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Research Article

Antimicrobial and Antioxidant Activities of Various Freeze-Dried Yogurt Fermented with The Addition of Pineapple: An *In Vitro* Study

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Abstract
As a fermented milk, yogurt is functional food which contains a lot of bioactive compounds beneficial for human health. Yogurt can also be made with the addition of fruit such as pineapple, where pineapple contains phytochemical compounds as antimicrobial and antioxidant. This research aims to determine the antimicrobial and antioxidant activity of freeze-dried yogurt with raw material skim milk, full cream, and soy powder with addition of pineapple juice. Antimicrobial activity was conducted using disc diffusion and macro-dilution methods. While antioxidants activity conducted using the DPPH method at a maximum wavelength of 516 nm and FTIR method. The results showed that the antimicrobial activity of freeze-dried yogurt macro-dilution method against *Escherichia coli* showed that most yogurt at a concentration of 1 g/mL could be determined as minimum bactericidal concentration (MBC). Antioxidant activity showed pineapple soy yogurt has the lowest IC_{50} namely 43.67 ± 0.21 µg/mL. Functional group analysis of samples with the highest antioxidant activity in pineapple soy yogurt showed C–H, C–C, C=O, N–H, C=C, and amide II groups. The antimicrobial and antioxidant activities of freeze-dried yogurt are influenced by the raw materials used and the addition of pineapple fruit.

Keywords: antimicrobial, antioxidant, FTIR, pineapple, yogurt

1. INTRODUCTION

With the development of food science and technology, there has been significant progress in the modification of processed foods to become functional, also known as functional foods. Functional food refers to food with specific physiological functions that provide health benefits. One example of functional food is yogurt, which has antimicrobial and antioxidant benefits [1][2]. Yogurt is a fermented product made from milk. Skim milk, full cream milk, and soy powder can be used as raw materials in yogurt, which can function as antimicrobial and antioxidant compounds [3]. When lactic acid bacteria are added during fermentation, they can produce lactic acid and acetic acid and act as antimicrobial agents by destroying the outer membrane of Gram-negative bacteria cells [4][5]. Additionally, bacteriocins or other metabolites can penetrate cell membranes, causing instability in bacterial intracellular activities and leading to the death of pathogenic bacteria [6]. Yogurt also contains antioxidant peptides, as well as vitamins E and C, which function as antioxidants [3][7][8].

Adding fruits to yogurt can enhance both antimicrobial and antioxidant activities. The inclusion of fruit in yogurt can increase its acidity, which helps in inhibiting pathogenic bacteria [9]. Moreover, fruit addition can boost antioxidant activity due to an increase in total phenolic content [10]. Adding pineapple to yogurt can specifically enhance antioxidant activity. Previous studies have shown that adding 60% pineapple (w/w) and 4% cinnamon results in an antioxidant activity of 47.8%, due to the presence of flavonoids, tannins, and vitamin C in pineapple [11]. Additionally, earlier research demonstrated that adding 60% pineapple and 4% cinnamon achieved an antimicrobial activity against *E. coli* of 6.33%, owing to the presence of polyphenols, saponins, and flavonoids as antimicrobial agents [12].

An innovation in yogurt as a functional food involves turning it into a powder. This change helps extend its shelf life, makes it easier to store and distribute, and preserves the lactic acid bacteria [13]. One method for drying yogurt is freeze-drying, which keeps the yogurt's taste, color, smell, nutrients, and lactic acid bacteria intact [14]. This

