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Research

The Microbiological and Chemical Characteristics of Yoghurt Incorporated With Watermelon Rind Powder

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ABSTRACT

Watermelon rind accounts for approximately one-third of the overall fruit mass. It is usually discarded due to its low commercial value. However, it is reported to contain valuable nutrients and is an effective source of pectin that can act as a potential prebiotic. This study aimed to study the effects of watermelon rind powder (WRP) on the growth of probiotic bacteria in yoghurt and its chemical characteristics. Watermelon rind was dried by using a dehydrator and ground into powder form before being incorporated into fresh yoghurt at 2% and 4% w/v. A sample with 0% w/v WRP was prepared as control. The effect of WRP on the growth of probiotic bacteria was determined by MRS plate count. Chemical analyses including titratable acidity, pH and Brix were conducted during the fermentation process. The results showed that the increase in WRP percentage resulted in a significant increase in bacterial growth with 7.20 ± 0.22 log CFU/mL for the control sample as compared to 8.42 ± 0.23 log CFU/mL for sample with 4% WRP after 30 hr of incubation. The fermentation time was also improved with the presence of WRP with a 0.22 h^{-1} increase in growth rate observed for the sample with 4% WRP as compared to the control sample. Furthermore, samples containing 4% WRP showed the highest increment in titratable acidity (12.47) and the highest percentage in Brix value reduction (51.04%) during the fermentation period as compared to the control sample. Biochemical analysis showed negative values for oxidase and catalase test while positive values were obtained for gram-staining indicating the presence of Lactic acid bacteria from the gram-positive group. This study demonstrates the high potential of WRP in promoting bacterial growth for yoghurt production which is beneficial to the food industry other than promoting the ongoing effort of food waste reduction.

Key words: Fermentation, lactic acid bacteria, prebiotic, probiotic, watermelon rind, yoghurt

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INTRODUCTION

Watermelon (*Citrullus vulgaris*) is indigenous to South Africa, where it has been grown and consumed for over 5,000 years and is widely cultivated in Malaysia with Terengganu as one of the main producers (Johnson *et al*., 2013). According to the Department of Statistics Malaysia (DOSM), watermelon is one of the most highly cultivated fruits in Malaysia with the highest self-sufficiency ratio (SSR) of 151.7% in 2017 and among the most highly consumed fruits in Malaysia with 3.3 kg/year of per-capita consumption (PCC). Watermelon is composed of three major parts: flesh, seed, and rind where the flesh is either yellow or red. It has been estimated that the rind accounts for approximately one-third of the overall fruit mass (Kumar, 1985; Romdhane *et al*., 2017). According to the United Nations Food and Agriculture Organization (FAO), it is reported that almost half of the world's horticultural produce originating from fruits, vegetables, and root crops is wasted resulting in economic loss and environmental problems (Hussain *et al*., 2020). Watermelon rind is the

major waste produced from watermelon processing and is regarded as having no commercial value in the community with approximately 36 million tons of this by-product being wasted annually (Al-Sayed & Ahmed, 2013; Petkowicz *et al*., 2017).

Despite this, watermelon rind contains nutrients such as vitamin C, dietary fibre, potassium, sterols, triterpenes, cucurbitacin, and a small amount of vitamin B and alkaloids. The high dietary fiber content in the WRP indicates its potential application for the enrichment of foods resulting in the production of value-added products. The major type of fibre found in WRP is insoluble fibre (Al-Sayed & Ahmed, 2013). In addition, it also contains antioxidants such as citrulline which converts to arginine, an important amino acid for the immune system (Lobo & Dorta, 2019). Moreover, several studies have been reported on the application of watermelon rind in food products (Dubey *et al*., 2021). Previous studies on the utilization of WRP in the production of noodles showed a gradual increase in nutritional content such as ash, crude fibre, crude fat, carbohydrate, and total phenolic content (TPC) with an increase in WRP (Lee-Hon & Norhidayah, 2016). Moreover, the addition of WRP in cookies and burgers also leads to an increase in nutrients such as dietary fibre and proteins (Naknaen *et al*., 2012; Najafi *et al*., 2022). A study by Al-Sayed and Ahmed (2013) on the substitution of flour and fat with WRP in the making of cake also showed an increase in fibre and bioactive compounds with an improved quality of cake during storage. Nevertheless, the use of food waste such as peel as a food ingredient is usually associated with toxicity concerns that limit its application. However, a study conducted by Arojojoye *et al*. (2018) on the toxicity of watermelon rind reveals that watermelon rind does not pose adverse effects on the kidney and liver of rats and thus may be safe for human consumption.

