


## Article

# The Ability of *Lacticaseibacillus paracasei* MSMC 36-9 Strain with Probiotic Potential to Ferment Coconut Milk and Produce a Yogurt-Type Beverage

Porntipha Vitheejongjaroen <sup>1</sup>, Pooyanee Phettakhu <sup>2</sup>, Wannicha Arsayot <sup>2</sup>, Malai Taweechotipatr <sup>1,3</sup> and Ulisa Pachekreapol <sup>1,2,\*</sup> 

<sup>1</sup> Center of Excellence in Probiotics, Strategic Wisdom and Research Institute, Srinakharinwirot University, Bangkok 10110, Thailand; porntipa.noei@gmail.com (P.V.); malai@g.swu.ac.th (M.T.)

<sup>2</sup> Division of Food Science and Nutrition, Faculty of Agricultural Product Innovation and Technology, Srinakharinwirot University, Nakhon Nayok 26120, Thailand

<sup>3</sup> Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Bangkok 10110, Thailand

\* Correspondence: ulisa@g.swu.ac.th

**Abstract:** The efficacy of the *Lacticaseibacillus paracasei* MSMC 36-9 strain with probiotic potential to ferment coconut milk and produce coconut milk yogurt-type beverages was examined. Tapioca starch was used as a stabilizer at concentrations of 0, 1.0, and 2.0% (*w/w*). The samples were stored at 4 °C for 21 days and analyzed for viability and resistance to in vitro gastrointestinal conditions of *L. paracasei* MSMC 36-9, pH changes, radical scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and apparent viscosity. The viability of the strain with probiotic potential in the samples remained stable during storage and ranged between 12 and 13 log CFU/g by the end of the storage period. The strain *L. paracasei* MSMC 36-9 from all samples survived under simulated gastrointestinal conditions. The pH levels of all samples decreased during storage due to post-acidification. The radical scavenging activity of the products fermented with *L. paracasei* MSMC 36-9 was higher than that of the sample fermented with the commercial yogurt starter culture. The addition of tapioca starch to fermented coconut milk increased the viscosity of the samples. The results suggested that *L. paracasei* MSMC 36-9 can be used as a starter culture in the production of coconut milk yogurt-type beverages with antioxidant potential.

**Keywords:** coconut; dairy alternative; probiotic; yogurt-type beverage; sustainable food production



**Citation:** Vitheejongjaroen, P.; Phettakhu, P.; Arsayot, W.; Taweechotipatr, M.; Pachekreapol, U. The Ability of *Lacticaseibacillus paracasei* MSMC 36-9 Strain with Probiotic Potential to Ferment Coconut Milk and Produce a Yogurt-Type Beverage. *Beverages* **2024**, *10*, 30. <https://doi.org/10.3390/beverages10020030>

Academic Editor: Senaka Ranadheera

Received: 12 March 2024

Revised: 12 April 2024

Accepted: 16 April 2024

Published: 18 April 2024



**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Dairy production has been identified as a substantial contributor to greenhouse gas emissions [1]. However, milk and dairy products remain widely consumed foods, particularly in developed countries, and the global demand for milk is predicted to increase by 35% by 2030 [2]. Thus, interest in plant-based milk has increased to provide more sustainable production with health benefits and reduced environmental impact. Plant-based milk alternatives prepared from many sources, including soy, almond, oat, rice, coconut, wheat, maize, sorghum, and quinoa [1], have become increasingly popular in the United States, Europe, Australia, and New Zealand [3]. This shift has also been driven in part by the prevalence of cow's milk protein allergies and lactose intolerance. Cow's milk protein allergy is one of the most frequent allergies in infants and children, with research showing that the allergy is often outgrown [4]. Lactose intolerance is caused by a decrease in the activity of intestinal  $\beta$ -galactosidase in the gastrointestinal tract, potentially leading to diarrhea, abdominal pain, bloating, flatulence, or nausea [5]. This condition affects 65% of the global population and between 70 and 100% of the East Asian population [6]. People often choose to substitute cow milk with plant-based options for health benefits and avoid consumption of milk fats, which are rich in saturated fatty acids, while plant-based sources are rich in dietary fiber, vitamins, minerals, and antioxidants.

Coconut (*Cocos nucifera*) is an important economic plant, particularly in tropical and subtropical regions. Coconut flesh is nutritious, containing 31–35% fat and 3.5–4.0% protein, essential amino acids, calcium, phosphorus, potassium, vitamin C, E, and B6 [7]. Coconut oil is known for its high concentration of medium-chain fatty acids, which are more easily digested than long-chain fatty acids found in other plant-based milk alternatives [8]. Clinical studies have proven that coconut oil exhibits preventive effects against hyperlipidemia, fatty liver disease, and diabetes [9]. Unlike other plant-based sources such as soy, oat, almond, and barley, allergies to coconut are rare [10].

Yogurt, a popular dairy product consumed worldwide, is fermented by lactic acid bacteria starter cultures, typically containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Probiotics are added to yogurts to provide therapeutic properties. Probiotics are defined as beneficial microorganisms that provide health benefits for the host when consumed in adequate quantities [11]. Probiotics are known to have inhibitory activities against pathogenic microorganisms, anti-carcinogenic, anti-tumor, and cholesterol-lowering characteristics [12].

The application of plant-based milk alternatives for the manufacturing of probiotic yogurt-like products has been studied. A blend of yogurt starter culture and *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Lacticaseibacillus paracasei* improved angiotensin-converting enzyme inhibitory activity in fermented soy-based products [13]. A study of probiotic oat-based yogurt showed that after 3-week refrigerated storage, *Lacticaseibacillus casei* and *Bifidobacterium* remained at therapeutic levels of  $\sim 10^6$  CFU/g [14]. However, scant information is available on probiotic coconut milk-based fermented products, especially the stability of probiotics during gastrointestinal passage. In our previous study, the potential of using coconut milk to produce yogurt-type beverages fermented with commercial yogurt starter cultures was explored, with tapioca starch incorporated into the products to obtain the yogurt-like viscous texture [15]. For a techno-functional purpose, the incorporation of stabilizers is necessary for the development of coconut milk yogurt-type beverages because coconut milk contains lower protein levels, which are necessary for the development of viscous texture. Tapioca starch is a widely utilized ingredient in many food products due to its high viscosity and clear appearance, while its low production cost makes it a highly cost-effective choice for food manufacturers [16]. Building upon our findings, we aimed to further investigate the ability of the *Lacticaseibacillus paracasei* strain with probiotic potential as a starter culture for the production of coconut milk yogurt-type beverages with antioxidant activity.