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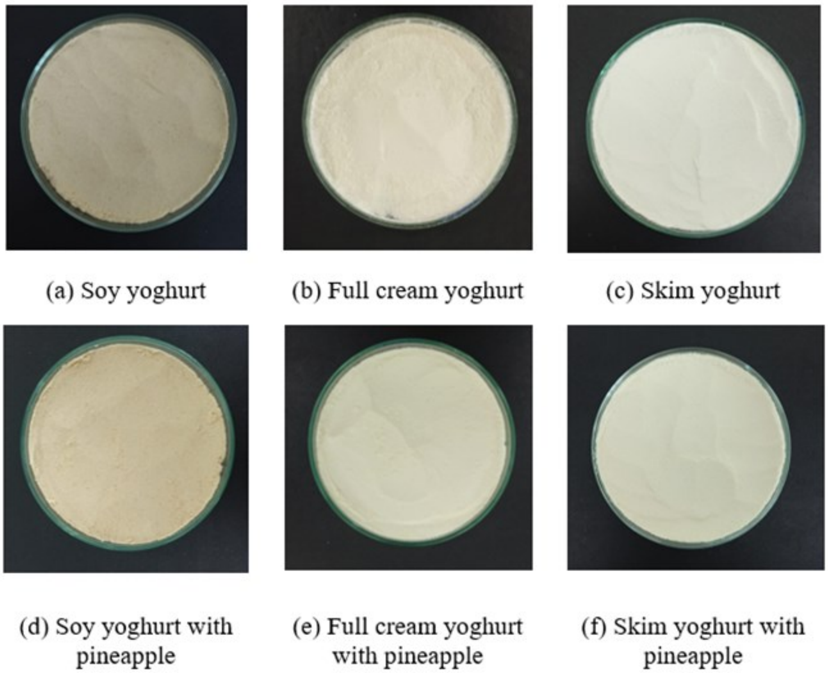

Figure 1. Results of freeze-dried yogurt.

study was carried out to test the antimicrobial and antioxidant properties of yogurt with added pineapple juice in its freeze-dried form.

2. MATERIALS AND METHODS

2.1. Materials

The materials used include honey pineapple from Sunpride, Dancow full cream milk powder, Indoprima skim milk powder, Mandala soy powder, commercial yogurt starter (containing bacterial cultures of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei*), tissue, and aluminum foil. Additionally, the following media and chemicals were used: potato dextrose agar (PDA), Tryptone Bile X-Glucuronic (TBX) medium, Mueller Hinton broth (MHB), Mueller Hinton agar (MHA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, methanol, and distilled water.

2.2. Methods

2.2.1. Apparatus Sterilisation Preparation

The sterilisation stage starts from the apparatus preparation stage to sample preparation. The apparatus sterilisation process uses an autoclave with a pressure of 1 atm, temperature 121 ^{o}C for 15 min [15]. While in the process of sterilisation on, the work table can be done by spraying 70% alcohol [16].

2.2.2. Pineapple Extract Preparation

The pineapple peel was removed, and the fruit was washed under running water. After that, the pineapple was cut into pieces and blended. The blended pineapple was then filtered using a clean cloth, pasteurized by heating for 5 min at 80 °C [17].

2.2.3. Preparation of Yogurt from Skim Milk Powder, Full Cream Milk, and Soybean Powder

The raw materials, including 15 g of skim milk powder, full cream milk powder, and soybean powder, were weighed. Then, 84.9 mL of water was added to each ingredient. The mixture was heated at 43 °C. After that, 0.1 g of yogurt starter was added, stirred, and incubated in a yogurt maker for 24 h [18][19].

2.2.4. Preparation of Yogurt from Skim Milk Powder, Full Cream Milk, and Soybean Powder with Pineapple Extract Addition

The raw materials, including 15 g of skim milk powder, full cream milk powder, and soybean powder, were weighed. Then, 69.9 mL of water and 15 mL of pasteurized pineapple extract were added.

The mixture was heated at 43 °C. Afterward, 0.1 g of yogurt starter was added, and the mixture was incubated in a yogurt maker for 24 h [18][19].