The classification of food ingredients as prebiotics relies on their ability to be a selective substrate for one or a limited number of potentially beneficial commensal bacteria in the colon and their inability to be hydrolyzed or absorbed in the upper part of the gastrointestinal tract. Following that, prebiotics must be able to stimulate the growth of bacteria, become metabolically activated, or both, and be capable of altering the colonic microflora to a healthier composition (Allgeyer *et al*., 2010). The selection of prebiotics to be incorporated into yoghurt remains a challenge as it may affect the physicochemical and organoleptic properties of yoghurt. In addition, it is also important to ensure the stability of the prebiotics used during the processing and storage of yoghurt to ensure its effectiveness (Prasanna & Rastall, 2017). Fructooligosaccharide (FOS) has been reported to be unstable in pasteurization temperatures (Klewicki, 2007) while insulin-like prebiotics showed poor solubility in water under room temperatures (Oliver *et al*., 2006) that limits its application in food products. Therefore, it is crucial to investigate the suitable source of prebiotics to produce yoghurt. Watermelon had a high potential to act as a prebiotic due to its high pectin content (Chatterjee *et al*., 2016) whereby its high molecular weight has been shown to particularly influence the growth of *Bifidobacterium spp* (Pascale *et al*., 2022). Furthermore, a study conducted by Toupal and Coşansu (2023) revealed that the addition of banana and watermelon peel powders improved the survival of probiotics under bile salt conditions. However, it is important to ensure the suitability of WRP to be incorporated in food products such as yoghurt to ensure its potential as a source of prebiotics.

Meanwhile, probiotics are defined as live microorganisms that are intended to cause health-promoting function in the human guts which are typically found in fermented foods such as miso, kefir and yoghurt. The functional and health-promoting properties of yoghurt depended on the viability of the probiotics from processing, up to consumption. The probiotic bacteria need to remain viable with approximately 10^8 CFU/g as it reaches the gut (Kechagia *et al*., 2013). The presence of prebiotics may improve the viability of probiotic bacteria (Meybodi *et al*., 2020). Thus, the addition of watermelon rind powder to dairy products such as yoghurt can be viewed as a cost-effective and functional option as it provides the possibility of prebiotic effects that can promote and enhance the viability of probiotics (Santos *et al*., 2017). Therefore, this study was designed to investigate the effect of watermelon rind on the growth of probiotics in yoghurt and the chemical properties of the watermelon rind powder incorporated in yoghurt prepared using commercial probiotic strains. This study may provide insight into the application of watermelon rind powder as a beneficial food ingredient.

MATERIALS AND METHODS

Watermelon rind powder preparation

Watermelon (*Citrullus vulgaris*) rinds were recovered from Kuala Terengganu's local fresh fruit processing establishments. The outer skin was removed, and the rind was cut into small pieces. The cleaned rinds were dried in a food dehydrator (74-1001-W, Weston, Southern Pines) at 50°C for 24 hr. The dried rinds were then cooled and ground by using a laboratory mill. To obtain a regular and homogeneous powder, the powder was sieved with a 0.5 mm sieve screen. The moisture content of

the resulting powder was approximately 17.05 ± 2.10% in the previous study by Zia *et al*. (2021) with the recorded moisture content of 9-17% and the study by Ho *et al*. (2016) with the recorded moisture content of 14.75-18.86% for watermelon rind powder. The watermelon rind powder (WRP) was then kept in hermetic containers before use.

