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The Effect of Audiosonic Waves on Microbial Activities in Yoghurt Fermentation

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Yoghurt is well known dairy product that is able to help digestion process. Its value can be increased using several innovation method, such as probiotic bacteria addition and unique physical treatment. This study found that the highest glucose level observed in yoghurt without any audiosonic exposure treatment was at 0.1656%, with the addition of 2000 Hz frequency was at 0.2128%, and with the addition of 8000 Hz frequency was at 0.114%. The highest protein content in yoghurt without any audio sonic treatment was found at 0.418%, with the addition of *Zymomonas mobilis* bacteria and 2000 Hz frequency was at 0.333%, and with the addition of *Streptomyces* sp. and 8000 Hz frequency was 0.328%. The highest fat content in yoghurt with *Zymomonas mobilis* bacteria was 18.29%, with 2000 Hz audiosonic treatment was at 10.58%, and with 8000 Hz frequency was at 9.333%. Based on the research, it can be concluded that the nutritional value of yoghurt with *Zymomonas mobilis* bacteria and *Streptomyces* sp. can be increased with audisonic exposure treatment.

Keywords: yoghurt; audiosonic; *Zymomonas mobilis*; *Streptomyces* sp.

I. INTRODUCTION

Refined-processed milk is well-known and has an important role on food processing in every corner of the country. It is known for its high nutrients, and is usually used as supplement food even though it only contains 10% of the total protein that needed by humans. Milk has a high nutritional value because it contains nearly all food substances such as carbohydrates, proteins, minerals, and vitamins. On the other hand, milk also contains protein, fats, lactose, and various vitamins and mineral that are needed by the bacteria to grow (Guetouache *et al.,* 2014). By controlling the process, the nutritional value can be conserved. One of this controlling processes is fermenting milk into yoghurt. Yoghurt is one of the processed products that went through the fermentation process by lactic acid bacteria at a temperature of 37-45 ℃ (Weerathilake *et al.,* 2014).

Yoghurt has higher nutritional value than fresh milk as a raw material. According to Tamime and Robinson, the type of milk and lactic acid bacteria starter are used to determine the quality of yoghurt, especially by the number of microbial life and the acidity of the yoghurt (Tamime & Robinson, 2007).

Innovations in yoghurt are being developed by adding probiotic bacteria in the body which can help maintain digestion. The addition of micronutrients and probiotic bacteria characterisation analysis has been done through the process of fortification of yoghurt (Zhen *et al*., 2017). According to Rahayu, variations of yoghurt with probiotic bacteria products dominated 36.6 % of Indonesia's processed milk market, making this type of yoghurt the largest contributors in the market (Rahayu, 2009). A review article, written by Pisoschi *et al.* said that the addition of antibacterial into food has an important role in the development of healthy diet, and *Streptomyces* sp. are commonly used as the producers of antibiotics and antibacterial because it is fairly safe (Aurelia *et al.,* 2018). Fermentation by *Z. mobilis* on extracts of tropical plants has been studied as one of the agents for the production of alcohol. *Z. mobilis* bacteria is usually used on bioethanol production process (Ajit *et al.,* 2017).

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Based on the research, the exposure of ultrasonic wave can maintain the pH of milk from dairy cows (Villamiel *et al.,* 2017) and it can also reduce the amount of microbes in Bouillon beef (Piyasena *et al.,* 2003). The exposure of audiosonic waves can be used as a tool to attract fish in which fish responds faster and closer to the source of the sound in the frequency range of 500-1000 Hz (Rosana *et al.,* 2018). However, up to current date, none of the researchers have studied the effect of audiosonic to the fermentation of yoghurt.

This study was performed by adding audiosonic wave to the fermentation process of yoghurt with the addition of probiotic bacteria *Streptomyces* sp. and *Z. mobilis*. These treatments can cause a change in the bacterial activity to produce lactic acid. The bacteria's activity can also be observed from nutritional changes and the pH of yoghurt.

