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ORIGINAL ARTICLE

Physicochemical and amino acid profiles of probiotic yoghurt with the addition of podang urang mango (*Mangifera indica* L.) extract

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Abstract

Yoghurt as a milk fermentation product has been popular for several years because of its probiotic properties. Probiotic yoghurt is processed by the heated milk then added with lactic acid bacteria and allowed to ferment. However, the series of processes makes some of the nutritional content of milk lost. Therefore, probiotic yoghurt needs to replace this nutritional loss and improve the yoghurt quality. The addition of herbs or natural ingredients could be an effective way to improve the functional properties of yoghurt, such as the addition of Podang Urang mango extract (*Mangifera indica* L.). This research aimed to recognize the physicochemical, and amino acid profiles of probiotic yoghurt through the addition of Podang Urang mango extract. The addition of Podang Urang mango extracts into yoghurt has significantly affected titratable acid (TA), viscosity, moisture, ash, protein, fat, and lactic acid bacteria (LAB). However, it did not significantly affect water activity and pH. The highest levels of amino acids in yoghurt with the addition of Podang Urang mango extract were leucine and lysine. There was a significant difference in alanine, arginine, tyrosine, proline, and histidine in the percentage of Podang Urang mango extract, while the other amino acids showed insignificant differences.

Keywords: Extraction; Lactic acid bacteria; *L. bulgaricus* RRAM-01; Natural ingredient; *S. thermophilus* RRAM-01.

Highlights

- Processing probiotic yoghurt by heating and fermenting milk, makes some milk nutritional content lost
- Podang Urang mango is one local fruit from Kediri Indonesia and has a lot of starch, high cellulose, hemicellulose, lignin, carotin, and organic acids, such as acetic acid, ascorbate, citrate, butyrate, propionate
- Yoghurt has a higher nutritional value than fresh milk mainly due to the increase in total solids, fat, and protein. The lactose component in yoghurt is less than fresh milk and the shelf life of yoghurt is longer than fresh milk



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- The addition of Podang Urang mango extracts in probiotic yoghurt significantly affected titratable acid (TA), viscosity, moisture, ash, protein, fat, and lactic acid bacteria (LAB)
- The addition of Podang Urang mango extracts in probiotic yoghurt showed significant differences in alanine, arginine, tyrosine, proline, and histidine, while the other amino acids showed insignificant differences

1 Introduction

Yoghurt is considered to be the most well-known dairy product in the world (Pomsanam et al., 2014). In the last few years, the trend of yoghurt consumption is gradually increased with consumer consciousness of functional drink healthy food (Ganato et al., 2010). Yoghurt is a fermented dairy product obtained from fermented milk by two species of lactic acid bacteria (LAB), *Lactobacillus bulgaricus* and *Streptococcus thermophilus* or another LAB as appropriate, with or without the addition of other foodstuffs and permitted food additives (Tewari et al., 2019). According to Mohammed et al. (2022), yoghurt has a higher nutritional value than fresh milk as a basic ingredient in the manufacture of yoghurt, mainly due to the increase in some components, such as total solids, fat, and protein. Trachopoulos (2002) reported that the lactose component in yoghurt was 1,7% less than the initial ingredients because LAB can ferment glucose, fructose, galactose and lactose to lactic acid. The accumulation of lactic acid in yoghurt causes a decrease in pH (Costa et al., 2013). This acidic atmosphere is not favored by pathogenic bacteria which causes the shelf life of yoghurt longer than fresh milk.

Yoghurt can be a probiotic drink if the added bacteria are probiotic bacteria. Previously, two Indonesian bacteria, *L. delbrueckii* subsp *bulgaricus* RRAM-01 and *S. salivarius* subsp *thermophilus* RR 261-01 were isolated from a local farm and met the requirements to be classified as probiotics (Arief et al., 2010). Probiotics, defined as live microorganisms once ingested in adequate amounts, have the potential to improve health and nutrition in consumers (Bajaj et al., 2015). *Lactobacillus bulgaricus* and *S. thermophilus* yoghurt bacteria are unable to survive in the gastrointestinal tract, so they cannot live properly in the human gastrointestinal tract (Lourens-Hattingh & Viljoen, 2001). Therefore, probiotic bacteria are added to adapt, multiply, and ferment substrates, and also produce antimicrobial substances in the human gastrointestinal tract, so as to maintain the stability of the intestinal microflora (Lee & Salminen, 2009). Probiotic yoghurt is processed by first heated milk, then adding LAB and left to ferment. However, the series of processes makes some of the milk's nutritional content lost due to the heating process.

