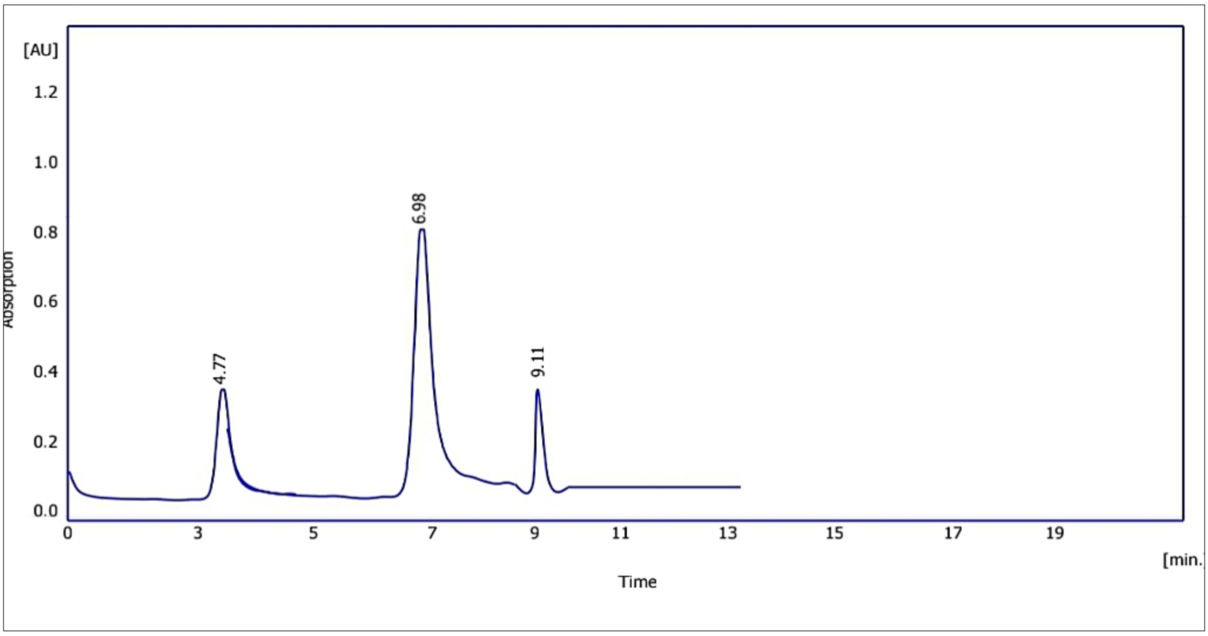

Figure 3-2: glyphost percentage in milk by HPLC

Table 3-3: Result chromatographly table (Uncal-F\ yoghurt)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.62	425.89	105.14	23.25	25.22	0.10	
2	6.80	9562.25	425.65	48.14	44.19	0.25	
3	9.02	1255.69	123.66	22.23	205	0.36	
	Total	11235.65	712.56	100.00	100.00		

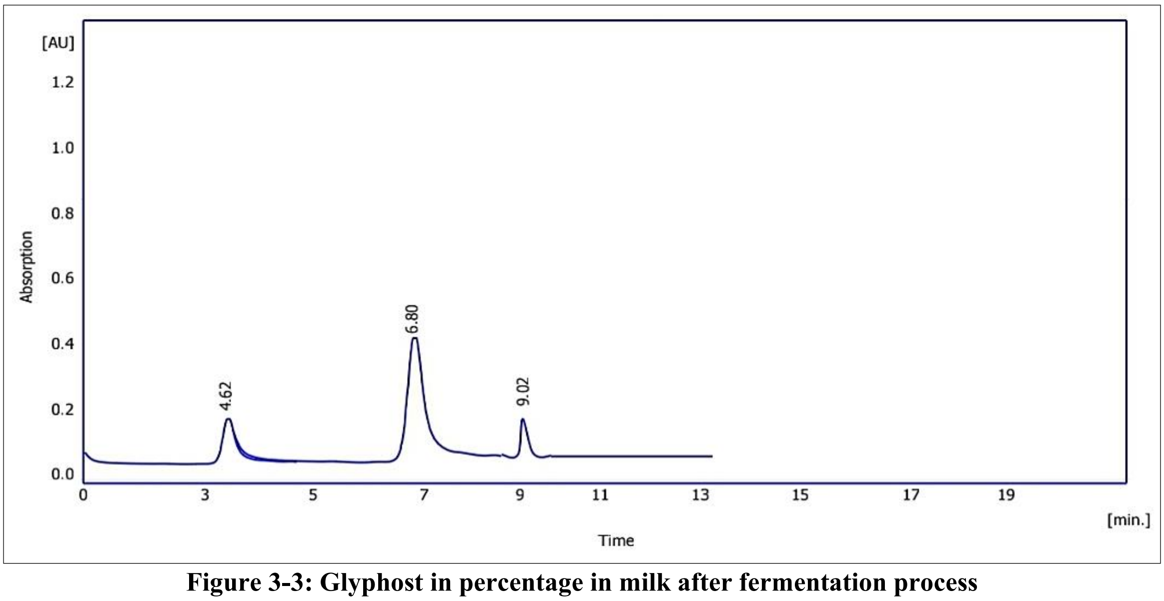

Figure 3-3: Glyphost in percentage in milk after fermentation process

Conclusion

Highlights the importance of quantifiable rate data, integrating enzymatic mechanisms, microbial ecology, and tools from metabolic biology to gain a more precise understanding of degradation processes and develop even more powerful field monitoring applications.

Microbial degradation plays a significant role in reducing pesticide persistence. It is a fundamental process in bioremediation and in the breakdown and detoxification of major pesticide classes in ecosystems. While microbial degradation shows great promise, challenges remain. The wide variety of pesticides and their metabolites, combined with varying environmental conditions, can interfere with the effectiveness of microbial treatment. In addition, the use of modified microorganisms creates concerns regarding environmental impacts and their regulatory frameworks. According to results obtain from this research. It could be concluded that LAB were capable to reduction of Glyphost pesticide residue by fermentation process.

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