Yoghurt preparation

Yoghurt samples containing different percentages of WRP were produced at 0, 2 and 4% (w/v). Based on a preliminary study, higher than 4% of the percentage may lead to a significant change in the organoleptic properties of the yoghurt. The powders were added into rehydrated skim milk (10% w/v). The mixture was then pasteurized at 85°C for 30 min by using a water bath and cooled in an ice bath to 42-45°C before inoculation with a mixture of commercial starter culture consisting of *Lactobacillus casei*, *Bifidobacterium longum*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus acidophilus* (Yogourmet, Lallemand, Canada) at the ratio of 1:1:1:1:1. The ratio of all cultures followed the general formulation in yoghurt making industry. Then, the inoculated milk samples were then incubated for 24-30 hr at 42-45°C. The incubation time was set to 30 hr as a longer fermentation time may lead to an increase in the production of metabolic by-products that may affect the growth of bacteria.

Chemical analysis

Moisture content analysis of watermelon rind powder

The moisture content of the prepared watermelon rind powder (WRP) was determined by using a halogen moisture analyzer (Mettler toledo HE73, United States). The operation of a moisture analyzer entails weighing and heating the sample at the same time whereby approximately 5 g of samples was placed on the aluminium dish for analysis.

Titratable acidity and pH

The titratable acidity and pH were determined in yoghurt samples at 0, 6, 24 and 30 hr of incubation time. Briefly, 5mL of yoghurt samples were mixed with 5 mL of distilled water and added with phenolphthalein as an indicator solution. The samples were then titrated with 0.1N NaOH standard solution while constantly stirred until a faint pink colour persisted. The percentage of titratable lactic acid was measured following Equation 1. The pH value of the samples was determined with the direct measurement method by using a pH Meter (500 Beckman Coulter, Fullerton).

Equation 1:

$$\%Lactic\ acid\ = \frac{Amount\ of\ 0.1\ M\ NaOH\ (ml) \times 0.9\ (conversion\ factor\ to\ lactic\ acid)}{Sample\ volume}$$

Total soluble solid

The total soluble solid of the yoghurt was determined as the degree of Brix directly by using a refractometer (MA871 Milwaukee, United States).

Microbiological analysis

Lactic Acid Bacteria (LAB) count using MRS agar

The fermentation of the yoghurt samples was monitored during the incubation period with samples taken at 0, 6, 24 and 30 hr to determine the viable count. Standard plate count of the yoghurt samples mixed in de Man, Rogosa and Sharpe (MRS) culture medium was conducted with pertinent dilutions. MRS agar was used for determining the total count of LAB as it has been designed to promote the growth of LAB cultures including *Lactobacillus* and *Streptococcus*. It has also been reported to be suitable for the growth of *Bifidobacterium* (Fachin *et al*., 2008). Serial dilution was conducted by pipetting 1.0mL of the mixture into 9 mL of saline water (0.85% salt) in a test tube. 0.1 mL of appropriate decimal dilutions of the sample was inoculated in triplicate onto the surface of MRS agar plates. The prepared plates were then incubated at 30°C for 24 hr under anaerobic conditions as a low level of oxygen is required for the optimum growth of LAB. The colony was then counted by using the colony counter and the viable cell count was calculated in log CFU/mL.

Bacterial growth rate and duplication time

Mean bacterial growth rate, k and duplication time, g was determined according to Willey *et al*. (2008) where the calculation was made according to Equations 2 and 3 as follows:

Equation 2:

$$k = \log \frac{logN_{30} - logN_0}{\log 2 \times 30}$$

Equation 3:

$$g = \frac{1}{k}$$

where N_{30} is the CFU/mL at the end of fermentation (30 hr) and N_0 is the CFU/mL at the start of fermentation (0 hr).

Biochemical analysis

Gram staining

Bacterial staining was conducted where a smear of the sample was placed on a glass slide and allowed to air dry completely. It was then stained for 1 min in crystal violet solution and 1 min in iodine solution. After that, it was rinsed for 10 s in ethanol and then counterstained for 1 min with safranin. The glass slide was then examined under a microscope with immersion oil.