In addition, an unattracted fermentation process can reduce the composition of milk. Aritonang (2007) stated that long and unprotected storage of yoghurt will reduce physicochemical quality. Probiotic yoghurt is processed by first heated milk, then adding LAB, and left to ferment. However, the series of processes makes some nutritional content lost due to the manufacturing process. Sterilization causes the loss of vitamins, and folic acid as well as causing denaturation of serum proteins (Aritonang, 2017). Therefore, there is a need for supplementation in probiotic yoghurt to increase the content of nutritional loss during the manufacturing process. In order to improve the quality, flavouring ingredients have been added during the yoghurt manufacturing process. Mostly, artificial flavours are used which are sometimes based on a chemical basis that is unfavourable to the body. The use of natural flavours is an alternative way that can improve organoleptic quality, and be more beneficial to the consumer. The addition of natural ingredients could be an effective strategy to improve yoghurt quality with respect to health benefits, food safety, and bio-preservation (Aswal et al., 2012). One of which is worth trying is the addition of Podang Urang mango extract (*Mangifera Indica* L.).

Podang Urang mango is one of the local fruits that are abundant in the Kediri Regency. According to the BPS (Badan Pusat Statistik, 2021), the amount of production of Mangoes in Kediri Regency was 227,127 quintals. Podang Urang mango is one of the specific flagship fruits in Kediri Regency, East Java. The highest fruit production occurs in the harvest season, which is from September until December (Baswarsati & Yuniarti, 2007).

Podang Urang mango has a lot of starch, high cellulose, hemicellulose, lignin and carotin (Paramita, 2012). High levels of beta carotene in podang urang mango are expected to have a lot of benefits for humans. Carotin

itself can be converted into vitamin A or it is referred to as provitamin A. Provitamin A is essential to be consumed to prevent blindness. In addition, beta carotene is also an antioxidant that can help to fight free radicals that can damage DNA and immune system function (Azqueta & Collins, 2012). Suwardike et al. (2018) reported antioxidant activity in mangoes depending on the variety and type, including ascorbic acid, carotenoids, and phenols. The diversity of antioxidants and acidity levels in Podang Urang mango can affect the physicochemical and amino acid profile. Based on the background above, it is important to conduct research on the addition of Podang Urang mango extract to recognize the benefits, physicochemical and amino acid profiles of probiotic yoghurt.

2 Material and method

2.1 Material

Cow milk, as the basic material, was collected from KUD Karya Bhakti Ngancar Kediri. Starter yoghurt (*L. delbrueckii* subsp *bulgaricus* RRAM-01 and *S. salivarius* subsp *thermophilus* RRAM-01 which were isolated from fresh milk of a local farm in Bogor) which also culture collection of Animal Product Technology Division, Faculty of Animal Science, Institut Pertanian Bogor (IPB) University. Podang Urang mango (*Mangifera Indica* L.), as the local fruit from Banyakan, Kediri.

2.2 Method

This research has been carried out in the Animal Science and Biology Laboratory, at Kadiri Islamic University according to the scheme (Figure 1).

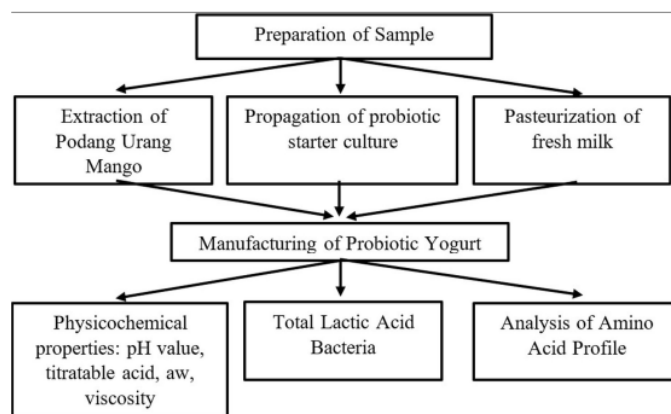


Figure 1. Research scheme for making yoghurt from Podang Urang Mango extract, probiotic culture starter, milk and the observed variables.

