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Characteristics and Antibacterial Activity of ZnO Nanoparticle Fortified Probiotic Yogurt

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ABSTRACT

Zinc deficiency is a major health issue in developing nations, especially in Southeast and South Asia. To address this, food fortification using nanotechnology, such as ZnO nanoparticles deemed safe by the US FDA, can enhance daily zinc intake essential for growth and immune health. This research emphasizes on yoghurt fortification with various types: ZnO nanoparticle fortification and the addition of probiotic bacteria *Lactiplantibacillus plantarum subsp. plantarum IIA-1A5* (Accession Number: OR473281) and aims to analyze the nutritional value, characteristics, total lactic acid bacteria (LAB) and antibacterial activity of probiotic yogurt fortified with ZnO nanoparticles. There were six levels of treatment designed in this study and each treatment was tested three times. The statistical test used in analyzing the nutritional value, characteristics, total LAB and antibacterial activity in this study is the Tukey test which is applied as a multiple range test while in organoleptic testing using the Kruskal-Wallis non-parametric test. The results showed that the fortification of yogurt with ZnO nanoparticles had significant interaction ($p<0.05$) on yogurt viscosity, LAB content, and color attributes in hedonic quality test. The antibacterial activity towards *Staphylococcus aureus* and *Escherichia coli* produced by six treatments were moderate, with inhibition zones ranging between 5 to 10 mm. This study concluded that the fortified yoghurt treatment met the Indonesian National Standard (SNI), demonstrating the potential of this approach in addressing zinc deficiency in at-risk populations. This research underscores the promise of nanotechnology in food fortification strategies to combat micronutrient deficiencies globally.

Keywords: Antibacterial, Characteristics, Fortification, Yogurt, Zn Nanoparticle

Introduction

Zinc deficiency is the most common micronutrient deficiency worldwide, especially in developing countries. It affects about 31% of the global population, with the highest rates in Southeast Asia (34%) and South Asia (73%) (Marlia *et al.,* 2016). Over 25% of Indonesian suffer from zinc deficiency (Darma *et al.,* 2019). Zinc deficiency, as noted by Purwandini and Atmaka (2023), increases the risk of stunting, where infectious diseases become a primary concern. This occurs as nutrients needed for growth are redirected to strengthen the immune system. The Recommended Dietary Allowance (RDA) for zinc is 5 mg daily for children aged four to eight, crucial for their growth and development. Women require 8 mg daily, while men need 11 mg daily, due to the body's inability to store zinc. Regular consumption is essential to maintain adequate levels, with food fortification offering a practical approach to increasing dietary zinc intake.

Nanotechnology offers a promising solution for safe and effective food fortification. According to the Indonesian Food and Drug Authority (BPOM), nanotechnology holds significant prospects in food processing, enhancing the physical and chemical properties of food and improving nutrient stability and availability (Patra *et al.,* 2018). Nanoparticles, with their vast surface area, heightened reactivity, and unique properties, are valuable in various sectors, including the food industry (Ariyarathna *et al.,* 2017). They serve as preservatives, flavor enhancers, encapsulating agents, antimicrobial sensors, and packaging material components. Additionally, nanoparticles enhance nutrition, prolong shelf life, and detect foodborne pathogens, ensuring food quality (Biswas, 2022). Their high absorption rate by the body further underscores their effectiveness (Hashem and Bulnes, 2020).

Zinc is also available in nanoparticle form as ZnO (zinc oxide), deemed safe for use as a food additive (Generally Recognized as Safe or GRAS) by the US Food and Drug Administration (US FDA)

(Cotton *et al.,* 2019). The absorption of zinc from ZnO reaches 49.9%, indicating its effectiveness as a zinc source (Wegmüller *et al.,* 2014). Zinc oxide nanoparticles (ZnO-NPs) have recognized antibacterial capabilities, inhibiting the growth of bacteria such as Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli (Mendes *et al.,* 2022). ZnO disrupts bacterial DNA structure and protein metabolism, preventing replication and growth. It also induces hydrogen peroxide production, enhancing its antibacterial properties. Smaller ZnO particles are particularly effective antimicrobials. A challenge in synthesizing nanometer-scale ZnO particles is preventing particle agglomeration, which is achievable by adding a capping agent. Tamrakar *et al.,* (2008) reported that a capping agent could limit agglomeration in nanoparticle crystals, resulting in smaller crystal sizes. Pectin serves as a capping agent as a capping agent during the production of ZnO-NP as it prevents nanoparticle aggregation and maintains their size and shape (Dmochowska *et al.,* 2020).