Oxidase test

A piece of filter paper was prepared and moistened with tetramethyl-p phenylenediamine, a chromogenic reducing agent. A small number of bacteria colonies were rubbed onto the moist paper by using a toothpick. The presence of a dark brown-purple colour indicates a positive result, while no colour change indicates a negative result (Shields & Cathcart, 2013).

Catalase test

The procedure was conducted according to Hitchins *et al*. (2022). Briefly, a small amount of colony was taken from isolates and placed on a slide. A few drops of hydrogen peroxide (H_2O_2) reagent were placed onto the sample using a Pasteur pipette. The reaction was observed for bubbles formation (positive reaction) whilst no bubbles formation indicated a negative reaction.

Statistical analysis

Statistical analysis was performed by using One-way Analysis of Variance (ANOVA) and the means were compared between groups using the Tukey test. All of the tests were done in triplicate and the mean ± standard deviation of the data was recorded (N=3). The IBM SPSS 27 software was used to analyze the data, and significant differences were identified at (p<0.05).

RESULTS AND DISCUSSION

Moisture content of watermelon rind powder

Moisture is an important factor in contributing to food spoilage related to microbial growth where low moisture can ensure the shelf life of a product. In this study, the moisture content of WRP was recorded at 17.50% ± 2.10. According to a previous study, the moisture content of watermelon rind powder was recorded at 9-17% (Zia *et al*., 2021). Research by Ho *et al*. (2018) states that the moisture contents of watermelon rind powders were between 14.75-18.86%. The percentage is also within the range of the recorded fruit and peel powders used for yoghurt enrichment ranging from 13.37-83.76% (Raihan Kabir *et al*. 2020; Sheikh *et al*. 2022). Therefore, the moisture content of the resultant powder is deemed suitable for incorporation into products such as yoghurt.

Total soluble solid (°Brix)

The Brix value of the yoghurt sample with different percentages of watermelon rind powder at different fermentation times was recorded in Table 1. According to Table 1, the increase in Brix value was observed with an increase in the percentage of WRP for fresh samples at 0hr. Significant reduction (p<0.05) was observed during the 30 hr of fermentation time for all samples. Figure 1 shows the percentage of Brix value reduction after 30 hr of fermentation time concerning 0 hr. A higher reduction in Brix value was observed for samples containing WRP as compared to control samples with only a 27.39% reduction. The percentage of Brix value reduction increases with an increase in WRP percentage whereby the sample containing 4% of WRP showed the highest reduction of 51.04% as compared to

the other samples (Figure 1). In general, a higher Brix value indicated high total soluble solid (TSS) content resulting in more concentrated yoghurt. The high TSS can be associated with the amount of sugar in the sample whereby WRP has been recorded to contain a high amount of carbohydrate of 42%-51% originated from sugars (Chakrabarty, 2020). Thus, it leads to an increase in TSS content with an increase in WRP percentage for freshly prepared yoghurt (0 hr) as observed in the current study. In terms of reduction in TSS with an increase in incubation time, a similar result was reported by Wijesinghe *et al*. (2016) where the total soluble solids (TSS) content of drinking yoghurt samples decreased with storage time. The decrease in total soluble solids is due to the sugar being used up by the starter culture during the fermentation process. Thus, higher utilization of sugar by the bacteria during the fermentation process may provide an indication of a higher fermentation process efficiency by the bacteria which was observed for samples containing WRP (Lu *et al*., 2006).

Table 1. Brix value of yoghurt with different percentages of watermelon rind powder during fermentation time

Percentage of WRP (%)	Fermentation time (hr)			
	0	6	24	30
0 (Control)	10.37 ± 0.06 $^{c, A}$	7.63 ± 0.06 $^{c, B}$	5.00± 0.20 $^{b, C}$	7.53± 0.15 $^{a, B}$
2	11.83 ± 0.06 $^{b, A}$	10.39± 0.01$^{b, B}$	7.80± 0.10 $^{a, C}$	7.73± 0.06 $^{a, C}$
4	15.87 ± 0.30 $^{a, A}$	15.03± 0.15 $^{a, B}$	7.63± 0.25 $^{a, C}$	7.77± 0.06 $^{a, C}$

