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Physicochemical properties of goat milk yoghurt with synbiotics from inulin of mangrove apple and *Lactobacillus plantarum*

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Abstract. Physicochemical changes could affect the quality of goat milk yoghurt. Yoghurt quality has been improved by incorporating a synbiotic derived from a prebiotic combined with probiotics. This study aimed to evaluate the effect of different concentrations of synbiotics (inulin of mangrove apple and *Lactobacillus plantarum*) in the physicochemical properties of goat's milk yoghurt. This study used a Completely Randomized Design (CRD) with 5 treatments and 4 replications, with differences in the concentration of synbiotics as much as 0, 2, 4, 6, and 8%. The addition of synbiotics was significant ($p < 0.05$) in the sugar reduction, colour, and syneresis of yoghurt products. They did not significantly affect ($p > 0.05$) the firmness of yoghurt texture. This study provides support for the addition of synbiotics to improve physicochemical properties of goat's milk yogurt.

Key words: inulin, mangrove apple, physicochemical, synbiotics, yogurt.

INTRODUCTION

The addition of synbiotics to yoghurt products is one of the development trends for dairy products in Indonesia. Yoghurt has benefited as a portion of healthy food due to the beneficial effects of its bacteria which are typically attributed to the presence of probiotics and bioactive peptides (Mantzourani et al., 2022; Wang et al., 2022; Zahid et al., 2022). *Streptococcus thermophilus* and *Lactobacillus bulgaricus* combine their bacterial cultures to produce yoghurt (Sharma & Ramanathan, 2021; Mitra et al., 2022). Synergistic synbiotics, which contain prebiotics that can specifically stimulate probiotic growth, also have beneficial effects on digestive health (Lim, 2018). Yoghurt that contains both beneficial bacteria (probiotic microorganisms) and indigestible carbohydrates (prebiotic compounds) to encourage the growth of those bacteria is known as a synbiotic yoghurt (El-Kholy et al., 2020; Sakr & Massoud, 2021).

Several studies were related to synbiotic yoghurt including synbiotic yoghurt using puree banana and *Lactobacillus* (Fidina et al., 2018), goat milk of synbiotic yoghurt with sorghum flour and *Lactobacillus acidophilus* (Sukarmimah et al., 2019), synbiotic yoghurt containing *Bifidobacterium* and konjac mannan oligosaccharides (Li et al., 2021b), and synbiotic yoghurt with lactitol and *Lacticaseibacillus paracasei* (Li et al., 2022). Synbiotics could increase the effectiveness of probiotics (Shafi et al., 2019, Li et al., 2022). Prebiotics including inulin can promote the development of probiotic product (El-Kholy et al., 2020). Mangrove apples (*Sonneratia caseolaris*), have an inulin content of up to 5.08% (Wibawanti et al., 2021).

Yoghurt is one of the most popular fermentation products made from goat's milk (Park et al., 2019). Yoghurt is the preferred dairy product because of its unique qualities (Ehsani et al., 2018). One of the determinants of yoghurt quality are its physicochemical properties yoghurt (Wibawanti & Rinawidiastuti., 2018; Khalifa & Zakaria, 2019; Prayitno et al., 2020). The texture and appearance of yoghurt are important indicators of its quality and structure (Li et al., 2021a; Hu et al., 2022; Mitra et al., 2022). Yoghurt quality also varies based on colour, affecting how well consumers will accept the product (Ścibisz et al., 2019; Marand et al., 2020). Therefore, this study aimed to evaluate the effect of different concentrations of synbiotics from inulin of mangrove apple and *Lactobacillus plantarum* on the physicochemical properties of goat's milk yoghurt.