II. MATERIALS AND METHOD

The equipment used in this research were glassware laboratory, autoclave, laminar flow, refrigerator, thermometer, pH meter. The instrument used was the IOS UV-Vis spectrophotometer. The materials used in this study included Nutrient Agar (NA) medium, Nutrient Broth medium (NB), distilled water, 70% alcohol, *Streptomyces sp.* bacterial strain, mineral water, yoghurt seedlings containing strains of *Lactobacillus bulgaricus* bacteria, *Streptococcus thermophilus* bacterial strains, and strains of Lactobacillus acidophilus, pure cow's milk, glucose, phenol, concentrated sulfuric acid, n-hexane, Coomasie Brilliant Blue, 70% ethanol, phosphoric acid, and Bovine Serum Albumin (BSA).

A. Regeneration of Zymomonas mobilis and Streptomyces sp. bacteria

Each bacterium was inoculated on a different petri dish containing Nutrient Agar which was sterilised using an autoclave at 121 °C for 15 minutes and incubated for 24 hours at 37 °C.

B. Preparation of Bacteril Starter Culture

Two sterilised 100 mL Erlenmeyer flasks were prepared. Then 10 mL pasteurised cow milk was added into each flasks and heated to 40 °C. A colony of *Z. mobilis* bacteria and *Streptomyces* sp., bacteria were inserted into each flasks, and then shaken slowly. Starter cultures of *Z. mobilis* bacteria and *Streptomyces* sp. bacteria were incubated at 30 °C for 24 hours.

C. Making Yoghurt

Yoghurt seeds in the form of powder containing 20 g of *Lactobacillus bulgaricus, Streptococcus thermophilus*, and *Lactobacillus acidophilus*, were mixed into 150 mL of mineral water. The mixture was whisked until the yoghurt seeds were dissolved. Then the mixture was incubated at 30 °C for 24 hours until the yoghurt starter thickened. In the next step, 50 mL of thick yoghurt starter was transferred into 1 litre of pasteurised milk, in order for the 150 mL yoghurt starter to be used for 3 litres of pasteurised milk. The mixture of yoghurt starter and the pasteurised milk were incubated for 12 hours at 30 °C. The product can be used directly afterwards.

D. Addition of Probiotic Microbes to Yoghurt without Additional Frequency

Approximately 50 mL yoghurt was added into three 100 mL Erlenmeyer which was labelled A, B, and C. Erlenmeyer A acted as a control. In Erlenmeyer B, 2.5 mL of starter culture of *Z. mobilis* was added, while 2.5 mL of starter culture of *Streptomyces* sp. was added to Erlenmeyer C. Then, Erlenmeyer A (control), Erlenmeyer B, and Erlenmeyer C were incubated at 30 °C for 24 hours. At 24 hours, pH testing, protein content testing, fat content, and glucose contents were carried out to control and to take samples.

E. Addition of Probiotic Microbes to Yoghurt with Additional Frequency

Yoghurt was poured for into 100 mL Erlenmeyer labelled A1, A2, B1, B2, C1 and C2 50mL each. Erlenmeyer A1 and A2 served as controls. In Erlenmeyer B1, B2 were added 2.5 mL *Z. mobilis* starter cultures and C1, C2 added 2.5 mL starter culture of *Streptomyces* sp. then, each Erlenmeyer A1, A2, B1, B2, C1 and C2 was closed and the cap was marked with 2000 Hz (A1, B1, and C1) and 8000 Hz (A2, B2, and C2). Furthermore, Erlenmeyer A1, A2, B1, B2, C1 and C2 were incubated at 30 °C for 24 hours. After 24 hours, pH testing, protein content testing, fat content, and glucose contents were carried out to control and for samples.

F. Measurement of pH

Controls and samples that had been made were put into a beaker glass and each pH was measured using a pH meter.

