



# The Characteristics of Goat Milk Synbiotics-Yogurt using *Lactobacillus plantarum* as Probiotic and Inulin of Mangrove Apple (*Sonneratia caseolaris*)

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**Abstract** | One of the sources of inulin comes from mangrove apple, which is potential as a prebiotic. Inulin combined with probiotics would be an ideal synbiotic for yogurt. By combining the synergistic effects of prebiotics and probiotics, synbiotic yogurts have the potential to be functional foods. This study was carried out to investigate the influence of synbiotics from the inulin of mangrove apple and *Lactobacillus plantarum* in the characteristics of goat milk. This study used a Completely Randomized Design (CRD) with five treatments and four replications, with differences in the addition of synbiotics inulin from extracted mangrove apple and *Lactobacillus plantarum*. Yogurts with no synbiotic were used as a control, while yogurts with synbiotics of 2, 4, 6, and 8% (v/v) were used in another treatment. The results of goat milk yogurt showed that the addition of various levels of synbiotic had a significant effect ( $p < 0.05$ ) on the total LAB, pH value, viscosity, total dissolved solid, and total soluble dietary fibre of yogurt. The yogurt with the addition synbiotic 8% was the highest ( $p < 0.05$ ) in the total Lactic Acid Bacteria (LAB) and the soluble dietary fibre compared to the other treatment. The addition of a synbiotic had no effect on the titratable acidity of the yogurt ( $p > 0.05$ ).

**Keywords** | Goat milk yogurt, Inulin, *Lactobacillus plantarum*, Mangrove apple, Synbiotics

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## INTRODUCTION

Yoghurt is a well-known and highly functional food of fermented dairy product (Mulyani et al., 2004; Wibawanti et al., 2018; Gu et al., 2021). Yogurt has grown in popularity, and it is regarded as a healthy food due to its high levels of essential nutrients (Qiu et al., 2021). Many synbiotic-based foods have been developed, including yogurt products made from probiotics and oats (Lim, 2017), sorghum flour (Sukarminah et al., 2019), and monk fruit

extract (Ban et al., 2020). Synbiotics are widely used in fermentation beverages because they improve beneficial bacteria and provide good nutrition in the gut (Li et al., 2021). Synbiotics are the combination of prebiotics and probiotics that has been formulated to stimulate the growth of microorganism (Dunislawska et al., 2017; Peng et al., 2020). They were developed to help probiotics survive in the gastrointestinal tract (Markowiak and Ślizewska, 2017). The synergistic activities of prebiotics and probiotics have had a greater impact on the effectiveness of gut microbiota than

individual use of prebiotics or probiotics (Sakr and Mas-soud, 2021).

The characteristics of yogurt depend on several factors such as fermentation process, starter cultures, probiotic strains and type of milk (Fazilah et al., 2018). Goat milk has a unique nutritional composition, and it has numerous health benefits (Wihansah et al., 2018). Goat milk is easily digestible and have been shown to improve biological functions (Shu et al., 2014; Sada et al., 2020).

Probiotics can be found in a variety of dairy products, including yoghurts. The probiotic products must have adequate amounts of live microorganisms ( $\leq 10^6$  CFU/g) at the time of consumption (Paseephol and Sherkat, 2009). Fruit dietary fibre has been proposed as an ingredient in probiotic dairy foods that improves the viability of the bacteria (Santo et al., 2012). The strain, dose, and components used to create a given probiotic product determine the effectiveness of probiotics (Markowiak and Ślizewska, 2017). *Lactobacillus plantarum* is a probiotic bacterium. *Lactobacillus* ability to use lactose and sucrose supplements in milk for metabolic activity is optimal, resulting in relatively high lactic acid production (Pramono et al., 2020). Prebiotics are natural, nondigestible food ingredients that promote the growth of probiotic bacteria (Khaled, 2021). Inulin is a type of prebiotic that can support the growth of probiotic products. Inulin has been obtained from the Mangrove apple (*Sonneratia caseolaris*), which contains up to 5.08% inulin (Wibawanti et al., 2021). As a result, the current study aimed to investigate the effect of adding inulin from a mangrove apple extract and *Lactobacillus plantarum* extract to the production of synbiotic goat milk yoghurt.