In the present study, *Lacticaseibacillus paracasei* MSMC 36-9 was isolated from healthy Thai human infant feces. In our preliminary study, *L. paracasei* MSMC 36-9 was screened for potential probiotic properties. The strain exhibited resistance to low acid and high bile, with high levels of hydrophobicity and non-hemolytic properties. In addition, *L. paracasei* MSMC 36-9 showed strong radical scavenging activity performed by the DPPH radical scavenging assay. The effect of tapioca starch as a stabilizer was also studied. Coconut milk yogurt-type beverages were manufactured by adding tapioca starch at 1.0 and 2.0% (*w/w*) for texture development, while the sample made without tapioca starch was used as the control. The viability and resistance to simulated gastrointestinal conditions of *L. paracasei* MSMC 36-9, the radical scavenging activity of the products, and their physical and rheological properties were analyzed during storage at 4 °C.

## 2. Materials and Methods

### 2.1. Culture Preparation

The strain, *L. paracasei* MSMC 36-9, was isolated from healthy infant feces and kept at  $-80$  °C (ethical approval: SWUEC 37/2551). A fresh culture was obtained by sub-culturing the frozen stock three times in de Man, Rogosa, Sharpe (MRS) broth (HiMedia Lab., Maharashtra, India) at 37 °C for 24–48 h under anaerobic conditions in an anaerobic jar with gas pack (AnaeroPack-Anaerobe, Mitsubishi, Japan). The number of bacterial cells was adjusted to 9.0 log CFU/mL for an OD600 of 1.0. The resultant culture was centrifuged

at  $4000 \times g$  for 5 min and washed with phosphate-buffered saline. The cell suspension was then centrifuged at  $4000 \times g$  for 5 min, and the cell pellets were resuspended in UHT coconut milk for use in the preparation of coconut milk yogurt-type beverages [17]. From the preliminary study, *L. paracasei* MSMC 36-9 exhibited probiotic potential, including resistance to low acid and high bile, having a high level of hydrophobicity, being non-hemolytic, and showing strong radical scavenging activity by DPPH radical scavenging assay.

## 2.2. Preparation of Coconut Milk Yogurt-Type Beverage

UHT coconut milk was supplemented with tapioca starch at concentrations of 1.0 (Pro-1) or 2.0% *w/w* (Pro-2), and the sample without the addition of tapioca starch was used as the control (Pro-0). At least three replications for each sample were performed. Sucrose (5% *w/w*) was added. The samples were heated to 90 °C for 3 min before cooling to 43 °C. The strain *L. paracasei* MSMC 36-9 was then added, and the samples were incubated at 43 °C overnight. One sample without the addition of tapioca starch (Yo-0) was fermented with a commercial yogurt starter culture, YF-L812, containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Chr. Hansen, Hørsholm, Denmark) to determine the effects of different bacterial cultures. Each inoculation was sufficient to attain the initial count of  $10^9$  CFU/g. After fermentation, the coconut milk yogurt-type beverages were kept at 4 °C for 21 days for subsequent analyses.

## 2.3. pH Changes during Storage

Changes in the pH levels of coconut milk yogurt-type beverages were evaluated on days 1, 7, 14, and 21 using a pH meter model Eutech pH 700 (Eutech Instrument, Vernon Hills, IL, USA).

## 2.4. Viability of the Strain with Probiotic Potential in Coconut Milk Yogurt-Type Beverages during Storage

The viability of *L. paracasei* MSMC 36-9 was determined on MRS agar. One gram of the samples was mixed with 9 mL of phosphate-buffered saline. The serial dilutions were prepared up to  $10^{-12}$ , and 0.1 mL of the dilutions were then spread on MRS agar and incubated at 37 °C for 24–48 h under anaerobic conditions in an anaerobic jar with gas pack (AnaeroPack-Anaerobe, Mitsubishi, Japan) [17]. Plates containing between 30 and 300 colonies were enumerated. The results were reported as log colony-forming units/g (log CFU/g).

## 2.5. Resistance of the Strain with Probiotic Potential to Simulated Gastrointestinal Conditions during Storage

Resistance of *L. paracasei* MSMC 36-9 to gastrointestinal conditions was determined using in vitro gastrointestinal digestion simulating sequential exposure to gastric and pancreatic juice as described by Mesquita et al. [18] with slight modifications. Ten grams of the products were mixed with 90 mL of 0.2% (*w/v*) NaCl, and pepsin (0.35% *w/v*) was added. The pH was adjusted to 3.0 using 1 M HCl. The digestion was carried out for 3 h at 37 °C. The products after exposure to gastric juice were added with trypsin (0.1%), bile salts (1.8%), NaCl, and  $\text{NaHCO}_3$ . The pH was adjusted to 8.0. The digestion was performed for 3 h at 37 °C. After gastrointestinal digestion, the number of viable cells was determined using MRS agar plates incubated at 37 °C for 24–48 h. The % survival was calculated according to Equation (1) by comparing the number of viable cells after in vitro digestion to the undigested sample as determined in Section 2.4.

$$\% \text{ Survival} = \frac{\text{Viable cells after digestion}}{\text{Viable cells of undigested sample}} \quad (1)$$

