Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.13123

Physicochemical profile, amino acid, and flavors of probiotic yogurt with the addition of nano ZnO food grade

Irma Isnafia ARIEF[1]*, Zaenal ABIDIN[2], Zakiah WULANDARI[1], Cahyo BUDIMAN[1], Reza ADIYOGA[1], Elfa Aida KAMILA[2]

Abstract

Yogurt is a functional food produced through milk fermentation by yogurt bacteria. The addition of *Lactiplantibacillus plantarum* IIA-1A5 in the fermentation of yogurt yielding a yogurt probiotic has been shown to exhibit some functional properties. The effects of adding ZnO on the overall properties of the yogurt are unknown. This study aimed to investigate the effect of ZnO nanoparticles on the characteristics of conventional yogurt and yogurt probiotics. Four sets of yogurts were prepared: conventional yogurt, yogurt with added ZnO, yogurt probiotic, and yogurt probiotic with added ZnO. Most of the physicochemical properties of all yogurts were found to be comparable, except for fat and solid non-fat contents. The addition of ZnO increased the total lactic acid bacteria (LAB) in the yogurt, but it apparently inhibited *L. plantarum* IIA-1A5 as indicated by a lower LAB population in the yogurt probiotic with added ZnO compared with the yogurt probiotic without ZnO. However, the combination of *L. plantarum* IIA-1A5 and ZnO in yogurt significantly enhanced the DPPH inhibition activity. Additionally, the positive effects of ZnO were also observed on the total amino acid content, which significantly modulate the flavor compounds. This indicates that, overall, ZnO contributed to the better characteristics of yogurts.

Keywords: yogurt; nanoparticle; functional food; ZnO.

Practical application: nano ZnO is applicable for improving the characteristics and functionality of yogurt.

1 INTRODUCTION

The safety and functional characteristics of agricultural products, particularly livestock, are closely tied to agro-maritime commodities that are competitive and meet the Sustainable Development Goals, which include zero hunger, health and welfare, infrastructure, industry and innovation, and sustainable production and consumption. Agro-maritime producers must prioritize quality, quantity, and continuity to remain competitive in the global market while also addressing functional and competitive advantages, as well as food safety and environmental concerns.

To achieve smart animal farming, a branch of animal husbandry that needs to be developed is prime meat, milk, and egg production, which incorporates the recent technologies, including omics and nanotechnology. Studies have shown that probiotic yogurt has functional properties as an anti-diarrheal, anti-hypertensive, anti-cholesterol, and anti-diabetic agent, as well as inhibits the growth of colon cancer cells. To increase the effectiveness of absorption of bioactive compounds in probiotic yogurt, it is essential to improve its functional properties using nanoparticle technology.

Nanomaterials with a size of approximately 1–100 nm have unique properties compared with their macroscale counterparts due to the high difference in surface-to-volume ratio and other physiochemical properties such as color, solubility, strength, diffusivity, toxicity, magnetic, optical, and thermodynamic, which in turn could affect the food properties (Rai et al., 2009; Gupta et al., 2016). Several studies have investigated the addition of nanomaterials to food systems, including yogurt. Some reports indicate that the application of nanoparticles in food processing can provide advantages such as improvements in bioavailability, taste, texture, and consistency (Cientifica Report, 2006). Additionally, the application of nanoparticles has been reported to increase the shelf-life of various food materials and reduce the extent of wastage caused by microbial infestation (Pradhan et al., 2015). Some nanoparticles that have been applied to food, including yogurt, are iron (Darwish et al., 2021; El-Saadony et al., 2021), zinc oxide produced through bacterial biosynthesis (El-Sayed et al., 2021), as well as various zinc compounds such as oxides, acetate, sulfate, citrate, and gluconate (Mishra et al., 2018). The process of adding these particles to food, known as fortification, involves incorporating essential components such as minerals, vitamins, and proteins to address nutrient deficiencies in the human population. This approach has been successful in correcting mineral deficiencies in humans and has been implemented in Indonesia (Ariningsih, 2016; Mishra et al., 2018). Zinc is one of the key nutrients, which is often added to yogurt as a fortificant.

Several types of zinc minerals, such as oxide, sulfate, nitrate, gluconate, chloride, and stearate, have been developed

Received Mar. 7, 2023.
Accepted Jul. 13, 2023.
[1]*IPB University, Faculty of Animal Sciences, Department of Animal Production and Technology, Jalan Agathis IPB Dramaga Campus, Bogor, Indonesia.*
[2]*IPB University, Faculty of Mathematics and Natural Sciences, Department of Chemistry, IPB Dramaga Campus, Bogor, Indonesia.*
*Corresponding author: isnafia@apps.ipb.ac.id

as fortifying ingredients. Previous studies have shown that the addition of zinc oxide and sulfate has good adsorption results in food (Mishra et al., 2018). However, zinc oxide (ZnO) is often used because it is considered a GRAS (generally recognized as safe) material by the US Food and Drug Administration (FDA) (Kim et al., 2022).