## MATERIALS AND METHODS

### STARTER CULTURE

As a starter culture, *Streptococcus thermophilus* (FNCC 0040) and *Lactobacillus bulgaricus* (FNCC 0041) bacteria were used. The culture collection at Gadjah Mada University provided the starter culture and *Lactobacillus plantarum* (FNCC 0026). The purified colonies were introduced to de Man Rogosa and Sharpe (MRS) broth and incubated in an anaerobic jar at 37°C for 48 h.

### SYNBIOTIC PREPARATION

The synbiotics were created by combining inulin extract of mangrove apple (IEMA) as a prebiotic and *Lactobacillus plantarum*, as described by Setyaningrum et al. (2019), with a few modifications. The synbiotics were prepared by mixing 10 ml *Lactobacillus plantarum* (viable bacterial load of  $> 10^8$  CFU/mL) and 9% of IEMA. They have incubated anaerobically in the MRS Broth for 24 hours at 37°C.

## YOGURT PREPARATION

Yogurt was prepared using Sharma and Ramanathan's (2021) method. The goat milk was pasteurized at 80°C for 15 min. It was cooled at temperature 45–42°C, which is inoculated with a 5% yogurt culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). The synbiotic from inulin extract of mangrove apple and *Lactobacillus plantarum* (FNCC 0026) were added to goat milk according to each treatment (0 as a control or T<sub>1</sub>, 2, 4, 6 and 8% (v/v) for T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub>, respectively). Separate goat milk and synbiotic mixtures were homogenized at 1000 rpm until all ingredients were dissolved. The incubation was performed at 42 ± 0.5°C for 5 hours. To calculate coagulation, goat yoghurt samples were stored at refrigeration temperature (4 ± 1°C). Four replicates of goat milk synbiotic-yogurt were made.

### INULIN EXTRACT OF MANGROVE APPLE

Inulin extract of mangrove apple (*Sonneratia caseolaris*) was prepared following the procedure of Wibawanti et al. (2021). The mangrove apple was cut into small pieces and heated to 90°C. Mangrove apple was extracted for 60 minutes at a 1:4 ratio (fruit: hot water at 90°C w/v). After being precipitated with 40% ethanol, the filtrates were stored at - 18°C. At room temperature, the filtrate was thawed. The supernatant was removed after centrifuging the filtrate inulin of mangrove apple for 5 minutes at 5000 rpm.

### DETERMINATION OF TOTAL OF LACTIC ACID BACTERIA

The spread plate technique and serial dilutions in normal saline were used to count the lactic acid bacteria (LAB) in yogurt (0.9% NaCl) (Abdel-Hamid et al., 2020). MRS agar (pH 5.4) was used to count LAB and the plates were anaerobically incubated at 37 °C for 48 h. Per ml of sample, the number of colony-forming units (CFU) was calculated in plates containing 25–250 colonies.

### DETERMINATION OF TITRATABLE ACIDITY (TA)

The titratable acidity (as percent percent lactic acid) of yoghurt was determined in triplicate using 0.1 M NaOH and the AOAC titration method 947.05 (AOAC, 2000).

### PH DETERMINATION OF GOAT MILK SYNBIOTIC-YOGURT

The pH of the goat milk synbiotic-yogurt was determined using a pH meter that had previously been calibrated with pH 7.0 and 4.0 standard buffers. All analyses were performed in duplicate at 20°C.

### DETERMINATION OF VISCOSITY

The viscosity of goat milk synbiotic-yogurt was measured with a viscometer (Brookfield R.V.T.) using the method described by Prayitno et al. (2020), with a few modifi-

cations. In a glass beaker, up to 100 ml of samples were placed. The sample viscosity was measured with a spindle no. 2 at 125 rpm and a readability of 85%. The sample was conditioned at room temperature after undergoing a two-minute viscosity test to achieve a stable condition.