2.2.5. Freeze Drying

A yogurt sample with a thickness of approximately 5 mm is placed in a petri dish with a diameter of 90 mm and stored in a freezer at a temperature of approximately -25 ºC for ±12 h. Freeze drying is carried out using a freeze dryer set to a temperature of -51±3 ºC and a pressure of 3±0.5 Pa., according to the minimum vacuum pressure achieved by the instrument [20]. Freeze drying is carried out for 12 h [21]. The dried sample is ground using a mortar and pestle until homogeneous. After that, it is filtered using a sieve with a no. 35 mesh [20]. Then, the powdered yogurt is stored in a sealed container with added food-grade silica gel.

2.2.6. Yield Analysis

The yield calculation was performed by weighing the final weight of the product after the processing and then comparing it to the initial weight before the processing took place [22]. The yield percentage was determined using the Formula 1.

$$\text{Yield (\%)} = \frac{Yoghurt\ freeze\ dried}{Yoghurt\ segar}\, x\ 100\% \qquad (1)$$

2.2.7. Analysis of Antibacterial Activity

Antimicrobial activity was determined using two methods: the agar disc diffusion method and macro-dilution. For agar disc diffusion method, sterile paper discs, moistened with yogurt, were placed on MHA media inoculated with the test bacteria and left for 30 min. Then, the petri dish was incubated for 24 h at 37 °C. To determine the antimicrobial activity of the sample, a clear zone will form around the disc paper, then the width of the diameter is measured with a caliper. The inhibition zone can be calculated using the following Formula 2 [23].

$$\text{Zone of inhibition} = \frac{(D1 - d1) + (D2 - d2)}{2} \qquad (2)$$

The antimicrobial activity of the macro-dilution method can be determined through the minimum concentration required to inhibit bacterial growth (MIC) and minimum bactericidal concentration (MBC) [23]. Two-fold dilution (1; 0.5; 0.25; 0.125; 0.063; 0.031 g/mL) of antimicrobial agent (freeze-dried yogurt) was prepared. Yogurt was placed in test tube 7 as positive control (without test microbes). About 100 µL of test microbe was added to test tube 8 as negative control. Furthermore, 100 µL of test microbes were added to test tubes 1–6. Each test tube was incubated at 37 ºC for 24 h. The MIC value was determined by observation based on turbidity in the screw tube compared to positive and negative control. The MBC value was determined by testing bacterial growth on PDA and TBX media after pouring 0.1 mL of suspension from each test tube and incubating at 37 ºC for 24 h [24].

2.2.8. Antioxidant Activity Analysis

Preparation of the DPPH solution involved weighing 5 mg of sample into a 100 mL volumetric flask and then mixing it with methanol in 100 mL volumetric flask. For positive control solution, 10 mg of ascorbic acid was weighed and then dissolved in methanol in 50 mL volumetric flask.

Table 1. Yield of freeze-dried yogurts.

Freeze-Dried Yogurt	Yield (%)
Skim yogurt	$15.44^{b} \pm 0.01$
Skim yogurt with pineapple	$18.55^{e} \pm 0.02$
Full cream yogurt	$16.03^{c} \pm 0.00$
Full cream yogurt with pineapple	$19.68^{f} \pm 0.01$
Soy yogurt	$14.60^{a} \pm 0.01$
Soy yogurt with pineapple	$16.99^{d} \pm 0.003$

Note: values in the same row carrying the same superscript are not significantly different ($p < 0.05$) according to Duncan's New Multiple Range Test.

Table 2. Antimicrobial activity of yogurt from different types of raw materials.

Freeze-Dried Yogurt	Diameter zone of inhibition (mm)	
	E. coli	*S. cerevisiae*
Skim milk	$3.35^{a} \pm 0.17$	$1.94^{a} \pm 0.15$
Skim with pineapple	$3.34^{a} \pm 0.24$	$3.65^{b} \pm 0.49$
Full cream milk	$3.86^{a} \pm 0.95$	$2.18^{a} \pm 0.07$
Full cream with pineapple	$3.77^{a} \pm 0.22$	$3.14^{b} \pm 0.60$
Soy milk	$3.25^{a} \pm 1.73$	$1.8^{a} \pm 0.15$
Soy milk with pineapple	$3.32^{a} \pm 0.27$	$2.25^{a} \pm 0.06$

Note: Values in the same row carrying the same superscript are not significantly different ($p < 0.05$) according to Duncan's New Multiple Range Test.