Dairy products also have the potential to be functional foods that positively impact health. Probiotic bacteria such as *Lactiplantibacillus plantarum subs plantarum IIA-1A5 / Lactobacillus plantarum IIA-1A5* (Accession Number: OR473281) can be added to enhance yogurt's functional properties. Research by Sihombing *et al.,* (2015) showed that *Lactobacillus plantarum IIA-1A5* is effective towards gram-positive and gram-negative bacteria. Therefore, research on the fortification of ZnO nanoparticles in probiotic yogurt is essential, as it is expected to create a combination that positively impacts health in preventing infections or certain diseases caused by zinc (Zn) deficiency and pathogenic bacteria. Research on the potential of zinc oxide nanoparticle fortification in yogurt has been conducted previously. The study concluded that zinc-fortified foods have the potential for human consumption and can combat problems related to zinc deficiency (Karmakar *et al.,* 2022). However, the study did not conduct antibacterial testing, and there is no addition of probiotic bacteria in the yogurt, which has good benefits. This study is the first research to analyze the characteristics and antibacterial activity of probiotic yogurt with probiotic bacteria *Lactobacillus plantarum IIA-1A5* fortified with ZnO nanoparticles. In this study, we used six levels of treatment; the treatment with the addition of ZnO was carried out by adding 7.5 mg of ZnO in 100 mL of yogurt. this is based on the level of zinc absorption possessed by ZnO and the daily requirement of zinc. This study aims to analyze the characteristics and antibacterial activity of probiotic yogurt fortified with ZnO nanoparticles.

Materials and Methods

Yogurt preparation and addition of nano ZnO

Yogurt processing is conducted using fresh cow's milk which is then heated at a temperature of 85 - 90°C for 35 min, following which it is then cooled to a range of 40 - 45°C. The probiotic strains *Streptococcus thermophilus* IFO 13957*, Lactobacillus bulgaricus* IFO 13953, and *Lactobacillus plantarum IIA-1A5* (Accession Number: OR473281) were introduced as starters into the cow's milk. The starter culture was introduced at a concentration of 3% (v/v) with a population exceeding 10^7 CFU mL^{-1}. The milk was subsequently subjected to incubation at a temperature of 37°C for a duration of 16 h. This time is needed to achieve the isoelectric point in yogurt so as to produce the desired pH as well as texture. Afterward, 7.5 mg of nano ZnO was added to 100 mL of yogurt and stirred using a stirring spoon until homogenous. Based on this composition, yogurt was divided into six different formulas: P1: Plain yogurt (*Streptococcus thermophilus* IFO 13957+ *Lactobacillus bulgaricus* IFO 13953), P2: Plain yogurt + *Lactobacillus plantarum IIA-IA5* (Probiotic Yogurt), P3: Probiotic Yogurt + commercial nano ZnO, P4: Probiotic Yogurt + synthetic nano ZnO with pectin, P5: Probiotic Yogurt + synthetic nano ZnO without pectin, and P6: Probiotic Yogurt + pectin. In the treatment that used pectin, pectin was added in the amount of 7.5 mg in 100 mL of yogurt.

Analysis procedure. Yogurt samples in this study were tested for characteristics such as proximate analysis, total LAB, pH, TAT (Titratable acidity), water activity (a_w), viscosity, and antibacterial activity. Proximate analysis, which includes water, ash, fat, and protein content, was performed using the AOAC (2005) method. Total LAB testing was conducted using the pour plate method, as Pelczar *et al.,* (2007) determined. pH measurement was performed using a Schoot Instrument pH meter (Germany) according to the method determined by Melanie *et al.,* (2018). Total Titratable Acid (TTA) testing was carried out using AOAC (2007). Water activity was measured using a Novasina a_w meter (Switzerland) and viscosity with a Rion VT04F viscometer (Japan). Antibacterial testing was performed using the disc diffusion method as determined by Mostafa *et al.,* (2018). The pathogenic bacteria used are *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. SEM analysis was conducted to confirm the damage to bacterial cells caused by ZnO nanoparticles (Aprilia, 2022), and *Escherichia coli* ATCC 25922 was used as a bacterial model. Organoleptic testing followed the method described by BSN (2006) and included 35 semi-trained panelists. Each panelist gave their consent and confirmed their availability by signing an informed consent form before participating. The organoleptic evaluation involved a hedonic test to assess the panelists' preferences and a hedonic quality test to evaluate the physical attributes of the sample based on their opinions. The parameters tested in this organoleptic evaluation were color, aroma, texture, and taste.