Nanosized ZnO is easier to absorb by the gastrointestinal tract due to its small size compared with its macroscale counterparts. Thus, they are more effective even at a lower dose (Feng et al., 2009). Nano ZnO also has the added advantage of being cheaper and more accessible, as they can be synthesized using physical, chemical, or biological methods (Swain et al., 2016). A previous study conducted by Santillán-Urquiza et al. (2017) investigated the effect of adding ZnO nanoparticles to yogurt. However, the study focused solely on the physical and sensory properties of the yogurt. There has been no comprehensive investigation into the physicochemical profile, amino acids, flavor, and sensory properties of yogurt fortified with ZnO nanoparticles. Notably, the yogurt used in Santillán-Urquiza et al.'s (2017) study was produced solely by a combination of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus.* Mega et al. (2020) previously reported that using additional probiotics such as *Lactiplantibacillus plantarum* or *Lactobacillus acidophilus* resulted not only in a yogurt with better characteristics but also in a higher probiotic content. Arief et al. (2015) had previously isolated local probiotics of *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4, which were shown to be good probiotic supplements for yogurt. The yogurt fortified with *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4, in addition to *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus,* is referred to as probiotic yogurt. However, no studies have reported on the potential for further enhancing this probiotic yogurt through the addition of ZnO.

Therefore, this study aims to investigate the effect of adding ZnO nanoparticles on the physicochemical, amino acid, flavor, and sensory profiles of probiotic yogurt. This study also compares the characteristics of probiotic yogurt with those of normal yogurt, which does not contain *L. plantarum* IIA-1A5 or *L. acidophilus* IIA-2B4. To the best of our knowledge, this is the first study to investigate the fortification of probiotic yogurt with ZnO nanoparticles, which may have promising applications for yogurt manufacturers.

2 MATERIALS AND METHODS

2.1 Starter bacteria preparation for yogurt production

The bacteria utilized in this study included *S. thermophilus* IFO 13957 and *L. delbrueckii* subsp. *bulgaricus* IFO 13953 which were obtained from the Food and Nutrition Culture Collection of Gadjah Mada University, Indonesia. In addition, the Indonesian probiotics of *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 were also used, which were from our own collection culture (Arief et al., 2015). For the fermentation process, these bacteria were prepared by inoculating the stock culture (10%) into sterile milk, which was autoclaved at 115°C for 3 min. The resulting product was incubated at 37°C for 18 h until coagulation formed, yielding a culture (Lee & Lucey, 2010).

2.2 Yogurt productions

The production was based on Afiyah et al. (2022) with some modifications. Briefly, the cow's milk was subjected to heating at 115°C for a duration of 3 min, and subsequently, the temperature was lowered to 40–45°C. The milk was then fermented by different combinations of starter cultures with or without nano Zn, as treatments. The first treatment was fermentation with *S. thermophilus* IFO 13957 and *L. delbrueckii* subsp. *bulgaricus* IFO 13953 with the addition of ZnO nanoparticles (yogurt + nano ZnO). The second treatment was fermentation with *S. thermophilus* IFO 13957, *L. delbrueckii* subsp. *bulgaricus* IFO 13953, *L. plantarum* IIA-1A5, and *L. acidophilus* IIA-2B4 without ZnO nanoparticles (yogurt + probiotic). The third treatment was fermentation with sterile milk supplemented with *S. thermophilus* IFO 13957, *L. delbrueckii* subsp. *bulgaricus* IFO 13953*, L. plantarum* IIA-1A5, and *L. acidophilus* IIA-2B4, with the addition of ZnO nanoparticles (yogurt + probiotics + nano ZnO). As a control, a normal yogurt was prepared by fermenting the milk with a mixture starter of *S. thermophilus* IFO 13957 and *L. delbrueckii* subsp. *bulgaricus* IFO 13953, without ZnO nanoparticles (yogurt).

2.3 Chemical composition analysis

A lactoscan milk (Model Lactoscan SL, Milkotronic Ltd, Bulgaria) at the Animal Products Technology Laboratory was used to analyze fat, protein, and water contents, according to the manufacturer's protocol. For this purpose, a total of 20 mL of the yogurt sample was prepared and then placed in a container. For the measurement, cow's milk was selected as a reference in the instrument.

2.4 Physical characteristic testing

2.4.1 pH value

To determine the pH value of the sample, a Schott pH meter (Schott Instruments GmbH, Hertfordshire, UK) was used, according to the manufacturer's protocol. The electrode tip of the tool was first calibrated with a buffer solution at pH 7–4. After that, it was rinsed with distilled water and dried using a tissue. A 10-mL sample was then prepared, and the meter was immersed into the sample. The reading on the meter was allowed to stabilize to obtain an accurate pH value.

2.4.2 a_w value

The a_w value (water activity) in this study was determined according to Meilanie et al. (2018) using an a_w meter (Novasina ms 1 Set-a_w, Novasina AG, Lachen, Switzerland). A yogurt sample was prepared based on the size of the chamber provided and then inserted close to the top. The results were read by the tool, and the values were allowed to stabilize (Meilanie et al., 2018).

2.4.3 Viscosity value

Viscosity value testing was carried out using a VT-04F Rion viscometer (Japan), according to the manufacturer's protocol. A total of 150 mL of the sample was placed in a vessel, which

has been prepared. Furthermore, the tool was installed above the sample until it rotated and the results were displayed.

