The Effect of Sorghum Flour (Sorghum bicolor L. Moench) Addition to Characteristic Quality of Goat Milk Sinbiotic Yoghurt Candidate

Een Sukarminah, Indira Lanti, Endah Wulandari, Elazmanawati Lembong, Ratih Utami

1Departement of Food Industrial Technology, Faculty of Agro-industrial Technology, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km 21. Jatinangor, Sumedang, 40600, Indonesia
(a)Corresponding author: e.sukarminah@yahoo.com

Abstract. Sorghum flour goat milk can be processed into functional food product, which is sinbiotic yoghurt. The aim of this study is to determine the appropriate concentration of sorghum flour to produce goat milk sinbiotic yoghurt with good characteristics. The method of this study is descriptive method and using Randomized Block Design with 4 treatments of sorghum flour addition by 2%, 3%, 4%, 5% (w/v) and 3 replications. The results showed that the addition of sorghum flour of 5% (w/v) was the selected treatment with microbiological quality, namely total acid resistant probiotic bacteria, total salt resistant probiotic bacteria, total lactic acid bacteria (BAL) in accordance with the standard of more than 7 log CFU/mL, pH and total titrated acid are in the acid range, total solids and viscosity tend to be in the yoghurt set range.

1. Introduction
Modern public awareness of health is increasing. Food philosophy has been shifting, where eating is not merely to fill the stomach, but more importantly is to achieve the optimal level of health and fitness.

Functional food is food that is in its natural form or processed has health effects if consumed because it contains one or more components that have physiological functions for health [1]. The market growth for functional food continues to increase, where it reaches 8.5% per year globally [2].

Yoghurt is one of the functional food products that have been known and popular among the community. Consuming yoghurt regularly will stimulate the growth and activity of friendly bacteria in the intestines. Beneficial effect of yoghurt consumption is prevention of weight gain and management of obesity [3]. The functional effect on yoghurt results from the formation of functional components by bacterial cultures of the Lactic Acid Bacteria (LAB) group during the fermentation process.

In its development, yoghurt has been developed into synbiotic yoghurt. Synbiotic yoghurt is fermented milk by probiotic microbes and given the addition of prebiotics as a food of the probiotics. According to [4] a combination of prebiotics and probiotics produces good benefits for the body.

Probiotics termed as live micro-organisms which, when administered in adequate amounts, confer a health benefit to the host [5]. Probiotics should have the ability to withstand difficult environmental conditions, for example stomach acid that has very low pH and bile salts before reaching the intestine. Prebiotics are defined as ‘non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the intestine’ [6].
Functional food in the form of sinbiotic yoghurt can be made by using a combination of sorghum flour that acts as a prebiotic and L. acidophilus bacteria as probiotics. Sorghum (Sorghum bicolor L. Moench) is a cereal plant that has the potential to be a source of prebiotics. Sorghum is a cereals and a staple food of more than 750 million people in Africa, Asia, and Latin America [7]. The chemical composition and nutrient of sorghum are similar to wheat and other cereals [8]. Nutrients contained in sorghum flour are 12.16% -15.12% total dietary fibre and 0.6% - 2.0% resistant starch [9].

Prebiotic sources in sorghum are oligosaccharides, dietary fibre, resistant starch and non-starch polysaccharides. Shorgum can be added to yoghurt in the form of flour which can increase the prebiotic component and solids in yoghurt. Based in previous study, the highest level of prebiotic component in sorghum is food fibre of 5.30% followed by inulin of 2.73% and FOS of 2.22%.

Saanen goat is one of the dairy goats that is often bred in Indonesia. Saanen goat has a good body performance as a milk producer. According to [10] Saanen breed produced more milk (p<0.05) than the other two breeds of goat that is british alpine and toggenburg. Saanen goat can produce milk about 5.17 kg/day.

Saanen milk goat is one of the type of milk that has the potential to be developed in Indonesia as an effort to diversify food. Goat milk has some different characteristic compared to cow milk, namely the color is whiter, its fat globules are smaller so that goat milk's fat is more easily digested, and can be drunk by people who are allergic to cow milk, lactose intolerance, or to people who experience various indigestion [11].

