**EFFECTS FERMENTATION OF STARTER *Lactococcus lactis* With *Streptococcus thermophilus* ON THE CHARACTERISTICS FRUITGHURT BLACK MULBERRY PROBIOTIC DRINK (Morus nigra L.)**

**ARTICLE**

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***Abstract***

*The purpose of this research was to find the optimum fermentation time of starter Lactococcus lactis and Streptococcus thermophillus toward the characteristics of probiotic drinks fruitghurt black mulberry.*

*The reasearch methods consists of two phases : a preliminary study and main study. The preliminary study did starter Lactococcus lactis and Streptococcus thermophiles, did the analysis raw materials of black mulberry (total sugars) and the determination of black mulberries formulations. The main study did the fermentation process of formulation selected from the preliminary study and the use the starter Lactococcus lactis and Streptococcus thermophilus has been made, the duration of the fermentation process for 24 hours, 48 hours, 72 hours, 96 hours, 120 hours and 144 hours. The response of this study was to measure the lactic acid content, pH (acidity), and viscosity are influenced by the duration of fermentation*

*The results of this research showed that the analysis of the raw materials of black mulberries have a total sugar content of 15.65%. Based on th starter Lactobacillus lactis and Streptococcus thermophilus was found that the total number of cells has increased in every hour. Formulations were selected in the preliminary study that is a ratio of 2: 1 (mulberry black: water) is in getting the value of lactic acid levels of 1.796%. Differences in duration of effect on the fermentation of lactic acid content, pH and viscosity. Where the optimum fermentation time is at a time of 72 hours with 1.08% lactic acid levels, pH 3.86 and viscosity 125 mPas.*

*Keywords: black mulberry, Lactococcus lactis, Streptococcus thermophilus, long fermentation, fruitghurt.*

1. **Introduction**

Yogurt is one of the probiotic processed food products containing Lactobacillus bulgaricus and Streptococcus thermophillus bacteria. Yoghurt is a milk-based product which has been added non-fat milk paste form which is then pasteurized and fermented by a mixture of lactic acid bacteria (BAL), commonly used is Lactobacillus delbrueckii ssp. Bulgaricus (LB) and Streptococcus salivarus ssp. Thermophilus (ST), to obtain a semisolid texture, a distinct degree of acidity, odor, and taste (Wong et al., 1988).

Murbei (Morus nigra L.) is a long-lived crop (perennial) and naturally adapt well to various soil types. Murbei is always associated with the sericulture industry where the quality and quantity of leaf production is very important for silkworms. Mulberry leaves are highly favored and easily digested by herbivorous animals and can also be used as monogastric fodder. Murbei (Morus nigra l.) Is a tree plant that has very good nutritional value and has a high crude protein content of 22.9-25.6% (Saddul et al., 2004).

Generally milk-based yoghurt so that for vegetarian groups can not consume yoghurt. So that the alternative is used for yoghurt can be consumed by all parties that is with the manufacture of probiotic non-diary product drink.

One functional food product that widely circulated in the market is fermented food products containing probiotics. Probiotics are living microbes that can affect health by balancing the microbes in the gut and inhibiting the growth of pathogenic microbes. The presence of lactic acid as a metabolite of lactic acid bacteria can inhibit the growth of pathogenic bacteria (Retnowati, 2014).

Probiotic drinks contain beneficial microbes that contribute to the digestion of humans, namely Befidobacterium which is found in many food and beverage products such as salami, yakult, yoghurt, cheese and others. Other bacteria including probiotic bacteria and already through clinical trials are Lactobacillus casei and L. acidophilus (Sukotjo, 2003).

In addition to microbial content, probiotic drinks are also very useful in adding nutrients and minerals needed by the body. This can be fulfilled because the basic ingredients used are whey still contains the materials (nutrients and minerals) needed by the body (Sukotjo, 2003).

Probiotics Fruitghurt Murbei is a combination of mulberry juice with probiotic bacteria. Commonly used probiotic bacteria are lactic acid bacteria Lactobacillus bulgaricus and Streptococcus thermophilus.

Selection of black mulberry as the basic ingredients of making fruitghurt is to utilize mulberry fruit into more diverse preparations. Besides mulberry fruit has a good enough nutritional content and has a high carbohydrate content that can be fermented into lactic acid.

The benefits of making a probiotic fruitghurt black mulberry fruit that is utilizing the fruit of mulberry as a fruit that has a high nutritional content. Provide an alternative probiotic drink to the community for those who can not consume milk or animal-based, minimize allergen and low fat.

The purpose of this research is to find the optimum fermentation time at starter Lactococcus lactis and Streptococcus thermophillus on probiotic fruit drink characteristics of black mulberry fruit.

