

Fayoum Journal of Agricultural Research and Development ISSN:1110-7790 ONLINE ISSN:2805-2528



Development and quality assessment of probiotic *Enterococcus* durans fermented milk beverage supplemented with perilla and psyllium seeds



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ABSTRACT

Plant ingredients like cereals, nuts and seeds are used in functional dairy products to enhance nutritional value and offer health benefits. the development and quality evaluation of a new probiotic fermented milk beverage using *Enterococcus durans* MT545074.1 and enriched with 1.0% psyllium, 1.0% perilla seeds, and a mixture of 1.0% psyllium and perilla seeds (1:1) during cold storage for 14 days was evaluated. The addition of perilla or psyllium seeds to fermented milk beverage significantly increased titratable acidity, total solids, protein, fat, ash, water holding capacity, total phenolic content, antioxidant activity and viability of *Ent. durans*. Psyllium seed significantly reduced the Syneresis of the product. Higher antioxidant activity was observed in beverages enriched with seeds. The highest viability of *Ent. durans* was found in beverages containing 1.0% perilla or psyllium seeds. The final product retained probiotic survival above 10⁷ cfu/g even after storage at 5 °C for 14 days. Fermented milk beverages enriched with 1.0% psyllium seeds exhibited higher acceptance from panelists during sensory evaluation.

Keywords: Enterococcus durans, supplementation, plant seeds, probiotics, prebiotic.

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1. INTRODUCTION

Probiotic fermented milk beverages enriched with plant ingredients have been a focus of recent research, aiming to create functional and nutritious dairy products. Studies have explored the fortification of fermented milk beverages with plant ingredients like soy protein, and barley, and rice (Gupta et al., 2023). These plant ingredients not only enhance the nutritional value of the beverages but also contribute to their functional properties by providing bioactive compounds, proteins, and antioxidants, which may help prevent several diseases (Liburdi et al., 2020). The addition of plant ingredients has shown promising results in improving nutritional sensory quality, probiotic content, viability, and overall health benefits of fermented milk beverages, making them a viable option for consumers seeking probiotic fermented beverages with added health benefits (Stanislav et al., 2019).

Probiotic microorganisms are mostly of human and animal origin as it lives normally in their gastrointestinal tract and are mostly isolated from their feces. In addition, fermented foods have vielded various probiotic bacteria. Most isolated probiotics, including Lactobacillus, Enterococcus, and Bifidobacterium, interact with gut flora. Lactobacillus, a broad genus of beneficial LAB bacteria in the human microbiota, has many probiotic uses. There are 250 Lactobacillus species. The most important probiotic species, Lactobacillus acidophilus and Lactobacillus casei, are thought to benefit human health (Mafra et al., 2020).

Enterococci species are the most important lactic acid bacteria that play an important role in the fermented food industry due to their functional properties (**Fugaban** *et al.*, 2021). They live in the gastrointestinal tracts of humans and animals, plants, soil, water, and food like cheese and fermented sausages. This genus includes different species and faecium, Enterococcus enterococcus durans, and Enterococcus faecalis are the most common Enterococcus species which can be found in dairy products (Dapkevicius et al., 2021).

Perilla and psyllium seeds can be incorporated into dairy products like ice milk or yogurt due to their functional and nutritional benefits. Perilla (Perilla frutescens), aromatic plant is an extensively used for culinary and medicinal purposes in Asian countries (Chandra et al., 2021). Perilla seeds are rich in ω -3 fatty acids, dietary fiber, amino acids, vitamins, and minerals, and offer various health benefits. Psyllium seeds contain (Plantago ovata) high polysaccharide content and soluble fiber that stimulates the growth of bacteria in the digestive tract (Kaialy et al., 2014). Research shows that incorporating psyllium seed in ice milk formulas increases total solids, protein, and ash content and improves sensory quality (Elwahsh et al., 2023). Similarly. supplementing skim buffalo's milk with psyllium seeds in stirred yogurt leads to increased total solids, protein, and ash content, improved water holding capacity, and enhanced viscosity, body and texture without affecting flavor (Sakr, 2019). Furthermore, the addition of psyllium probiotic yogurt enhances seeds in titratable acidity, ash content, sensory, and storage stability.