Experimental design and data analysis. Data were processed using variance analysis (ANOVA). Further data testing was conducted if significant differences were found ($p<0.05$) using the Tukey Test with six levels of treatment and three repetitions per treatment. Statistical testing of organoleptic test data was processed using the non-parametric Kruskal-Wallis test.

Results and Discussion

Proximate value of yogurt

The chemical composition or proximate value of yogurt tested in this study included moisture, ash, fat, and protein content. The analysis of variance indicated that the fortification of yogurt had no significant impact on its chemical composition (Table 1). These results align with research by El-Sayed *et al.* (2021) and Urquiza *et al.* (2017), stating that fortification with ZnO nanoparticles does not significantly affect yogurt's water, ash, protein content, and protein content. The moisture content obtained in this study ranged from 87.34% to 87.86%, slightly higher compared to the findings of Urquiza *et al.,* (2017), who reported moisture content between 83% and 84%. This was because the test conducted by Urquiza *et al. (2017) performed* standardization on milk by adding milk powder to increase the value of solids contained in yogurt so that the water content value is lower. The ash content ranged from 0.70% to 0.73%, consistent with the SNI standard of less than 1%. According to the National Standardization Agency (2009), the minimum fat content in yogurt is 3%. The analysis revealed fat content that varied from 3.22% to 3.63%, which satisfies the established standards. All yogurt treatments' protein contents ranged from 2.98% to 3.23%, meaning that each treatment's protein content surpassed the SNI minimum requirement of 2.7%.

Microbiological characteristics of yogurt

The amount of lactic acid bacteria (LAB) is a microbiological quality indicator of fermented dairy products. LAB plays a crucial role in yogurt production, fermenting lactose in milk to produce lactic acid. The analysis of variance revealed that fortification with ZnO nanoparticles significantly affected the total LAB ($p<0.05$) (Table 2). Based on the table, it can be concluded that the probiotic yogurt (P2) treatment has the largest LAB population compared to plain yogurt (P1), probiotic yogurt with commercial nano ZnO addition (P3), probiotic yogurt with synthetic nano ZnO addition with pectin (P4), yogurt with synthetic nano ZnO addition without pectin (P5), and probiotic yogurt with pectin (P6).

The table shows that the plain yogurt treatment (P1) has less LAB than P2 even though ZnO is not added; this is because P1 doesn’t add probiotic bacteria; the addition of probiotic bacteria L. Plantarum IIA - 1a5 will increase the amount of LAB in yogurt (Arief *et al.,* 2023). The P1 treatment was also not significantly different from the P3, P4 and P5 treatments. The lower LAB content in samples enriched with nano ZnO can be caused by the antibacterial properties of ZnO-NPs (Sirelkhatim *et al.,* 2015). Wang *et al.,* (2021) attribute the decrease in LAB to ZnO nanoparticles' impact on the cytoplasm and cell membranes of LAB. The negative charge of the LAB and the positive charge of the ZnO nanoparticles interact electrostatically to produce this process, which could affect the stability of the bacterial cell membrane and cytoplasmic function, inhibiting the growth of LAB.

The minimal quantity of LAB required for yogurt quality is 7 log CFU/mL, determined by the Indonesian National Standard (SNI) 01-2981-2009. In this study, the total LAB values for all treatments ranged from 8.16 to 8.44 log CFU/mL. These results indicate that all treatments met the quality criteria set by BSN (2009), with LAB values exceeding the minimum limit of 10^7 CFU/mL.