## 2.6. Radical Scavenging Activity of Coconut Milk Yogurt-Type Beverage

To investigate the antioxidant activity of *L. paracasei* MSMC 36-9 grown in coconut milk, the DPPH radical scavenging assay was performed on the samples after 21 days

of storage following the method described by Shori et al. [19] with slight modifications. Ten grams of the samples were mixed with 2.5 mL of distilled water. The pH value was adjusted to 4.0 by the addition of 0.1 M HCl, and the sample was incubated in a water bath at 45 °C for 10 min. The mixture was then centrifuged at  $5000 \times g$  at 4 °C for 10 min, and the supernatant was collected and used for analysis. DPPH reagent was prepared by dissolving DPPH in 95% ethanol. The supernatant was mixed with DPPH reagent and incubated for 30 min in the dark at ambient temperature. Absorbance was measured at 517 nm against the control (ethanol) using a spectrophotometer. DPPH radical scavenging activity was calculated as shown in Equation (2). The sample prepared using a commercial yogurt starter (Yo-0) was used to compare the effect of different starter cultures on the antioxidant activity of the products.

$$\% \text{ Radical scavenging activity} = \left( \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \times 100 \quad (2)$$

### 2.7. Viscosity

Apparent viscosity was determined using a controlled stress rheometer (HAAKE Mars 40, Thermo Fisher Scientific, Karlsruhe, Germany) equipped with a plate and cone geometry (35 mm diameter, 1 mm gap). The pre-shear step with a shear rate of  $500 \text{ s}^{-1}$  was first applied to the samples for 30 s. The samples were then left for structural recovery for 300 s. The apparent viscosity was measured at the shear rate, which logarithmically increased from 0.01 to  $100 \text{ s}^{-1}$  [20]. All experiments were performed at 25 °C.

### 2.8. Data Analysis

Data analysis was conducted using SPSS 16.0 (Version 16.0, IBM®SPSS® Statistics, Chicago, IL, USA). A one-way analysis of variance (ANOVA) was used to compare the mean values of different samples. The differences between means were analyzed using Tukey's honestly significant difference (HSD) at the  $p < 0.05$  level. All experiments were performed at least in triplicate.

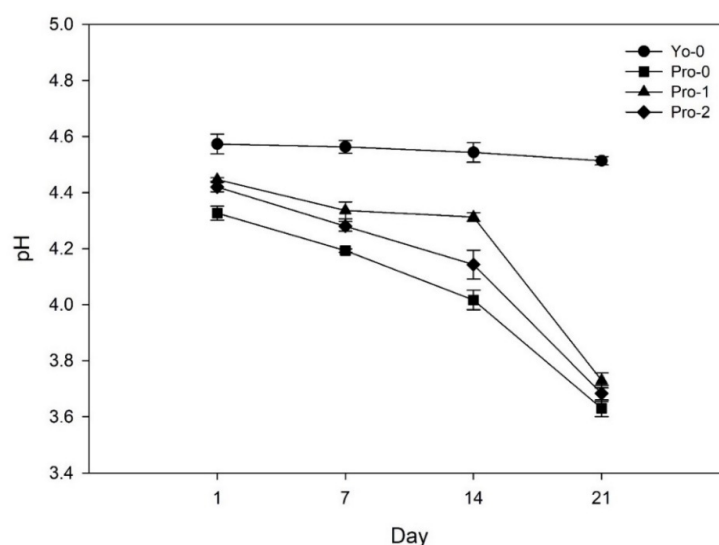
## 3. Results and Discussion

### 3.1. pH Changes during Storage

A decrease in pH during storage at 4 °C was observed in all samples, as shown in Figure 1. Other studies on fermented dairy alternatives also showed a decrease in pH levels during storage [15,21]. The samples fermented with *L. paracasei* MSMC 36-9, Pro-0, Pro-1, and Pro-2, showed significantly lower pH levels compared to the sample made with a regular yogurt starter culture, Yo-0, for the entire storage period. The lower pH values were possibly attributed to the production of organic acids by *L. paracasei* MSMC 36-9 presenting in the samples. According to Lourens-Hattingh and Viljoen [22], at lower pH, the growth rate of *S. thermophilus* declined and was inhibited at pH around 4.4, whereas lactobacilli still grew at pH values ranging from 3.5 to 3.8. Therefore, *S. thermophilus* in the sample Yo-0 might be inhibited and produce lactic acid to a lesser extent during storage.

Coconut milk contains 3.3 g of sugar in 100 g, of which 2.3 g are sucrose [8], together with added sucrose during manufacturing. Thus, sucrose is the main sugar in coconut milk yogurt-type beverages. The reduction in pH may indicate that the strain with probiotic potential used in the present study is able to metabolize sucrose as described by Huang et al. [23]. The authors found that probiotics with the presence of sucrose utilization genes acidified almond milk-based alternatives more efficiently compared to dairy yogurt starter cultures because the main sugar in almond milk was sucrose. However, additional study is required to confirm whether *L. paracasei* MSMC 36-9 possesses sucrose utilization genes. The effect of tapioca starch addition on the pH levels of the samples was not observed at the end of the storage period. Amaya-Llano et al. [24] also found no difference in pH values during storage between yogurts made with and without the addition of starch. However, Altemimi [25] found that the addition of 1.0% potato starch resulted in

higher pH levels compared to the control sample. The author suggested that reduced water availability when starch was added limited bacteria growth and lactic acid production.



**Figure 1.** pH values of coconut milk yogurt-type beverages fermented with a commercial yogurt starter culture (Yo-0), *L. paracasei* MSMC 36-9, without the addition of tapioca starch (Pro-0), with the addition of 1.0% tapioca starch (Pro-1), and with the addition of 2.0% tapioca starch (Pro-2) during storage at 4 °C. Points represent the mean, and error bars represent the standard deviation of three replicates.

It should be noted that the pH levels of fermented coconut milk in the present study were lower than those reported for fermented dairy products in other studies. Wang et al. [26] recorded pH values between 4.25 and 4.35 in yogurts made from cow milk after 21 days of storage, while pH levels of 3.79 to 3.91 were observed in fermented soy milk. The high pH buffering capacity of milk was responsible for the higher final pH. Proteins and small constituents (inorganic phosphate and organic acids) are the main factors contributing to the high pH buffering capacity of milk [26].