G. Measurement of Glucose Contents

Standard glucose solutions were carried out with variations in concentrations from 0 ppm to 70 ppm. 1 mL was taken from each concentration and 1 mL of 5% phenol solution was added and the containers were shaken. After shaking it, the 5 mL of concentrated sulfuric acid was added quickly and after that, the test tube was soaked in water for 10 minutes. Then, the absorbance is measured at a wavelength of 485 nm. The procedure above was repeated again by replacing the standard glucose solution into a control solution and yoghurt sample. Glucose contents can be determined by first determining the standard glucose solution regression equation of various concentrations, with the regression line equation as in Equation (2.1):

$$y = a X + b \quad (2.1)$$

where, a = slope

b = intercept

X= glucose contents

Y= absorbance

After obtaining an X value, the value is substituted into Equation (2.2)

$$\%glucose = \frac{glucosecontents(ppm)xDilutionFactor}{10000} \quad (2.2)$$

H. Measurement of Protein Contents

Determination of protein content was carried out by the Bradford method. The Bradford reagent was made by dissolving 0.01 g of Coomassie Briliant Blue into 5 mL of 95% ethanol. Next, 85 mL of phosphoric acid was added and filtered. Then stored in a dark bottle at low temperatures. Before taking it for usage, the Bradford reagent must be diluted five times. BSA (Bovine Serum Albumin) was used for standard solution. To make 1000 ppm, 0.05 g of Bovine Serum Albumin (BSA) was dissolved in 50 mL distilled water. After that, a 1000 ppm BSA solution was diluted to 0, 40, 80, 120, 60, 200, and 240 ppm. 100 µL of BSA solution from each concentration was taken and then added 2 mL of Bradford reagent, then homogenised and left for 5 minutes at room temperatures. The solution was measured using a spectrophotometer at a wavelength of 595 nm. The procedure is repeated by replacing the BSA solution into a control solution and yoghurt sample. Protein contents can be determined by first determining the standard protein solution regression equation of various concentrations, with the regression linear equation in equation 2.1. After obtaining the X value, the substituting it into Equation (2.3):

$$\% \text{ protein} = \frac{\text{protein contents (ppm)x Dilution Factor}}{10000} \quad (2.3)$$

I. Measurement of Fat Contents

Control and yoghurt samples were dried. 3g of sample and control were wrapped using filter paper. Then, it was macerated using n-hexane solvent until all filter paper is submerged for 2 hours. The fat solution has been collected in a bottle, then evaporated at 68 ° C using rotatory evaporator to separate the solvent and fat. The fat was dried in the oven until it became constant. From the results of weighing, the percentage of fat in yoghurt can be calculated by Equation (2.4):

$$\% \text{ fat} = \frac{\text{Total Mass} - \text{Flask Mass}}{\text{Dry Weight}} \text{ x } 100\% \quad (2.4)$$

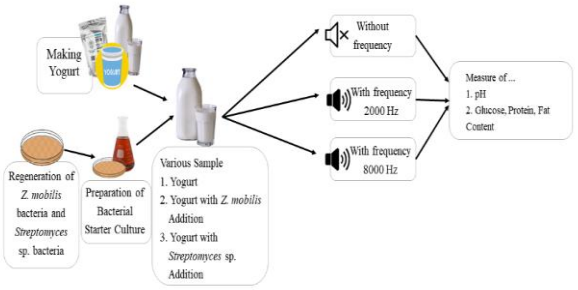

Figure 1. Research scheme

III. RESULT AND DISCUSSION

A. Regeneration of Zymomonas mobilis bacteria and Streptomyces sp.

Z. mobilis is a facultative anaerobic gram negative bacteri.a As one of the few facultative anaerobic bacterias, it can degrade glucose by Entner-Doudoroff (Baratti and Bu'lock, 1986). *Streptomyces* is a gram-positive bacteria that can grow

in various environments. *Streptomyces* is similar to fungi. *Streptomyces* and fungi can be differentiated because *Streptomyces* has a formation of hyphae layer that can be differentiated into a chain spores. *Streptomyces* has a special ability in which that it can produce bioactive secondary metabolites such as antifungal, antiviral, antitumoral, anti-hypertensives, and mainly antibiotics and immunosuppresives (Omura *et. al.,* 2001; Khan *et. al.,* 2011; Patzer & Braun, 2009). *Z. mobilis* and *Streptomyces* sp. has a similar function which can produce compound to inhibit o other bacterial growth.