MATERIALS AND METHODS

Synbiotic preparation

The synbiotics were prepared by combining *Lactobacillus plantarum* and inulin extract of mangrove apple as a prebiotic according to Setyaningrum et al. (2019), with a few modifications. Mangrove apple inulin was extracted using the procedure reported by Wibawanti et al. (2022). The mangrove apple was divided into tiny pieces and heated to 90 °C. Mangrove apple was extracted in hot water at 90 °C for 60 minutes using a 1:4 ratio of fruit to water. The filtrates were kept at -18 °C after being precipitated with 40% ethanol. The filtrate was thawed to room temperature. The filtrate of the mangrove apple's inulin was centrifuged at 5,000 rpm for five minutes before the supernatant was taken out. The synbiotics were made from 10 mL *Lactobacillus plantarum* and 9% inulin from mangrove apples. They were incubated anaerobically in Man Rogosa and Sharpe (MRS) Broth for 24 hours at 37 °C.

Yoghurt preparation

Freeze-dried bacterial cultures, namely *Lactobacillus bulgaricus* (FNCC 0041) (2.89×10^8 CFU/mL), *Streptococcus thermophilus* (FNCC 0040) (9.6×10^7 CFU mL^{-1}), and *Lactobacillus plantarum* (FNCC 0026) (2.25×10^8 CFU mL^{-1}), were purchased from Gadjah Mada University. These strains were thawed at room temperature in a Man Rogosa and Sharpe (MRS) Broth at the ideal temperature (37 °C) before the experiments to produce fresh cultures from frozen stocks (Fan et al., 2022).

Five different yoghurts were produced using a combination of the *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains, with four replications. Synbiotic yoghurt was done by method Sharma & Ramantha's (2021). The fresh milk from the goat was heated at 80 °C for 15 min. It was cooled at a temperature of 45–42 °C for 20 minutes. The starter culture consisting of *Streptococcus thermophilus* and

Lactobacillus bulgaricus was added with the ratio of maximum 5%. The treatment of synbiotics (*Lactobacillus plantarum* and inulin of mangrove apple) was applied with different concentrations of as much as 0 (control), 2, 4, 6, and 8% (v/v). For five hours, the incubation was carried out at 42 °C. The yoghurt synbiotic was kept at refrigeration temperature (4 ± 1 °C) to calculate coagulation.

Reducing sugar

Reducing sugar in the synbiotic yoghurt was determined using the Somogyi - Nelsen method previously described by Wibawanti et al. (2022). One gram of synbiotic yoghurt samples was prepared. The samples were added to the volume with distilled water until it reached 100 mL. The samples were centrifuged and then filtered. One mL of the filtrate was dissolved in one mL of Nelson reagent. The solution was heated over a waterbath with a temperature of 100 °C for 30 minutes. The mixture was cooled to 25 °C, 1 mL of the arsenomolybdate reagent was added, and it was agitated. Then, 10 mL of distilled water was added. The absorbance of the vortex solution was then measured using a spectrophotometer with a wavelength of 540 nm.

The firmness texture

The firmness texture of yoghurt were analysed using a texture analyser, according to Al-Sahlany et al. (2022), with modification. The yoghurt samples were served in cylindrical glass cups (50 mm diameter, 75 mm high) and then measured immediately after being removed from the fridge (4 °C). The sample compression was performed to 50% of their original height with probe P/35. A 50-kg load cell was used at a crosshead speed of 1 $mmVs^{-1}$.

Colour measurement

The yoghurt samples were poured into tubes and colour analysis was carried out using Colorimeter as previously described by Qiu et al. (2021). Values of L* (lightness), a*(red-green) and b* (yellow-blue) were evaluated.

Syneresis yoghurt

The syneresis of the yoghurt was evaluated using the centrifugation method, which was modified from the method proposed by Pereira et al. (2021).Yoghurt samples of 10 mL were placed in 15 mL Falcone tubes previously weighed and centrifuged at 3,500 rpm for 15 min at 4 °C. The syneresis index was calculated as a percentage of the weight of the serum in relation to the initial weight of the yoghurt after the supernatant serum was removed and weighed.

Statistical analysis

The mean value of four measurements was taken for each parameter assessed in the study. Data obtained from the Completely Randomized Design (CRD) study were evaluated statistically using variance analysis (ANOVA) and Duncan's new multiple-range tests. SPSS 16.0 software was used to statistically analyze all of the results.