### 3.2. Viability of the Strain with Probiotic Potential in Coconut Milk Yogurt-Type Beverage during Storage

The viability of *L. paracasei* MSMC 36-9 in coconut milk yogurt-type beverages during storage is shown in Table 1. The viability of the commercial yogurt culture in the sample Yo-0 decreased during storage, while the number of *L. paracasei* MSMC 36-9 did not significantly change. The acidic nature of the samples potentially inhibited bacterial growth during storage. However, *L. paracasei* MSMC 36-9 still survived even in the acidic environment in this study. These results may be attributed to the production of essential growth factors in the form of peptides and amino acids by the strong proteolytic activity of lactobacilli [21].

The number of *L. paracasei* MSMC 36-9 in samples Pro-0, Pro-1, and Pro-2 was significantly higher than commercial cultures found in the Yo-0 sample. These findings indicated that *L. paracasei* MSMC 36-9 was possibly more suitable for use in the fermentation of coconut milk that contained sucrose as the main sugar. Our results corresponded to the changes in pH of the samples, with lower pH levels found in samples fermented with the strain with probiotic potential. However, further study may be required to confirm the viability of *L. paracasei* MSMC 36-9 after 21 days of storage.

The number of *L. paracasei* MSMC 36-9 of approximately 10–13 log CFU/g found in Pro-0, Pro-1, and Pro-2 is in agreement with the study conducted by Szparaga et al. [27]. They recorded an increase in the number of the probiotic strain, *Lactobacillus casei* subsp. *rhamnosus*, in fermented coconut milk from 11 log CFU/mL (determined immediately after inoculation of probiotics at 10 log CFU/mL) to 13 log CFU/mL after a 6-hour fermentation.

**Table 1.** Viability of *L. paracasei* MSMC 36-9 (log CFU/g) in coconut milk yogurt-type beverages without addition of tapioca starch (Pro-0), with addition of 1.0% tapioca starch (Pro-1), and with addition of 2.0% tapioca starch (Pro-2) compared to the sample fermented with commercial yogurt starter cultures (Yo-0) during storage at 4 °C. Data are presented as the mean  $\pm$  SD of three replicates.

Sample	Viable Counts (log CFU/g)			
	Day 1	Day 7	Day 14	Day 21
Yo-0	9.20 $\pm$ 0.16 <sup>cA</sup>	8.71 $\pm$ 0.05 <sup>bA</sup>	8.00 $\pm$ 0.07 <sup>bB</sup>	7.78 $\pm$ 0.11 <sup>bB</sup>
Pro-0	10.42 $\pm$ 0.11 <sup>bA</sup>	11.17 $\pm$ 0.08 <sup>aA</sup>	9.94 $\pm$ 2.35 <sup>aA</sup>	12.39 $\pm$ 3.90 <sup>aA</sup>
Pro-1	13.35 $\pm$ 0.32 <sup>aAB</sup>	13.25 $\pm$ 0.12 <sup>aAB</sup>	12.32 $\pm$ 0.23 <sup>aB</sup>	13.56 $\pm$ 0.35 <sup>aA</sup>
Pro-2	13.25 $\pm$ 0.44 <sup>aA</sup>	11.92 $\pm$ 0.93 <sup>aA</sup>	12.25 $\pm$ 0.58 <sup>aA</sup>	13.83 $\pm$ 0.04 <sup>aA</sup>

<sup>a-c</sup> Different superscripts in the same column indicate a significant difference ( $p < 0.05$ ). <sup>A,B</sup> Different superscripts in the same row indicate a significant difference ( $p < 0.05$ ).

The incorporation of tapioca starch at 1.0 and 2.0% ( $w/w$ ) did not affect the viability of bacteria. In probiotic food products, the probiotic concentration is a key factor, and probiotic food products should include at least 6 log CFU/g of probiotics, and 8 to 9 log CFU/g of probiotic microorganisms is the recommended dosage for daily consumption [28]. Thus, our coconut milk yogurt-type beverages fermented with potential probiotic strains are in agreement with the recommended dose.

### 3.3. Resistance of the Strain with Probiotic Potential to Simulated Gastrointestinal Digestion

The ability of probiotics to survive in the low pH condition of the stomach and in bile salts released in the small intestine is one of the key selection criteria for probiotic usage in food products. Studies of simulated gastrointestinal digestion under the typical conditions of the human digestive system, such as pH and bile salts, allow evaluation of the influence of these conditions on bacterial viability [29]. The number of bacterial cells that survived after sequential exposure to simulated gastrointestinal conditions and the survival rate are shown in Table 2. After sequential exposure to gastric and pancreatic juice, the commercial yogurt starter cultures (Yo-0) survived the simulated gastrointestinal condition only on day 1, while *L. paracasei* MSMC 36-9 in all samples survived the gastrointestinal conditions throughout the refrigerated storage. Wang et al. [26] reported a similar trend of *L. casei* reduction in fermented cow and soy milk after simulated digestion, with no significant changes in cell viability during refrigerated storage. According to the authors, changes in cell viability were not observed during storage, but a decrease in cell resistance to gastric acidity was detected.

**Table 2.** Number of *L. paracasei* MSMC 36-9 (log CFU/g) in coconut milk yogurt-type beverages without addition of tapioca starch (Pro-0), with the addition of 1.0% tapioca starch (Pro-1), and with the addition of 2.0% tapioca starch (Pro-2) compared to the sample fermented with a commercial yogurt starter culture (Yo-0) after sequential exposure to simulated gastrointestinal digestion and survival (%). Data are presented as the mean  $\pm$  SD of three replicates.