### DETERMINATION OF TOTAL DISSOLVED SOLID OF GOAT MILK SYNBIOTIC-YOGURT

Goat milk synbiotic-yogurt was assessed using a refractometer according to the method by Santos et al. (2020). The sample of synbiotic yogurt was homogenized into glass beaker. One drop of the sample was placed in the refractometer. The result was accumulated as °Brix (the value (%)) of total dissolved solid. The result was calculated as °Brix (the value (percentage)) of total dissolved solid.

### DETERMINATION OF THE TOTAL OF SOLUBLE DIETARY FIBRE

A multienzyme analysis was used to determine the total soluble dietary fibre of goat milk synbiotic-yogurt in accordance with the AOAC (1995).

### STATISTICAL ANALYSIS

All results were statistically analysed using SPSS 16.0 software. One-way analysis of variance (ANOVA) was used in the statistical analysis, followed by Duncan's test to determine the difference between mean values.

## RESULTS

### THE TOTAL LAB OF GOAT MILK SYNBIOTIC-YOGURT

Figure 1 depicts the addition of synbiotic inulin extract mangrove apple (IEMA) with *Lactobacillus plantarum* at various concentrations. According to the results of the total LAB analysis, yogurt with various additional synbiotic treatments (IEMA with *Lactobacillus plantarum*) had a significant effect ( $P < 0.05$ ). The addition of synbiotics was found to increase total LAB in the yogurt product. Goat milk synbiotic yogurt (GMS-Y) with 8% synbiotic addition shows the highest LAB result ( $10.54 \pm 0.39$  Log CFU/mL). The total LAB of GMS-Y with 4 and 6% was  $10.19 \pm 0.16$  and  $10.35 \pm 0.37$  Log CFU/mL, respectively. The total LAB of the control sample and yogurt with a 2% synbiotic addition showed no significant difference ( $p > 0.05$ ) ( $9.52 \pm 0.23$  and  $9.16 \pm 0.89$  Log CFU/mL, respectively).

### THE TITRATABLE ACIDITY OF GOAT MILK SYNBIOTIC-YOGURT

Titrate acidity (TA) of GMS-Y is shown in Figure 2. The addition of synbiotics of inulin extracted of mangrove apple and *Lactobacillus plantarum* to yogurt resulted in no significant differences in titrate acidity ( $p > 0.05$ ). The

titrate acidity values of yogurt with the addition of 0, 2, 4, 6, and 8% synbiotics ranged from  $1 \pm 0.08$ ,  $1.07 \pm 0.07$ ,  $1.04 \pm 0.06$ ,  $1.04 \pm 0.07$ , and  $1.02 \pm 0.04$ %, respectively.