2.4.4 Value of total titrated acid

Titratable acidity was measured by the titration method (SNI 2981:2009). Adequate amounts of 0.1 N NaOH and phenolphthalein (PP) were used to conduct the complete acid titration test. A 25-mL vessel was used to prepare the sample, to which 3–5 drops of PP were added. The sample was then exposed to a 0.1 N NaOH solution until a color change occurred or the pH reached neutrality. The total amount of acid was calculated based on the Equation 1:

$$\text{Acid amount (\%)} = (V.N.90)/W \times 100\% \quad (1)$$

where:
V: volume of NaOH solution (mL);
N: normality of the NaOH solution;
90: lactic acid equivalent weight;
W: sample weight (mg);

2.4.5 Antioxidant activity

The experimental procedure was based on Aloglu and Oner's (2011) method for measuring the radical scavenging activity of yogurt using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. In brief, 250 µL of the samples were mixed with 3 mL of 60 µM DPPH and ethanol, and their absorbance was measured at a wavelength of 517 nm until it reached a constant value. A control solution was also prepared, which included 250 µL of distilled water in place of the extract. The results were calculated using the Equation 2:

$$\%\text{inhibitor} = \{(A517\ \text{Control} - A517\ \text{Extract})/A517\ \text{Control}\} \times 100\% \quad (2)$$

2.5 Amino acid testing

2.5.1 Sample preparation

The protein content of the sample was analyzed using the Kjeldahl method. To do so, 6 mg of protein was placed in a screw tube and mixed with 2 mL of 6 N HCl. The tube was then purged with nitrogen gas for 0.5–1 min and immediately sealed. Next, the tube was heated to 110°C and left for 24 h to complete the hydrolysis stage. After cooling the sample to room temperature, the solution was transferred to a rotary evaporator flask. The tube was rinsed 2–3 times with Aquadest, and the rinsing solutions were added to the flask. The samples were then dried using a rotary evaporator and made up to a volume of 10 mL with 0.01 N HCl, ready for analysis using HPLC.

2.5.2 Reagent preparation

To prepare the OPA reagent, a stock solution was first made by mixing 25 mg of OPA with 2 mL of methanol, 0.020 mL of mercaptoethanol, 0.050 mL of 30% Brij-30 solution, and 0.5 mL of 1 M borate buffer at pH 10.4. The solution was gently shaken to mix the ingredients. The resulting reagent was stored in a dark-colored bottle at 4°C and remained stable for 5 days. For daily use, the derivatization reagent was freshly prepared by mixing one part of the stock solution with two parts of potassium borate buffer at pH 10.4.

2.5.3 Mobile phase preparation and HPLC parameters

To prepare Buffer A, Na acetate (pH 6.5) was added at a concentration of 2 g (0.02%), along with 0.5 g of Na-EDTA (0.005%), 90 mL of methanol (9.00%), and 15 mL of THF (1.50%) in 1 L of HP water. The solution was filtered through a 0.45-µm millipore paper and allowed to stabilize for 5 days in a dark-colored bottle filled with He or nitrogen gas at room temperature. Buffer B was prepared by mixing 95% methanol and HP water, and the resulting solution was filtered through a 0.45-µm millipore paper. This solution is expected to remain stable indefinitely. The HPLC conditions were set as follows: the column was Thermo Scientific ODS-2 Hyersil, the mobile phase flow rate was 1 mL/min, and the detector was Fluorescence. The mobile phase consisted of a mixture of Buffer A and Buffer B.

2.6 Amino acid analysis

After hydrolyzing the sample in 10 mL of 0.01 N HCl, the solution was dissolved and filtered with a millipore paper. It was then added to potassium borate buffer with a pH of 10.4 in a 1:1.3 ratio. Next, 5 µL of the resulting solution was transferred to a clean vial, and then 25 µL of OPA reagent was added. The mixture was left to complete the derivatization process for 1 min. Subsequently, 5 µL of the sample was injected into the HPLC column and allowed to run until the separation of all amino acids was complete. The entire process took 25 min to complete.

2.7 Metabolite profiling using GC-MS

A total of 1 µL of the sample was injected with the Agilent 7683 autosampler in split-less mode onto a DB-5MS GC capillary column (Agilent Technologies, 60 m × 0.250 mm × 0.25 µm; Agilent 7890 GC) coupled to a 5975 mass selective detector (Agilent Technologies, Palo Alto, CA, USA) with an electron ionization ion source, which was set at 230°C. Furthermore, the inlet temperature was set at 250°C, while that of the oven was 50°C for 2 min. The subsequent temperature gradient was 5°C per minute until a final of 315°C was obtained and held for 3 min. Each sample was run in duplicates beginning with a blank sample consisting of 200 µL of methanol. Additionally, ultra-pure helium gas (Stillwater Steel, Stillwater, OK) was used as a mobile phase, and mass spectra ranging from 50 to 650 *m/z* were recorded.

2.8 Principal component analysis for the flavor compounds

The Unscrambler X 10.4 (CAMO Analytics, Oslo, Norway) was used to perform principal component analysis (PCA). The peak area values of the 51 identified compounds were considered variables. The data were imported into Excel and preprocessed by performing centering and scaling on the resulting data. The results were then grouped using PCA to obtain a minimum of 70% for both principal components.

2.9 Data analysis

A randomized block design with five biological replications was used in the study. The data are presented as the mean with standard deviation. Differences among the means were examined using analysis of variance along with Tukey's post-hoc test.

3 RESULTS AND DISCUSSION

The ZnO used in this research is commercial food grade nano ZnO. Typically, ZnO nanomaterials come in various shapes such as nanorods, nanospheres, nanowhiskers, flower-like structures, agglomerated structures, and other structures, as reported by several researchers (Alfarisa et al., 2018; Venu Gopal & Kamila, 2017). The final concentration of yogurt with the addition of ZnO nanoparticles in this study was 3 mg/200 mL yogurt, which is within the safe limit for human consumption based on the Recommended Dietary Allowance (RDA). The RDA recommends a maximum daily consumption of 40 mg/day, with an average requirement of 9 mg/day (Mishra et al., 2018). To determine the effect of the addition of ZnO nanoparticles on yogurt, several types of tests were conducted, including physical and chemical evaluations. The physical and chemical characteristics of the yogurt product were assessed by determining the value of a_w, pH, total titrated acid (TTA), viscosity, as well as the fat, protein, and water content.