Its nutrients and benefits are very potential to be developed. Goat milk consumed by people is still very small in number, because it is still considered unusual for consumption. This is due to the distinctive aroma of goat milk which makes many people less like it.

The distinctive aroma of goat milk is caused by the content of goat milk that is rich in volatile fatty acids i.e caproate, caprylate and caprate which contribute to the formation of specific tastes and odors [12]. The smell of prengus can be reduced by a way of fermentation. The fermentation process releases gases or volatile compounds such as acetaldehyde and diacetyl which serve as compounds that reduce the scent of prengus. The original aroma of milk will decrease if the acetaldehyde content is high [13]. Diacetyl has a role to give the aroma of fermented milk, the diacetyl compound is not detected in fresh milk and will show its existence when the milk is fermented [14].

According to [15] a product can be regarded as a synbiotic product in if there is a probiotic bacteria at least 7 Log CFU g-1 which is expected to develop into 12 Log CFU g-1 in human intestines. The probiotic bacteria must be able to pass unfavourable conditions on the body such as stomach acid and bile salts. Therefore the number of probiotic bacteria in the resulting synbiotic yoghurt should be more than 7 Log CFU g-1 or 7 CFU Log/ml and must also comply to applicable standards.

Based on the description above, it is necessary to do a research on the effect of the addition of the right sorghum flour to produce synbiotic saanen goat milk yoghurt that meets the standard of synbiotic product.

2. Materials and Methods
The materials used in this research include fresh goat milk obtained from breeders in Cilayung Jatinangor Sub-district, white sorghum flour Bandung local cultivars type obtained from farmers in Soreang Regency Bandung, freeze dried commercial yoghurt starter L. bulgaricus, S. termophilus and L.acidophilus. aquades, MRSA (deMann Rogosa Sharpe Agar), MRSB (deMann Rogosa Sharpe Broth), NaCl physiological 0.85%, HCl 1N, bile salt, and buffer pH 4 and 7.

The tools used are refrigerator, stove, incubator, oven, colony counter, vortex mixer, pH meter, petri dish, test tube, measuring cup, beaker glass, erlenmeyer, measuring flask, measuring pipette, bulb pipette, micro pipette, finn tip , thermometer, spatula, stirring rod, pot, spoon, crucible tong, scales, glass jar, zip lock plastic, aluminum foil, clingwrap, spoon, pipette, tissue, plastic cup, and cloth.

The research method used is experimental method followed by Descriptive and Random Block Design (RBD) with confidence interval α = 0.05 with four treatments namely the addition of sorghum
flour on yoghurt by 2%. 3%, 4% and 5% (w/v) and repeated three times. The method analysis using SPSS Statistics Ver. 17 software.

2.1. The Production Process of Synbiotic Yoghurt
Production process of saneen goat milk sinbiotic yoghurt with the addition of sorghum flour can be seen in Figure 1.

![Diagram of the production process of saanen goat milk sinbiotic yoghurt with the addition of sorghum flour](image)

Figure 1. Diagram of the production process of saanen goat milk sinbiotic yoghurt with the addition of sorghum flour

2.2. The Procedures for the Calculation Analysis of the Total Lactic Acid Bacteria
Samples of 1 ml were diluted to 9 ml of 0.85% NaCl physiologic solution. A dilution is done to $10^9$ aseptically. A sample of 1 ml each was taken from the last 3 dilutions and inserted into a petri dish. MRS agar (Merck) was poured as much as 15 ml. Petri dishes were incubated in a reverse for 48 hours at 37°C. The number of colonies grown on the order is calculated using the BAM calculation method.

$$N = \frac{\Sigma C}{[(1 \times n_1) + (0.1 \times n_2)] \times d}$$  \hspace{1cm} (1)