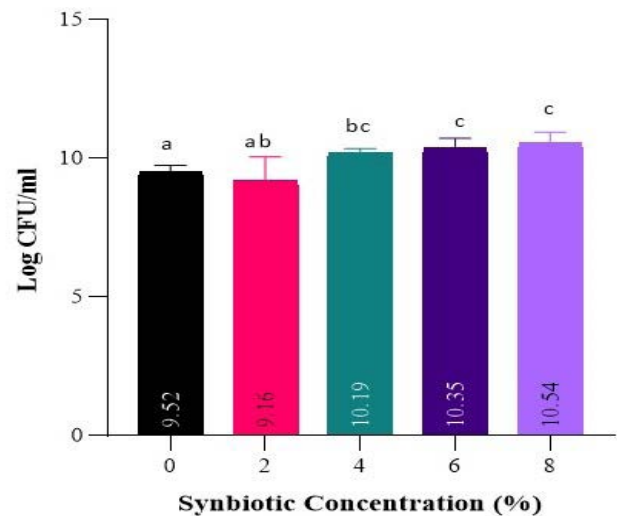


Figure 1: The total LAB of goat milk synbiotic-yogurt

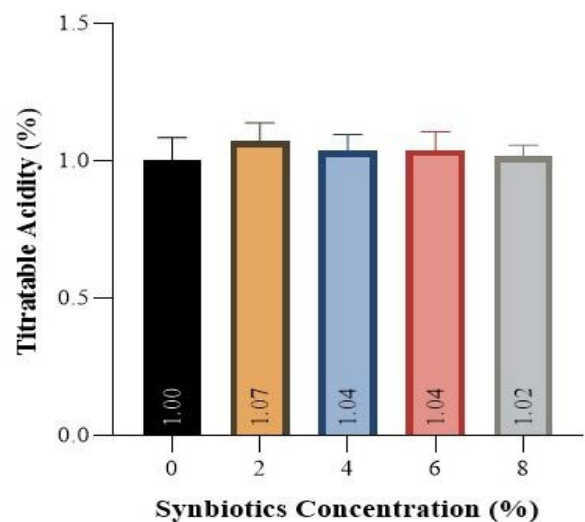


Figure 2: The titrate acidity of goat milk synbiotic-yogurt

### THE PH OF GOAT MILK SYNBIOTIC-YOGURT

Figure 3. shows the pH of GMS-Y with different treatments for the addition of synbiotic (IEMA with *Lactobacillus plantarum*). Based on the pH results, yogurt with different synbiotic addition treatments (IEMA with *Lactobacillus plantarum*) had a significant effect ( $P < 0.05$ ). The pH value of GMS-Y with synbiotic addition was significantly lower ( $p < 0.05$ ) when compared to the control. The highest pH value of GMS-Y was found in the control sample with a pH value of  $5.34 \pm 0.06$ . Meanwhile, the lowest pH value of GMS-Y was found in the addition of 8% synbiotic with a pH value of  $5.09 \pm 0.05$ . The addition of 2, 4, and 6% synbiotic showed no significant differences ( $p > 0.05$ ) with the pH value yogurt of  $5.24 \pm 0.06$ ,  $5.27 \pm$

0.05, and  $5.21 \pm 0.05$ , respectively.

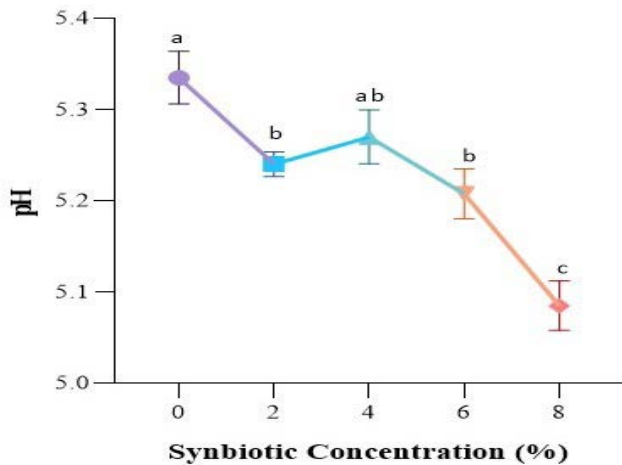


Figure 3: The pH value of goat milk synbiotic-yogurt

**THE VISCOSITY OF GOAT MILK SYNBIOTIC-YOGURT**

The viscosity of the GMS-Y was measured at a constant shear rate. The viscosity of goat milk yogurt with different treatments for the addition of synbiotic (IEMA with *Lactobacillus plantarum*) was presented in Figure 4. The statistical analysis showed a significant effect of the addition of synbiotic ( $p < 0.05$ ) on the viscosity of yoghurt. Significant differences were observed in the viscosity of yogurt synbiotic ( $p < 0.05$ ). The addition of synbiotic resulted in a significantly ( $p < 0.05$ ) lower value (2630.1 cP) of viscosity compared to the control sample of yogurt (3763.7 cP). There were no significant differences in the viscosity between yogurts containing 0% synbiotic to 2% synbiotic (3667.6 cP) treatment ( $p > 0.05$ ). The addition of GMS-Y with 4% and 6% synbiotic has viscosity values about 3172.1 and 2817.4 Cp, respectively.