Z. mobilis and *Streptomyces* sp. bacteria must be regenerated before application. The purpose of regeneration is to multiply and rejuvenate the age of bacteria. *Z. mobilis* and *Streptomyces* sp. bacteria were inoculated by ose needles using the quadrant stroke method into a petri dish which was filled with sterile nutrient agar (NA) media. NA is an inoculation medium that has complete content and its accordance with the nutritional needs of bacteria in the breeding process and NA media has a pH 6,8 – 7,0 where the condition is suitable for most bacteria. NA media contains meat extract, peptone, NaCl, nitrogen, and agar. Meat extracts contain water-soluble animal tissue substances, including carbohydrates, organic nitrogen compounds, vitamin B complexes, and mineral salts such as calcium, sulfur, phosphate, and potassium (Atlas, 2010). Carbohydrates serve a source of carbon in the formation of energy needed in metabolism and bacterial growth (Jorgensen, 2009). Pepton is a product that produced from ingredients that contain proteins such as meat, casein, and gelatin. In NA media, peptone serves the main source of organic nitrogen and as a support for bacterial growth. Nitrogen is a precursor to synthesising amino acids, proteins, and enzymes and to form new cells by bacteria while NaCl is a provider of sodium and as a factor that can increase osmosis pressure and psychochemical balance in bacterial cells. Agar is not contribute to the bacterial growth. Agar is used as a medium for compacting media and its not a source of nutrients (Nishiyama & Saito, 2012).

Z. mobilis and *Streptomyces* sp. bacteria were incubated at 30 °C which was the optimum temperature for the growth of *Z. mobilis* and *Streptomyces* sp. (Kusmiyati *et. al.,* 2016; Palanichamy *et al.,* 2011). After 24 hours of incubation, *Z. mobilis* colonies were formed. The *Z. mobilis* colonies had a very fine-looking stem with large amount while the colony of *Streptomyces* sp. will be seen clearly in the second or third-day incubation. *Streptomyces* sp. colonies looked firmly attached to the surface of the media and its structure is rough or mealy.

B. Yoghurt, Starter Culture Zymomonas mobilis and Streptomyces sp. Bacteria, and Addition of Probiotic Microbes to Yoghurt

There are two stages to make yoghurt. The first on was making yoghurt starter. It was made by dissolving yoghurt powder containing *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus* into 150 mL of mineral water, then shaken until completely dissolved and incubated at 30 °C for 24 hours. After 24 hours of incubation, the yoghurt starter becomes thick and white in color. The next step of making yoghurt was by mixing the starter yoghurt and pasteurised milk. 150 mL yoghurt starter can be used for 3 litres of pasteurised milk, thus yoghurt was produced by mixing 50 mL of yoghurt starter with 1 litre of pasteurised milk then incubated for 12 hours at 30 °C. After 12 hours of incubation, the yoghurt was finished because its color had changed into white and its texture thickened. It also has a distinctive sour odor.

The starter culture was made before adding it into the yoghurt. Bacterial starter cultures were made to help the process of bacterial adaptation, so that when the fermentation process begins, the adaptation phase in the fermentation media becomes faster. The process of making bacterial starter cultures is by inoculating *Z. mobilis* and *Streptomyces* sp. bacteria which has been regenerated into the media. The media used to make bacterial starter cultures was pasteurised cow's milk. Then bacterial starter cultures were incubated for 24 hours at 30 °C. The starter culture of the bacteria was white and had a thicker texture than pasteurised milk.

The effect of audiosonic wave test was in Erlenmeyer. Audiosonic waves were produced by setting a series of piezo speakers with the frequency of 2000 and 8000 Hz which were arranged in an Erlenmeyer lid that contains yoghurt. Erlenmeyers were marked A1, A2, B1, B2, C1, and C2. Figure 1 shows the instrument used for audiosonic test. A1 and A2 Erlenmeyer are control, yoghurt without the addition of

probiotic bacteria. Starter culture of *Z. mobilis* was added to B1 dan B2 Erlenmeyer while *Streptomyces* sp.was added toC1 and C2 Erlenmeyer. Erlenmeyer with code A1, B1, and C1 were exposed to 2000 Hz and A2, while B2, and C2 were exposed to 8000 Hz. After 24 hours incubation, the yoghurt became thick liquid, has a distinctive yoghurt smell, and homogenous.