The objective of this study was to develop a novel *Enterococcus durans* probiotic milk beverage enriched with psyllium or perilla seeds and evaluate the physicochemical, water holding capacity, Syneresis, total phenolic content, antioxidant activity, the viability of *Ent*.

durans and sensory characteristics of the 2. MATERIALS AND METHODS 2.1. Materials

Skimmed buffalo milk was obtained from our dairy pilot plant, Fac. of Agric., Fayoum Univ., Egypt. The perilla seeds were obtained from the Quiko Egypt Company and the psyllium seeds were obtained from Imtenan Company, Egypt. The probiotic Enterococcus durans MT545074.1 was used in this study which was isolated and identified by Nasr et al. (2023).

2.2. Experimental design

Briefly, four different treatments of probiotic fermented milk beverages were made from skimmed buffalo's milk. This skimmed buffalo's milk (4 L) was divided into four equal parts. The first part was left without any seeds to be served as a control and the last three parts were individually enriched with 1.0% perilla, 1.0% psyllium and 0.5% perilla plus 0.5% psyllium of skim milk, respectively. All treatments were pasteurized at 90 °C/10 min, then cooled to 40 °C and inoculated with 1.0% Ent. durans until a complete fermentation. The inoculum levels of Ent. durans were determined to give a final concentration of approximately $8-9 \log_{10} cfu/g$ in milk after inoculation and incubated at 40 °C until the final pH value reached 4.7. Following fermentation, the strawberry juice was added to each treatment at a rate of 15%. The fermented milk treatments were immediately filled into suitable plastic bottles (100 ml volume) and then the fermented milk samples were kept in the refrigerator at 5±1 °C for 14 days.

All fermented milk treatments were analyzed in triplicate on 1st day for determination of their physiochemical and at 1, 7 and 14 days during cold storage for their total phenolic content, antioxidant activity, water holding capacity, syneresis,

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product during cold storage for 14 days. viability of probiotic counts, and sensory characteristics.

2.3. Methods of analysis

2.3.1. Physicochemical analysis

The titratable acidity, fat, protein, total solids, ash and fiber contents of the samples were determined as described in **AOAC (2013).** The pH of the fermented milk beverages was measured using a digital pH meter (Heidolph, Germany).

Water-holding capacity was determined according to Ladjevardi *et al.* (2018) and syneresis was determined according to García-Pérez *et al.* (2005). The total phenolic contents and the antioxidant activity were determined spectrophotometrically at 725 nm and 517 nm, respectively, (Shori and Baba (2014).

2.3.2. The viability of *Enterococcus durans*

The probiotic strain used in this study is *Enterococcus durans* MT545074.1. This strain was isolated and identified by **Nasr et al. (2023)**. The viability of *Ent. durans* was estimated on MRS agar after incubation at 37 °C for 48 h.

2.3.3. Sensory evaluation

The milk beverages samples were organoleptically evaluated for order, taste, consistency, appearance, whey separation, and overall acceptance, using a 7-point hedonic scale at 1, 7 and 14 days during a cold storage period by fifteen untrained panelists in the Dairy Department, Faculty of Agriculture, Fayoum University, Egypt. **2.3.4. Statistical analysis**

Data were statistically analyzed with SPSS 17.0 software (2008). Two-way analysis of variance was used to study significant differences between means at P < 0.05.

3. RESULTS AND DISCUSSION 3.1. Physicochemical characteristics

The results regarding the total solids, protein, fat, ash, fiber, pH and acidity of probiotic fermented milk beverage enriched with perilla and psyllium seeds are presented and are shown in Table 1. It can be observed that the plant seeds led to a significant increase $(P \le 0.05)$ in the total solids, protein, fat, ash and fiber content. Perilla seeds are mainly rich in protein, fatty acids, dietary fiber, amino acids, and minerals, which explains the increase of all previous values.