Different range concentrations were prepared for each sample to achieve a 50% inhibition value in every sample. The standard concentration series was made into 1, 2, 4, 6, and 8 µg/mL.The concentration series of soybean powder samples included 90, 150, 200, 300, and 400 µg/mL. The concentration series of full cream milk powder included 100, 200, 300, 400, 550, and 750 µg/mL, while the concentration series of skim milk powder included 150, 250, 350, 450, 550, and 750 µg/mL. Furthermore, for yogurt samples, the concentration series of soy yogurt powder includes 60, 80, 100, 120, and 150 µg/mL. Furthermore, the concentration series of full cream milk yogurt includes 90, 100, 120, 140, and 160 µg/mL, and the concentration series of skim milk yogurt includes 120, 140, 150, and 160 µg/mL. In soy yogurt with added pineapple, the concentration series used included 15, 20, 30, 40, and 50 µg/mL. While in pineapple full cream yogurt the concentration series used included 35, 50, 60, 70, and 80 µg/mL, and the pineapple skim yogurt concentration series used included 55, 65, 75, 85, and 100 µg/mL. In the antioxidant activity test, DPPH and samples were mixed using a 1:4 ratio into a vial tube, then The mixture was blended and stored in the dark for 30 min. Then, the absorbance was recorded at the wavelength of 516 nm. Furthermore, the inhibition percentages were calculated using the Formula 3 [25].

$$\text{Inhibition (\%)} = \frac{Abs\ K - Abs\ P}{Abs\ K} x\ 100\% \quad (3)$$

The IC_{50} value was determined by plotting percent inhibition against the natural logarithm of the concentration on a calibration curve. The regression equation used for this calculation indicates that y represents the percent inhibition at 50, while x signifies the IC_{50} value [25].

2.2.9. FTIR Analysis

In the use of FTIR, ATR installation is carried out before the test is carried out. then the ATR crystal is cleaned using water and 96% ethanol with a tissue before and after testing. in testing with FTIR instruments carried out by placing a sample of 0.1 g on the ATR crystal. Then the FTIR spectrum was obtained in the range of wave numbers from 400 to 4,000 cm^{-1} [26].

2.2.10. Data Analysis Process

Data analysis was carried out using Two-way ANOVA with a significance of 5%. If the p-value is less than 0.05, it indicates a significant difference. If the p-value is greater than 0.05, it suggests there is no significant difference. If there is a difference between treatments, it is continued with the Duncan Test with a significance of 5% [27][28].

3. RESULTS AND DISCUSSIONS

3.1. Yield of Powdered Yogurt

The results of the freeze-dried yogurt can be seen in Figure 1. The freeze-dried yogurt shows different colors. The type of milk used affects the yogurt's color. Soy yogurt powder and full cream yogurt powder are yellow, while skim yogurt powder is white. This is because the fat content in the milk influences the yogurt's color; more fat results in a different color [29]. There are also differences in the texture of the powders produced.

Adding pineapple juice can make the yogurt appear more yellow due to the beta-carotene it contains. Quantitative color testing of the addition of pineapple juice to yogurt using a colorimeter with Commission International de I Eclairage (CIE) coordinates, showed a ΔE value greater than 6.0, indicating that the addition of pineapple juice affected the colour of the yogurt [17].

Based on the yield results in Table 1, the average, and standard deviation for yogurt with and without pineapple, a higher yield indicates a greater weight of the sample after drying [30]. Previous studies reported that the yield of powdered yogurt using foam mat drying and freeze-drying methods were 25.26% and 26.13%, respectively [31][32]. These differences are due to the inclusion of filler materials and variations in drying temperatures, which can increase both the product weight and yield [32][33]. Additionally, calculations for standard deviation was performed to validate the precision of the data obtained [34]. The result of the two way anova test showed a value $p < 0.05$, so there is significant between raw material and addition pineapple in yogurt. It is continued with the Duncan test with a significance of 5% provided in Table 1.