1. 1. Preparation of samples and extraction of Podang Urang mango (Wijana & Titik, 2017)

Podang Urang mango fruits were obtained from the district of Banyakan Kediri. The mango was taken on 7-20 days after harvest, Podang Urang mangoes were extracted using hexane: acetone: ethanol solution at a ratio of 2:1:1 until a filtrate was formed. The filtrate was then evaporated to obtain a dry extract

2. 2. Preparation of probiotic starter cultures (Suharto et al., 2016)

Starter cultures used in this research were *L. delbrueckii* subsp *bulgaricus* RRAM-01 and *S. salivarius* subsp *thermophilus* RRAM-01. Starter rejuvenation was carried out by inoculating yoghurt culture as much as 10%

into sterilized milk first on the autoclave at a temperature of 115 °C for three minutes. Furthermore, it was incubated at a temperature of 37 °C for 18-24 hours until coagulated so that a work culture could be obtained.

3. 3. Making probiotic yoghurt with the addition of Podang Urang mango extract (Sihombing et al., 2015)

Yoghurt was first produced by refreshing the bacteria of *L. delbrueckii* subsp *bulgaricus* RRAM-01 and *S. salivarius* subsp *thermophilus* RRAM-01. 3% of such bacterial cultures were added to pasteurized milk at a temperature of 85 °C for 24 hours and 35 minutes. Podang Urang mango extract added as much as 0% (P1), 2% (P2), 4% (P3), 6% (P4), 8% (P5) and 10% (P6). The Yoghurt was then placed in an incubator until thickened.

4. 4. Physicochemical properties

a. pH value (Matela et al., 2019)

A total of 10 g of samples was dissolved in 100 mL of distilled water in a glass cup. The mixture was determined by pH meter. The tool was calibrated using 2 buffer solutions representing a low pH (4.00) and high pH (7.00). The pH meter electrode was dipped in the sample until some number could be read on the pH meter screen and classified that it was a pH value. Measurement of pH values was done duplo for each sample.

b. Titratable acid (TA) (Ibrahim et al., 2019)

The percentage of lactic acid was measured through titration methods for a sample of 10 mL added 2 drops of phenolphthalein 1% indicator then titrated with NaOH solution 0.1 N until it was neutralized and turned pink. Each sample was titrated three times.

c. Water Activity (aw) (Wihansah et al., 2018)

Water activity was measured using aw meters (Novasiana-Ms1). The tool was first calibrated with a saturated NaCl solution before being used. The saturated NaCl solution was inserted into the measurement chamber, and then the device was turned on by pressing the start button and waiting until the aw read 0.750 to 0.752. Next, the sample was entered into the chamber, and then the start button was pressed and waited until the aw value could be read. Samples were duplo-measured.

d. Viscosity (Lindasari, 2013)

The viscosity of yoghurt was analyzed using a viscometer. The sample was put in a container and arranged at room temperature (25 °C to 27 °C).

5. 5. Total Lactic Acid Bacteria (LAB) (Hanifah et al., 2016)

The 1 mL of *L. delbrueckii* subsp *bulgaricus* RRAM-01 and *S. salivarius* subsp *thermophilus* RRAM-01 were added with buffer peptone water (BPW) until the concentration of LAB reached 10%. This solution was diluted until the concentration of 10 to 10⁸ times. The dilution from 10⁶ to 10⁸ was poured into a petri dish that was added with MRSA and then incubated at 37 °C for 24 to 48 hours.

6. 6. Analysis of Amino Acid Profile (Gandjar & Rohman, 2007).

The amino acid composition in yoghurt was analyzed using Ultra Performance Liquid Chromatography (UPLC).

7. 7. Statistical analysis

The experimental design used in this research was a completely randomized design (CRD) and all the data was analysed using Analysis of Variance (ANOVA) with Duncan's Multiple Range Test at a significance level of 0.05% ($p < 0.05$).