The titratable acidity (TA) of probiotic fermented milk beverages containing the plant seeds (perilla or psyllium) was significantly higher and their pH values were significantly lower than that control sample. This might be attributed to perilla or psyllium seeds improving the growth of probiotic bacteria contained in the fermented milk beverage.

Similar results was found by Atwaa *et al.* (2022). They found that the use of psyllium seeds or husks in yoghurt formulations has been linked to increased total solids, protein, and ash content. Moreover, **Bhat** *et al.* (2018) found that 0.5 of Psyllium husk can be added to yogurt to fortify it with fiber without compromising quality or acceptability. **Islam et al. (2021)** evaluated the chemical yogurt-like product fortified with 0, 0.4, 0.8, and 1.2 % psyllium husk. The addition of psyllium husk significantly increased the crude fiber content of the product. Also, **Elwahsh** et al. (2023) investigated the use of psyllium seed husk powder as a fat replacer in ice milk formulas. Results showed that adding powder increased total solids, protein and ash content.

On the other hand, perilla seeds have substantial nutritional value. So, they may also be used in the food industry as functional dietary supplements. Zheng et al. (2022) studied the physical and chemical indices of 1%, 2%, 3%, and 4% perilla fortified yogurt stored at 4°C for 28 days. It's promoted the pH value of potato blueberry yogurt. Fermentation and Perilla improved the composition of fatty acids in vogurt and made it more nutritive value. Also, Chen et al. (2023) assessed the benefits of adding perilla seed oil to yogurt concerning nutrition and health. They indicated that adding it considerably affected fat, acidity and viability of Bifidobacterium animalis subsp. lactis.

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Parameter	Pro	- Significance			
	0.0% (control)	1.0% Psy.	1.0% Per.	1.0% Per. 0.5% Psy. +0.5% Per.	
Total solids (%)	9.61 ^d	10.84 ^c	11.21 ^a	10.97 ^b	***
Protein (%)	4.96 ^d	5.04 ^c	5.46 ^a	5.25 ^b	***
Fat (%)	0.41 ^d	0.44 ^c	0.82 ^a	0.63 ^b	***
Ash (%)	0.76^{d}	0.91°	0.98 ^a	0.95 ^b	***
Fiber (%)	0.06 ^b	0.19 ^a	0.21 ^a	0.20 ^a	***
рН	4.60 ^a	4.33 ^b	4.13 ^c	4.19 ^c	***
TA (%)	0.68 ^d	0.72 ^c	0.82 ^a	0.76 ^b	***

Table 1. The physicochemical characteristics of probiotic *Enterococcus durans* milk beverages enriched with psyllium (Psy.) and perilla (Per.) seeds:

a, b,... and d: Means having different superscripts within each row are significantly different ($p \le 0.05$). TA: titratable acidity.

Stability of probiotic beverages during refrigerated storage

The pH and titratable acidity of the beverages were determined after 1, 7, and 14 days of storage and the results were presented in Table 2. Probiotic milk beverages contain 0.0%. 1.0% psyllium, 1.0% perilla and 0.5% psyllium + 05% perilla after 1 day of storage had pH values, respectively equal to 4.60, 4.33, 4.13 and 4.19. The differences between the initial pH values of the beverages could be related to the plant seeds, which allowed a little post-acidification.

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Thereafter, after 14 days of storage pH slightly decreased and were 4.09, 4.00, 3.66 and 3.96 for 0.0%, 1.0% psyllium, 1.0% perilla and 0.5% psyllium + 0.5% perilla, respectively. The lowest pH value was 3.66 for the probiotic milk beverages supplemented with 1% perilla seeds at 14 days. Probiotic milk beverages supplemented with 1.0% perilla seeds and kept for 14 days had the highest titratable acidity (TA%) of 1.46%. These findings are consistent with (Kowaleski et al., 2020).