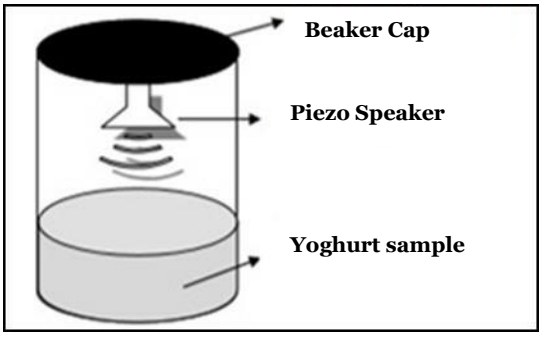

Figure 2. Instrument used for audiosonic test

C. Measurement of pH

The pH measurement on yoghurt aims to determine the acidity of yoghurt so that the level of quality of yogurt can be estimated. A low pH value indicates that a lot of lactose has been converted into lactic acid (Zourari *et al.,* 1992). Levels of lactic acid in the product are influenced by the ability of the starter to form lactic acid or is determined by the number and type of starter bacteria used. Based on Figure 3, it can be seen that the pH of yoghurt changes during the fermentation process. In this study the pH value of yoghurt tends to decrease during storage. This is caused by the accumulation of organic acid resulting from fermentation of lactose to acid by lactic acid bacteria.

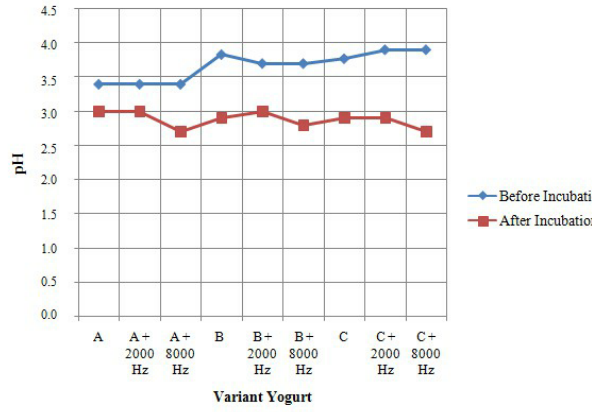

Figure 3. pH of yoghurt

After incubation for 24 hours, the pH value of each yoghurt decreased. In yoghurt, the highest pH is at 2000 Hz. In yoghurt with the addition of *Z.mobilis*, the lowest pH is at 8000 Hz exposure, while in yoghurt with the addition of *Streptomyces* sp. is at 8000 Hz frequency exposure. This shows that at a frequency of 8000 Hz, the ability of lactic acid bacteria to convert lactose to lactic acid increase, while the addition of *Z. mobilis* and *Streptomyces* sp. bacteria had no effect on pH values. The differences of pH values involving bacteria against frequency are caused by sound stimuli between different strains of the same species, species of the same genus, and genus of the same family. Intermittent exposure to sound frequencies will cause a decrease in bacteria and minimal damage to the cell wall in weak bacteria, but the sound frequency exposure does not cause the number of bacteria to be 0 or die all. The stronger bacteria will act faster and absorb more energy when the vibrational frequency given matches the natural frequency of vibration (Reguera, 2011). According to Australia's New Zealand Food Standards (FSANZ) (Zealand, 2014), a good pH of yoghurt has a value of 4.5.

In this study, however, the pH of yoghurt did not meet the standards set by the Food Standards Australia New Zealand, this was due to the increased ability of lactic acid bacteria to produce lactic acid.

D. Measurement of Glucose Content

Glucose is a type of monosaccharide with the molecular formula $C_6H_{12}O_6$. Glucose is determined by the total sugar (TS) method. The phenol-sulfuric acid method is used to determine the total carbohydrate concentration in food. Glucose levels in yoghurt are obtained from the decomposition of lactose that occurs during the fermentation process (McKevith & Shortt, 2003). During the fermentation process, lactose and lactic acid levels will increase. Lactose levels continue to decrease because it is used by cells to grow and to form lactic acid so that lactose which is hydrolysed to glucose is also small. Fermentation glucose to lactic acid showed in Figure 4.