According to Ikhsan and Yulyanti (2015) in a study entitled fruitghurt fermented with fruit peel varieties, it was stated that a mixture of Lactobacillus bulgaricus and Streptococcus thermophilus with a 1: 1 ratio in mango skin fruitghurt with time of 96 hours produced more lactic acid with lactic acid 1 , 36% compared to lactic acid produced by starter mixture with a ratio of 0: 1 and 1: 0.

1. **Materials and Research Methods**

**Materials**

The ingredients used in the study of mulberry fruitghurt manufacture include two-month-old black mulberry fruit or ripe ones, mineral water, allumnium foil, filter paper, plain yoghurt, starter Lactococucs lactis and Streptococcus thermophillus which have previously been cultured through fruit pulp Mulberry with a ratio of 1: 1.

Chemicals used for the analysis is aquadest, phenolphthalein indicator, 0.1 N NaOH, amylum, I2 0.01 N, Luff Schoorl, H2SO4 6 N, KI, Na2S2O3 1 N, HCl 9.5 N, concentrated HCl, 30% NaOH, PDA, YGA, sterile water, N-Hexan.

The tools used in the manufacture of fruitghurt are scales, pans, stoves, incubators, spoons, thermometers, glass jars, 250 ml erlenmeyer flask and funnel.

The tools used for chemical analysis are 100 ml erlenmeyer flask, measuring cylinder, stirring rod, volumetric pipette, dropper, digital balance, biuret, beaker, funnel, pumpkin, stove, bunsen, stative, clamp, bath, viscotester , Pikno meter, reflux, and pH meter.

**Research Methods**

The implementation of the research conducted preliminary research and primary research**.**

**Preliminary Research**

The preliminary study consisted of three stages namely the introduction of stage 1 making starter Lactococcus lactis and Streptococcus thermophilus, the preliminary phase 2 analysis of raw material of total sugar content in mulberry fruit. Making starter is intended as a starter preparation that will be used for the fermentation process in the main research, by first determining the total sugar content in mulberry fruit, and determine the number of living cells in probiotic fruit fruitghurt. The next stage 3 introduces the comparison of formulations between mulberry and water. This study aims to determine the comparison between the mulberry fruit and water that is appropriate for use in fermentation with lactic acid content analysis parameters and using a microbial starter that has been made before.

**Main Research**

The main research is a follow-up study of preliminary research covering: treatment design, experimental design, analysis design and response design that observed lactic acid levels during fruitghurt manufacture and pH and viscosity measurement. The main research results will be determined the selected sample by means of analysis of Vitamin C content in fruit fruit mulberry fruit. The purpose of the main study was to determine the optimum time of starter growth in producing lactic acid according to the standard.

**The Experimental Design**

Treatment design consists of one factor that is difference of fermentation time, with level:
The fermentation time used with 6 levels and temperature 40oC, namely:

t1 = 24 hours
t2 = 48 hours
t3 = 72 hours
t4 = 96 hours
t5 = 120 hours
t6 = 144 hours

The experimental model used in the manufacture of mulberry fruit Fruitghurt is Simple Linear Regression.

The experimental method for this study is as follows:

Y = a + bx

Information :
Y = Bound Variable
X = Free Variable
A = Constant Number (regression line intercept)
B = Regression Coefficient (regression line slope)

A and b values can be calculated by a simple formula. To get the value of a can be used the formula:

a =(Σy) (Σx²) – (Σx) (Σxy)
           n(Σx²) – (Σx)²

While the value of b can be calculated using the formula:

b =   n(Σxy) – (Σx) (Σy)
           n(Σx²) – (Σx)²

**Response**

Responses conducted in this study are:

**Chemical response**.

Chemical response was made by determination of lactic acid alkalimetry titration method, determination of iodimiter vitamin C level, pH measurement, and alcohol test of distillation method.

**Physical Response**.

The Physical Response performed on the main research is the determination of the viscosity of the fruitghurt. Measurement of viscosity is done after fermentation process.

**Microbiological Response.**

Microbiological response carried out in preliminary research by TPC (Total Plate Count) method.

**3.** **Result and Discussion**

**Preliminary Research**

Preliminary Phase 1 study aims to determine total sugar content in black mulberry raw material, so it can be known the amount of sucrose sugar to be added to match the amount of carbohydrate in the yoghurt fermentation condition.