Table 2. Changes in pH and titratable acidity of probiotic *Enterococcus durans* milk beverages enriched with psyllium (Psy.) and perilla (Per.) seeds during storage period at 5 ± 1 °C

	Storage (d)	Probiotic milk beverages enriched with seeds at							
Parameter		0.0% (control)	1.0% Psyllium	1.0% Perilla	0.5% Psy. +0.5% Per.	Significance			
	1	4.60 ^a	4.33 ^b	4.13 ^{cde}	4.19 ^c				
pН	7	4.16 ^{cd}	4.12 ^{cde}	3.99 ^{ef}	4.04 ^{def}	***			
	14	4.09 ^{cdef}	4.00 ^{ef}	3.66 ^g	3.96 ^f				
	1	0.68 ⁱ	0.72^{i}	0.82^{g}	$0.76^{\rm h}$				
TA (%)	7	0.85 ^g	0.91 ^f	1.13 ^d	1.03 ^e	***			
	14	1.01 ^e	1.21°	1.46 ^a	1.33 ^b				

a, b,... and i: Means having different superscripts within each parameter are significantly different ($p \le 0.05$). TA: titratable acidity

3.3. Water-holding capacity (WHC) and Syneresis

Table 3 shows the effect of psyllium or perilla seeds on water holding capacity and syneresis of the samples during the storage The data indicated that the period. probiotic milk beverages without any seeds added had lower WHC and higher syneresis compared to beverages that contained seeds, especially psyllium seeds, throughout the storage period. The samples containing plant seeds exhibited lower syneresis (%) values in comparison to the control samples without seeds. The probiotic milk beverage enriched with 1% psyllium seeds had the lowest syneresis percentage, followed by the beverage with 0.5% psyllium and 0.5% perilla, the beverage with 1% perilla seeds, and lastly the control beverage.

These results were similar to those of **Basiri** *et al.* (2018) who reported that stabilizer supplements such as plant seeds and guar can benefit syneresis in fermented milk, whose neutral hydrocolloids reduce syneresis.

Psyllium is a naturally occurring material that has a high-water absorption capacity and provides a similar effect to xanthan gum when mixed with starchy items. Psyllium gum is a hydrocolloid that is present in the outer covering of seeds. Psyllium husk (the outer cover of seeds) is primarily comprised of fiber, specifically arabinoxylans (**Belorio and Gómez, 2021; Jimenez-Gomez and Beaulieu, 2022**).

The increases in WHC and decreases in syneresis were seen in probiotic milk beverages supplemented with psyllium then followed by perilla seeds, which were - 89 -

caused by increasing protein and fiber content and led to a decrease in water separation in probiotic milk beverages (Marand *et al.*, 2020). These results were in line with those of Sakr (2019), who found that psyllium seed content almost significantly improved its water-holding capacity and viscosity compared to control. Based on the findings obtained from this study, the plant seeds supplementation to milk beverages improved the water holding capacity thereby decreasing syneresis. The high fiber and protein content of seeds might be related to these results. Fermented milk's high protein content may promote syneresis because of its strong binding strength (**Kycia** *et al.*, **2018**)

Table 3. Changes in water holding capacity (WHC) and syneresis of *Enterococcus durans* milk beverages enriched with psyllium (Psy.) and perilla (Per.) seeds during storage period at 5 ± 1 °C

Parameter	Storage (d)	Probioti	Significance			
		0.0% (control)	1.0% Psy.	1.0% Per.	0.5%Psy.+ 0.5% Per.	-Significance
WHC (%)	1	37.18 ^j	59.38°	44.74 ^g	51.21 ^f	
	7	36.83 ^j	61.12 ^b	43.46 ^h	53.44 ^e	***
	14	34.71 ^k	63.81 ^a	42.41 ⁱ	54.31 ^d	
Syneresis (%)	1	46.00 ^b	28.00^{fg}	37.00 ^c	30.27 ^e	
	7	47.00a ^b	25.27 ^h	35.93 ^{cd}	29.00 ^{ef}	***
	14	47.53 ^a	24.20 ^h	34.73 ^d	26.93 ^g	

a, b,.... and k: Means having different superscripts within each parameter are significantly different ($p \le 0.05$).