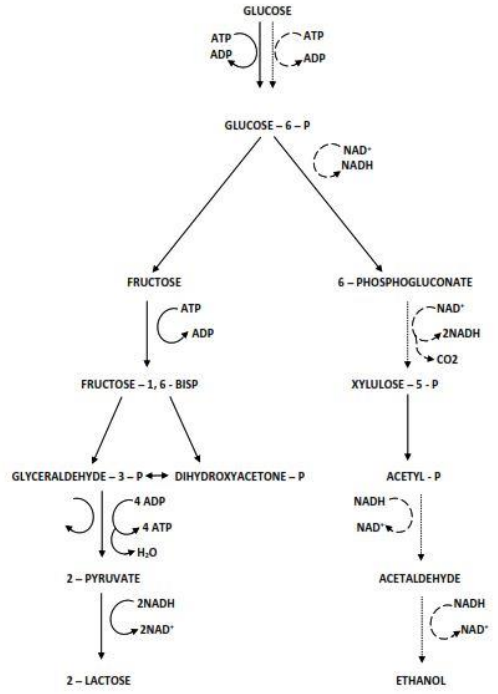

Figure 4. The pathway metabolism of glucose to lactose and glucose to ethanol

Control yoghurt with or without frequency has a low glucose content compared to yoghurt which are added with probiotic microbes. Control yoghurt without the addition of probiotic bacteria has a glucose content of 0.1656% while yoghurt with addition of *Z. mobilis* and *Streptomyces* sp. increased by 0.2832% and 0.2448%. The results of testing glucose contents with the addition of 2000 Hz frequency showed that the lowest glucose content in the control yoghurt was 0.2128% while the yoghurt with the addition of *Z. mobilis* and *Streptomyces* sp. bacteria increased by 0.2648% and 0.2404%. As for the glucose contents with the addition of frequency of 8000 Hz, the control yoghurt has a very small value between control yoghurts without the addition frequency and the addition of a frequency of 2000 Hz by 0.114% while the glucose content from yoghurt added with *Z. mobilis* and *Streptomyces* sp. that is 0.2404% and 0.2584%. The graph of glucose contents in each yoghurt can be seen in Figure 4.

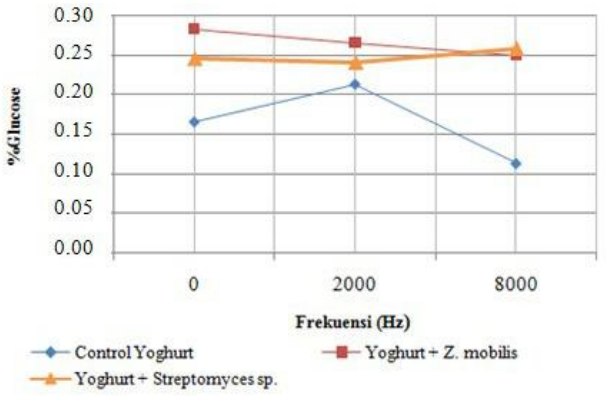

Figure 5. Glucose contents of yoghurt

E. Measurement of Protein content

In this study the protein content in yoghurt was determined by the Bradford method. The Bradford method is a test to measure total protein concentration by colorimetry in a solution (Bradford, 1976). Bradford's test involved a Coomassie Brilliant Blue (CBB) dye which binds to proteins in an acidic solution to give bluish colour. The Bradford method was chosen because it is faster and more accurate, involves fewer mixing steps, does not require heating, and provides a more stable colourimetric response compared to other methods (John, 2000). The principle of the Bradford method is based on direct binding of Coomasie Brilliant Blue dye by proteins containing amino acid residues with aromatic side chains (tyrosine, tryptophan, and phenylalanine) or alkaline (arginine, histidine, and leucine). The free Bradford reagent is red-brownish in colour, whereas in the atmosphere, the Bradford reagent acid will be in the form of anion which will bind the protein to form a blue color with a maximum wavelength of 595 nm.