Sample	Day 1		Day 7		Day 14		Day 21	
	Viable Cells (log CFU/g)	Survival (%)	Viable Cells (log CFU/g)	Survival (%)	Viable Cells (log CFU/g)	Survival (%)	Viable Cells (log CFU/g)	Survival (%)
Yo-0	6.53 $\pm$ 0.10 <sup>b</sup>	71	ND	-	ND	-	ND	-
Pro-0	8.08 $\pm$ 0.03 <sup>a</sup>	78 <sup>A</sup>	8.38 $\pm$ 1.42 <sup>a</sup>	75 <sup>A</sup>	6.68 $\pm$ 0.05 <sup>a</sup>	67 <sup>A</sup>	6.77 $\pm$ 0.11 <sup>b</sup>	55 <sup>B</sup>
Pro-1	7.34 $\pm$ 0.69 <sup>a</sup>	58 <sup>B</sup>	8.70 $\pm$ 1.15 <sup>a</sup>	66 <sup>A</sup>	6.85 $\pm$ 0.01 <sup>a</sup>	56 <sup>B</sup>	7.99 $\pm$ 0.61 <sup>a</sup>	59 <sup>B</sup>
Pro-2	7.46 $\pm$ 0.23 <sup>a</sup>	56 <sup>B</sup>	8.15 $\pm$ 1.08 <sup>a</sup>	68 <sup>A</sup>	7.28 $\pm$ 0.33 <sup>a</sup>	59 <sup>B</sup>	6.92 $\pm$ 0.05 <sup>b</sup>	50 <sup>C</sup>

ND: Not detected. <sup>a,b</sup> Different superscripts in the same column indicate significant differences ( $p < 0.05$ ). <sup>A-C</sup> Different superscripts in the same row indicate significant differences in survival rate during storage ( $p < 0.05$ ). No significant differences between the number of cells after exposure to simulated gastrointestinal digestion within the same sample throughout the storage period.

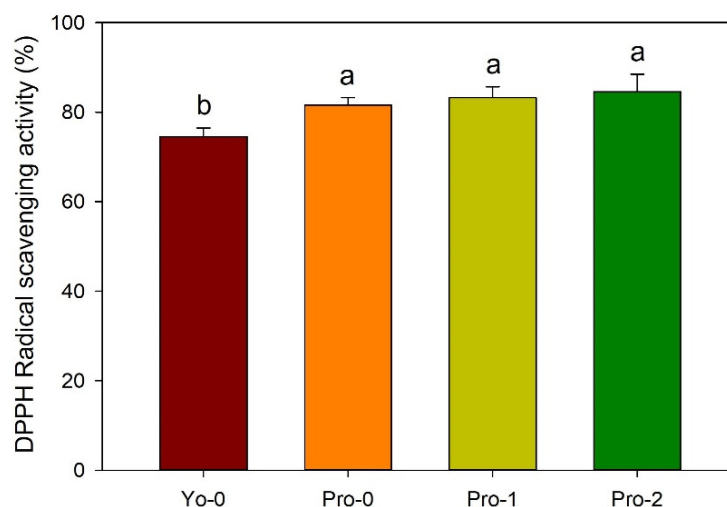
In the present study, a decrease in the pH of coconut milk yogurt-type beverages during storage at 4 °C was possibly responsible for the increased tolerance to the sequential exposure to gastric and pancreatic juice, with an increase in survival rate after day 1 of storage for samples fermented with the strain with probiotic potential. It is possible that during storage, the strain adapted to acidic conditions, potentially triggering resistance mechanisms against simulated gastric juice exposure [18]. However, after 14 days of storage, the sharp decrease in the pH value of the sample may limit the survival rate of *L. paracasei* MSMC 36-9.

Survival of the cells may be due to the protective effect of the high fat content in coconut milk, which increases cell resistance to gastrointestinal stress [30]. Moreover, the tolerance of bacteria to gastrointestinal stress has strain-dependent characteristics and depends on various intrinsic factors. Lactic acid bacteria with the ability to produce the bile salt hydrolase enzyme could generally reduce the toxicity of bile salts [18]. Exopolysaccharides (EPS) produced intrinsically during fermentation have also been associated with increased tolerance to gastric juice. Therefore, the EPS test may need to be carried out to confirm whether *L. paracasei* MSMC 36-9 is an EPS-producing strain.

Our results confirmed that *L. paracasei* MSMC 36-9 could be potentially used as a probiotic culture for therapeutic purposes as well as a starter culture for the production of coconut milk yogurt-type beverages.

### 3.4. Radical Scavenging Activity

The DPPH radical scavenging activity of coconut milk yogurt-type beverages is shown in Figure 2. The radical scavenging activity of Yo-0 was significantly lower than Pro-0, Pro-1, and Pro-2.



**Figure 2.** DPPH radical scavenging activity (%) of coconut milk yogurt-type beverages fermented with yogurt starter culture (Yo-0), *L. paracasei* MSMC 36-9 without the addition of tapioca starch (Pro-0), with the addition of 1.0% tapioca starch (Pro-1), and with the addition of 2.0% tapioca starch (Pro-2) measured on day 21 during refrigerated storage at 4 °C. Columns represent the mean, and error bars represent the standard deviation of three replicates. <sup>a,b</sup> Different superscripts indicate significant differences ( $p < 0.05$ ).