Where :
- $N$ : The number of colonies in product (colony/ml or colony/gram)
- $\Sigma C$ : The number of colonies grown on petri dish
- $n_1$ : The amount of petri dish in the first dilution
- $n_2$ : The amount of petri dish in the calculated second dilution
- $d$ : The dilution rate obtained from the first calculated dish
2.3. The Calculation of the Total Number of Acid-Resistant Probiotics Bacteria

10 ml MRSB was poured into the test tube. 1% HCl was added until it reached pH 4. 1 ml sample was inserted into the reaction tube containing MRSB. The reaction tube was incubated at 37°C for 2 hours. 1 ml of suspension was diluted to 9 ml of 0.85% physiological NaCl solution. Then it was diluted aseptically up to 10⁻⁸. A sample of 1 ml each was taken from the last 3 dilutions, then put into a petri dish. MRS Agar was poured as much as ± 15 ml into a petri dish. The petri dish was incubated in a reverse position at 37°C for 48 hours. The number of colonies grown on the agar was calculated using BAM calculations.

2.4. The Calculation of the Total Number of Bile Salt-Resistant Probiotics Bacteria

10 ml MRSB was poured into the test tube. Bile Salt of 0.3% (b/v). 1 ml sample was inserted into the reaction tube containing MRSB. The reaction tube was incubated at 37°C for 2 hours. 1 ml of suspension was diluted to 9 ml of 0.85% physiological NaCl solution. Then dilution was done aseptically up to 10⁻⁸. A sample of 1 ml each was taken from the last 3 dilutions, then put into a petri dish. MRS Agar was poured as much as ± 15 ml into a petri dish. The petri dish was incubated in reverse position at 37°C for 48 hours. The number of colonies grown on the agar was calculated using BAM calculations.

2.5. pH Testing

The pH meter was calibrated using pH 4 and 7 buffers. The pH meter electrode was dipped in the sample for a while until the tool worked for reading. The pH value of the sample was read from the tool display.

2.6. Total Titrated-Acid Testing

10 ml of the sample was diluted to 100 ml in a volumetric flask. 10 ml of the diluted sample was put into erlenmeyer. 2-3 drops of 1% phenolphthalein indicator were dropped on the sample. Samples were titrated with 0.1N NaOH until the sample turned pink. The total of the titrated acid is calculated using the formula.

\[
\frac{\text{NaOH Volume} \times N_{\text{NaOH}} \times 90.08 \times \text{Dilution Factor}}{\text{Initial Volume} \times 1000} \times 100\% \quad (2)
\]

2.7. Viscosity Testing Using Reypa Rotational Viscometer RP-1-L

The viscometer was turned on by pressing the on button on the back of the tool. The sample was inserted into the viscometer cup up to ¾ part. Number 3 spindle was attached to the viscometer. The spindle speed was set to 60, 20, 12 and 6 rpm. The viscometer was run by pressing start. The data was read from the tool display (value taken with an accuracy of 20-90%). All data on the screen was noted.

2.8. Total Solids Test

The moisture content was calculated by weighing 5 ml of the sample and putting the sample into an evaporating porcelain dish which empty weight was known. The dish was put into the oven at 105°C for 3-5 hours. The dish was cooled in a desiccator and weighed until it was constant. The moisture content was calculated using the formula

\[
\frac{(b-c)}{c} \times 100\% \quad (3)
\]

Where:

- a : Empty dish weight
- b : sample + dish weight
- c : dish + dry sample weight

The total of the solid was calculated using the formula

\[
100\% - \text{Moisture Content} \quad (4)
\]
3. Result and Discussion

3.1. The Total of Lactic Acid Bacteria (LAB)

The effect of the addition of sorghum flour on the production of synbiotic yoghurt can be seen in Table 1.

| Treatment | Mean (Log CFU g⁻¹) |
|-----------|-----------------|
| A (2%)    | 9.36            |
| B (3%)    | 10.02           |
| C (4%)    | 10.22           |
| D (5%)    | 11.41           |

According to Table 1, the average number of LAB in all treatments of synbiotic yoghurt ranged from 9.36 to 11.41 Log CFU g⁻¹. The amount has met the requirements of [16] which is at least 7 Log CFU g⁻¹ or 10⁷ colonies/g. Treatment D showed the highest total LAB in the synbiotic yoghurt compared to other treatments, indicating that the more prebiotic addition in the form of sorghum flour in this study, then the higher the number of LAB.