Table 1. Result of Analysis of Black Murbei Raw Material

|  |  |  |
| --- | --- | --- |
| **No** | **Type of Carbohydrate** | **Sugar Level (%)** |
| 1 | Monosaccharide | 6,84 |
| 2 | Discharges | 8,80536 |
| 3 | Total Sugar | 15,65 |

Table 2. Results of Making Pure Culture Starter

|  |  |
| --- | --- |
| **Type** | **Amount** |
| Black Mulberry fruit | 93 grams |
| Pure Culture Starter | *Lactococcuc lactis* dan *Streptococcus thermophilus* (1:1) |
| Sterile water | 5 ml |
| Total sugar content | 15, 65 % |
| Sucrose | 2 gram |

In the preliminary study the starter was first made with the goal as a starter preparation that will be used for the fermentation process in the main research, by first determining the total sugar content in mulberry fruit, and determine the number of living cells in fruitghurt probiotic drink. Starter is made to give adaptation time to bacterial growth from media to liquid medium. So that the fermentation will be run more optimal.

Total sugar content in black mulberry fruit of 15.65% which means it has met the requirement of carbohydrate for fermentation. In the formulation, 2% of sucrose sugar is added to increase carbohydrate content to optimize starter action during fermentation.

If the glucose concentration is greater then the speed of fermentation will decrease and inhibit the activity of microbial Lactobacillus bulgaricus, lactococcus lactis, and Streptococcus thermophilus so that the fermentation process will be longer. This happens because if the concentration of glucose is too large will happen plasmolisis on the cell wall of microorganisms cause the cell wall will break. If glucose is lower than 10% then the resulting product will be less because nutrition and media for microorganisms are too few (Ardiyawati and Fitriyah, 2015).

**Growth of Lactococcus lactis and Streptococcus thermophilus for 24 hours**

Figure 1. Graph of L.lactis and S.thermophilus Growth

Table 3. Analysis of activity of bacterial cell count on pure fruitghurt culture starter

|  |  |  |
| --- | --- | --- |
| **Time(J)** | **Dilution** | **∑ cell** |
| **10-1** | **10-2** | **10-3** |
| **J1** | 15 | 3 | 3 | 150 |
| **J2** | 13 | 5 | 3 | 130 |
| **J3** | 9 | 3 | 1 | 90 |
| **J4** | 20 | 7 | 4 | 200 |
| **J5** | 23 | 8 | 6 | 230 |
| **J6** | 76 | 31 | 16 | 760 |
| **J7** | 37 | 9 | 11 | 900 |
| **J8** | 131 | 70 | 17 | 1310 |
| **J9** | 169 | 89 | 23 | 1690 |
| **J10** | 42 | 19 | 12 | 1900 |
| **J11** | 225 | 106 | 57 | 2250 |
| **J12** | 281 | 96 | 61 | 2810 |

Growth growth curve aims to measure changes in the amount of lactic acid bacteria contained within the fruit mulberry black mulberry for 24 hours.

Based on the above growth chart shows that there is a change in the growth of L.lactis and S.thermophilus bacteria at every hour. In the first hour until the third hour decreased growth. This is because the need for lactic acid bacteria to adapt to liquid media previously located on agar media. At four o'clock to 12 o'clock it increases. Growth charts show that lactic acid bacteria are increasing in amounts logarithmically it indicates lactic acid bacteria can utilize the existing substrate optimally. Lactic acid bacteria utilize carbohydrates contained in black mulberry fruit in the form of glucose and fructose as an energy source. In addition, the addition of 2% sucrose affects the growth of lactic acid bacteria due to the addition of an energy source as a lactose substitute. Streptococcus thermophilus is thought to be the only lactic acid bacteria that can use sucrose to produce energy. Sucrose is thought to be broken down into glucose and fructose, then Lactococcus is thought to use glucose that is formed to produce energy.

**Determination of Formulation**

Table 4. Result of Formulation Determination Analysis

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Mulberry Fruit(gram) | Water (gram) | Type of comparison | Starter(ml) | Sucrose(gram) | % Lactic Acid |
| 69,75 | 23,25 | 3 : 1 | 4,75  | 2 | 4,5 |
| 62 | 31 | 2 : 1 | 4,75 | 2 | 1,796 |
| 46,5 | 46,5 | 1 : 1 | 4,75 | 2 | 0,89 |
| 93 | 0 | 1 : 0 | 4,75 | 2 | 0,63  |

Based on the above fermentation results can be selected 2: 1 ratio that has the best lactic acid during fermentation 3 days. The lactic acid level was determined by titrimetric titration using 0.1 N NaOH. The lactic acid content in the 2: 1 ratio was 1, 796%. This result is in accordance with Indonesian National Standard (SNI) for lactic acid probiotic milk (yoghurt) is 0.5% - 2.0%.

