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ORIGINAL ARTICLE

ZnO and lactic acid bacteria interaction in yogurt: sensory, surface morphology, and functional group analysis

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Abstract

This study, a novel exploration into the interaction of zinc oxide (ZnO) and lactic acid bacteria (LAB) in yogurt, aims to analyse the morphology and consumer acceptance by organoleptic test effects through yogurt, yogurt probiotics, and yogurt mixtures containing zinc oxide (ZnO). The Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDX) and Fourier Transform Infrared (FTIR) were used to analyse the morphology and functional group of the samples. Four samples were prepared: A) yogurt; B) yogurt + ZnO; C) yogurt + probiotics; D) yogurt + probiotics + ZnO. The addition of ZnO to the yogurt structure increased the compactness and homogeneity of the structure, according to microscopic visual inspection (yogurt B and D). The addition of ZnO did not affect the functional group of the yogurt. Importantly, the organoleptic testing, which includes hedonic and hedonic tests carried out by semi-trained panelists, resulted in overwhelmingly positive responses to the product, indicating its potential for consumer acceptance.

Keywords: FTIR; Organoleptic test; Probiotic; SEM-EDX; Zinc fortification.

Highlights

- The addition of ZnO improved the yogurt structure and homogenity
- ZnO did not alter the yogurt's functional group composition
- ZnO addition had a significant effect on yogurt viscosity

1 Introduction

A century ago, fermented food, particularly yogurt, was a staple. It has evolved into a widely consumed supplement and a healthy diet. To enhance its nutritional value, yogurt is often fortified with probiotics. These probiotics, in addition to improving gut health by restoring the balance of gut microorganisms and producing antimicrobial compounds, have shown promising results in recent clinical trials. These trials have demonstrated that yogurt with probiotic addition can lower blood pressure in adults with hypertension, reduce infectious disease in children, temporarily improve bone health markers in patients with osteoporosis, and increase the vitamin content of food (Gul & Durante-Mangoni, 2024).

One aspect of animal production that needs to be developed to implement smart animal farming is "prime and future milk products", the dairy innovation that may incorporate technological, advancements in sustainability, or nutritional enhancement. Adding essential micro-minerals in milk and yogurt is also important to increase the effectiveness of bioactive compound absorption in probiotic yogurt. Zinc is a material known to improve the quality of food products and is known as a micro-mineral that the body needs (Roohani et al., 2013). Zinc is important in cellular metabolic processes, including catalytic, structural, and immune system functions (Kiouri et al., 2023). Zinc can be obtained from zinc oxide (ZnO) compounds, which have also been widely used as plastic fillers that can be consumed and used in meat, cheese, fruit and vegetables, sweets, bakery products, and food (Abdullah et al., 2018). Fortifying milk with ZnO becomes an opportunity to promote optimal health and support immune function. With the addition of ZnO in the probiotic yogurt, hopefully, this combination could help to improve human immunity and meet daily nutritional needs, especially for children, to minimize stunting and help their growth.

Fortification is the process of adding or incorporating bioactive components (nutrients or non-nutrients) into edible products (Ganthal et al., 2022). Fortification may alter the fermentation process and cause chemical reactions that could change the physicochemical properties, such as syneresis, rheological features, and off-flavors (Santillán-Urquiza et al., 2017). Incorporating probiotics and ZnO into food products can significantly influence their technological and sensorial properties Probiotics, being live microorganisms, can impact food items' texture, taste, and shelf life. Zinc fortification might affect the color, texture, or even the nutrient bioavailability of the final product (Radi & Doosh, 2023). However, this alteration can often contribute positively to the sensory experience by introducing unique flavors or enhancing the overall palatability of the food. Yogurt fortification with probiotics and ZnO formulations necessitates careful consideration of their interactions with the food matrix to maintain desired sensory qualities while ensuring improved nutritional content and technological stability. Balancing these factors is crucial in creating food products that offer enhanced health benefits and retain favorable taste, texture, and overall consumer acceptance.

Previously, the results of making yogurt containing ZnO have been done. We were also already testing the physicochemical profile for the sample (Arief et al., 2023). However, the effects of structure morphology and sensory properties of the probiotics yogurt fortified with ZnO have not been explored. This study aimed to investigate another effect of ZnO fortification on probiotic yogurt based on morphology and sensory properties.