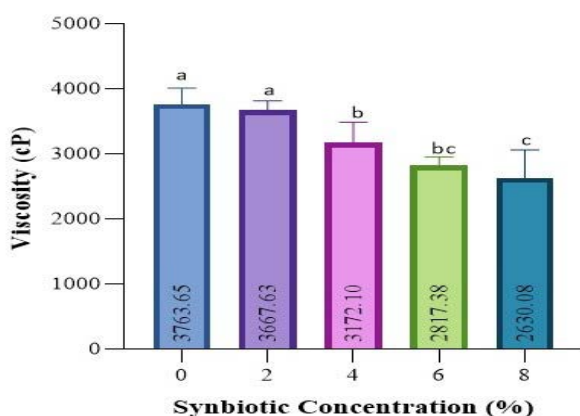


Figure 4: The viscosity value of goat milk synbiotic-yogurt

**THE TOTAL DISSOLVED SOLID OF GOAT MILK SYNBIOTIC-YOGURT**

The total dissolved solid of GMS-Y with different treatments for the addition of synbiotic (IEMA with *Lactoba-*

*cillus plantarum*) was presented in Figure 5. The statistical analysis showed a significant effect of the addition of synbiotic ( $p < 0.05$ ) on the total dissolved solid of yoghurt. The total dissolved solid value of yogurt synbiotic with different treatments for synbiotic addition was reduced. The addition of synbiotic resulted in a significantly ( $p < 0.05$ ) lower total dissolved solid compared with the control sample of yogurt. The total dissolved solid of yogurt without treatment (control) was valued at  $11.7 \pm 0.18$  %° Brix. The total dissolved solid in yogurt synbiotic (2%) was significantly different ( $p > 0.05$ ) from the yogurt synbiotic (4%), which had values of  $9.85 \pm 0.31$  %° Brix and  $10 \pm 0.32$  %° Brix, respectively. The total dissolved solid in yogurt synbiotic (8%) was not significantly different ( $p > 0.05$ ) from the yogurt synbiotic (6%), which had values of  $9.38 \pm 0.35$  %° Brix and  $9.43 \pm 0.54$  %° Brix, respectively.

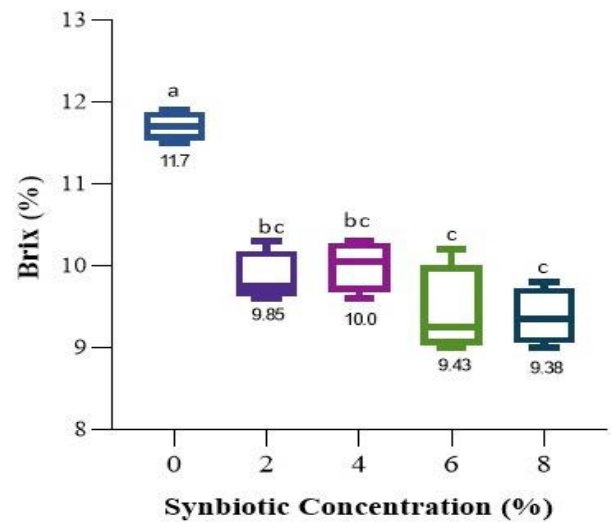
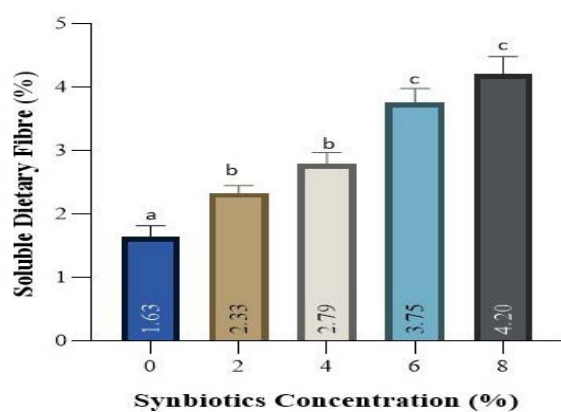