3.4. Total phenolic content and antioxidant activity

The results are presented in Table 4 showed that the highest content of TPC was determined in the samples enriched with perilla or psyllium seeds, while the lowest content was observed in the control sample. A significant increase in the TPC content during storage was observed in the case of perilla or psyllium milk beverages. The probiotic milk beverage containing 1% psyllium seeds had the highest TPC (32.94 mg GAE/100 g) after 14 days of storage. The difference in TPC between this beverage and others was not statistically significant ($P \le 0.05$). Psyllium seeds are a rich source of phenolic compounds, flavonoids, and sulfur-containing amino acids (Shah et al., 2020).

The same trend was observed, as shown in Table 4, the antioxidant activity as measured by the DPPH (2,2-diphenyl-1picrylhydrazyl) radical scavenging activity method was significantly higher ($P \le 0.05$) in seeds-added samples than in the control. This might be attributed to the higher phenolic content in seeds-added samples than in the control. During storage, the antioxidant activity of the samples presented the same pattern as observed for the total phenolic content, which increased as the time of storage increased. The results indicate that the drinks containing perilla seeds had a greater level of antioxidant activity, both when fresh and after being stored for 14 days (63.16% and 71.93%, respectively).

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Table 4. Changes in total phenolic content and antioxidant activity of *Enterococcus durans* milk beverages enriched with psyllium (Psy.) and perilla (Per.) seeds during storage period at 5 ± 1 °C

	Storage (d)	Probio	- CI - 101			
Parameter		0.0% (control)	1.0% Psy.	1.0% Per.	0.5% Psy. + 0.5% Per	- Significance
Total showska contout	1	28.16 ^g	29.72 ^e	31.56 ^b	30.65 ^{cd}	
Total phenolic content (mg GAE/100 g)	7	29.23^{f}	30.33 ^d	32.69 ^a	30.90 ^c	***
(ing GAL/100 g)	14	29.44 ^{ef}	30.38 ^d	32.94 ^a	31.59 ^b	
	1	42.46 ^h	57.11 ^f	63.16 ^{cd}	59.86 ^e	
Antioxidant activity (%)	7	44.27 ^h	60.88 ^{de}	67.40 ^b	63.88 ^c	***
	14	47.56 ^g	64.96 ^c	71.93ª	67.74 ^b	

a, b,... and g: Means having different superscripts within each parameter are significantly different ($p \le 0.05$).

3.5. The viability of *Enterococcus durans*

Table 5 presents the effect of adding plant seeds (perilla or psyllium) on the viability of Enterococcus durans in milk beverages during 14 days of storage. The results showed that there was an increase in the log count of Enterococcus durans in treatments enriched with perilla or psyllium seeds reaching the highest count after 14 days of storage. Results show that supplementation with plant seeds significantly $(P \le 0.05)$ improves the viability of the probiotic starter Ent. durans. The probiotic milk beverages supplemented with 1% perilla seeds that were stored for 14 days recorded the significantly highest viability (255.80×10^9) cfu/g).

The probiotic milk beverages supplemented with 1.0% perilla seeds that were stored for 7 days recorded the significantly highest viability (255.80×10⁹ cfu/g). While the lowest viability numbers were recorded for all trials when fresh. Whereas, the lowest viability numbers (0.04, 0.27 and 14.66 ×10⁹ cfu/g) were recorded for probiotic milk beverages without any seeds added and stored for 1,7 and 14 days.

The findings of Arabshahi and Sedaghati (2022) indicate psyllium seeds are high in dietary fiber, a prebiotic substance that can increase the survival of probiotic bacteria, and indigestible oligosaccharides. Additionally, considerable of number insoluble polysaccharides, or parts of the cell wall, such as cellulose and hemicellulose, are present in perilla seeds (Hwang and Yoon, 2020).