The standard solution used is Bovine Serum Albumine (BSA). BSA solution is made with concentrations of 0, 40, 80, 120, 60, 200, and 240 ppm. Then 100 µL of BSA solution was taken from each concentration and 2 mL of Bradford reagent were added, and left for 5 minutes. After that, the absorbance is measured by using a spectrophotometer at a wavelength of 595 nm. Protein content in yoghurt is influenced by the protein content contained in raw materials, the more raw materials that contain protein, the higher protein produced in yoghurt products.

Yoghurt that had not been given any additional probiotic microbe has a protein content of 0.4175%, protein content of

yoghurt was given the addition of *Z. mobilis* bacteria is decreased to 0.4% and protein content of yoghurt which was given the addition of bacteria *Streptomyces* sp. is decreased to 0.37875%.

Protein content of yoghurt which was given with additional 2000 Hz frequency and probiotic microbe is 0.2675%, protein content of yoghurt was given the addition of *Z. mobilis* bacteria increased to 0.3325%, and protein contents of yoghurt was given the addition of *Streptomyces* sp. increased to 0.32875%.

Protein content of yoghurt which was given additional 8000 Hz frequency and any additional probiotic microbe is up to 0.3125%, protein content of yoghurt was given the addition of *Zymomonas mobilis* bacteria is decrease to 0.3%, and protein content of yoghurt given the addition of *Streptomyces* sp. is increase to 0.3275%.

However, this is not in accordance with the quality standards of good quality yoghurt according to the National Standardisation Agency which explains that the yoghurt protein content of good quality is at least 2.7%. The graph of the results of testing the protein content in yoghurt can be seen in Figure 5.

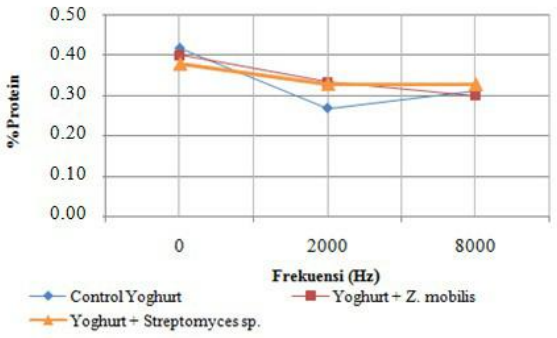

Figure 6. Protein contents of yoghurt

F. Measurement of Fat

Fat is a very important source of nutrition because its functions improves texture and taste (Akbari *et al.,* 2019). In this research, the analysis of fat used extraction method with n-hexane. A sample of each variation was taken and weighed as much as 3 g dry-weight. Before maceration, sample wrapped with filter paper. n-hexane was used on the maceration process because of non-polar solvents and fats are soluble in n-hexane. After evaporating maceration process of solvent by using a rotatory evaporator temperature 68 ^{o}C to separate between fat and solvents. Because the solvent n-hexane has a boiling point temperature 68 ^{o}C while boiling point's fat at the temperature about 200^{o}C. The reason of evaporating in 68 ^{o}C aims not to damage the fat and to successfully separate solvent with evaporation. The graph of the results of testing the fat content in yoghurt can be seen in Figure 6.

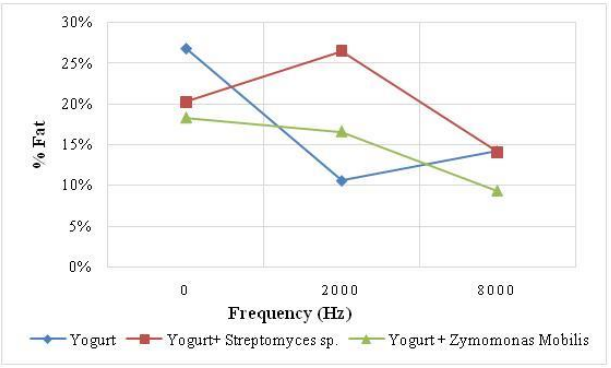

Figure 7. Fat Contents of yoghurt

As seen in the chart above, it shows that the addition of both bacterias produce a decrease in percentage of fat less than 26.7967% fat in yoghurt without the addition. For the percentage of fat produced by yoghurt with the additional Z. *mobilis* bacteria after being incubated is 20.203%, while the percentage of fat produced by yoghurt with additional *Streptomyces sp*. bacteria after incubated is 18.2967%. The results of *Z. mobilis* and *Streptomyces* sp. Bacteria indicated activity in the yoghurt. Fat in yoghurt acts as food for both bacterias contained in order to decrease fat in yoghurt. Audiosonic exposure treatment was done against yoghurt without the addition of probiotic bacteria and yoghurt with the addition of probiotic bacteria (*Z. mobilis* and *Streptomyces* sp.).