3 Results and discussion

Raw cow's milk used in this study was obtained from a local farm in Kediri Indonesia. This milk was analyzed for pH and nutrient content as shown in Table 1. In the current study, the measured pH value was 6.74 ± 0.01 , moisture $88.96\% \pm 0.16\%$, ash $0.86\% \pm 0.08\%$, protein $4.06\% \pm 0.12\%$, and fat $3.41\% \pm 1.10\%$. The results of this study are in accordance with Moghaddam et al. (2014). The varying composition of cow's

milk was influenced by nutritional factors, environment, lactation period, and season (Park, 2006). Most of these parameters matched the Indonesian quality standard of the National Standardized Agency regarding the quality standard of fresh milk, including pH, moisture, ash, protein and fat (Ariani et al., 2021)

Table 1. Composition of raw cow's milk.

Items	Values
pH	6.74 ± 0.01
Moisture (%)	88.96 ± 0.16
Ash (%)	0.86 ± 0.08
Protein (%)	4.06 ± 0.12
Fat (%)	3.41 ± 1.10

Podang Urang mango extract is a tropical fruit with a yellow to orange colour (Afiyah et al., 2022a). After the Podang Urang mango was extracted, then it was analyzed and the measurement results can be seen in Table 2. The measurement results of pH value and TA showed that Podang Urang mango extract had a pH of 5.11 ± 0.01. This result was slightly higher than the research by Hidayat et al. (2013) that showed mango extract pH was 4.42. The low pH is due to organic acids, such as acetic acid, ascorbate, citrate, butyrate, and propionate component in mango (Utama et al., 2016).

Table 2. Composition of Podang Urang mango extract.

Items	Values
pH	5.11 ± 0.01
Titrateable acid (TA)	0.12 ± 0.01

The yoghurt physicochemical composition with the addition of Podang Urang mango extract is shown in Table 3. The first composition measure as pH. The results indicated that the pH of yoghurt was not affected ($p > 0.05$) by the Podang Urang mango extracts. This is due to the relatively low water content in the extracts. This pH result was similar to that research of Amadou et al. (2018) who obtained a non-significant of pH reduction due to the addition of Podang Urang mango extract. The pH values of this yoghurt were similar to the normal pH value which is between 4.0 and 4.1 and in appropriate with the standard of the Food and Drug Administration (FDA), comprising values ≤ 4.6 (Food and Drug Administration, 2009). This result indicated the ability of *L. delbrueckii* subsp *bulgaricus* RRAM-01 and *S. salivarius* subsp *thermophilus* RRAM-01 to convert lactose into lactic acid if incubated at their optimum temperature (40-45 °C). In general, the pH value of the yoghurt depends on milk composition such as lactose, as well as the ingredients that were added, and the activity of LAB added (Afiyah et al., 2022c).

Table 3. Composition of yoghurt with the addition of Podang Urang mango extract.

Parameters	P1	P2	P3	P4	P5	P6
pH	4.20 ^a ± 0.03	4.17 ^a ± 0.01	4.15 ^a ± 0.01	4.13 ^a ± 0.07	4.13 ^a ± 0.08	4.13 ^a ± 0.07
Titrateable acid	1.2 ^a ± 0.01	1.03 ^a ± 0.01	1.03 ^a ± 0.01	1.04 ^b ± 0.01	1.04 ^b ± 0.01	1.04 ^b ± 0.01
aw	0.96 ^a ± 0.01	0.96 ^a ± 0.01	0.96 ^a ± 0.01	0.97 ^a ± 0.01	0.97 ^a ± 0.01	0.96 ^a ± 0.01
Viscosity (dpa)	2.42 ^a ± 0.51	2.44 ^a ± 0.15	2.45 ^a ± 0.36	2.89 ^a ± 0.30	2.90 ^a ± 0.79	2.98 ^a ± 0.66
Moisture (%)	86.54 ^b ± 0.30	86.52 ^b ± 0.35	86.50 ^b ± 0.45	86.23 ^{ab} ± 0.81	86.19 ^a ± 0.81	85.30 ^{ab} ± 0.85
Ash (%)	0.82 ^d ± 0.01	0.81 ^d ± 0.01	0.79 ^c ± 0.01	0.78 ^b ± 0.01	0.78 ^b ± 0.01	0.76 ^a ± 0.01
Protein (%)	3.67 ^a ± 0.06	3.68 ^a ± 0.06	3.70 ^a ± 0.05	3.79 ^{ab} ± 0.06	3.89 ^b ± 0.06	3.89 ^b ± 0.18
Fat (%)	3.19 ^c ± 0.23	3.09 ^{bc} ± 0.17	3.01 ^{abc} ± 0.14	2.98 ^{abc} ± 0.15	2.91 ^{ab} ± 0.05	2.81 ^a ± 0.04
LAB (log cfu/mL)	10.88 ^a ± 0.82	9.56 ^a ± 0.31	10.00 ^a ± 0.00	11.36 ^b ± 0.82	11.79 ^b ± 0.86	11.59 ^b ± 0.51