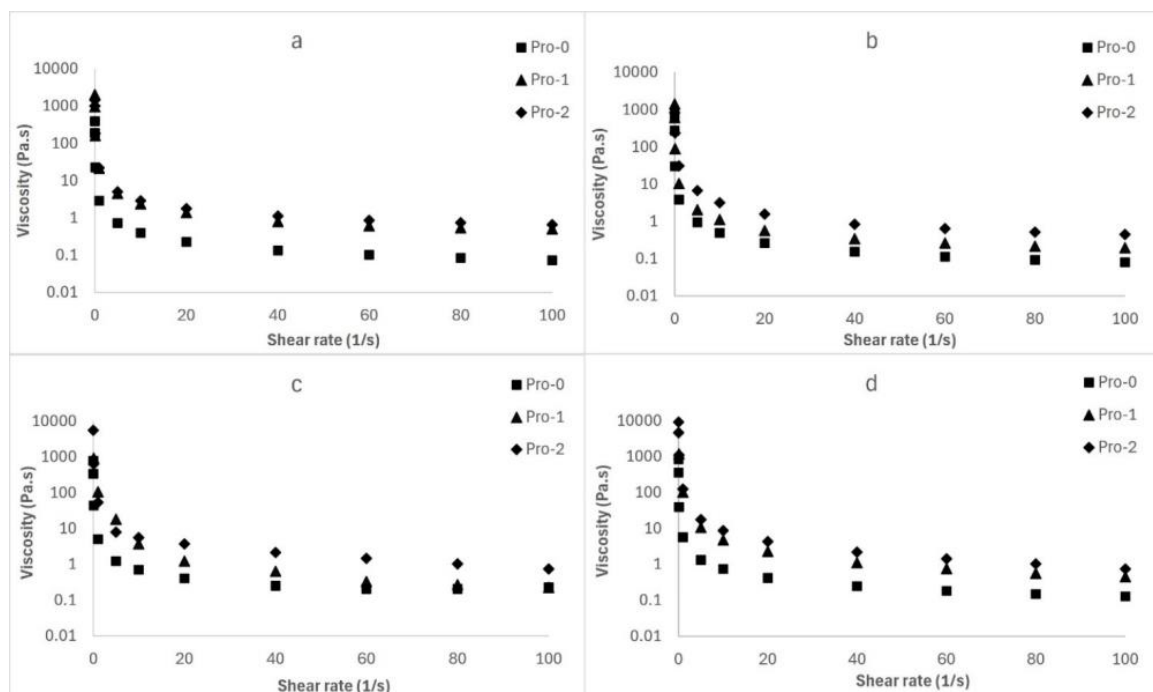
No significant differences in radical scavenging activity were found between samples fermented with *L. paracasei* MSMC 36-9 at different concentrations of tapioca starch. The values of antioxidant activity determined from Pro-0, Pro-1, and Pro-2 were 81.56%, 83.24%, and 84.59%, respectively, while the sample Yo-0 possessed a value of 74.55%. The antioxidant activity of these fermented coconut products may partly be due to the phenolic compounds present in coconut oils, such as p-coumaric acid, ferulic acid, caffeic acid, and catechin acid [31]. Specific metabolites produced during the fermentation process of the

strain with probiotic potential can also enhance antioxidant activity. However, Soumya et al. [32] found lower radical scavenging activity of around 40% in fermented coconut milk after 21 days of storage. The lower value of radical scavenging activity could be attributed to various parameters, including different coconut milk preparation methods and starter cultures used in the fermentation.

Shori et al. [21] recorded enhanced antioxidant activity of cashew nut yogurts by co-fermentation with three strains of *Lactobacillus* spp., which increased the flavonoid and phenolic contents. Heydari et al. [33] used Iranian *Bifidobacterium lactis* in the manufacturing of dairy yogurt and found that *B. lactis* produced bioactive peptides with strong antioxidant activity. In the present study, our obtained antioxidant activity values are higher than those of dairy yogurt fortified with ingredients rich in phenolic compounds, such as mulberry pomace [34] and pomegranate juice powder [35]. Therefore, *L. paracasei* MSMC 36-9 showed high potential for producing bioactive substances with therapeutic effects in fermented coconut milk.

### 3.5. Viscosity

The apparent viscosity of all samples decreased with an increase in shear rate (Figure 3), indicating that coconut milk yogurt-type beverages possessed shear-thinning behavior [20]. Table 3 presents the viscosity values of samples measured at a shear rate of  $40\text{ s}^{-1}$ . Increasing tapioca starch concentrations resulted in higher viscosity, with sample Pro-2 the most viscous. According to these findings, adding tapioca starch to the samples strengthened the network as the starch granules swelled and absorbed water in the continuous phase during heating at  $90\text{ }^{\circ}\text{C}$ , thereby enhancing particle-particle interactions [36]. The viscosity values found in the present study were comparable to the viscosity values found in probiotic coconut beverages reported by Soumya et al. [32]. According to Mishra and Rai [37], tapioca starch was found to be stable in acidic conditions, indicating that it is suitable for use in the formulation of yogurt-type beverages made from plant-based milk.



**Figure 3.** Apparent viscosity of coconut milk yogurt-type beverages fermented with yogurt starter culture (Yo-0), *L. paracasei* MSMC 36-9 without the addition of tapioca starch (Pro-0), with the addition of 1.0% tapioca starch (Pro-1), and with the addition of 2.0% tapioca starch (Pro-2) was measured on days 1 (a), 7 (b), 14 (c), and 21 (d).

**Table 3.** Apparent viscosity (Pa.s) of coconut milk yogurt-type beverages measured at a shear rate of  $40 \text{ s}^{-1}$ . Data are presented as the mean  $\pm$  SD of three replicates.

Sample	Apparent Viscosity (Pa.s)			
	Day 1	Day 7	Day 14	Day 21
Pro-0	$0.14 \pm 0.03$ <sup>bB</sup>	$0.15 \pm 0.02$ <sup>bB</sup>	$0.25 \pm 0.06$ <sup>bA</sup>	$0.24 \pm 0.04$ <sup>cA</sup>
Pro-1	$0.80 \pm 0.11$ <sup>aB</sup>	$0.34 \pm 0.05$ <sup>bC</sup>	$0.64 \pm 0.06$ <sup>bB</sup>	$1.11 \pm 0.18$ <sup>bA</sup>
Pro-2	$1.12 \pm 0.20$ <sup>aB</sup>	$0.85 \pm 0.12$ <sup>aB</sup>	$2.15 \pm 0.40$ <sup>aA</sup>	$2.18 \pm 0.09$ <sup>aA</sup>

<sup>a-c</sup> Different superscripts in the same column indicate significant differences ( $p < 0.05$ ). <sup>A-C</sup> Different superscripts in the same row indicate significant differences ( $p < 0.05$ ).

#### 4. Conclusions

This study highlighted the ability of the strain with probiotic potential, *L. paracasei* MSMC 36-9, to be used as a starter culture in the production of coconut milk yogurt-type beverages with the addition of tapioca starch as a stabilizer. The strain with probiotic potential was isolated from healthy infant feces. The strain survived in the simulated gastric juice and bile salts for the entire storage period of 21 days. At the end of storage, the number of *L. paracasei* MSMC 36-9 in all samples was above the range of  $10^6$  CFU/g recommended for human consumption. The radical scavenging activity of samples fermented with *L. paracasei* MSMC 36-9 was in the range of 80%. The addition of tapioca starch modified the viscosity of the products because of its swelling property. Our results suggested that *L. paracasei* MSMC 36-9 could be used as a starter culture for the manufacturing of fermented coconut milk beverages. However, since the strain *L. paracasei* MSMC 36-9 is of human origin, further safety assessments, i.e., assessment of D-lactate production and determination of antimicrobial resistance factors, are required before introducing it into products intended for human or animal consumption.