Audiosonic exposure on this research was conducted at a frequency of 8000 Hz and 2000 Hz. After exposure of the audiosonic, the results show that yoghurt without adding probiotic bacteria and yoghurt with *Z. mobilis* bacteria has decreased before the exposure. This suggests audiosonic's exposure makes the activity of bacteria work more than usual. So the audiosonic's exposure makes the product of yoghurt has less fat. As for the yoghurt with *Streptomyces* sp., it is increased in the frequency of 2000 Hz, but not on the frequency of 8000 Hz that could decrease it like yoghurt without addition of probiotic bacteria. Audiosonic exposure

inhibits the activity of *Streptomyces* sp.bacteria so that the fat of yoghurt is almost the same as fat of yoghurt before the bacteria probiotics wereadded.

Table 1. Result of additional bacteria and audiosonic exposure to yoghurt

	Yoghurt	Yoghurt + *Streptomyces* sp.	Yoghurt + *Z. mobilis*
-Sound			
Glucose	0.166 %	0.245 %	0.283 %
Protein	0.418%	0.379%	0.400 %
Fat	26.790%	20.200 %	18.290 %
Final pH	3.0	2.9	2.9
+2000 Hz			
Glucose	0,213 %	0.265 %	0.240 %
Protein	0.268 %	0.329%	0.333 %
Fat	10.582 %	26.466 %	16.604 %
Final pH	3.0	2.9	3.0
+8000 Hz			
Glucose	0.114 %	0.258 %	0.250 %
Protein	0.313 %	0.328 %	0.300 %
Fat	14.190%	14.050 %	9.323 %
Final pH	2.7	2.7	2.8

IV. CONCLUSION

Based on the research, it can be concluded that the addition of *Z.mobilis* and *Streptomycess* sp. bacteria can increase glucose content and reduce the protein and fat content of yoghurt. The glucose content of yoghurt control, yoghurt with addition of *Z.mobilis*, and yoghurt with addition of *Streptomyces* sp. bacteria in a row is 0.1656%; 0.2832%; and 0.2448%. The highest glucose content found on yoghurt which added with Z. *mobilis* without frequency exposure, while the lowest glucose found on yoghurt control with 8000 Hz frequency exposure is 0.114%. Frequency exposure to yoghurt with addition of *Streptomycess* sp. does not different significantly.

Addition of *Z. mobilis* and *Streptomyces* sp. bacteria reduces contents protein in yoghurt. Protein content in control yoghurt, yoghurt with the addition of *Z. mobilis*, and *Streptomyces sp*. in a row of 0.334%; 0.32%; and 0.303%. In protein measurement, the addition of frequency exposure also affects the decrease in protein contents in yoghurt. At a frequency of 2000 Hz, the highest protein content is in yoghurt with the addition of *Z. mobilis* by 0,3325% while at a frequency of 8000 Hz, the highest protein content is in yoghurt with the addition of *Streptomyces* sp.

Fat contents in yoghurt with the addition of *Z. mobilis* and *Streptomyces* sp. bacteria also decreased by 26.8%; 20.2%; and 18.3%, respectively. The frequency exposure of 2000 Hz and 8000 Hz in the control yoghurt decreased with values of 10.58% and 14.19%, respectively. In yoghurt with the addition of *Z. Mobilis*, the frequency exposure decreased at 2000 and 8000 Hz which results in the fat contents 16.61% and 9.32%, respectively. Whereas in *Streptomyces* sp., fat content increased at a frequency of 2000 Hz by 26.47% and decreased at 8000 Hz frequency exposure.

V. ACKNOWLEDGEMENT

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