Treatment of the addition of sorghum flour gives the effect in the form of the amount of nutrients or food for LAB to fulfil its growth and provide energy to ferment. Bacteria can grow well on a medium if there are enough energy, carbon and nutrient sources and are in appropriate environmental conditions [17].

The LAB contained in the synbiotic yoghurt comes from several naturally existing good species in milk, as well as those derived from the addition of starter, but the number of LAB derived from the starter has a greater amount and dominates in the total amount of LAB contained in the product. The LAB used and contained in the starter added to the production of yoghurt include *S. thermophilus*, *L. bulgaricus* and *L. acidophilus*.

The viability of *S. thermophilus* can be increased when added oligosaccharide i.e FOS [18]. *L. bulgaricus* may metabolize some prebiotic carbohydrates i.e. resistant starch [19]. Meanwhile, *L. acidophilus* compared to the other two bacteria are more flexible in fermenting different types of carbohydrates such as monosaccharide, disaccharide and oligosaccharide such as glucose, fructose, galactose, sucrose, mannose, rafinose, stakiose, lactosukrosa, FOS, GOS, inulin, XOS, MOS and IMOs [20].

3.2. The Total of Acid-Resistant Bacteria

The effect of the addition of sorghum flour to the production of synbiotic yoghurt can be seen in Table 2.

| Treatment | Mean (Log CFU g⁻¹) |
|-----------|-----------------|
| A (2%)    | < 7.40          |
| B (3%)    | < 7.40          |
| C (4%)    | 7.56            |
| D (5%)    | 7.89            |

According to Table 2, the average number of probiotic bacteria in synbiotic yoghurt ranges from < 7.40 to 7.89 Log CFU g⁻¹. In the treatment of A and B, the number of bacteria can not be ascertained because at the time of observation the colony was below the BAM calculation range of 25 - 250. In the treatment of C and D the number of probiotic bacteria has met the [21], where a product can be regarded
as a probiotic product if it contains probiotic bacteria at least 7 Log CFU g⁻¹ and is expected to develop up to 12 Log CFU g⁻¹ in the intestine. Treatment D showed the highest probiotic acid-resistant bacteria in synbiotic yoghurt compared to other treatments, it shows that the more prebiotic addition of sorghum flour in this study, the higher the number of probiotic acid-resistant bacteria in it.

The total probiotic bacteria contained in synbiotic yoghurt is influenced by temperature, storage time, and prebiotic content added to the process of the production of synbiotic yoghurt. The longer the storage of the yoghurt, hence the number of probiotic bacteria decreases, which according to [22], synbiotic yoghurt stored at 4°C storage temperature undergoes a decrease in total probiotic bacteria \emph{L. acidophilus} approximately 2 Log from day one to day 28.

The amount of prebiotics which is sorghum flour is added to the process of making synbiotic yoghurt that serves as a provider of nutrition and food to the probiotic bacteria so that the more addition of sorghum flour, the more probiotic bacteria in the product. The availability of nutrients contained in the product is required for the probiotic bacteria for life and growth to protect absorption in the small intestine reaches the colon, where most probiotics breeds.

The condition of gastric acidity serves as the first stage to perform bacterial selection before entering the intestine. Tolerance to pH or acid levels is one of the important requirements of a LAB to be regarded as a probiotic.