3.1 Physicochemical characteristics

The physical characteristics that were measured in this study included water activity (a_w), pH, TTA, and viscosity. The results showed that the a_w values ranged from 0.904 to 0.906. As yogurt is a liquid product, it typically has a relatively high a_w value. However, the a_w values obtained in this study were still within the acceptable range for food quality, as they were below the recommended maximum of 0.95 by the IFT/FDA (2001). The viscosity of the probiotic yogurt was found to be affected by the type of bacteria inoculated, while the addition of ZnO nanoparticle did not have any significant impact on the viscosity of the product. Yogurt inoculated with only *S. thermophilus* IFO 13957 and *L. delbrueckii* subsp. *bulgaricus* IFO 13953 had lower viscosity compared with other samples. The acidity of the product was primarily caused by the release of lactic acid into the medium by lactic acid bacteria (LAB) (Santillán-Urquiza et al., 2017). Both the pH and TTA of the samples were measured, and it was observed that the yogurt without ZnO nanoparticle tended to be more acidic, as indicated by its lower pH and higher amount of lactic acid. However, the addition of ZnO nanoparticle was found to reduce the acidity in the yogurt, as shown in Table 1.

Table 1 revealed that there were no significant differences in the lactose content among all the samples, which is consistent with the pH and total titratable acids data. This suggests that the yogurt starters used in all samples have a similar fermentation rate for lactose. The variations in pH and total acids, resulting from the formation of lactic acid during lactose fermentation, indicate differences in the fermentation rates. Moreover, the study suggests that ZnO has no impact on the lactose fermentation rate. This is noteworthy as previous research by Boyaval (1989) reported that Zn had an inhibitory effect on lactic acid fermentation by LAB. However, the absence of any negative effects in this study confirms that ZnO is safe for LAB metabolism. Another interesting finding is that there were no significant differences observed in the protein content of all the samples (Table 1). This suggests that the ZnO did not interfere with the protein metabolism of the yogurt starters. According to Savijoki et al. (2006), the proteolytic system of LAB plays a vital role in casein utilization, providing cells with essential amino acids during growth in milk. This system is also significant in the development of the organoleptic properties of fermented milk products and has industrial importance.

The fat concentration in yogurt is relatively low, typically ranging from 1 to 1.4%, owing to the use of low-fat milk in the formulation. Furthermore, the addition of ZnO nanoparticle has been found to decrease its concentration in the products. Meanwhile, the water content of the yogurt was measured by determining the solid non-fat/SNF and fat content, thereby enabling the determination of the total solids. A higher total solids content is associated with lower water content, which can significantly influence the physical properties of the yogurt, such as its thickness and texture. However, the addition of ZnO nanoparticle has been found to decrease the SNF and fat content, leading to a higher volume of moisture. Syneresis is a common phenomenon of phase separation in suspension that occurs in dairy products. In this regard, the addition of zinc has been shown to increase the syneresis value, which may explain the increase in water content observed in the yogurt, as reported by Santillán-Urquiza et al. (2017). It is worth noting

Table 1. Physicochemical, microbiology, and antioxidant activity of the yogurts.

	Yogurt	Yogurt + Zn nano	Yogurt+probiotics	Yogurt+probiotic +nano ZnO
pH	4.15±0.024	4.16±0.005	4.17±0.44	4.18±0.13
a_w	0.870±0.017	0.871±0.004	0.866±0.33	0.871±0.14
TAT (%)	10.63±0.023	10.37±0.002	10.46±0.27	10.23±0.06
Viscosity (dPas)	2.26±0.023	2.25±0.004	2.43±0.43	2.42±0.07
Lactose (%)	4.09±0.05	4.09±0.05	4±0.03	4.02±0.07
Protein (%)	2.732±0.042	2.732±0.046	2.682±0.039	2.685±0.044
Fat (%)	3.72±0.14 [a]	3.56±0.1 [b]	3.65±0.19 [ab]	3.7±0.11 [a]
Solid nonfat (%)	7.49±0.1 [a]	7.47±0.09 [a]	7.29±0.06 [b]	7.31±0.12 [b]
Salt (%)	0.61±0.01	0.61±0.01	0.59±0.005	0.59±0.009
Density	26.05±0.34	26.06±0.38	25.49±0.32	25.47±0.4
Total lactic acid Bacteria (log cfu/mL)	9.39±0.22 [a]	9.96±0.33 [b]	11.02±0.30 [c]	9.34±0.32 [ab]
Inhibition of DPPH (%)	86.36±0.18 [b]	84.85±0.46 [a]	87.14±0.49 [c]	88.24±0.62 [c]

*Different letters following the means in the same row indicate the significant difference ($p<0.05$).

that the greater syneresis observed in the yogurt containing ZnO nanoparticle may have resulted in reduced consumer acceptance. However, this study did not conduct sensory tests to address this issue. Nevertheless, Bierzuńska et al. (2019) have shown that syneresis does not affect sensory acceptance according to a panel. Their study demonstrated that yogurt with and without syneresis had similar sensory qualities. Therefore, whether the yogurt with ZnO nanoparticle has any sensory issues remains to be investigated experimentally. Additionally, El-Sayed et al. (2021) revealed that the addition of ZnO can increase the amount of protein, fat, ash, and dry matter compared with the control.