$^{a, b, c}$ Means with the different letters (low case letter) within the same column differ significantly (p<0.05) while $^{A, B, C}$ Means with different letters (upper case letter) within the same row differ significantly (p<0.05). WRP = Watermelon rind powder

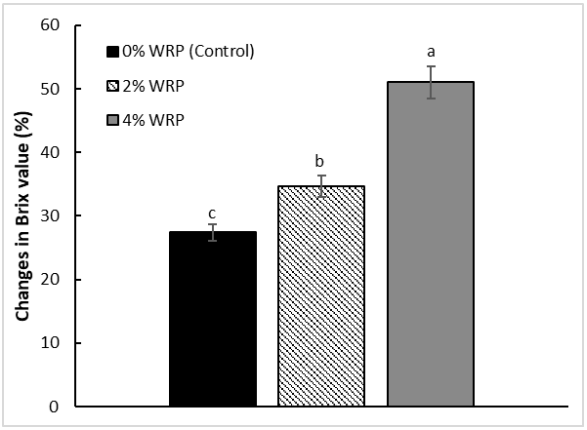

Fig. 1. Changes in Brix value (%) of yoghurt samples prepared at different percentages of WRP after 30 hr of incubation concerning 0 hr. Bars represent mean ± standard deviation (N=3) with different letters indicating significant difference at p<0.05. WRP= Watermelon rind powder.

Changes in pH value

Table 2 showed that significant changes in the pH value were observed for all of the yoghurt samples whereby samples with 4% w/v WRP showed the highest reduction in pH value with 44.76% reduction as compared to the control and samples containing 2% w/v of WRP after 30 hr of incubation. The pH of yoghurts decreased significantly (p<0.05) with an increase in incubation time and watermelon rind powder content (%). After 30 hr of incubation, the highest pH value was observed for the control sample at 3.63 while samples containing 4% of WRP showed the lowest pH value of 3.53.

The reduction in pH is due to the conversion of sugar found in milk which is lactose into lactic acid by bacterial cultures, giving the product the desired tartness and texture. The fermentation endpoint for yoghurt fermentation is the pH value of 4.5–4.6 (Soukoulis *et al*. 2007) whereby further reduction in pH may result in a decrease in the viability of LAB. Figure 2 shows the changes in pH value with incubation time at different percentages of WRP. According to Figure 2, it is shown that the fermentation time for the yoghurt to achieve the pH value of 4.5 was the fastest for samples containing 4% and 2% (w/v) of WRP as compared to the control sample. These indicated that the presence of WRP may help to shorten the fermentation time by encouraging the growth of LAB. The finding in this investigation was consistent with the prior findings by Dabija *et al*. (2018) which reported a higher decrease in the pH value of yoghurt samples over time during incubation for samples containing various plant-based raw materials. Besides, the final pH of the yoghurt was also determined by the lactic acid starter culture, as each bacterium has unique lactose consumption and acidification capabilities (Zanhi & Jideani, 2012).

Therefore, mixed culture was used in the current study to obtain the desired characteristic of yoghurt whereby the addition of WRP leads to higher bacterial growth and an increase in the conversion of lactose to lactic acid that leads to a rapid reduction in pH.

Table 2. pH value of yoghurt with different percent of watermelon rind powder during fermentation time

Percentage of WRP (%)	Fermentation time (hr)			
	0	6	24	30
0 (Control)	6.05 ± 0.02 $^{c, A}$	6.04 ± 0.01 $^{c, A}$	3.77 ± 0.05 $^{a, B}$	3.63 ± 0.01 $^{a, C}$
2	6.23 ± 0.03 $^{b, A}$	6.19 ± 0.01 $^{b, B}$	3.55 ± 0.03 $^{b, C}$	3.57 ± 0.03 $^{b, C}$
4	6.39 ± 0.01 $^{a, A}$	6.36 ± 0.02 $^{a, A}$	3.54 ± 0.05 $^{b, B}$	3.53 ± 0.02 $^{c, B}$

$^{a, b, c}$ Means ± standard deviation with different letters (lower case letters) within the same column differ significantly (p<0.05) while $^{A, B, C}$ Means ± standard deviation with different letters (upper case letters) within the same row differ significantly (p<0.05). WRP = Watermelon rind powder.