3.3. \emph{The Total of Bile Salt-Resistant Bacteria}

The effect of the addition of sorghum flour to the production of synbiotic yoghurt can be seen in Table 3.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|}
\hline
\textbf{Treatment} & \textbf{Mean (Log CFU g⁻¹)} \\
\hline
A (2\%) & 7.64 \\
B (3\%) & 7.78 \\
C (4\%) & 9.38 \\
D (5\%) & 10.30 \\
\hline
\end{tabular}
\caption{The Effect of the Addition of Sorghum Flour to the Total Number of Bile Salt-resistant Bacteria on Synbiotic Yoghurt}
\end{table}

According to Table 3, the average number of probiotic bacteria in the synbiotic yoghurt ranges from 7.64 to 10.30 log CFU g⁻¹. The number of probiotic bacteria has met the International Standard of [15], where a product may be considered to be a probiotic product if it contains at least 7 Log CFU g⁻¹ probiotic bacteria and is expected to grow up to 12 Log CFU g⁻¹ in the intestine. Treatment D showed the highest bile salt-resistant probiotic bacteria in the synbiotic yoghurt compared with other treatments, indicating that the more prebiotic addition in the form of sorghum flour, the higher the probiotic bile salt-resistant bacteria there is. This shows that the more prebiotic addition of sorghum flour in this study, the higher the number of acid-resistant probiotic bacteria.

The effect of prebiotics i.e. sorghum flour to the amount of salt-resistant probiotic bacteria produced has the same mechanism as the acid-resistant probiotic bacteria, where prebiotics act as a source of nutrients and foods to fulfil the growing needs of probiotic bacteria.

Bile salt is a sodium salt such as taurocholate and glycholate that function in fat emulsions into micelles so that they are easily absorbed in the intestinal mucosa. According to [23] bile salts are the most serious barrier to probiotic resistance in the small intestine. The resistance of lactic acid bacteria to bile salts is related to bile salt hydrolase (BSH) enzyme which is capable of hydrolysing conjugated bile salts thereby reducing the toxic effects on cells [24].

All paragraphs must be indented. All paragraphs must be justified, i.e. both left-justified and right-justified.
3.4. pH Value

Based on the statistical test of the addition of sorghum flour as a prebiotic on the production of synbiotic saanen goat milk yoghurt gives a significant different effect to the pH of synbiotic yoghurt. The effect of the addition of sorghum flour on the production of synbiotic yoghurt can be seen in Table 4.

Table 4. The Effect of the Addition of Sorghum Flour to the pH Value on Synbiotic Yoghurt

| Treatment | Mean   |
|-----------|--------|
| A (2%)    | 4.05 ±1.14 a |
| B (3%)    | 3.97 ±1.11 b |
| C (4%)    | 3.92 ±0.96 b |
| D (5%)    | 3.91 ±0.91 b |

The average treatment values which were marked with different letters were not significantly different at the 5% test level according to Duncan's Test.

Based on the results in Table 4, the average pH of synbiotic saanen goat milk yoghurt ranged from 3.91 to 4.05. These results are consistent with the statement of [25] which states that good yoghurt has a pH of 3.8 to 4.5. In addition, according to [26] the standard pH for yoghurt ranges from 3.5 to 4.5. The results of the study indicate that the greater the addition of sorghum flour to 5% (w/v) the pH will be significantly different.

The pH value is influenced by the number of prebiotics added to the process of the making of yoghurt. The fermentation process causes the pH value to decrease, this is because BAL will utilize the carbohydrates contained in prebiotics, causing lactic acid formation which is characterized by a decrease in pH. In addition, BAL will also metabolize milk lactose into lactic acid. BAL will break down lactose so that lactose levels will decrease by 40-50%. Lactose will first be hydrolyzed by the D-galactosidase enzyme in the bacterial cells into glucose and galactose. Glucose is then metabolized by bacteria into pyruvic acid which is then converted to lactic acid [25].

At the beginning of fermentation, *S. thermophilus* will grow faster and initiate the fermentation process which causes the pH to drop below 5.5. *L. bulgaricus* bacteria begin to grow faster and cause a decrease in the growth of *S. thermophilus*. *L. bulgaricus* is the cause of the formation of acetyldehides in yoghurt [27].