Furthermore, Table 1 shows the total LAB population in all yogurts containing ZnO was higher than that of normal yogurts. However, yogurt + probiotics without ZnO exhibited a higher LAB population. This suggests a few things. First, the LAB used in yogurt fermentation was not affected by Zn toxicity. As reported by Yusof et al. (2020), several LAB have developed mechanisms to tolerate Zn^{2+} by preventing their toxicity and the production of ZnO nanoparticle. Second, adding *L. plantarum* IIA-1A5 to yogurt was able to increase the LAB population, but the susceptibility of this bacterium to ZnO is apparently higher than that of *S. thermophilus* IFO 13957 and *L. delbrueckii* subsp. *bulgaricus* IFO 13953. This explains why the LAB population in yogurt + probiotics + ZnO was lower than in yogurt + probiotics samples.

The inhibition of DPPH activity was also measured for four different samples as shown in Table 1. The results revealed that the highest inhibition of DPPH activity was observed in the yogurt samples with *L. plantarum* IIA-1A5, both with and without ZnO (88.24 and 87.14%, respectively). On the other hand, the lowest DPPH activity was observed in the yogurt sample with ZnO. These findings suggest that the addition of *L. plantarum* IIA-1A5 to yogurt may have a positive impact on its antioxidant properties, which is consistent with previous research that has demonstrated the antioxidant potential of *L. plantarum* (Kachouri et al., 2015). The lower DPPH activity in the sample of yogurt with ZnO suggests that ZnO may inhibit the antioxidant activity of *S. thermophilus* IFO 13957 and *L. delbrueckii* subsp. *bulgaricus* IFO 13953. Perna et al. (2014) noted that the antioxidant activity of yogurt is influenced by bacterial fermentation, which leads to the release of several bioactive peptides. The relationship between antioxidant activity and the concentration of low-molecular-weight peptides has been reported in many studies.

3.2 Amino acid composition

To determine the percentage and score of different types of amino acids, the composition of the sample was subjected to testing. The types and percentages of amino acids are presented in Table 2, while the corresponding scores are shown in Table 3. Based on the total number of amino acids, it was observed that the yogurt sample containing ZnO had a higher total amino acid content (3.11%) compared with the other samples. The sample of yogurt enriched with both probiotics and ZnO had a similar total amino acid content to the control yogurt, but significantly higher than the total amino acids in the yogurt enriched with only probiotics. This indicates a few things: the addition of another probiotic (*L. plantarum* IIA-1A5) during fermentation did not contribute to the total amino acid content and ZnO has a significant effect on increasing the total amino acid content in the yogurt enriched with *L. plantarum* IIA-1A5. Interestingly, in human studies, ZnO has been shown to increase amino acid metabolism (Zhang et al., 2018). Applying this idea to the yogurt, it is possible that the ZnO also plays a role in increasing the amino acid content of the LAB, although this remains to be experimentally confirmed. Further analysis of the amino acid score showed that all samples had zero scores for tryptophan, as shown in Table 3. Moreover, high scores for the essential amino acids, namely, valine and phenylalanine + tyrosine, were observed in all samples.

Table 2. Total amino acids.

Parameters	**Result (%w/w)**			
Amino Acids	**Yogurt**	**Yogurt + nano ZnO**	**Yogurt + probiotis**	**Yogurt + probiotics + nano ZnO**
Aspartic acid	0.24±0.007^{ab}	0.26±0.007^{a}	0.21±0.007^{c}	0.22±0^{bc}
Glutamic acid	0.69±0.021^{ab}	0.72±0.007^{a}	0.60±0.021^{c}	0.63±0.014^{bc}
Serine	0.16±0.007	0.17±0.014	0.14±0.007	0.17±0.007
Histidine	0.08±0	0.08±0	0.07±0.007	0.08±0.007
Glycine	0.07±0.007	0.07±0.007	0.06±0	0.07±0.007
Threonine	0.12±0^{ab}	0.14±0.007^{a}	0.11±0.007^{b}	0.12±0.007^{ab}
Arginine	0.11±0.007	0.11±0	0.10±0.007	0.11±0.007
Alanine	0.12±0.007	0.13±0.007	0.11±0.007	0.12±0
Tyrosine	0.15±0^{ab}	0.17±0.007^{b}	0.14±0.007^{b}	0.15±0 ^{ab}
Methionine	0.05±0^{b}	0.08±0.007^{a}	0.05±0^{b}	0.05±0^{b}
Valine	0.20±0.007^{ab}	0.21±0^{a}	0.18±0.007^{b}	0.19±0.007^{b}
Phenylalanine	0.16±0^{ab}	0.19±0^{a}	0.14±0.014^{b}	0.16±0.007^{b}
I-Leucine	0.16±0	0.17±0.007	0.14±0	0.15±0.014
Leucine	0.31±0.021^{ab}	0.33±0.007^{a}	0.27±0^{b}	0.29±0.007^{ab}
Lysine	0.34±0.021	0.33±0.014	0.32±0.035	0.32±0.042
Total amino acids	2.92±0.085^{ab}	3.11±0.01^{a}	2.58±0.071^{c}	2.80±0.106^{b}

*Different letters following the means in the same row indicate the significant difference ($p<0.05$).