The results of the Duncan test, which analyzed the interaction between variations in raw materials and the addition of pineapple juice, revealed a significant difference. The viscosity levels of yogurt, when subjected to freeze-drying, led to varying yields in the drying process. This finding aligns with previous research, which indicates that the water content of the material prior to drying and its structure—whether solid or viscous—can influence the efficiency of the freeze-drying process, thereby improving yield. Additionally, material loss during the pre-drying weighing process is another factor contributing to the inconsistency in drying yields [35].

3.2. The Results of Microbiological Analysis

Antimicrobial activity testing using 2 methods, namely agar disc diffusion and macro-dilution methods. antimicrobial activity with agar disc diffusion method can be known from the formation of inhibition zone around the disc. The larger the inhibition zone formed, the higher the antimicrobial activity [36]. Antimicrobial activity testing carried out on yogurt products against *E. coli* bacteria and *S. cerevisiae* yeast can be seen in Table 2.

The ANOVA results indicated that variations in raw materials and the addition of pineapple treatment ($p>0.05$) did not significantly affect the antimicrobial activity of the yogurt product against *E. coli* bacteria, a finding further supported by the Duncan test. However, these factors did have a significant impact on the antimicrobial activity against *S. cerevisiae* ($p < 0.05$). The antimicrobial activity against *E. coli* bacteria was categorized as moderate. On the other hand, the greatest antimicrobial activity against *S. cerevisiae* yeast was observed in skimmed milk yogurt with pineapple addition and full cream with pineapple, measuring 3.65 and 3.14 mm also categorized as moderate. The antimicrobial activity was classified based on the zone's diameter as follows: a weak response for diameters between 0 and 3 mm, a moderate response for diameters ranging from 3 to 6 mm, and a strong response for diameters greater than 6 mm [37]. Based on previous research, the antimicrobial activity of yogurt with the addition of

Table 3. MBC determination of freeze-dried yogurt against *E. coli* bacteria.

Sample	Concentration (g/mL)					
	1	0.5	0.25	0.125	0.063	0.031
Skim milk	-	+	+	+	+	++
Skim with pineapple	-	+	+	+	++	++
Full cream milk	-	+	+	+	+	+
Full cream with pineapple	-	+	+	+	+	+
Soy milk	+	+	+	++	++	++
Soy milk with pineapple	-	+	+	+	+	++

Note: (-) = no growth of *E. coli*; (+) = minimal growth of *E. coli;* (++) = high growth of *E. coli*

Table 4. MBC determination of freeze-dried yogurt against the *S. cerevisiae* yeast.

Sample	Concentration (g/mL)					
	1	0.5	0.25	0.125	0.063	0.031
Skim milk	++	++	++	++	++	++
Skim with pineapple	+	++	++	++	++	++
Full cream milk	++	++	++	++	++	++
Full cream with pineapple	+	++	++	++	++	++
Soy milk	+	++	++	++	++	++
Soy milk with pineapple	++	++	++	++	++	++

Note: (-) = no SC growth; (+) = minimal growth of SC; (++) = high growth of SC

a combination of pineapple juice and cinnamon was 6.27 mm. The difference in these results is due to the active bromelain enzyme in pineapple and the addition of cinnamon which has antimicrobial properties [12].

The formation of a clear zone in testing antimicrobial activity against *E. coli* bacteria and *S. cerevisiae* yeast occurs due to the formation of lactic acid bacteria (LAB) isolate interactions that inhibit the growth of these microbes. The process of inhibiting the growth of *E. coli* bacteria and *S. cerevisiae* yeast by LAB can be through several mechanisms such as the formation of organic compounds such as lactic acid, acetic acid, hydrogen peroxide, bacteriocins [38]. Bacteriocins produced by LAB can disrupt the defence system of *E. coli* bacteria and *S. cerevisiae* yeast by damaging the permeability membrane and entering the cell to disrupt the cell's metabolic system so that it can damage cell activity resulting in cell death [39].