2 Materials and methods

2.1 Yogurt production

The raw cow's milk was purchased from farms in the holy city of Bogor, Indonesia. The ZnO food grade was obtained from the commercial store with nanosize, 1-10 nm. The yogurt was made according to the method adopted by Arief et al. (2023) as follows: Cow's milk used in this study was heated to the temperature of 115 °C for 3 min and lowered to 40-45 °C. Four different treatments of the samples were conducted in the production of yogurt. Four different starter cultures were also used: *Streptococcus thermophilus* IFO 13957, *Lactobacillus bulgaricus* IFO 13953, probiotic *Lactiplantibacillus plantarum* subsp. *plantarum* strain IIA-1A5, and probiotic *Lactobacillus acidophilus* IIA 2B-4 (Arief et al., 2015). Treatment A was pasteurized milk supplemented with *S. thermophilous*, *L. bulgaricus* (yogurt). Treatment B was yogurt + ZnO. Treatment C was yogurt + probiotics, which was pasteurized milk supplemented with *S. thermophilus*, *L. bulgaricus*, and *L. plantarum* subsp. *plantarum* IIA-1A5, and *L. acidophilus* IIA 2B-4. Treatment D was yogurt + probiotics + ZnO, from pasteurized milk supplemented with *S. thermophilus*, *L. bulgaricus*, and ZnO.

The concentration of the microorganism used in this experiment was 10^7 CFU/mL for 5 mL culture per microorganism. The population of LAB was measured based on the Total Plate Count using de Man Rogosa Sharp Agar. The yogurt production was conducted in three repetitions, and the testing was carried out in duplicates for each repetition. The analysis was carried out at time 0 with 24 hours of fermentation time. The shelf life of the samples was not analyzed in this study.

2.2 Sample preparation

Scanning Electron Microscopy (SEM) analysis was carried out using a powdered sample from each sample. The liquid yogurt was dried to powder using a freeze dryer, with temperatures for primary drying ranging from -40 °C to -50 °C (-40 °F to -58 °F) for 72 hours. Secondary drying, with temperatures ranging from 0 °C to 30 °C (32 °F to 86 °F) for 48 hours, followed. The powdered yogurt was then homogenized and ready to observe the yogurt structure using SEM.

2.3 SEM-EDX analysis

The surface layer of the yogurt was examined using SEM. 0,1 mg of the sample was placed on a conductor adhesive, coated with gold, and observed under SEM at a voltage of 10 kV with the magnification of 10,000x, thus the structure of LAB and materials embedded in the yogurt matrix could be easily examined. The chemical composition of the samples was analyzed using energy-dispersive X-ray spectroscopy (EDX).

2.4 Functional group characterization

Functional groups were characterized by Fourier transform infrared (FTIR) spectroscopy. The materials were mixed with KBr with a ratio of 1:1. The mixtures were then formed into pellets and scanned with the wavenumber range 4000-400 cm^{-1}. The data was processed using OriginPro 2016 software.

2.5 Organoleptic test

An organoleptic test, including a hedonic test, was performed with 40 semi-trained panelists for replications. Furthermore, the attributes tested include color, taste, thickness, and aroma with a scale of 1 to 6, ranging from the least preferred for the smallest number to the most preferred for the biggest number. The hedonic test was conducted using only two classes, which are likes and dislikes. Meanwhile, the hedonic quality test assesses samples with more variables. The data obtained was calculated on average for each panelist. The sensory test was conducted right after the product was prepared. Before starting the sensory test, the panelists obtain approval by filling out an informed consent form (ICF).

2.6 Statistical test

Statistical tests on the organoleptic tests used Minitap17 to investigate the Analysis of Variance (ANOVA) value based on the result. Besides, the data from the organoleptic test were analyzed qualitatively using the non-parametric Kruskal-Wallis test.

3 Results and discussion

3.1 SEM-EDX results

ZnO materials have various shape, such as rods, spheres, and whiskers, flower-like, agglomerated structures, and other structures that have been stated by researchers (Alfarisa et al., 2018; Gopal & Kamila, 2017). The morphology of commercial ZnO samples has more diverse shapes and sizes as shown in the SEM results in Figure 1. The cubic, rectangular, and irregular round shapes dominate more than the spherical shapes of ZnO particles. The size of the particle is around 100 nm to more than 1500 nm. Particles with smaller sizes form more aggregates than larger particles.