3.3 Volatile/flavor components

Prior to conducting PCA grouping, preprocessing of the peak area values for the 51 identified compounds was performed, as presented in Table 4. The preprocessing method utilized was automatic center and scale calibration, with the center transformation employing the average value and the scale utilizing the standard deviation. The objective of automatic scaling is to establish a dataset with improved distribution. Additionally, PCA is frequently utilized to summarize complex data, allowing for the visualization of the diversity of variance and differentiation of the sample from others. In this analysis, cluster formation on a specific PC is the most influential function (Hasan and Abdulazeez, 2021). The PCA score plot displays the grouping of each sample based on the variable chromatogram peak area. Typically, Components 1 (PC1) and 2 (PC2) are employed in PCA (Jollife and Cadima, 2016). The score plot acquired in this study exhibited a data diversity of 65% from the two PCs, indicating that 65% of the data's variability can be explained by the chromatogram peak area variable. The values of the two PCs suffer from poor two-dimensional visualization due to the diversity of PC1 and PC2 being less than 70%.

Figure 1 illustrates that all samples cannot be appropriately grouped, as the closer one group is to another, the greater the similarity of existing metabolite compositions. The inability to properly explain the grouping of the ST-LB-LP-LA sample (yogurt + probiotics) from the PCA predictions is suspected to be due to several similarities in the composition of the same metabolites between these samples and the ST-LB-LP-LA-ZnO

Table 3. Amino acid chemistry score.

Essential amino acids	Amino acid chemistry score			
	Yogurt	Yogurt + nano ZnO	Yogurt + probiotics	Yogurt + probiotics + nano ZnO
Isoleucine	76	81	67	71
Leucine	56	60	49	53
Lysine	35	35	33	33
Methionine + cysteine	7	12	7	7
Phenylalanine + tyrosine	94	100	85	94
Threonine	13	15	12	13
Tryptophan	-	-	-	-
Valine	100	100	100	100

Table 4. List of compounds that were identified in GC/MS.

Compounds	Code
1-Methoxyacetone	1
2-Acetylpropane	2
2-Butyne, 1-methoxy-	3
2-Furanmethanol	4
2-Heptanol	5
2-Heptanol, 6-methyl-	6
2-Heptanone	7
2-Nonanol	8
2-Nonanone	9
2-Pentanol	10
2-Pentanone	11
2-Propanol, 1-ethoxy-	12
2-Tridecanone	13
2-Undecanone	14
3-Ethylcyclobutanone	15
3-Heptanol	16
3-Hydroxybutanal	17
3-Pentanol, 2-methyl-	18
4-Penten-2-ol	19
4-Pentenal, 2-methyl-	20
Acetic acid	21
Acetoin	22
Benzene, 1,2-dichloro-	23
Benzeneacetaldehyde	24
Benzoic acid	25
Butanoic acid	26
Butylated hydroxytoluene	27
Capric acid	28
Caproic acid	29
Caprylic acid	30
Cyclobutanol	31
Eugenol	32
Furan, 2-pentyl-	33
Furan, tetrahydro-2,5-dimethyl-	34
Isobutyl methyl ether	35
Isopropyl ethoxyacetate	36
Limonene	37
Methyl glyoxal	38
Naphthalene	39
Octane, 4-ethyl-	40
Oxime-, methoxy-phenyl-	41
p-Methoxybenzyl azidoformate	42
sec-Isoamyl alcohol	43
Undecane, 5,5-dimethyl-	44
α-Pinene	45
α-Terpinene	46
α-Terpineol	47
α-Terpinolene	48
β-Cymene	49
γ-Terpinene	50
γ-Undecalactone	51

sample (yogurt + probiotics + ZnO). This possibility can be eliminated by developing a database, which would enable more variables to be explained through PCA. Furthermore, it would facilitate better preprocessing searches or testing through PLS-DA, thereby making correlations and groupings more visible. However, further studies are required to analyze the effect of adding zinc to yogurt. Another output from PCA, in addition to the score plot, is the biplot. The biplot on PCA is a combination of the score and loading plots, and it explains the identifying variables in the main components. The loading plot indicates the measurement variables used, namely, the peak area. Influential parameters can be determined by their proximity to the sample grouping (Jollife and Cadima, 2016). Compound numbers 21, 46, and 8 had a significant effect on the ST-LB-Zn sample (yogurt + ZnO), while for the ST-LB (yogurt) sample, the volatile compound variables that had a major impact were 19, 24, 29, and 38. For the ST-LB-LP-LA-Zn sample, the influential compounds were 6, 22, and 11. However, for the ST-LB-LP-LA sample, influential compounds cannot be accurately predicted, as further treatment for their grouping is still required, as shown in Figure 2.

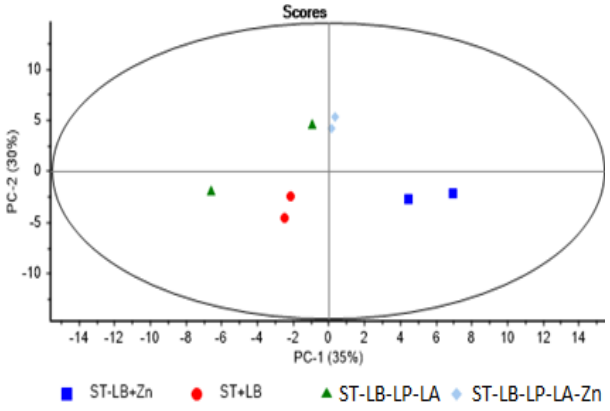

Figure 1. Plot of PCA scores from PC1 and PC2 samples ST-LB-Zn ( ), ST-LB ( ), ST-LB-LP-LA ( ), and ST-LB-LP-LA-Zn ( ).