Based on the results, most of the samples exhibited stronger antimicrobial activity against *E. coli* bacteria compared to *S. cerevisiae* yeast. This is due to the difference in the composition of the cell wall between the microba. *E. coli* bacteria are Gram -negative bacteria which are composed of peptidoglycan which is thinner than gram-positive bacteria, while *S. cerevisiae* yeast has several main components that make up the cell wall, namely: mannoprotein, glucan, and chitin. The fibrous β-1,3 -glucan-chitin complex is the major constituent of the inner cell wall. The β-1,6-glucan branch is a component of the connecting component between the inner and outer cell walls. The outer cell wall is composed of mannoproteins which are arranged tightly and compactly and functions to limit the entry and exit of a solution or compound through the cell wall [40]. So that the bacteriocin produced by LAB is more more difficult to penetrate the cell wall of *S. cerevisiae* than *E. coli.*

Determination of the lowest concentration to inhibit microbial growth, both bacterial and yeast species can be seen from the turbidity of the results after incubation for one day. The MIC value in this study cannot be observed, this is due to bias or interference from the colour of the sample, namely freeze-dried yogurt dilution which causes the results of the dilution series before incubation to be cloudy. Other studies also experienced bias in determining MIC microbial heavy metal resistance using the turbidimetry method [41][42].

The determination of MBC in the macro-dilution method of antimicrobial testing against *E. coli* bacteria is conducted on selective agar media, specifically TBX media. TBX media can selectively indicate the presence of *E. coli* bacteria by turning the agar a greenish color. TBX media contains complex sugars, notably 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG). When *E. coli* bacteria consume the sugar, the β-D-glucuronidase enzyme in the bacteria separates the bonds in the complex sugar, specifically glucuronide and chromogen. The glucuronide bond is absorbed by the *E. coli*, while the chromogen bond is released, resulting in the greenish coloration [43][44].

Based on the research data in the Table 3, it can be observed that the MBC value in most of the freeze-dried yogurt samples, with or without pineapple treatment, shows that only at a concentration of 1 g/mL can bacterial growth be inhibited. However, the freeze-dried soy yogurt sample without pineapple does not inhibit or kill *E.*

coli bacteria at any concentration. This may be attributed to the sample's dilution concentration being too low and need to be improved.

Based on Table 4, the antimicrobial testing using the macro-dilution method on all freeze-dried yogurt samples, whether with or without pineapple treatment, showed no ability to eliminate *S. cerevisiae* yeast at the concentrations tested. This is suspected to be due to the insufficient concentration of the diluted sample and a reduction in the total LAB, leading to a decrease in antimicrobial metabolites. As a result, *S. cerevisiae* yeast was able to grow at all concentrations on PDA media.

This lack of activity is attributed to the highly compact structure of the *S. cerevisiae* cell wall. The total mass of the *S. cerevisiae* cell wall is about 10–25% of the total cell mass. The yeast cell wall is composed of polysaccharide layers that serve as protection for the outer layer. These polysaccharides are composed of 1,3-β-glucan branched chains. These chains only form hydrogen bonds, resulting in a compact and continuous structure, commonly referred to as a network or three-dimensional network [45]. Additional study suggests that *S. cerevisiae* exhibits better acid tolerance than *E. coli*. In this study, yogurt samples, which are inherently acidic, were used, which may have contributed to the observed differences in antimicrobial activity [46].

3.3. Antioxidant Analysis Results

Antioxidant activity can be determined based on the IC_{50} value; a lower IC_{50} value indicates higher antioxidant activity [47]. The results of the antioxidant activity test are shown in Table 5.

In this study, a positive control was used to validate the methodology. The antioxidant activity of ascorbic acid, which is classified as very strong, was represented by an IC_{50} value of 4.39 μg/mL. Similar results were observed in other studies that used ascorbic acid as a standard solution, which also categorized it as very strong [48].