RESULTS AND DISCUSSION

Reducing Sugar

Statistical analysis showed that the addition of synbiotics had a significant effect ($p < 0.05$) on reducing sugar from yoghurt. The results of the reducing sugar is presented in Fig. 1. Goat milk yoghurt with synbiotic treatment had a significant effect ($p < 0.05$)on the value of reducing sugar. The addition of synbiotics resulted in lower reducing sugar content ($p < 0.05$) compared to the yoghurt control. Reducing sugar in yoghurt without treatment (control) of 4.06% ± 0.28 was not significantly different ($p > 0.05$) with the addition of 2% or 4% synbiotic with a value of 3.86% ± 0.15. The addition of synbiotics with a concentration of 6% showed results that were not significantly different ($p > 0.05$) with a concentration of 8% with a value of 3.53% ± 0.16 and 3.68 ± 0.08, respectively.

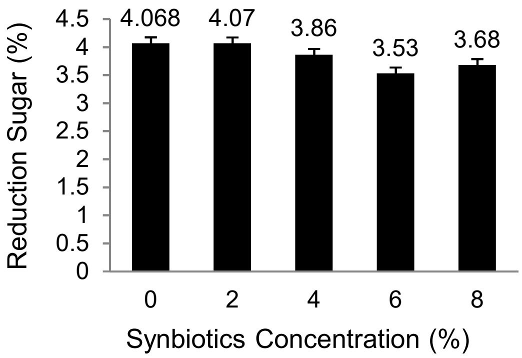

Figure 1. The sugar reduction of synbiotic yoghurt.

The reduced sugar content of synbiotic goat milk yoghurt was significantly lower than that of yoghurt without synbiotics. The decrease in sugar reduction value is thought to be due to inulin from mangrove apple extract contained in prebiotics used as a source for the growth of lactic acid bacteria, both *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus plantarum* in yoghurt, in order to increase the viability of probiotics. Krasaekoopt & Watcharapoka, (2014) reported that the use of prebiotics is a factor that can increase the viability of probiotics. Hartati et al. (2012) reported that the total amount of reduced sugar in yoghurt can support the growth of lactic acid bacteria.

The firmness texture of synbiotics yoghurt

Firmness texture is one of the determining factors for assessing the physical characteristics of yoghurt. Firmness texture is used to measure the maximum force of the material at a certain deformation in the sample, which directly reflects the yoghurt's gel strength (Li et al., 2022). The firmness results are shown in Fig. 2. Statistical analysis revealed that the addition of synbiotics did not have a significant ($p > 0.05$) effect on the firmness of yoghurt. The addition of 0, 2, 4, 6, and 8% synbiotics showed results that were not significantly different with value of 10.64 ± 2.25, 10.67 ± 1.00, 11.65 ± 1.44, 10.65 ± 1.53, and 8.5 ± 0.73 g, respectively.

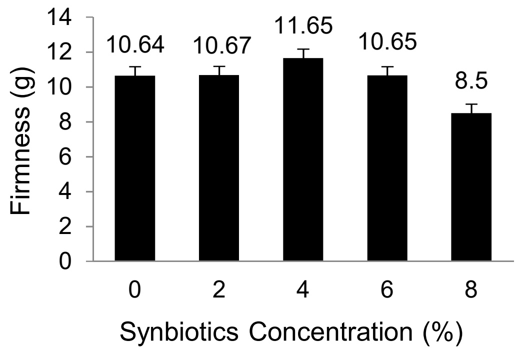

Figure 2. The Firmness texture of synbiotic yoghurt.

The inulin content of mangrove apples as a polysaccharide in synbiotics influences the texture of yoghurt. Synbiotic yoghurt's firmness is also influenced by the protein content. Nadtochii et al. (2020) reported that polysaccharides could be used in dairy products to modify the rheological properties. The texture of yoghurt is directly dependent on the type of protein, the protein content, and the total solids yoghurt (Ścibisz et al., 2019). The texture is also caused by the interaction of proteins, fats, and water in the food matrix (Mitra et al., 2022). Hu et al. (2022) reported that during yoghurt production, stabilizers were added to bind free water as hydration and stabilize protein molecules in the network through covalent or electrostatic interactions.