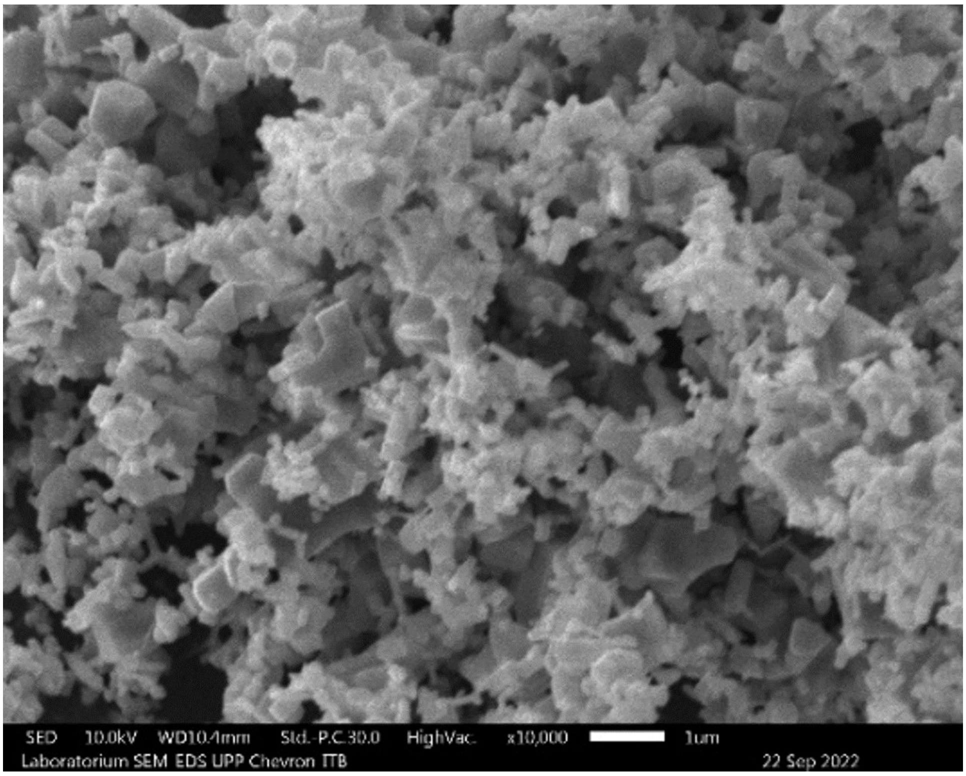
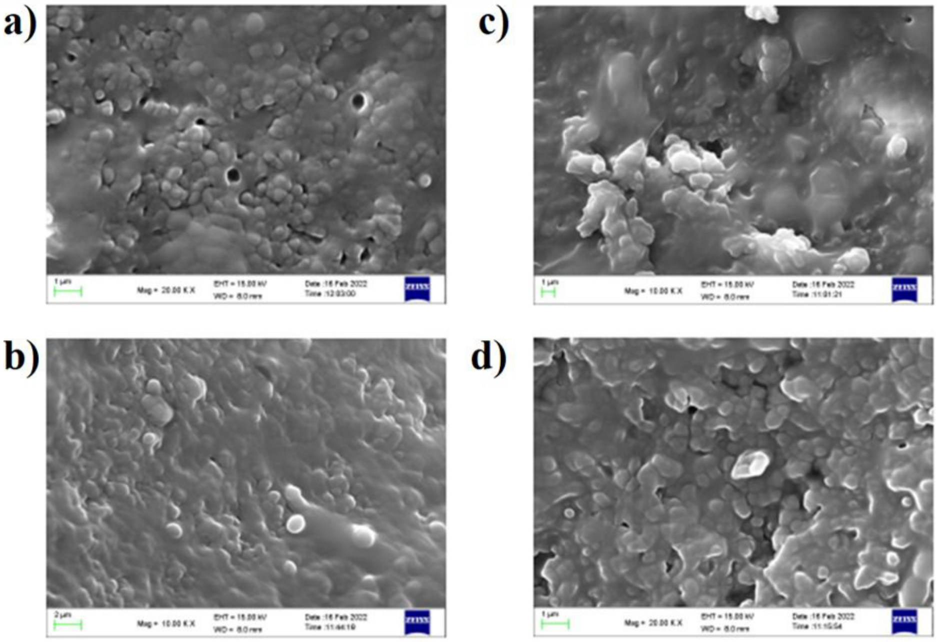


Figure 1. Morphology of ZnO in 10.000x magnification.