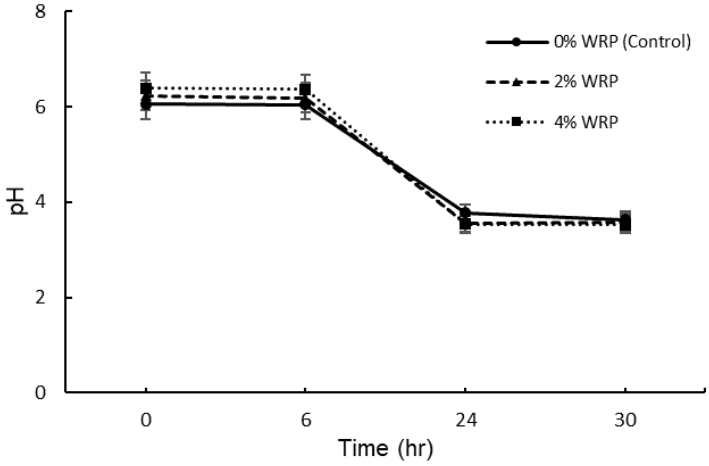

Fig. 2. Changes in pH value with Time (hr) for yoghurt samples prepared at different percentages of WRP. Bars represent mean ± standard deviation (N=3). WRP = Watermelon rind powder

Titratable acidity of yoghurt

According to Table 3, the titratable acidity increased significantly (p<0.05) during the 30 hr of incubation period for all of the samples. The lowest titratable acidity value was observed for the control sample, whereas a significantly (p<0.05) higher value was observed for the 4% WRP yoghurt after 30 hr of incubation. The result showed that the yoghurt sample containing WRP produced lower pH values and higher titratable acidity as compared to the control. These results were similar to the findings by Dabija *et al*. (2018) whereby yoghurt samples containing herb extracts showed significantly lower pH values (p<0.05) and higher acidity (p<0.001) than the control sample. This result was attributed to the presence of herb extracts that promote the growth of microorganisms in the yoghurt samples (Dabija *et al*., 2018). In addition, it was also reported that the titratable acidity in yoghurts containing passion fruit peel powder was significantly higher than its respective control, a behaviour expected by the homolactic metabolism of the lactic acid bacteria which was enhanced with the presence of passion fruit peel powder (Do Espírito Santo *et al*., 2012). Thus, the increase in titratable acidity values for yoghurt samples prepared with WRP may indicate an increase in the metabolic activity of the lactic acid bacteria that resulted in a higher production of lactic acid.

Effects of watermelon rind powder on probiotic LAB growth

Bacterial cell counts revealed that samples containing WRP have a higher rate of bacterial growth as compared to yoghurt samples prepared without the addition of WRP (Table 4). The bacterial growth rate of samples containing WRP was 0.36-0.47 h^{-1} as compared to the control sample with 0.25 h^{-1}. This is due to the shorter duplication time of samples with WRP (2.13-2.81 hr) as compared to the control sample with 3.92 hr of duplication time. The Log CFU/mL value of yoghurt with different percent of watermelon rind powder during fermentation time is shown in Figure 3 whereby yoghurt with 4% WRP showed a significantly higher increase (p<0.05) in bacterial counts during 30 hr of the incubation period with 4.25 increase in log CFU/mL as compared to 3.21 and 2.30 increase in log CFU/mL for samples