Colour property

The lightness (L) of synbiotic yoghurt is shown in Fig. 3. Results based on the research showed that the addition of synbiotics had an effect on the colour of the lightness (L) of yoghurt ($p < 0.05$). The addition of synbiotics showed significantly different results ($p < 0.05$) on the lightness colour of synbiotic yoghurt. The addition of synbiotics with concentrations of 0, 2, 4, and 6% showed no different results between treatments ($p > 0.05$) with average values of 88.41 ± 3.81; 88.53 ± 1.03; 89.19 ± 0.56; 89.65 ± 0.14, respectively. The addition of synbiotics with a concentration of 8% showedsignificantly different results ($p < 0.05$) when compared to other treatments with a value of 81.31 ± 8.53.

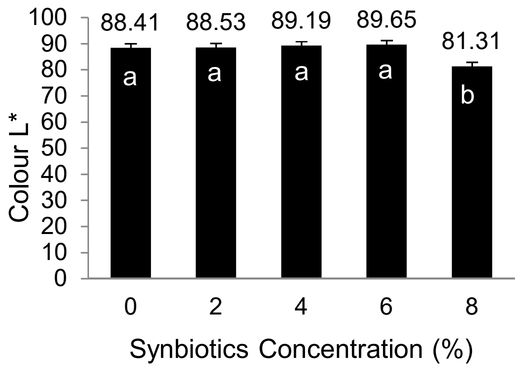

Figure 3. The lightness (L) colour of synbiotic yoghurt.

The results showed that an 8% synbiotic concentration decreased the lightness value of yogurt. This is because inulin from mangrove apples has a brownish color, which causes the lightness value to be lower when added to goat's milk yogurt. Qiu et al. (2021) reported that the the addition of rose extracts can reduce the lightness of the yogurt color.

The redness (a*) colour of synbiotic yoghurt is presented in Fig. 4. The addition of synbiotics showed no significant ($p > 0.05$) effect on the redness colour of symbiotic.

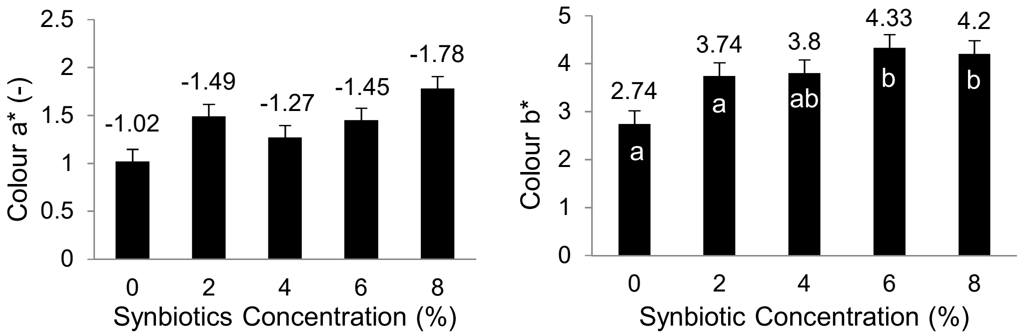

Figure 4. The redness (a*) and yellowness (b*) color of synbiotic yoghurt.