Among the raw materials tested, soy powder exhibited the highest antioxidant activity. This is attributed to its higher vitamin E content compared to cow's milk, as well as the presence of polyphenols, particularly isoflavones, which are unique to soy powder and contribute to its antioxidant properties [7][8][49][50]. Furthermore, full cream milk demonstrated greater antioxidant activity than skim milk due to its higher content of lipophilic antioxidants [3].

Fermentation was found to enhance antioxidant activity [7]. This is because fermentation releases antioxidant peptides from probiotic strains, which help neutralize free radicals [3][51][52]. The increase in antioxidant activity from soy powder to soy yogurt is due to the conversion of isoflavone glucosides into more bioavailable aglycone compounds through fermentation [53]. Similarly, the rise in antioxidant activity from cow's milk powder to yogurt is attributed to the increased amount of acids produced by lactic acid bacteria, which is reflected by a decrease in pH [54]. Additionally, adding pineapple enhances antioxidant activity in yogurt [38]. Pineapple

Table 5. Antioxidant activity of freeze-dried yogurt from different types of raw materials.

Types of Raw Materials	*Treatment*	Average IC_{50} ± SD (μg/mL)
Ascorbic acid	Positive control	4.39 ± 0.33
Soy	Before fermentation	374.88 ± 1.35
Full cream		483.86 ± 1.66
Skim		512.02 ± 4.16
Soy	After fermentation	117.69 ± 0.14
Full cream		130.81 ± 0.21
Skim		146.05 ± 0.44
Soy	After fermentation with pineapple	43.67 ± 0.21
Full cream		70.03 ± 0.15
Skim		83.23 ± 0.14

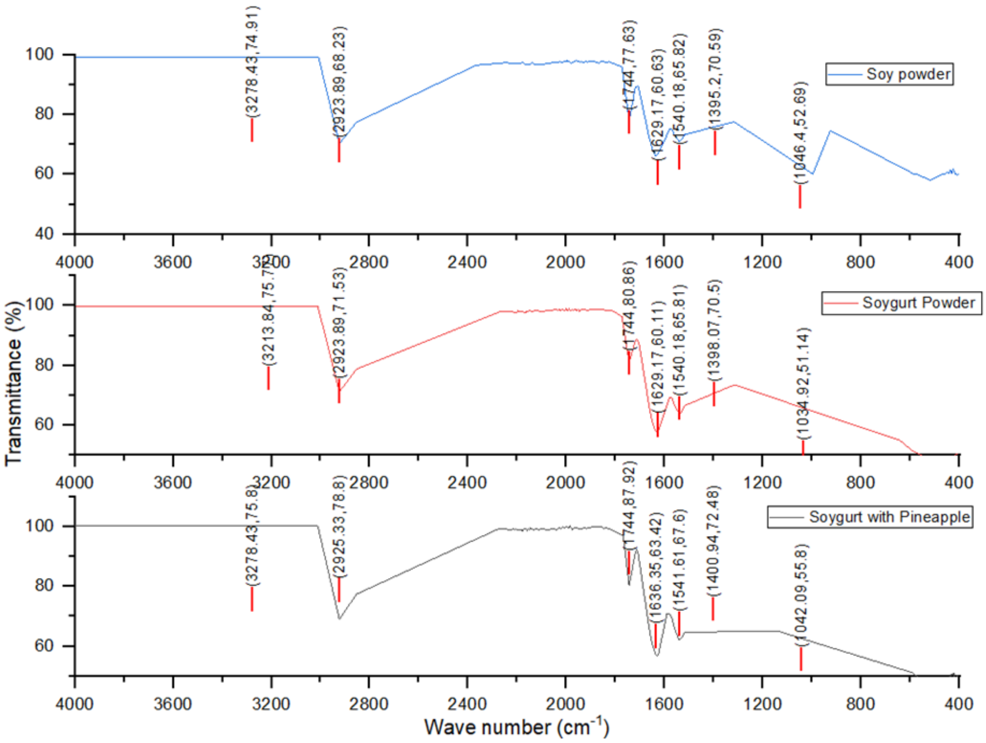

Figure 2. The FTIR spectrum of soy yogurt with pineapple.