Yogurt, yogurt+probiotics, and a mixture of them with ZnO are used as samples with A) yogurt; B) yogurt + ZnO; C) yogurt + probiotics; and D) yogurt + probiotics + ZnO as the sample code. Physically, ZnO does not affect the color and aroma of the yogurt. The effect of adding ZnO into yogurt and probiotic yogurt is shown in Figures 2B and 2D. The addition of ZnO to the yogurt structure increased the compactness and homogeneity of the structure, according to microscopic visual inspection (yogurt B and D). EDX analysis of samples B and D did not show the presence of ZnO. This issue is probably caused by the ZnO being used for bacterial growth. Based on the measured number of bacteria that has grown in the samples, the total number of bacteria in sample A was 9.39 log CFU/mL, after adding ZnO the bacterial population increased to 9.69 log CFU/mL (sample B). Sample C from 9.34 log CFU/mL to 11.02 log CFU/mL (sample D) (Arief et al., 2023). The effect of ZnO addition into the yogurt are increasing the dry matter, protein, ash, and fat content compared with the control yogurt (El-Sayed et al., 2021).

Dry matter refers to the solid components present in the yogurt, excluding water. The addition of ZnO may contribute to this increase by adding additional solid particles to the yogurt matrix. Protein content in yogurt can also increase with the addition of ZnO. This increase may occur due to the presence of protein-bound zinc, or other mechanisms facilitated by the interaction between zinc oxide and yogurt components. Ash content represents the mineral content of yogurt, including essential minerals like zinc. Adding ZnO can raise the ash content of yogurt, as zinc oxide itself contributes to the mineral composition of the product. The fat content of yogurt may also experience an increase when supplemented with ZnO. This elevation could be due to various factors such as changes in the yogurt's texture, consistency, or lipid interactions induced by the addition of zinc oxide particles.



Element	Mass (%)			
	A	B	C	D
C	69.27	76.74	62.68	69.53
O	21.29	21.68	31.41	26.50
Zn	0.26	0.00	0.08	0.00

Figure 2. Morphology and EDX spectra of (a) sample A (yogurt); (b) sample B (yogurt+ZnO); (c) sample C (yogurt+probiotics); and (d) sample D (yogurt+probiotics+ZnO) in 10.000x magnification.

3.2 FTIR analysis

The FTIR analysis was conducted 2 days after sample preparation. The samples were stored at a low temperature of 4 °C before the analysis. FTIR spectral data was collected from samples C, D, and ZnO commercial samples. The infrared spectra of commercial ZnO were observed at 549 cm^{-1} dan 1011 cm^{-1} (Figure 3a). The peak at 549 cm^{-1} is corresponding to Zn–O stretching vibration, while the peak observed at 1011 cm^{-1} is corresponding to the existence of ZnO and organic groups, which could be due to the presence of zinc hydroxy acetates. This means that the ZnO commercial used for the mixture is not pure ZnO (Vijayalakshmi et al., 2016).

Both sample probiotic yogurt (C) and probiotic yogurt + ZnO (D) are shown in the peaks that indicate the functional group of yogurt (Figure 3b). The functional group is observed at 3275 and 1640 cm^{-1} reflecting water (O–H); whereas 2290 and 2852 cm^{-1} correspond to C−H asymmetric vibration; 1742 and 1245 cm^{-1} are corresponding to amide III, C–N stretch, N-H bend, and C=O–N bend; 1632 cm^{-1} corresponding to amide I band functional group; 1542 cm^{-1} corresponding to amide II band; 1460 cm^{-1}corresponding to CH_2 functional group; 1165-1065 cm^{-1} corresponding to lipid, nucleic acid, and polysaccharides; 1032 cm^{-1} corresponding to polysaccharides (C–O stretch) and amines (CN stretch); and peak at 800-500 cm^{-1} were observed as the presence of α-glycosidic bonds. (Jaya, 2009; Naibaho et al., 2022; Papadopoulou et al., 2021). The FTIR spectra from probiotic yogurt and probiotic yogurt + ZnO have different intensities at wave number 750-500 cm^{-1}, which could happen due to the effect of adding ZnO to the sample. The sharp peak in the probiotic yogurt + ZnO sample indicated the interaction of Zn–O at 515 cm^{-1}. However, this effect does not affect the major functional group that composes the yogurt or yogurt fortified with ZnO (Figure 3b).