Shree *et al.* (2017) reported that the probiotic buttermilk can be produced using psyllium husk with probiotic *Lactobacillus acidophil*us and *Lactobacillus bulgaricus*. This product serves as a functional food and is suitable for commercial use. Furthermore, (Zheng *et al.*, 2022) found that a concentration of 1.0% perilla oil is optimal for promoting the viability of yogurt bacteria. Perilla oil enhances the viable count of yogurt, hence enhancing the probiotic effect of yogurt. The results indicated that the addition of the perilla and psyllium in beverages might improve the viability of bacteria.

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Table 5. The viability of *Enterococcus durans* in probiotic fermented milk beverage enriched with psyllium (Psy.) and perilla (Per.) seeds during the storage period at $5\pm1^{\circ}$ C

Parameter	Store as (d)	Probiot	Ciquificance			
	Storage (d)	0.0% (control)	1.0% Psy.	1.0% Per.	0.5%Psy. + 0.5% Per.	- Significance
Viability of	1	0.04 ^h	0.94 ^h	1.64 ^h	1.22 ^h	
bacteria	7	0.27^{h}	50.90^{f}	255.80 ^a	121.10 ^d	***
(×10 ⁹ cfu/ml)	14	14.66 ^g	95.33°	249.00 ^b	154.33°	

a, b,... and h: Means having different superscripts within the parameter is significantly different ($p \le 0.05$)

3.6. Sensory evaluation

The scores of sensory attributes of the probiotic fermented milk beverages enriched perilla or psyllium seeds during the storage period are shown in Fig. 1. Generally, all sensory attributes were decreased with increasing storage period. The samples with 1.0% perilla had the lowest scores in terms of appearance, odor, taste, and overall acceptability, while they had the highest score of consistency.

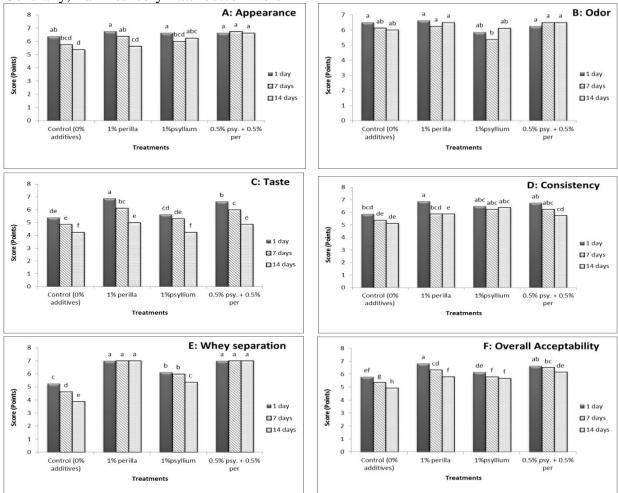


Fig. 1. Scores of sensory properties of probiotic *Enterococcus durans* milk beverages enriched with psyllium and perilla seeds during the storage period at 5 ± 1 °C. A: appearance, B: odor, C: taste, D: consistency, E: whey separation and F: overall score.

Among the probiotic fermented milk beverages, those containing perilla were found to be less desirable and they had a distinctive taste. The perillacontaining fermented milk was not preferred by panelists because it had a plant odor, while psyllium-containing beverages were more appreciated because of their taste, odor and whey separation.

It was generally found that probiotic fermented milk beverages enriched with psyllium seeds had desirable consistency, odor and taste; however, beverages with the seeds additives showed better acceptability because of their taste. It can be concluded that fermented milk beverages with psyllium seeds were preferred

4. CONCLUSIONS

The addition of psyllium or perilla probiotic fermented milk to seeds beverages led to improved physiochemical properties, total phenolic content, antioxidant activity, water holding capacity, Syneresis, the viability of Enterococcus durans, and also positively affected the sensory characteristics of the beverage. Overall. milk this study highlights the potential for the development of milk beverage fermented with Ent. durans as a new probiotic strain enriched with 1.0% psyllium seeds to improve the functional and physiochemical properties of the product.

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