with 2% WRP and control sample respectively. This is due to the presence of fibre in WRP, which acts as a source of prebiotics and promotes bacterial growth. Several studies reported on the high fibre content of WRP, whereby this fibre is beneficial as a source of prebiotics (Naknaen *et al*., 2016; Hao *et al*., 2021). According to a previous study by Naknaen *et al*. (2016), watermelon rind powder (WRP) is a rich source of dietary fibre and bioactive compounds, therefore it has a high potential to be utilized in foods such as cookies. A study conducted by Toupal and Coşansu (2023) reported that the addition of banana (BPP) and watermelon peel powder (WPP) in MRS broth leads to an increase in the growth of lactic acid bacteria whereby the bacterial count for *L.plantarum* was 0.52 - 1.13 log CFU/mL higher for samples containing WPP as compared to samples without the addition of WPP while the bacterial population for *L.acidophilus* were 2.47-2.49 log CFU/mL higher in samples with WPP as compared to without WPP. Moreover, Hao *et al*. (2021) reported that the yellow watermelon peel has a higher prebiotic potential as compared to honeydew and papaya peels due to its high yield, high amount of reducing sugar and indigestible non-starch polysaccharide. Nevertheless, the yellow watermelon peels also showed an effectively quick stimulation of probiotic growth and are resistant to digestion by gastric juice and amylase enzyme. Besides that, other plant-based flour such as mango peel flour has also been reported to promote the growth of lactic acid bacteria as it contains high dietary fibre and total soluble sugars (Pérez-chabela *et al*., 2021). Apple and banana peel fibres have also been reported to improve probiotic viability during storage where it was shown for the first time that fruit fibres can improve the fatty acid profile of probiotic yoghurts (Do Espírito Santo *et al*., 2012).

According to Figure 3 for control samples, a slight reduction in bacterial growth between 24 to 30 hr of incubation time may be attributed to the depletion of nutrients as the bacterial cultures only depend on lactose as a source of nutrients but for samples with WRP, additional nutrients were provided by the watermelon rind powder that supports the bacterial growth. A reduction in bacterial growth was also observed for samples with 4% WRP between 24 to 30 hr of incubation. The reduction of bacterial count is due to the accumulation of bacterial metabolic products. The high percentage of WRP leads to rapid bacterial growth resulting in a high accumulation of by-products. This creates an unfavourable condition for bacterial growth. The high content of by-products resulted in a further reduction of pH that is not suitable for bacterial growth. Therefore, as the pH reaches 4.5, the yoghurt must be kept at a cool temperature to stop the fermentation to maintain an optimal bacterial count (Kim *et al*., 2009).

Table 3. Titratable acidity value of yoghurt with different percent of watermelon rind powder during fermentation time

Percentage of WRP (%)	Fermentation time (hr)			
	0	6	24	30
0 (Control)	1.13± 0.25 $^{c, C}$	1.13± 0.06 $^{c, C}$	6.80± 0.36 $^{c, B}$	7.40± 0.30 $^{c, A}$
2	2.13± 0.32 $^{b, C}$	1.93± 0.15 $^{b, C}$	10.03± 0.12 $^{b, B}$	10.67±0.31 $^{b, A}$
4	2.73± 0.15 $^{a, C}$	2.73± 0.15 $^{a, C}$	11.77± 0.38 $^{a, B}$	12.47±0.35 $^{a, A}$

$^{a, b, c}$ Means with different letters (lower case letters) within the same column differ significantly (p<0.05) while $^{A, B, C}$ Means with different letters (upper case letters) within the same row differ significantly (p<0.05). WRP = Watermelon rind powder

Table 4. Growth rate and duplication time of bacteria in yoghurt with different WRP percentages

WRP %	Growth rate (h^{-1})	Duplication time (hr)
0 (Control)	0.25	3.92
2	0.36	2.81
4	0.47	2.13

WRP = Watermelon rind powder.

Biochemical test of probiotic LAB

Table 5 shows the biochemical test results of the bacteria isolated from the samples of control, 2% and 4% WRP-formulated yoghurt. The tests comprising gram-staining, catalase and oxidase tests were conducted to confirm the presence of lactic acid bacteria in the samples and to ensure that the samples were free from contamination.