**Author Contributions:** Conceptualization, U.P.; methodology, U.P., P.V. and M.T.; investigation, U.P., P.P., W.A. and P.V.; writing—original draft preparation, U.P. and P.V.; writing—review and editing, U.P., P.V. and M.T.; supervision, U.P.; funding acquisition, U.P. and M.T. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the Fundamental Fund, Thailand Science Research and Innovation, and Innovation and the the Strategic Wisdom and Research Institute of Srinakharinwirot University (grant number 005/2565).

**Data Availability Statement:** Data are available upon request from the authors.

**Conflicts of Interest:** The authors declare no conflicts of interest.

#### References

- Cardello, A.V.; Llobell, F.; Giacalone, D.; Roigard, C.M.; Jaeger, S.R. Plant-based alternatives vs dairy milk: Consumer segments and their sensory, emotional, cognitive and situational use responses to tasted products. *Food Qual. Prefer.* **2022**, *100*, 104599. [\[CrossRef\]](#)
- Adesogan, A.T.; Dahl, G.E. Dairy production in developing countries. *J. Dairy Sci.* **2020**, *103*, 9677–9680. [\[CrossRef\]](#)
- Munekata, P.E.S.; Domínguez, R.; Budaraju, S.; Roselló-Soto, E.; Barba, F.J.; Mallikarjunan, K.; Roohinejad, S.; Lorenzo, J.M. Effect of innovative food processing technologies on the physicochemical and nutritional properties and quality of non-dairy plant-based beverages. *Foods* **2020**, *9*, 288. [\[CrossRef\]](#)
- Santos, A.; Dias, A.; Pinheiro, J.A. Predictive factors for the persistence of cow's milk allergy. *Pediatr. Allergy Immunol.* **2010**, *21*, 1127–1134. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sharp, E.; D'Cunha, N.M.; Ranadheera, C.S.; Vasiljevic, T.; Panagiotakos, D.B.; Naumovski, N. Effects of lactose-free and low-lactose dairy on symptoms of gastrointestinal health: A systematic review. *Int. Dairy J.* **2021**, *114*, 104936. [\[CrossRef\]](#)
- Aydar, E.F.; Tutuncu, S.; Ozelik, B. Plant-based milk substitutes: Bioactive compounds, conventional and novel processes, bioavailability studies, and health effects. *J. Funct. Foods* **2020**, *70*, 103975. [\[CrossRef\]](#)
- Góral, M.; Kozłowicz, K.; Pankiewicz, U.; Góral, D.; Kluza, F.; Wójtowicz, A. Impact of stabilizers on the freezing process, and physicochemical and organoleptic properties of coconut milk-based ice cream. *LWT* **2018**, *92*, 516–522. [\[CrossRef\]](#)