Physicochemical characteristics of yogurt

This study's physical characteristics tested included pH, water activity (a_w), viscosity, and TTA. The variance analysis indicated that adding ZnO nanoparticles significantly influenced the viscosity of probiotic yogurt (Table 3). The table shows that pH values in each treatment are not significantly different, with values ranging from 4.38 to 4.44, so it can be concluded that fortification with nano ZnO particles did not significantly affect the pH of yogurt. The low pH value is caused by LAB present in the fermentation process which converts lactose into lactic acid so that the pH of yogurt becomes

Table 1. Chemical composition of yogurt

Chemical analysis	Treatments						Yoghurt Quality Standard (SNI)
	P1	P2	P3	P4	P5	P6	
Water (%)	87.34±0.23	87.83±0.07	87.83±0.16	87.80±0.07	87.86±0.05	87.73±0.10	–
Ash (%bb)	0.73±0.004	0.71±0.018	0.72±0.007	0.72±0.025	0.70±0.00	0.71±0.011	Max. 1.0
Fat (%bb)	3.22±0.10	3.32±0.1	3.48±0.33	3.57±0.21	3.47±0.34	3.63±0.19	Min. 3.0
Protein (%bb)	3.23±0.02	2.98±0.02	3.15±0.16	3.01±0.07	3.17±0.14	3.16±0.07	Min. 2.7

P1: Plain yogurt; P2: Probiotic yogurt; P3: Probiotic yogurt + commercial nano ZnO; P4: Probiotic yogurt + synthetic nano ZnO with pectin; P5: Probiotic yogurt + synthetic nano ZnO without pectin; P6: Probiotic yogurt + pectin.
Different letters on the same row indicate statistical differences ($p<0.05$) among treatments.

Table 2. Microbiological quality of yogurt

Treatment	P1	P2	P3	P4	P5	P6
Total of LAB (log cfu/ml)	$8.16±0.07^c$	$8.44±0.05^a$	$8.24±0.05^c$	$8.16±0.07^c$	$8.16±0.04^c$	$8.32±0.05^b$

P1: Plain yogurt; P2: Probiotic yogurt; P3: Probiotic yogurt + commercial nano ZnO; P4: Probiotic yogurt + synthetic nano ZnO with pectin; P5: Probiotic yogurt + synthetic nano ZnO without pectin; P6: Probiotic yogurt + pectin.
Different letters on the same row indicate statistical differences ($p<0.05$) among treatments.

Table 3. Physical characteristics of yoghurt

Physical Characteristic	Treatments						Yoghurt Quality Standard (SNI)
	P1	P2	P3	P4	P5	P6	
pH	4.43±0.07	4.38±0.04	4.40±0.12	4.41±0.06	4.44±0.03	4.39±0.19	3.80–4.50
a_w	0.85±0.003	0.85±0.003	0.86±0.003	0.85±0.004	0.85±0.007	0.86±0.001	0.85–0.86 (Melanie. 2018)
Viscocity (dPa)	2.30±0.13 ^{ab}	2.72±0.16 ^{a}	2.18±0.13 ^{b}	2.12±0.08 ^{b}	2.08±0.12 ^{b}	2.40±0.22 ^{ab}	–
TTA (%)	0.58±0.002	0.61±0.001	0.59±0.001	0.58±0.001	0.55±0.010	0.60±0.005	0.5 – 2.0%

P1: Plain yogurt; P2: Probiotic yogurt; P3: Probiotic yogurt + commercial nano ZnO; P4: Probiotic yogurt + synthetic nano ZnO with pectin; P5: Probiotic yogurt + synthetic nano ZnO without pectin; P6: Probiotic yogurt + pectin.
Different letters on the same row indicate statistical differences ($p<0.05$) among treatments.

low (Winarno and Fernandez, 2007). According to BSN 2009, good quality yogurt should have a pH value between 3.80 and 4.50; therefore, the entire treatment in this study met the SNI standard.