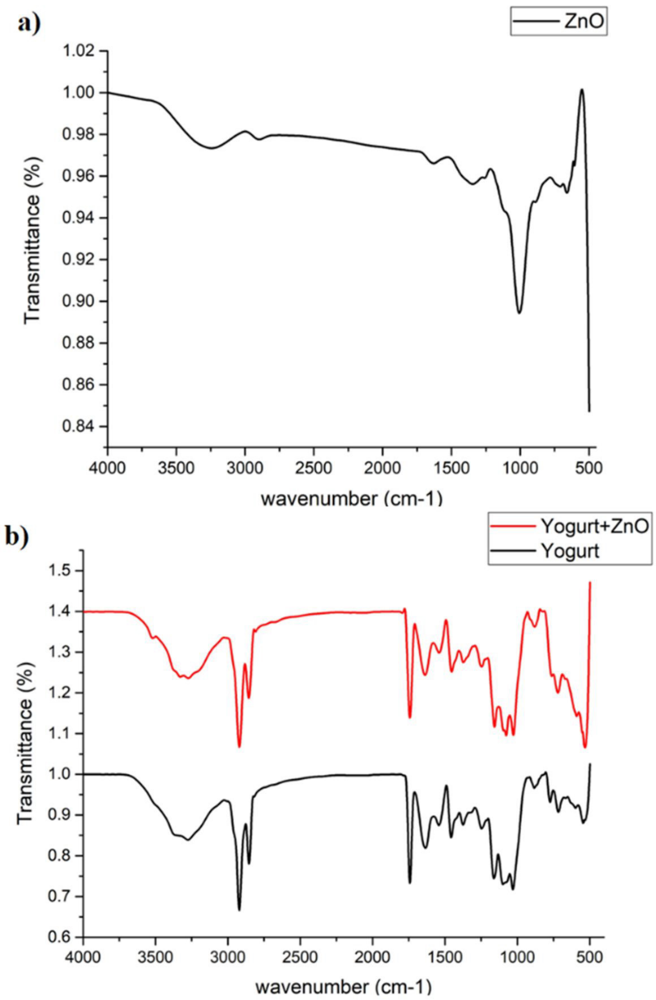


Figure 3. FTIR spectra of (a) ZnO commercial (b) probiotic yogurt + ZnO (top) and probiotic yogurt (bottom).

3.3 Organoleptic test results

For this purpose, an organoleptic test was conducted through a hedonic and hedonic quality test by panelists. The samples that we used are yogurt, yogurt+ZnO, yogurt+probiotic, and yogurt+probiotic+ZnO. Both hedonic and hedonic quality examined the color, taste, thickness, and aroma of the samples that are shown in Table 1 and Table 2, respectively.

3.3.1 Hedonic test of cow's milk probiotic yogurt with ZnO fortification hedonic test results

The results of the hedonic test that were examined included color, taste, thickness, and aroma. The panelists judged that the samples had a different aroma and taste based on the addition of the material increased this attribute (ANOVA). The different taste of the yogurt was mainly caused by the addition of probiotics, namely *L. acidophilus* IIA-2B4 *and Lactiplantibacillus plantarum* subsp. *plantarum* IIA-1A5. These probiotics are LAB known for their ability to ferment lactose, the primary sugar in milk (Arief et al., 2015). During fermentation, these bacteria metabolize lactose into lactic acid, thus contributing to yogurt's characteristic tangy taste. The presence of other organic acids, such as acetic acid and citric acid produced during fermentation, further influences the flavor profile of the yogurt. For the thickness attribute, the panelists preferred those without probiotic bacteria. These results showed that the treatment only had a significant effect on the taste parameter, as shown in Table 1. The testing was carried out using Kruskall-Wallis and the Mann-Whitney tests as a follow-up.