Figure 5: The total of sugar of goat milk synbiotic-yogurt

**THE TOTAL OF SOLUBLE DIETARY FIBRE OF GOAT MILK SYNBIOTIC-YOGURT**

The soluble dietary fibre is shown in Figure 6. The statistical analysis showed a significant effect of the addition of synbiotic of inulin extracted mangrove apple and *Lactobacillus plantarum* ( $p < 0.05$ ) in the soluble dietary fibre of yoghurt. The addition of synbiotic was significantly different ( $p < 0.05$ ). The inclusion of synbiotics resulted in an increase in total soluble dietary fibre. Yogurt with the addition of 8% synbiotic had a higher value ( $4.20 \pm 0.56$ %) compared to other treatments. The sample control had the lowest value ( $1.63 \pm 0.36$ %) of soluble dietary fibre of yogurt. The addition of 6% synbiotic ( $3.75 \pm 0.46$ %) compared to the concentration of 8% synbiotic was not significantly different ( $p > 0.05$ ). The addition of 2% synbiotic ( $2.33 \pm 0.24$ %) compared to the concentration of 4% synbiotic ( $2.79 \pm 0.36$ %) was not significantly different ( $p > 0.05$ ).



**Figure 6:** The total of soluble dietary fibre of goat milk synbiotic-yogurt

## DISCUSSION

### THE TOTAL LAB OF GOAT MILK SYNBIOTIC-YOGURT

The addition of synbiotic with different concentrations can be increased and help the growth of the total of LAB. Inulin of mangrove apple could be used as an energy source for LAB during fermentation. Synbiotics with IEMA prebiotics that promote probiotic growth have a synergistic effect. *Lactobacillus plantarum* probiotics produce total lactic acid that is enriched with inulin extracted from mangrove apple. Markowiak and Ślizewska (2017) reported that the synbiotics promote probiotic organism growth by providing the probiotic organism with a specific substrate for fermentation. Hosseini and Behbahani (2021) pointed out that in sheep yogurt enriched with *P. ferulaceae* extract, the total lactic acid produced by *Lactobacillus plantarum* increased. The results of this study were similar to Lim (2018) that the number of LAB in yogurt synbiotics prepared with various types of probiotics strain was significantly increased by supplementary with prebiotic from oat flour. Yoha et al. (2020) studied that the spray freeze-drying of synbiotic improved *Lactobacillus plantarum* viability. In Voragen (1998), saccharides chemical structure (linear or branched), degree of polymerisation (DP), monomer unit composition, and water solubility all have an impact on microorganism ability to use them. According to Lim (2018) lactic acid lowers the pH of the product, allowing some milk proteins to coagulate, and allowing yogurt to be made. Due to the protonation of its amino acid residues, the tertiary structure of casein, a hydrophobic protein, is broken down when the pH falls below 5. The denatured protein reassembles through other hydrophobic molecules and casein intermolecular interactions. They contributed to the semisolid texture of yogurt.

### THE TITRATABLE ACIDITY OF GOAT MILK SYNBIOTIC-YOGURT

Based on this study, the addition of synbiotics (IEMA

with *Lactobacillus plantarum*) had no effect on the titratable acidity. The value of titratable acidity has been affected by pH reduction during fermentation by lactic acid bacteria due to an increase in the amount of lactic acid. El-Kholy et al. (2020) reported that the titratable acidity of yogurt synbiotics containing 2% inulin was value 0.967%. In the literature, Lim (2018) said that acidity levels of 1.0-1.1 % are known to be the best for improving yogurt quality. The difference in titratable acidity was caused by lactic acid produced by the hydrolysis of lactose during the fermentation by LAB as primer metabolic (Melia et al., 2021).