According to United States Department of Agriculture (1995), water activity (a_w) refers to the availability of unbound water molecules in a food product that can support the growth of microorganisms. Food deterioration from microbial activity is more likely in foods with a higher a_w value. The study demonstrated that the fortification of yogurt had no significant impact on a_w ($p>0.05$), with values ranging from 0.85 to 0.86. This finding is in line with previous studies, such as those conducted by Melanie (2018) and Juandini *et al.,* (2024), which reported a_w values for yogurt in the range of 0.850 to 0.861 and 0.83 to 0.87. The a_w value produced in this study is of good value because all treatments have a_w values below 0.90 so that they can inhibit the growth of pathogenic bacteria such as *Salmonella*, *Escherichia coli*, and *Clostridia* which can grow at $a_w > 0.91$. Viscosity is a measure that expresses the level of thickness of a liquid (Oktaviani *et al.,* 2024). The research results indicated that fortification significantly impacted ($p<0.05$) yogurt's viscosity, whereas treatments P3, P4, and P5 that used ZnO fortification in yogurt produced low viscosity values. The measured viscosity of the six yogurts ranged from 2.08 to 2.72 dPa, with the highest value belonging to P2 (yogurt + probiotics). The viscosity value can be influenced by yogurt's total LAB. The increase in yogurt viscosity is caused by the LAB and the higher total acid, forming a gel during fermentation (Harjiyanti *et al.*, 2013) This is in accordance with the results obtained where the highest viscosity value is owned by P2, which has the highest total LAB. Adding ZnO nanoparticles to yogurt decreased the amount of LAB, resulting in a reduced viscosity compared to probiotic yogurt despite ZnO nanoparticles. In this study, the addition of pectin did not affect the viscosity due to the amount of pectin added only in small amounts (no more than 0,1%) and had the aim of protecting the content of ZnO nanoparticles.

In this study, it can be seen that the TTA value is inversely related to the pH value where the higher the TTA value, the lower the pH value. This is also supported by the statement of Oktaviana *et al.* (2018) and Toffanin *et al.* (2015) which states that TTA in yogurt indicates acidity is generally inversely related to the pH value. Based on testing, there was no significant difference between treatments in yogurt regarding TTA ($p>0.05$). The TTA values in the six treatments ranged from 0.55% to 0.61%. The TTA values obtained in this study still meet the standard set in the BSN (2009), which is between 0.5% and 2.0%.

Antibacterial activity of yogurt

In this study, antibacterial activity testing was carried out by calculating the inhibition of bacterial growth of *Escherichia coli* and *Staphylococcus aureus* with the disc diffusion method. This method indicates the presence of antibacterial activity by forming a clear zone. The value of antibacterial activity produced by all treatments against *Escherichia coli* and *Staphylococcus aureus* ranged between 5 and 10 mm (Table 4). Davis and Stout (1971) established that antibacterial strength is determined by the presence of an inhibitory zone with a diameter of 5 mm or less, which is classified as weak. It is categorized as moderate if within the 5 –10 mm range. A diameter ranging from 10 to 20 mm is classified as essential, whilst a diameter over 20 mm is classified as extremely powerful. Based on these criteria, the antibacterial activity of the six treatments against *Escherichia coli* and *Staphylococcus aureus* falls into the moderate category.

Based on the results of the study, it is known that probiotic yogurt (P2), probiotic yogurt with the addition of commercial ZnO nanoparticles (P3) and the addition of synthetic ZnO nanoparticles (P5) have the best antibacterial effectiveness against *E. coli* bacteria. The high P2 value is due to the content of *L. Plantarum* IIA - 1A5 which has antibacterial activity (Arief *et al.*, 2015) while in the P3 and P5 treatments it can be concluded that ZnO

Table 4. Antibacterial activity of yogurt is indicated by the diameter of the inhibition zone (mm)

Microbes	Treatments					
	P1	P2	P3	P4	P5	P6
E..coli (mm)	6.43±0.04^{c}	10.2±0.27^{a}	10.1±0.92^{a}	7.07±0.58^{b}	9.01±0.99^{a}	7.38±0.62^{b}
S.aureus (mm)	6.59±0.32^{c}	9.65±0.039^{a}	9.17±0.86^{a}	9.00±0.21^{a}	9.09±0.97^{a}	8.68±0.21^{ab}

P1: Plain yogurt; P2: Probiotic yogurt; P3: Probiotic yogurt + commercial nano ZnO; P4: Probiotic yogurt + synthetic nano ZnO with pectin; P5: Probiotic yogurt + synthetic nano ZnO without pectin; P6: Probiotic yogurt + pectin. Different letters on the same row indicate statistical differences ($p<0.05$) among treatments.