The result showed that the lactic acid bacteria present in all of the samples belong to the group of Gram-positive bacteria. The catalase test showed a negative result whereby no formation of air bubbles was observed for the tested samples. Some of the features of lactic acid bacteria are gram-positive, non-spore-forming rods, do not generate the catalase enzyme which converts hydrogen peroxide to water and oxygen, and are mostly non-motile (Sheeladevi and Ramanathan, 2011). Gram-staining tests of all the samples produced a blue-purple colour with staining as observed under the microscope,

indicating that the culture was Gram-positive bacteria. In addition, an oxidase test was conducted to determine the ability of the test organism to produce oxidase enzymes by detecting the presence of cytochrome C. A positive result is shown by the development of a purple colour due to the oxidation of the reagent when it was directly applied to the test organism while negative results were shown by the absence of a purple colour (Hemraj *et al*., 2013). The test conducted on the control yoghurt samples and yoghurt containing WRP shows negative results for the oxidase test that confirms the presence of *Lactobacillus sp*. According to Thakur *et al*. (2017), this is due to the lack of cytochrome C which indicates that the isolates were anaerobic organisms that allow the use of free oxygen in their energy metabolism.

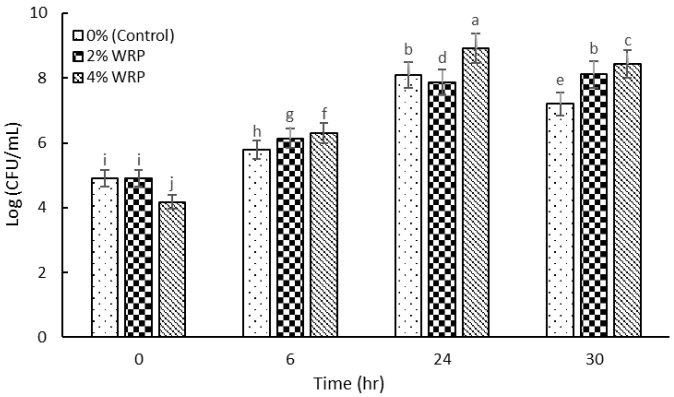

Fig. 3. Log CFU/mL values of yoghurt containing different percentages of watermelon rind powder during 30 hr of fermentation. Bars represent mean ± standard deviation taken from three replicates (*N*=3). Means with different letters differ significantly ($p<0.05$). WRP = Watermelon rind powder, CFU = Colony form unit.

Table 5. Gram staining, oxidase test and catalase test of yoghurt samples with different WRP percent at time 0 and 24 hr of fermentation

Time (hr)	WRP (%)	Gram staining	Oxidase test	Catalase test
0	0%	+	-	-
	2%	+	-	-
	4%	+	-	-
24	0%	+	-	-
	2%	+	-	-
	4%	+	-	-

CONCLUSION

This study was conducted to determine the effect of watermelon rind powder on the growth of probiotic bacteria in yoghurt whereby significant effects were observed for samples incorporated with 2% and 4% of WRP as compared to control samples. Evaluation of the Brix value of the yoghurt showed an increase in the percentage change of Brix value with an increase in watermelon rind powder concentration whereby samples containing 4% WRP showed 51.04% Brix reduction as compared to 27.39% reduction for control samples. In addition, yoghurt samples with watermelon rind powder have lower pH values ($p<0.05$) and higher titratable acidity ($p<0.05$) after 30 hr of incubation as compared to the control sample.

The results obtained from this study revealed the high potential of WRP as a prebiotic source in fermented dairy products. The addition of watermelon rind powder helped to support bacterial growth and improve the fermentation time of the yoghurt. An increase in bacterial growth rate was observed with an increase in WRP concentration and the required pH of 4.5 for yoghurt was achieved faster for samples containing WRP as compared to the control samples. This study also shows that due to the higher bacterial growth rate for samples containing 4% WRP, prolonged fermentation time has resulted in a decrease in bacterial growth due to high acidity content and nutrient depletion. Based on this study, the suitable time for WRP-incorporated yoghurt fermentation is between 8 to 12 hr to achieve 4.5 pH and maintain maximum bacterial growth. However, further studies are still required to determine the effects of WRP concentration on the fermentation time to determine the best fermentation time for WRP-incorporated yoghurt to achieve optimal probiotic content.

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ETHICAL STATEMENT
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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