Table 1. Hedonic test scores.

Samples	Hedonic Test Parameters			
	Color	Aroma	Taste*	Viscosity
A	4.6 ± 0.92	4.4 ± 1.10	4.3 ± 1.19^{a}	4.2 ± 1.24
B	4.5 ± 0.86	4.5 ± 0.93	4.2 ± 1.21^{a}	4.4 ± 1.20
C	4.5 ± 0.96	4.4 ± 0.93	4.2 ± 1.21^{a}	4.3 ± 1.19
D	4.6 ± 0.89	4.5 ± 0.97	4.6 ± 1.25^{b}	4.4 ± 1.19

Score:
1 = Very dislike; 4 = Rather like
2 = Dislike; 5 = Like
3 = Rather dislike; 6 = Really like

*indicates the significantly affected hedonic test parameter; numbers in the same column followed by different superscript letters indicate that the results are significantly different (P < 0.05).

3.3.2 Hedonic quality test of dairy's milk probiotic yoghurt with ZnO fortification

The results of the hedonic quality test that were examined include color, taste, thickness, and aroma. There was a difference in the color attribute (ANOVA), but no variation was observed in the aroma (ANOVA). Statistical analysis, specifically ANOVA, indicated a notable difference in the color attribute among the samples. This implies that color variations were observed between different treatments of samples tested. Conversely, ANOVA analysis showed no significant variation in the aroma attribute across the tested samples. This suggests that the addition of probiotic bacteria and zinc did not impact the aroma profile of the yogurt samples in a discernible manner.

In the taste attribute, there is a difference (ANOVA) found in samples with probiotic bacteria that had a more yogurt-like taste. Samples treated with probiotic bacteria exhibited a taste profile more reminiscent of traditional yogurt, indicating that the addition of probiotics contributed to a characteristic yogurt-like taste. Furthermore, yogurt subjected to probiotic and zinc treatment exhibited a thick texture, while showing no discernible variance in color and aroma attributes. These results showed the treatment only had a significant effect on viscosity (Table 2). The test used the Kruskall-Wallis and Mann-Whitney tests as a follow-up.

Table 2. Score of hedonic quality test.

Samples	Hedonic Quality Test Parameters			
	Color	Aroma	Taste	Viscosity
A	3.1 ± 0.74	3.5 ± 1.30	4.0 ± 1.25	3.3 ± 1.03^{a}
B	3.1 ± 0.75	3.7 ± 1.28	4.5 ± 1.00	4.1 ± 1.01^{b}
C	3.1 ± 0.67	4.0 ± 1.12	4.4 ± 1.07	4.4 ± 1.00^{c}
D	3.2 ± 0.65	3.7 ± 1.29	4.3 ± 1.24	3.8 ± 1.04^{d}

Color Score:
1 = So white; 4 = Somewhat brown
2 = White; 5 = Brown
3 = Rather white; 6 = Very brown

Aroma Score:
1 = Very milky; 4 = Slightly yogurt flavored
2 = Milk scented; 5 = Yogurt-scented
3 = Slightly milky; 6 = Very flavorful yogurt

Viscosity Score:
1 = Very watered down; 4 = It is kinda thick
2 = Diluted; 5 = Thick
3 = Somewhat watered down; 6 = Very thick

Taste Score:
1 = Strongly tastes of milk; 4 = Slightly tastes of yogurt
2 = Taste of milk; 5 = Taste of yogurt
3 = Slightly milky taste; 6 = Very yogurt taste

[a,b,c,d] indicate that the results are significantly different (P < 0.05)

4 Conclusion

The ZnO particles that are added to the yogurt have cubic, rectangular, and irregular round shapes. ZnO is trapped and used by bacteria for growth, so it is not detected by SEM-EDX because it is used for bacterial growth. Adding ZnO can improve the compactness and homogeneity of the yogurt structure microscopically. The FTIR spectra analysis showed that the addition of ZnO affects the intensity of the sample probiotic yogurt peak at ~500 cm^{-1}. Besides, this effect did not affect the main typical functional group of yogurt and indicated the ZnO functional group was detected. Although the ZnO and probiotic addition affect the product's homogeneity, the organoleptic result showed that panelists had a positive response to the ZnO yogurt product.

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