8. Tulashie, S.K.; Amenakpor, J.; Atisey, S.; Odai, R.; Akpari, E.E.A. Production of coconut milk: A sustainable alternative plant-based milk. *Case Stud. Chem. Environ. Eng.* **2022**, *6*, 100206. [\[CrossRef\]](#)
9. Narayanankutty, A.; Illam, S.P.; Raghavamenon, A.C. Health impacts of different edible oils prepared from coconut (*Cocos nucifera*): A comprehensive review. *Trends Food Sci. Technol.* **2018**, *80*, 1–7. [\[CrossRef\]](#)
10. Anagnostou, K. Coconut allergy revisited. *Children* **2017**, *4*, 85. [\[CrossRef\]](#)
11. Fan, X.; Shi, Z.; Xu, J.; Li, C.; Li, X.; Jiang, X.; Du, L.; Tu, M.; Zeng, X.; Wu, Z.; et al. Characterization of the effects of binary probiotics and wolfberry dietary fiber on the quality of yogurt. *Food Chem.* **2023**, *406*, 135020. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Fawzi, N.Y.; Abdelghani, D.Y.; Abdel-azim, M.A.; Shokier, C.G.; Youssef, M.W.; Gad El-Rab, M.K.; Gad, A.I.; Abou-Taleb, K.A. The ability of probiotic lactic acid bacteria to ferment Egyptian broken rice milk and produce rice-based yoghurt. *Ann. Agric. Sci.* **2022**, *67*, 107–118. [\[CrossRef\]](#)
13. Donkor, O.N.; Henriksson, A.; Vasiljevic, T.; Shah, N.P. Probiotic strains as starter cultures improve angiotensin-converting enzyme inhibitory activity in soy yogurt. *J. Food Sci.* **2005**, *70*, M375–M381. [\[CrossRef\]](#)
14. Walsh, H.; Ross, J.; Hendricks, G.; Guo, M. Physico-chemical properties, probiotic survivability, microstructure, and acceptability of a yogurt-like symbiotic oats-based product using pre-polymerized whey protein as a gelation agent. *J. Food Sci.* **2010**, *75*, M327–M337. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Pachekrepapol, U.; Kokhuenkhan, Y.; Ongsawat, J. Formulation of yogurt-like product from coconut milk and evaluation of physicochemical, rheological, and sensory properties. *Int. J. Gastron. Food Sci.* **2021**, *25*, 100393. [\[CrossRef\]](#)
16. Pongsawatmanit, R.; Chantaro, P.; Nishinari, K. Thermal and rheological properties of tapioca starch gels with and without xanthan gum under cold storage. *J. Food Eng.* **2013**, *117*, 333–341. [\[CrossRef\]](#)
17. Vitheejongjaroen, P.; Kanthawang, P.; Loison, F.; Jaisin, Y.; Pachekrepapol, U.; Taweechotipatr, M. Antioxidant activity of *Bifidobacterium animalis* MSMC83 and its application in set-style probiotic yoghurt. *Food Biosci.* **2021**, *43*, 101259. [\[CrossRef\]](#)
18. Mesquita, M.C.; dos Santos Leandro, E.; Rodrigues de Alencar, E.; Botelho, R.B.A. Survival of *Lactobacillus paracasei* subsp. *paracasei* LBC 81 in fermented beverage from chickpeas and coconut in a static in vitro digestion model. *Fermentation* **2021**, *7*, 135.
19. Shori, A.B.; Albalawi, A.; Al Zahrani, A.J.; Al-sulbi, O.S.; Baba, A.S. Microbial analysis, antioxidant activity, and sensory properties of yoghurt with different starter cultures during storage. *Int. Dairy J.* **2022**, *126*, 105267. [\[CrossRef\]](#)
20. Tan, P.Y.; Tan, T.B.; Chang, H.W.; Tey, B.T.; Chan, E.S.; Lai, O.M.; Baharin, B.S.; Nehdi, I.A.; Tan, C.P. Effects of storage and yogurt matrix on the stability of tocotrienols encapsulated in chitosan-alginate microcapsules. *Food Chem.* **2018**, *241*, 79–85. [\[CrossRef\]](#)
21. Shori, A.B.; Aljohani, G.S.; Al-zahrani, A.J.; Al-sulbi, O.S.; Baba, A.S. Viability of probiotics and antioxidant activity of cashew milk-based yogurt fermented with selected strains of probiotic *Lactobacillus* spp. *LWT* **2022**, *153*, 112482. [\[CrossRef\]](#)
22. Lourens-Hattingh, A.; Viljoen, B.C. Yogurt as probiotic carrier food. *Int. Dairy J.* **2001**, *11*, 1–17. [\[CrossRef\]](#)
23. Huang, W.; Dong, A.; Pham, H.T.; Zhou, C.; Huo, Z.; Wätjen, A.P.; Prakash, S.; Bang-Berthelsen, C.H.; Turner, M.S. Evaluation of the fermentation potential of lactic acid bacteria isolated from herbs, fruits and vegetables as starter cultures in nut-based milk alternatives. *Food Microbiol.* **2023**, *112*, 104243. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Amaya-Llano, S.L.; Martínez-Alegria, A.L.; Zazueta-Morales, J.J.; Martínez-Bustos, F. Acid thinned jicama and maize starches as fat substitute in stirred yogurt. *LWT* **2008**, *41*, 1274–1281. [\[CrossRef\]](#)
25. Altemimi, A.B. Extraction and optimization of potato starch and its application as a stabilizer in yogurt manufacturing. *Foods* **2018**, *7*, 14. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Wang, J.; Guo, Z.; Zhang, Q.; Yan, L.; Chen, W.; Liu, X.M.; Zhang, H.P. Fermentation characteristics and transit tolerance of probiotic *Lactobacillus casei* Zhang in soymilk and bovine milk during storage. *J. Dairy Sci.* **2009**, *92*, 2468–2476. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Szparaga, A.; Tabor, S.; Kocira, S.; Czerwińska, E.; Kuboń, M.; Plóciennik, B.; Findura, P. Survivability of probiotic bacteria in model systems of non-fermented and fermented coconut and hemp milks. *Sustainability* **2019**, *11*, 6093. [\[CrossRef\]](#)
28. Kechagia, M.; Basoulis, D.; Konstantopoulou, S.; Dimitriadi, D.; Gyftopoulou, K.; Skarmoutsou, N.; Fakiri, E.M. Health benefits of probiotics: A review. *ISRN Nutr.* **2013**, *2013*, 481651. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Simões da Silva, T.M.; Piazzentin, A.C.M.; Mendonça, C.M.N.; Converti, A.; Bogsan, C.S.B.; Mora, D.; de Souza Oliveira, R.P. Buffalo milk increases viability and resistance of probiotic bacteria in dairy beverages under in vitro simulated gastrointestinal conditions. *J. Dairy Sci.* **2020**, *103*, 7890–7897. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Verruck, S.; Prudêncio, E.S.; Vieira, C.R.W.; Amante, E.R.; Amboni, R.D.d.M.C. The buffalo Minas Frescal cheese as a protective matrix of *Bifidobacterium* BB-12 under in vitro simulated gastrointestinal conditions. *LWT* **2015**, *63*, 1179–1183. [\[CrossRef\]](#)
31. Sandupama, P.; Munasinghe, D.; Jayasinghe, M. Coconut oil as a therapeutic treatment for alzheimer's disease: A review. *J. Future Foods* **2022**, *2*, 41–52. [\[CrossRef\]](#)
32. Soumya, M.P.; Suresh, A.; Parameswaran, R.; Nampoothiri, K.M. Physico-chemical and organoleptic evaluation of probiotic plant-milk yogurt-type beverages as a functional alternative to dairy yogurts. *Biocatal. Agric. Biotechnol.* **2024**, *57*, 103060.
33. Heydari, S.; Hosseini, S.E.; Mortazavian, A.M.; Taheri, S. Extraction of bioactive peptides produced in probiotic yoghurt and determination of their biological activities. *Int. Dairy J.* **2023**, *139*, 105544. [\[CrossRef\]](#)
34. Du, H.; Wang, X.; Yang, H.; Zhu, F.; Tang, D.; Cheng, J.; Liu, X. Changes of phenolic profile and antioxidant activity during cold storage of functional flavored yogurt supplemented with mulberry pomace. *Food Control* **2022**, *132*, 108554. [\[CrossRef\]](#)
35. Pan, L.H.; Liu, F.; Luo, S.Z.; Luo, J.P. Pomegranate juice powder as sugar replacer enhanced quality and function of set yogurts: Structure, rheological property, antioxidant activity and in vitro bioaccessibility. *LWT* **2019**, *115*, 108479. [\[CrossRef\]](#)

- 
36. Grasso, N.; Alonso-Miravalles, L.; O'Mahony, J.A. Composition, physicochemical and sensorial properties of commercial plant-based yogurts. *Foods* **2020**, *9*, 252. [[CrossRef](#)] [[PubMed](#)]
  37. Mishra, S.; Rai, T. Morphology and functional properties of corn, potato and tapioca starches. *Food Hydrocoll.* **2006**, *20*, 557–566. [[CrossRef](#)]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.