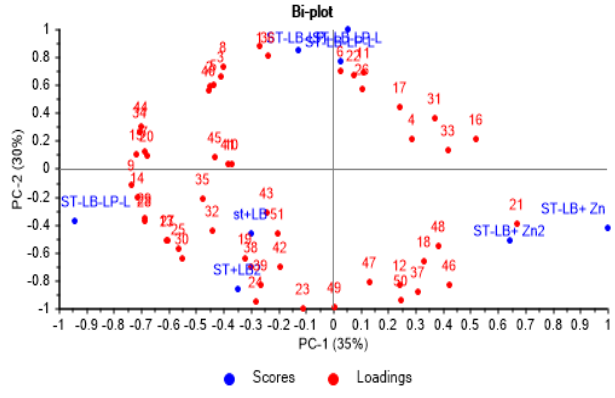

Figure 2. PCA biplot of the sample.

4 CONCLUSION

This study demonstrated that the use of nano ZnO was able to improve the characteristic of the yogurt, particularly in the total LAB and amino acid content, and modulate the flavor compounds, leading to better characteristics of the yogurt. The functionality of yogurt was also increased by the addition of ZnO, in particular for its antioxidant activity. However, caution should be taken when adding zinc to yogurt probiotic as it may inhibit additional probiotic added into the product.

ACKNOWLEDGMENTS

The authors acknowledge support from the Indonesian Ministry of Education Research and Technology for the research funding through Higher Education Applied Research (PTUPT) scheme with contract number 3889/IT3.L1/PT.01.03/P/B/2022-2023 and Agromaritime Research Scheme of IPB University with contract number 7825/IT3.L1/PT.01.03/P/B/2021

REFERENCES

Afiyah, D. N., Sarbini, R. N., & Huda, M. S. (2022). Analysis of the Yogurt Nutrient Content and Antioxidant Activity by Adding Podang Urang Mango Juice (Mangifera Indica L.). *Jurnal Ternak*, *13*(2), 47-52. https://doi.org/10.30736/jt.v13i2.144

Alfarisa, S., Lumban Toruan, P., Atina, A., Dwandaru, W. S. B., & Safitri, R. N. (2018). Morphological and Structural Studies of ZnO Micro-Nanorod Structures Synthesized Using a Low-Cost Hydrothermal Method. *Makara Journal of Science*, *22*(2), 59-66. https://doi.org/10.7454/mss.v22i2.8243

Aloglu, H. S. & Oner, Z. (2011). Determination of antioxidant activity of bioactive peptide fractions obtained from yogurt. *Journal of Dairy Science*, *94*(11), 5305-5314. https://doi.org/10.3168/jds.2011-4285

Arief, I. I., Jenie, B. S. L., Astawan, M., Fujiyama, K., & Witarto, A. B. (2015). Identification and probiotic characteristics of lactic acid bacteria isolated from Indonesian local beef. *Asian Journal of Animal Sciences*, *9*(1), 25-36. https://doi.org/10.3923/ajas.2015.25.36

Ariningsih, E. (2016). *Prospects of Nanotechnology Application in Agriculture and Food Processing in Indonesia*. Badan Standarisasi Nasional. SNI Yogurt (SNI 01-2981-2009). (2009). Dewan Standar Indonesia.

Bierzuńska, P., Cais-Sokolińska, D., & Yiğit, A. (2019). Storage Stability of Texture and Sensory Properties of Yogurt with the Addition of Polymerized Whey Proteins. *Foods*, *8*(11), 548. https://doi.org/10.3390/foods8110548

Boyaval, P. (1989). Lactic acid bacteria and metal ions. *Le Lait*, *69*(2), 87-113.

Cientifica Report (2006). *Nanotechnologies in the Food Industry*. Retrieved from http://www.cientifica.com/www/details.php?id47

Darwish, A. M. G., Soliman, T. N., Elhendy, H. A., & El-kholy, W. M. (2021). Nano-encapsulated Iron and Folic Acid-Fortified Functional Yogurt Enhance Anemia in Albino Rats. *Frontiers in Nutrition*, *8*, 654624. https://doi.org/10.3389/fnut.2021.654624

El-Saadony, M. T., Sitohy, M. Z., Ramadan, M. F., & Saad, A. M. (2021). Green nanotechnology for preserving and enriching yogurt with biologically available iron (II). *Innovative Food Science and Emerging Technologies*, *69*, 102645. https://doi.org/10.1016/j.ifset.2021.102645

El-Sayed, H. S., El-Sayed, S. M., & Youssef, A. M. (2021). Novel approach for biosynthesizing of zinc oxide nanoparticles using *Lactobacillus gasseri* and their influence on microbiological, chemical, sensory properties of integrated yogurt. *Food Chemistry*, *365*, 130513. https://doi.org/10.1016/j.foodchem.2021.130513

Feng, M., Wang, Z. S., Zhou, A. G., & Ai, D. W. (2009). The effects of different sizes of nanometer zinc oxide on the proliferation and cell integrity of mice duodenum-epithelial cells in primary culture. *Pakistan Journal of Nutrition*, *8*(8), 1164-1166. https://doi.org/10.3923/pjn.2009.1164.1166

Gupta, A., Eral, H. B., Hatton, T. A., & Doyle, P. S. (2016). Nanoemulsions: formation, properties and applications. *Soft Matter*, *12*, 2826-2841. https://doi.org/10.1039/c5sm02958a

Hasan, B. M. S., & Abdulazeez, A. M. (2021). A Review of Principal Component Analysis Algorithm for Dimensionality Reduction. *Journal of Soft Computing and Data Mining*, *2*(1), 20-30.