improves antioxidant activity due to its content of antioxidant vitamins E and C [11]. Based on IC_{50}, the antioxidant activity of pineapple is classified as very strong at 46.3 µg/mL [55]. Previous studies also showed that yogurt with 60% (w/w) honey pineapple and 4% (w/w) cinnamon had increased antioxidant activity [11][56].

Functional groups are those that determine the properties of organic compounds and provide information about the characteristics of the compounds present in the sample [57]. The functional group spectrum was showed in Figure 2. The spectra generally show similar characteristics, including C–H groups in the wavenumber range of 2915–2935 cm^{-1}, C=O groups in the range of 1740–1760 cm^{-1}, C=C groups in the range of 1629–1680 cm^{-1}, N–H groups in the range of 1590–1650 cm^{-1}, and amide II (N–H and C–N) groups in the range of 1700–1550 cm^{-1}. However, the spectrum of pineapple-added soy yogurt shows C–C groups in the wavenumber range of 700–1300 cm^{-1} [58]-[60].

Soy powder contain isoflavones, which transform from glycosides to aglycones with higher bioavailability during fermentation. Previous research indicates that the presence of C–H and C=O groups can signal the presence of isoflavones [60]. Additionally, soy is known for its vitamin E content, identified by C–H groups, while vitamin C is marked by C=O groups.

Soy powder contains a low concentration of free amino acids; however, the fermentation process enhances the levels of free amino acids and generates bioactive peptides with antioxidant effects [61]-[63]. Key antioxidant amino acids include His, Tyr, Thr, and Lys [62]. The antioxidant peptide found in yogurt is KAVPYPQ [63]. Additionally, soy powder contains oligosaccharides such as stachyose and raffinose. During fermentation, these substances are transformed into lactic acid by lactic acid bacteria [64]. Oligosaccharides are characterized by methyl (C–H) groups [65], while lactic acid is identified by the C=O group [66].

Additionally, the FTIR spectrum of pineapple soy yogurt is similar to that of soy powder and soy yogurt. This is because pineapple contains flavonoids, tannins, vitamin E, vitamin C, bromelain, and peptides, which result in similar functional groups being observed in the spectra of soy powder and soy yogurt [67]. Vitamin E is identified by its methyl (C–H) and C–O groups [68], while vitamin C is marked by its C=O group [69]. Previous studies have shown that flavonoids have C=C, C=O, and C–H group, and tannins also display C–H, C=O, and C=C groups [70]. However, the pineapple soy yogurt spectrum shows a new functional group, C–C. This indicates that adding pineapple to the yogurt introduces fructose, which was not present before, as evidenced by the new C–C group [71]. Based on the spectra of soy powder, soy yogurt, and pineapple soy yogurt, similar functional groups are observed due to the common functional groups present in the sample compositions [72]. However, a new functional group, C–C, appears, which indicates that fructose is present in the soy yogurt after pineapple is added. This results in a unique peak in the spectrum after pineapple is included.

4. CONCLUSIONS

Antimicrobial properties of freeze-dried yogurt showed moderate activity with no significant difference effect for each raw material used against *E. coli* and significant difference effect on *S. cerevisiae*. MBC against *E. coli* for each treatment except soy milk is 1 g/mL. Antioxidant activity in raw materials after fermentation and addition of pineapple is higher than raw materials. Soybean powder is a raw material that has the highest antioxidant activity. The functional groups produced in the spectrum of pineapple soy yogurt include O–H, C–H, C=O, N–H, C=C, amide II, and carboxylic (C=O and C–OH) groups.

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Conflicts of Interest

The authors declare no conflict of interest.

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