has good antibacterial activity. This is in accordance with the statement of Dizaj *et al.,* (2014) stated that ZnO nanoparticles possess strong antibacterial properties. El Sayed *et al.,* (2021) noted that antimicrobial activity begins with the physical contact between bacteria and metal nanoparticles. As shown result of SEM analysis in Figure 1, ZnO nanoparticles attach to the bacterial cell membrane, resulting in structural damage to the bacterial cell characterized by the emergence of protrusions (blebs) on the surface of the bacterial cell. A bleb represents a membranous protrusion that forms as a result of the internal pressure within a cell (Sens and Plastino, 2015).

In the treatment of Probiotic yogurt + synthetic nano ZnO with pectin (P4), it can be seen that the antibacterial activity against *E. coli* is reduced, it can be concluded that the pectin content affects the performance of zinc. This is in accordance with the statement of Khotimchenko *et al.,* (2008), which states that the chemical and physicochemical structure of pectin substances affects zinc binding activity where the lower the degree of esterification of pectin, the more Zn2+ ions are bound to the active site of pectin. On other hand, pectin basically functions as a protective agent that will protect zinc oxide from changing size and shape (Dmochowska *et al.,* 2020) but this results in reduced antibacterial activity, beside that, the pH that changes when adding pectin can also affect the activity and effectiveness of zinc.

Organoleptic characteristics

Hedonic tests and hedonic quality tests were among the organoleptic tests carried out in this research. The results show a statistically significant impact of the treatments administered on the flavor assessed in the hedonic quality test (Table 5). Based on the hedonic test results, the panelists generally liked the color of all treatments, with scores ranging from 3.47 to 3.63. Meanwhile, according to the hedonic quality test, the panelists considered all treatments white. However, there were some variations in the intensity of the white color, with P2 being considered closest to somewhat white. This is in accordance with the color of probiotic greek yogurt found in Juandini *et al.,* (2024), which is white in color. Aroma assessment was carried out using the sense of smell, as this sensory tool will detect volatile compounds in the product. The hedonic test revealed that the aroma scores varied between 3.13 and 3.40, indicating the panelist's overall enthusiasm for the aroma from all treatments. The hedonic quality test results show that all treatments are considered to have a yogurt aroma. However, there are some variations in the intensity of the aroma, mentioning slightly aromatic yogurt. From this, it can be concluded that the addition of zinc also does not change the color or aroma of yogurt. This is in accordance with the results in the research of Samarathunga *et al.,* (2020) that there is no significant difference between fortified yogurt

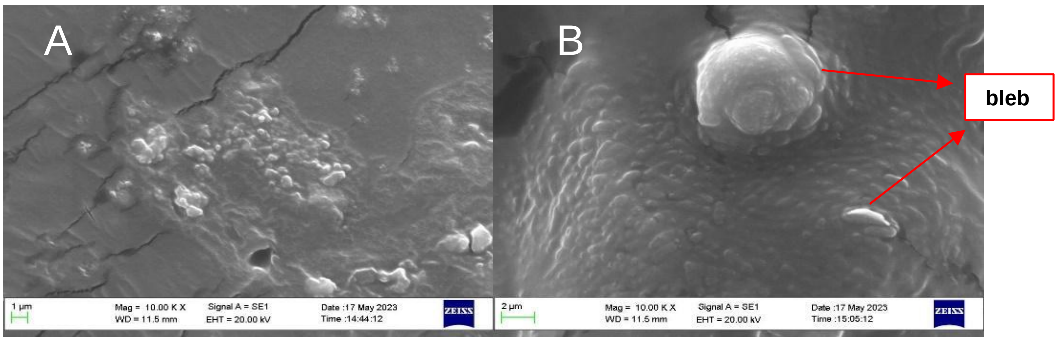

Figure 1. SEM Enlargement at 1000X of Control E. coli (A), E. coli Treated with ZnO Nanoparticles without Pectin (P5), resulting in a Rough Surface (B).