Different letters following values on the same line indicate statistically significant differences ($p < 0.05$).

There is an increase in yoghurt TA according to the increase of Podang Urang mango extract concentration which is similar to those observations by Njoya et al. (2016). The higher Podang Urang extract added, the higher TA value. This is because Podang Urang mango has sugars which are material for the production of lactic acid which leads to an increase of titratable acid. The TA of yoghurt depends on the activity of LAB, the nutrients and water activity.

Table 3 presents that increasing Podang Urang mango extract led to an increase in the viscosity of the yoghurt. Yoghurt with a high amount of Podang Urang mango extract had a higher viscosity. The differences in viscosities because of high formulations of Podang Urang mango extract had enough fermenting sugar from milk and Podang Urang mango required by LAB. The presence of Podang Urang mango extract helped fermentation to produce more lactic acid (Kpodo et al., 2014).

The increase of Podang Urang mango extract led to a decreasing moisture of yoghurt. Increasing moisture of yoghurt related to the low-water content of Podang Urang mango extract is due to *L. delbrueckii* subsp *bulgaricus* RRAM-01 and *S. salivarius* subsp *thermophilus* RRAM-01, which were associated with a homofermentative type of LAB who produces lactic acid and does not produce water or air.

In general, the dry matter content of yoghurt with the addition of Podang Urang mango extract was caused by many factors including the milk composition, quantity, and ingredients used. The results of this study that the yoghurt water content obtained ranged from 85% to 86% was not much different from the result of Hartini (2016) about the addition of cinnamon bark extract in the yoghurt. The water content of this yoghurt was much higher than commercial yoghurt. According to Sagita et al. (2020), the moisture content of commercial yoghurt was 75-80%. This was due to a lot of additional ingredients which could make the water content of commercial yoghurt have lower value.

The plain of the yoghurt sample presented the highest value of ash content. Yoghurt ash content was reduced with the increase of Podang Urang mango extract and it could be due to the low ash content of the Podang Urang mango extract. The yoghurt ash content depends on many factors, including the milk composition, the quantity, and the nature of the ingredients used. According to Hanifah et al. (2016), the addition of Podang Urang mango extracts changed some compounds in yoghurt, especially for ash and fat contents. Fat content was slightly reduced by the addition of Podang Urang mango extract, but ash content had slightly increased. This might be due to the metabolism of LAB in yoghurt or the presence of Podang Urang mango extract.

The plain yoghurt sample showed the highest ($p < 0.05$) fat content. Fat content was reduced with the increase of Podang Urang mango extract concentration in yoghurt. This fat content reduction was because of the low fat content of the Podang Urang mango extract (Njoya et al., 2016). There was only a minimal starter role in breaking milk fat into free fatty acids. According to Smith et al. (2016), during the fermentation, the bacteria through three main reactions, decomposed lactose into lactic acid (fermentation), hydrolysing casein into peptides and free amino acids (proteolysis), and break down the milk fat into free fatty acids (lipolysis).

Based on the measurement of protein content above, it shows that the increase in protein content is proportional to the addition of Podang Urang mango extract. The addition of Podang Urang mango extract caused high protein content. The crude protein content of the yoghurt with Podang Urang mango extract had increased due to the breakdown of protein by proteolytic organisms, producing a larger amount of free amino groups (Li et al., 2019).