Institute of Food Technologists for the Food and Drug Administration of the United States Department of Health and Human Services (IFT/FDA) (2001). Evaluation and Definition of Potentially Hazardous Foods. *Comprehensive Reviews in Food Science and Food Safety*, *2*(2). Retrieved from https://www.fda.gov/files/food/published/Evaluation-and-Definition-of-Potentially-Hazardous-Foods.pdf

Jollife, I. T., & Cadima, J. (2016). Principal component analysis: A review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *374*(2065). https://doi.org/10.1098/rsta.2015.0202

Kachouri, F., Ksontini, H., Kraiem, M., Setti, K., Mechmeche, M., & Hamdi, M. (2015). Involvement of antioxidant activity of Lactobacillus plantarum on functional properties of olive phenolic compounds. *Journal of Food Science and Technology*, *52*(12), 7924-7933. https://doi.org/10.1007/s13197-015-1912-2

Kim, I., Viswanathan, K., Kasi, G., Thanakkasaranee, S., Sadeghi, K., & Seo, J. (2022). ZnO Nanostructures in Active Antibacterial Food Packaging: Preparation Methods, Antimicrobial Mechanisms, Safety Issues, Future Prospects, and Challenges. *Food Reviews International*, *38*(4), 537-565. https://doi.org/10.1080/87559129.2020.1737709

Lee, W. J., & Lucey, J. A. (2010). Formation and Physical Properties of Yogurt. *Asian-Australasian Journal of Animal Sciences*, *23*(9), 1127-1136. https://doi.org/10.5713/ajas.2010.r.05

Mega, O., Jahidin, J. P., Sulaiman, N. B., Yusuf, M., Arifin, M., & Arief, I. I. (2020). Total Count of Lactic Acid Bacteria in Goats and Cows Milk Yoghurt using Starter S. thermophilus RRAM-01, L. bulgaricus RRAM-01 and L. acidophilus IIA-2B4. *Buletin Peternakan*, *44*(1), 50-56. https://doi.org/10.21059/buletinpeternak.v44i1.42311

Meilanie, R. T., Arief, I. I., & Taufik, E. (2018). Karakteristik Yogurt Probiotik dengan Penambahan Ekstrak Bunga Rosella (*Hibiscus sabdariffa L*) Selama Penyimpanan Suhu Dingin. *Jurnal Ilmu Produksi Dan Teknologi Hasil Peternakan*, *6*(1), 36-44. https://doi.org/10.29244/jipthp.6.1.36-44

Mishra, S. K., Kaur, R., & Mishra, K. K. (2018). Fermented Dairy Products as Zinc Fortification Vehicle. *International Journal of Fermented Foods*, *7*(1), 55-63. https://doi.org/10.30954/2321-712X.01.2018.7

Perna, A., Intaglietta, I., Simonetti, A., & Gambacorta, E. (2014). Antioxidant activity of yogurt made from milk characterized by different casein haplotypes and fortified with chestnut and sulla honeys. *Journal of Dairy Science*, *97*(11), 6662-6670. https://doi.org/10.3168/jds.2013-7843

Pradhan, N., Singh, S., Ojha, N., Srivastava, A., Barla, A., Rai, V., & Bose, S. (2015). Facets of nanotechnology as seen in food processing, packaging, and preservation industry. *BioMed Research International*, *2015*, 365672. https://doi.org/10.1155/2015/365672

Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, *27*(1), 76-83. https://doi.org/10.1016/j.biotechadv.2008.09.002

Santillán-Urquiza, E., Méndez-Rojas, M. Á., & Vélez-Ruiz, J. F. (2017). Fortification of yogurt with nano and micro sized calcium, iron and zinc, effect on the physicochemical and rheological properties. *LWT*, *80*, 462-469. https://doi.org/10.1016/j.lwt.2017.03.025

Savijoki, K., Ingmer, H. & Varmanen, P. (2006). Proteolytic systems of lactic acid bacteria. *Appl Microbiology and Biotechnology*, *71*, 394-406. https://doi.org/10.1007/s00253-006-0427-1

Swain, P. S., Rao, S. B. N., Rajendran, D., Dominic, G., & Selvaraju, S. (2016). Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. *Animal Nutrition*, *2*(3), 134-141. https://doi.org/10.1016/j.aninu.2016.06.003

Venu Gopal, V. R., & Kamila, S. (2017). Effect of temperature on the morphology of ZnO nanoparticles: a comparative study. *Applied Nanoscience*, *7*(3-4), 75-82. https://doi.org/10.1007/s13204-017-0553-3

Yusof, H. M., Mohamad, R., Zaidan, U. H., & Rahman, N. A. (2020). Sustainable microbial cell nanofactory for zinc oxide nanoparticles production by zinc-tolerant probiotic Lactobacillus plantarum strain TA4. *Microbial Cell Factories*, *19*(1), 10. https://doi.org/10.1186/s12934-020-1279-6

Zhang, W., Zhao, Y., Li, F., Li, L., Feng, Y., Min, L., Ma, D., Yu, S., Liu, J., Zhang, H., Shi, T., Li, F., & Shen, W. (2018). Zinc Oxide Nanoparticle Caused Plasma Metabolomic Perturbations Correlate with Hepatic Steatosis. *Frontiers in Pharmacology*, *9*:57. https://doi.org/10.3389/fphar.2018.00057