The redness (a*) colour of synbiotic yoghurt is presented in Fig. 4. The addition of synbiotics showed no significant ($p > 0.05$) effect on the redness colour of synbiotic yoghurt. The redness of yoghurt with the different concentrations of 0, 2, 4, 6, and 8% was the value -1,02 ± 0.52; -1.49 ± 0.23; -1.27 ± 0.26; -1.45 ± 0.43; and -1.78 ± 1.9, respectively. The yellowness (b*) colour of synbiotic yoghurt is shown in Fig. 4. The addition of synbiotics showed significant results ($p < 0.05$) on the yellowness colour of synbiotic yoghurt. The yellowness colour of yoghurt with the different concentrations of 0, 2, and 4% was the value 2.74 ± 1.0; 3.74 ± 0.52; 3.8 ± 0.28. The addition of synbiotics at concentrations of 6 and 8% did not result in a statistically significant difference, with values of 4.33 ± 1.0 and 4.2 ± 0.5. The addition of synbiotics at concentrations of 0 and 2% resulted in a statistically significant difference when compared to concentrations of 6 and 8%. This is due to the presence of pigments in mangrove apples as a source of prebiotics. Mangrove apples contain phenol components that may contribute to the colour of yoghurt. Pourjavid et al. (2022) reported a change in the colour parameters of a synbiotic yoghurt in the presence of natural prebiotics and *Lactobacillus paracasei* microencapsulation. The colour of yoghurt can also be influenced by adding natural plants that contain pigments (Fan et al., 2022). Ścibisz et al. (2019) reported that the colour of yoghurt indicates the concentration of pigments in the product. The water-soluble plant pigments anthocyanins are among the phenolic compounds (Khoo et al., 2017).

Yoghurt syneresis

The results of the study on the syneresis value of synbiotic yoghurt are presented in Fig. 5. The results showed that the synbiotic addition of inulin of mangrove apple with *Lactobacillus plantarum* with different concentrations had a significant effect ($p < 0.05$) on the syneresis value of yoghurt. It caused may be due to the extensive protein-carbohydrate interactions produced by inulin. It can extend its branched structure more evenly into casein aggregates. According to Gomes et al. (2022) that protein and carbohydrate interaction results in a decrease in syneresis and an increase in gel stability. Milk protein exposed to lactic acid (LA) produced by lactic acid bacteria coagulates to form a compact structure (Hosseini & Behbahani, 2021). Zakaria et al. (2020) reported that the decreased syneresis in the yoghurt can occur with an increase in total solids content and absorption of excess whey by lactoferrin powder.

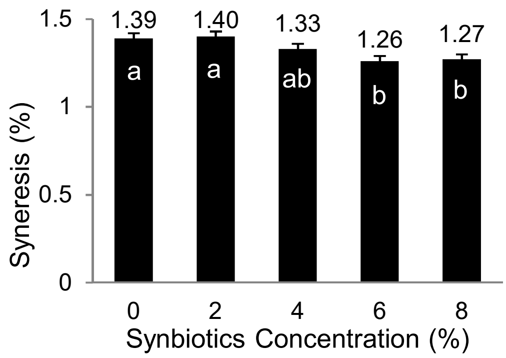

Figure 5. The syneresis of synbiotic yoghurt.

The addition of synbiotics showed significantly different results ($p < 0.05$) on the syneresis value of yoghurt. The addition of synbiotics with concentrations of 0, 2, and 4% showed non-significant results between treatments ($p > 0.05$) with syneresis values of 1.39 ± 0.01, 1.4 ± 0.13, and 1.33 ± 0.11%. The addition of synbiotics with concentrations of 6 and 8% showed significantly different results ($p < 0.05$) when compared to other treatments with syneresis values of 1.26 ± 0.05 and 1.27 ± 0.01%.

The addition of synbiotics to yoghurt showed lower syneresis results. The addition of synbiotics to yoghurt with various concentrations affects the total dissolved solids, thus causing differences in syneresis yoghurt. The syneresis was lower in yoghurts with the addition of synbiotics 6 and 8% compared with control yoghurt. Inulin in the synbiotics has a stabilizer in yoghurt products. Benmeziane et al. (2021) reported that stabilizers like pectin, starches, gums, or whey protein concentrate can be added to prevent syneresis. Setyawardani et al. (2020) stated that high-quality fermented products with low syneresis. Park et al. (2019) reported that syneresis can be avoided by increasing stabilizers such as pectin, starches, gums, or whey protein concentrate. In the literature, Celik & Temiz, (2022) reported that syneresis in yoghurt was associated with the dry matter content, more specifically the protein content.

CONCLUSIONS

The addition of 8% synbiotic (inulin of mangrove apple and *Lactobacillus plantarum*) to goat milk yoghurt could improve the quality of physicochemical properties including reduced sugar, colour, and syneresis. However, the addition of synbiotics to the firmness texture was not significant.

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