**Main Research**

The overall main research includes physical response, and chemical response. Based on fermentation results conducted from 24 hours, 48 hours, 72 hours, 96 hours, 120 hours and 144 hours. The results are as follows:

Table 5. Results of Main Study Analysis Using Starter Culture L. lactis with S. thermophilus

|  |  |  |  |
| --- | --- | --- | --- |
| **Time (Hour)** | **Lactic Acid (%)** | **pH** | **Viscosity(mPa.s)** |
| 24 | 0,81 | 4,23 | 175 |
| 48 | 0,99 | 4,07 | 140 |
| 72 | 1,08 | 3,86 | 125 |
| 96 | 1,345 | 3,71 | 121 |
| 120 | 1,44 | 3,48 | 115 |
| 144 | 1,62 | 3,13 | 102 |

Based on table 5. can be concluded linear regression result of each parameter:

Table 6. Old Fermentation Analysis Against Lactic Acid

|  |  |
| --- | --- |
| **Time (hour)** | **Lactic Acid (%)** |
| 24 | 0,81 |
| 48 | 0,99 |
| 72 | 1,08 |
| 96 | 1,345 |
| 120 | 1,44 |
| 144 | 1,62 |

|  |
| --- |
|  |

Figure 2. Graph of Old Fermentation Effect on Lactic Acid

The graph depicting the correlation of incubation time to lactic acid formation is a 2nd order polynomial graph. This is because lactic acid is affected by two factors: incubation time and unpredictable microbial behavior.

Levels of lactic acid formed increases with the longer incubation time so that the smell of acid in the turmeric also increasingly sting. Levels of lactic acid increased up to 6 days incubation so that the smell of acid in the turmeric also increasingly sting.

Table 4. Old Fermentation Analysis of pH

|  |  |
| --- | --- |
| **Time (hour)** | **pH** |
| 24 | 4,23 |
| 48 | 4,07 |
| 72 | 3,86 |
| 96 | 3,71 |
| 120 | 3,48 |
| 144 | 3,13 |

Figure 3. Old Fermentation Effect on pH

Figure 3. above shows the longer incubation time then the pH level decreases. This is because changes in the pH value of the media can alter the nutritional composition found in the mulberry fruit. Lactic acid bacteria will break down the glucose contained in the substance producing lactic acid.

The increase in acid total is indicated by a decrease in pH. The longer the fermentation of more active microorganisms, resulting in more lactic acid. Lactic acid produced by bacteria will be excreted out of the cell to accumulate in the fermentation fluid (Aswatan, 2008).

Table 5. Old Fermentation Analysis of Viscosity

|  |  |
| --- | --- |
| **Time (hour)** | **Viscosity(mPa.s)** |
| 24 | 175 |
| 48 | 140 |
| 72 | 125 |
| 96 | 121 |
| 120 | 115 |
| 144 | 102 |

Figure 4. The Effect of Old Fermentation on Viscosity

Based on Figure 4. above shows the longer fermentation will result in lower viscosity. Making fruitghurt does not make viscosity increase because at breaking its reaction only glucose produces lactic acid. While on yoghurt fermentation the longer the fermentation the viscosity will increase. This is because the protein contained in the milk will be disturbed to form a coagulant blob that resulted in increased viscosity and texture of milk will thicken.

According to fernandez, (2007). That the fermentation product referring to yoghurt has a viscosity between 8.23-13.00 cP. This may be due to acidic pH conditions. The pH value can decrease the solubility of casein, resulting in hydrophobic interaction between casein micelles to form the structure and consistency of yogurt drink which causes the yoghurt drink to become thicker so that the viscosity rises.

Table 6. Analysis of Vitamin C and Alcohol Test Fruitghurt black mulberry with natural starter and plain yoghurt starter

|  |  |  |
| --- | --- | --- |
| **Starter** | **Amount Vitamin C** | **Amount of Alcohol** |
| *Lactococcus lactis* dengan *Streptococcus thermophilus* | 532,4215 mg vit C/100  | 0,00 %  |
| Plain yoghurt (*Lactobacillus bulgaricus)* | 324, 959 mg vit C/100  | 0,33 % |

Based on Table 6. shows the amount of vitamin C that is formed during the fermentation takes place. In fruitghurt using pure culture of vitamin C that is formed larger than fruitghurt by using plain yoghurt. Because the manufacture of fruitghurt using pure culture in the absence of a mixture of milk so that vitamin C in the fruit can be measured. While fruitghurt using plain yoghurt has a milk content though with very small content.

**4. Conclusion**

Based on the research results can be concluded that:

1. The duration of fermentation has an effect on the increase of lactic acid by using pure starter Lactococcus lactis and Streptococcus thermophilus (1: 1) of 1.08%. Old Fermentation effect on the increase of lactic acid by using plain yoghurt of 0.76% at the chosen time of 72 hours.
2. The duration of fermentation has an effect on the decrease of the pH of the black mulberry fruitghurt by using natural starter L. Lactis with S.thermophilus (1: 1) and by fermentation using plain yoghurt.
3. The length of fermentation affects the viscosity of the black mulberry fruitghurt. The longer the fermentation time the viscosity will decrease

**5.Refrence**

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