Table 5. Hedonic and hedonic quality tests of probiotic yoghurt fortified with ZnO nanoparticles

Parameter	Treatments					
	P1	P2	P3	P4	P5	P6
Hedonic						
Color	3.53±0.51	3.63±0.50	3.47±0.57	3.47±0.57	3.47±0.57	3.50±0.57
Aroma	3.33±0.61	3.27±0.59	3.27±0.59	3.40±0.56	3.13±0.51	3.33±0.061
Texture	3.33±0.67	3.50±0.57	3.33±0.66	3.37±0.62	3.50±0.57	3.47±0.63
Flavour	2.93±0.74	2.83±0.75	2.70±0.70	3.07±0.59	2.80±0.71	3.07±0.69
Hedonic quality						
Color	3.17±0.65	2.97±0.81	3.13±0.62	3.13±0.68	3.2±0.61	3.07±0.74
Aroma	3.07±0.58	2.97±0.77	2.87±0.51	3.07±0.83	2.83±0.79	2.67±0.92
Texture	2.53±0.82	3.10±0.76	3.00±0.78	2.70±0.84	2.50±0.77	2.73±1.02
Flavour	2.17±0.87^{ab}	2.07±0.83^{ab}	2.23±0.77^{ab}	2.43±1.07^{ab}	2.20±0.99^{ab}	2.77±0.81^{a}

P1: Plain yogurt; P2: Probiotic yogurt; P3: Probiotic yogurt + commercial ZnO nanoparticles; P4: Probiotic yogurt + synthetic ZnO nanoparticles with pectin; P5: Probiotic yogurt + synthetic ZnO nanoparticles without pectin; P6: Probiotic yogurt + pectin.
Different letters on the same row indicate statistical differences ($p<0.05$) among treatments. The hedonic test scale used is 1 (dislike very much), 2 (dislike), 3 (like), 4 (like very much); Hedonic quality scale for Color is 1 (Yellowish white), 2 (Somewhat White), 3 (White), 4 (Very White); Aroma Intensity: 1 (Not aromatic of yogurt), 2 (Slightly aromatic of yogurt), 3 (Aromatic of yogurt), 4 (Very aromatic of yogurt); Texture: 1 (Not thick), 2 (Somewhat thick), 3 (Thick), 4 (Very thick); Flavor: 1 (Not sour), 2 (Somewhat sour), 3 (Sour), 4 (Very Sour).

and regular drinking yogurt in color and aroma. Based on the hedonic test results, the panelists generally liked the texture of all treatments, with scores ranging from 3.33 to 3.50. In the hedonic quality test, there was significant variation in texture scores. P2 is considered the thickest, while P5 is considered the least thick. These results also align with the viscosity values, with the highest value in probiotic yogurt (P2) and the lowest in P5.
In this study, the results of the analysis of variance of the hedonic quality test showed a significant interaction ($p<0.05$) of the treatment on the taste of yogurt where yogurt with pectin (P6) was the most acidic yogurt compared to other yogurts with a value of 2.77 ± 0.81 while other yogurts were below it. This is in accordance with the research of Arioui *et al.,* (2017), which concluded that acidity was increased with increasing of the pectin rate.

Conclusion

Proximate analysis reveals that ZnO nanoparticle fortification does not modify the chemical composition of yogurt. While yogurt fortified with ZnO nanoparticles demonstrates physicochemical properties, LAB levels, and antibacterial effects comparable to standard probiotic yogurt, they align with the SNI Yogurt in these aspects. ZnO nanoparticle fortification also did not affect the color, aroma and texture of the yoghurt and had the same level of liking for color aroma texture and taste as the others. Regarding antibacterial performance, probiotic yogurt enhanced with ZnO nanoparticles exhibits a moderate inhibitory effect on *Escherichia coli* and *Staphylococcus aureus* growth. Consequently, ZnO nanoparticles can serve as a reinforcing element in yogurt formulations to address zinc deficiency.

Conflict of interest

The authors have no conflict of interest to declare. All authors have seen and agree with the contents of the manuscript.

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Author's contribution

The authors confirm contribution to the paper as follows: study conception and design: ZA, PES, and IIA; data collection: PES; analysis and interpretation of results: PES, ZA, CB and IIA; draft manuscript preparation: ZA, PES, IIA, and CB. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval

There are human subjects in this article and informed consent is applicable.

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