### THE PH OF GOAT MILK SYNBIOTIC-YOGURT

The addition synbiotic (IEMA with *Lactobacillus plantarum*) exhibited a lower pH value than the control sample. The addition of synbiotics has produced yogurt with a pH value of 5. However, the total LAB in the yogurt has a number of bacteria above the standard minimum of yogurt products, which was  $10^7$ . In our view, this was due to the difference in concentration of inulin extract from mangrove apple combined *Lactobacillus plantarum* could be attributed to the acidity. Some of the IEMA components are required for LAB biosynthesis. The lower pH of yogurt synbiotic was linked to LAB growth, implying that the probiotics could use carbohydrates as their primary carbon source and produce acid. During the fermentation process, lactose transforms into lactic acid, which causes a decrease in pH in yogurt. This result was similar to those of previous studies Jang et al. (2018) who recorded that the pH of yogurt probiotic of *Lactobacillus plantarum* and ginseng extract have values of 4.5 to 6.4. Lim (2018) reported that during yoghurt production, lactose in milk is degraded by the lactase enzyme of LAB and converted to lactic acid and acetaldehyde. Lactic acid causes some milk proteins to coagulate by lowering the pH of the product.

### THE VISCOSITY OF GOAT MILK SYNBIOTIC-YOGURT

The viscosity of goat milk yogurt was decreased with the addition of synbiotic. It was caused by the fact that synbiotic cultures have lower total solids than yogurt products. Furthermore, synbiotics have been added to yogurt products in liquid form, thereby reducing the viscosity. The viscosity value is also influenced by the exopolysaccharide produced of LAB. El-Kholy et al. (2020) pointed out the ability of the inulin to influence the water structure of low-fat synbiotics yogurt. Wihansah et al. (2018) discovered that lactic acid bacteria's exopolysaccharides influenced the viscosity of yogurt containing Rosella extract.

### THE TOTAL DISSOLVED SOLID OF GOAT MILK SYNBIOTIC-YOGURT

Based on this study, the total dissolved solid value may be due to the fact that inulin from extracted mangrove apple prebiotics was used to boost the growth of LAB in the pro-

duction of lactic acid. Ismawati et al (2016) reported that the LAB fermentation process produces metabolites in the form of lactic acid. The addition of inulin extracted mangrove apple and *Lactobacillus plantarum* promotes growing nutrients for lactic acid bacteria in the yogurt. Krasaekoopt and Watcharapoka (2014) reported that prebiotics is a factor in enhancing probiotic viability.

### THE TOTAL SOLUBLE DIETARY FIBRE OF GOAT MILK SYNBIOTIC-YOGURT

The total soluble dietary fibre of GMS-Y was affected by using different level concentrations of synbiotic. The total soluble dietary fibre increase may be due to inulin from extracted mangrove apples. Inulin is one of the sources of soluble dietary fibre. The soluble dietary fibre content was found in the inulin of mangrove apples at 9.67% (Wibawanti et al., 2021). As a result, adding inulin from extracted mangrove apple to yogurt could increase the amount of soluble dietary fibre.

### CONCLUSION

The study indicated that adding 8% synbiotic of inulin extracted mangrove apple and *Lactobacillus plantarum* in the GMS-Y had the highest effect on total LAB and the total soluble dietary fibre. The viability of lactic acid bacteria of GMS-Y was  $10^{10}$  CFU/mL with 8% synbiotic. Further, the addition of 8% synbiotic had the lowest pH value, viscosity, and total dissolved solid. However, the addition of synbiotic does not affect the titratable acidity of yogurt.

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### CONFLICT OF INTEREST

The authors clarify no conflict of interest with any financial, personal, or other relationships with other people or organisations related to the material discussed in the manuscript.

### NOVELTY STATEMENT

The study of goat milk synbiotics-yogurt using *Lactobacillus plantarum* and inulin of mangrove apple (*Sonneratia caseolaris*).

Jeki Mediantari Wahyu Wibawanti: contributed and implementation of the research. Sri Mulyani, Rudy Hartanto and Anang Mohamad Legowo were involved in planning and supervised the work. Ahmad Ni'matullah Al-Baarri, Yoyok Budi Pramono: helped wrote the manuscript in consultation. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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