The total LAB of yoghurt in this research is presented in Table 3. There was a significant increase in the total LAB with the addition of Podang Urang mango extract. According to Askar & Sugiarto (2005), acidity in yoghurt is the major product of LAB fermentation. The number of LAB is an indicator of the microbiological quality of a fermented milk product. LAB is a group of gram-positive bacteria that do not form spores and can ferment carbohydrates to produce lactic acid. However, yoghurt with Podang Urang Mango extracts has fulfilled the quality requirements specified by [Afiyah et al., 2022b] at least 10^7 CFU mL⁻¹.

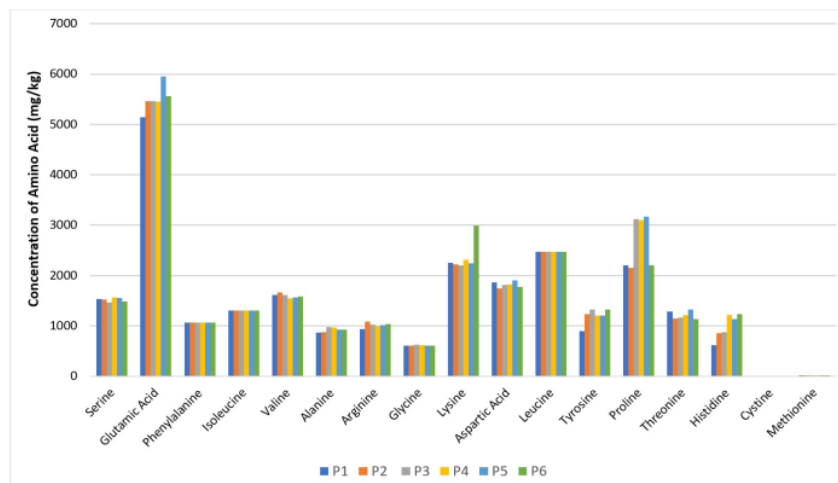


Figure 2. Amino acid profiles of yoghurt with Podang Urang mango extract.

The amino acids are the building blocks of protein, and their composition is unique for each type of protein. Protein can be broken down into simpler units (amino acids) through the hydrolysis process (Nelson & Cox, 2004). The amino acid composition of yoghurt with the addition of Podang Urang mango extract is presented in Figure 2. The detectable amino acids are serine, glutamic acid, phenylalanine, isoleucine, valine, alanine, arginine, glycine, lysine, aspartic acid, leucine, tyrosine, proline, threonine, histidine, and methionine. This amino acid can be detected in yoghurt because of the manufacturing process and LAB activity. The first step of amino acid changes is the pasteurization fermentation process resulting in the denaturation of protein. Germani et al. (2014) reported that free amino acids are used by *S. thermophilus* and *L. bulgaricus* especially: glutamic acid, histidine, tryptophan, arginine, tyrosine, large amounts of branch chain amino acid (valine, leucine, isoleucine) and sulfur amino acids (methionine and cystine).

The high levels of amino acids in yoghurt with the addition of Podang Urang mango extract are leucine and lysine. The results showed that there was a significant difference in alanine, arginine, tyrosine, proline, and histidine in the percentage of Podang Urang mango extract, while the other amino acid showed insignificant differences. The different percentage of yoghurt was caused by the raw material used and the activity of LAB. In fact, LAB in the fermentation process required protein as an energy source (Chalid et al., 2021). Germani et al. (2014) reported that *S. thermophilus* uses especially histidine, arginine, tyrosine, valine, leucine, isoleucine, methionine and cystine to produce acetaldehyde, a molecule which contributes to the development of the aroma in the yoghurt. With this bacteria activity, if there is still an increase in amino acids, it means the activity of releasing amino acids is greater than their utilization.

4 Conclusion

The results of this research allow us to draw the conclusion that the milk utilized in this study met the standards for pH, moisture, ash, protein, and fat. Podang Urang mango extract had low pH due to organic acids, such as acetic acid, ascorbate, citrate, butyrate, and propionate. The addition of Podang Urang mango extracts in yoghurt significantly affected TA, viscosity, moisture, ash, protein, fat, and LAB. However, it did not significantly affect water activity and pH. The highest levels of amino acids in yoghurt with the addition of Podang Urang mango extract are leucine and lysine. The results showed that there was a significant difference in alanine, arginine, tyrosine, proline, and histidine in the percentage of Podang Urang mango extract, while the other amino acid showed insignificant differences.

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