3.5. Total of Titrated Acid Value

Based on the statistical test of the addition of sorghum flour as a prebiotic on the production of synbiotic saanen goat milk yoghurt gives a significant different effect to the total of titrated acid on synbiotic yoghurt. The effect of the addition of sorghum flour on the production of synbiotic yoghurt can be seen in Table 5.

Table 5. The Effect of the Addition of Sorghum Flour to the Total of Titrated Acid Value on Synbiotic Yoghurt

| Treatment | Mean (%) |
|-----------|----------|
| A (2%)    | 0.99 ± 0.091 b |
| B (3%)    | 1.06 ± 0.067 b |
| C (4%)    | 1.33 ± 0.153 a |
| D (5%)    | 1.40 ± 0.200 a |

The average treatment values which were marked with different letters were not significantly different at the 5% test level according to Duncan's Test.

Based on the results in Table 5, the average of total titrated acid in saanen goat milk synbiotic yoghurt ranged from 0.99 to 1.40%. This value has met the [28] where the titrated acid content in
yoghurt must be more than 0.9%. The results show that the greater the addition of sorghum flour up to 5% (b/v), the total titrated acid will be significantly different.

The effect of adding sorghum flour to the total value of titrated acid has the same mechanism with the pH value, where the carbohydrates contained in sorghum flour which acts as a prebiotic will act as food or nutrient intake for BAL which will ferment the lactose in milk into the main product namely lactic acid \cite{29}.

According to \cite{27}, the level of acidity/total acid can affect the pH value of yoghurt which caused by an increase in the amount of lactic acid which will be followed by an increase in the concentration of hydrogen ions (H+) which causes the pH value of yoghurt to decrease. Therefore, the titrated acid value will be inversely proportional to the pH.

### 3.6. Total Solids Value

Based on the statistical test of the addition of sorghum flour as a prebiotic on the production of synbiotic saanen goat milk yoghurt gives a significant different effect to the total solid of synbiotic yoghurt. The effect of the addition of sorghum flour on the production of synbiotic yoghurt can be seen in Table 6.

**Table 6. The Effect of the Addition of Sorghum Flour to the Total Solids Value on Synbiotic Yoghurt**

| Treatment | Mean (%)          |
|-----------|------------------|
| A (2%)    | 20.51 ± 0.37 c   |
| B (3%)    | 20.73 ± 0.39 c   |
| C (4%)    | 21.55 ± 0.44 b   |
| D (5%)    | 22.18 ± 0.24 a   |

The average treatment values which were marked with different letters were not significantly different at the 5% test level according to Duncan's Test.

Based on the results in Table 6, the average of the total solids in saanen goat milk synbiotic yoghurt ranged from 20.51-22.18%. These results have met the requirements \cite{30} concerning yoghurts and sweetened yoghurts, where the minimum of total solids is 11.2%. The results shows that the more addition of sorghum flour up to 5% (b/v), the more significantly different the total solids value in saanen goat milk synbiotic yoghurt.

The total solids will increase with the formation of lactic acid during fermentation. The lactic acid produced will work to increase the nutritional value of protein and fat, with the increase of nutritional value, the total solids will also increase. High total solid values indicate the amount of protein, fat, carbohydrates, vitamins, and minerals in synbiotic yoghurt products are high.

### 3.7. Viscosity Value

Based on the statistical test of the addition of sorghum flour as a prebiotic on the production of synbiotic saanen goat milk yoghurt gives a significant different effect to the viscosity of synbiotic yoghurt. The effect of the addition of sorghum flour on the production of synbiotic yoghurt can be seen in Table 7.

**Table 7. The Effect of the Addition of Sorghum Flour to the Viscosity Value on Synbiotic Yoghurt**

| Treatment | Mean (cPs)          |
|-----------|---------------------|
| A (2%)    | 3296.67 ± 427.7 d   |
| B (3%)    | 4700.00 ± 1129.4 c  |
| C (4%)    | 6823.33 ± 724.7 b   |
| D (5%)    | 8380.00 ± 95.4 a    |

The average treatment values which were marked with different letters were not significantly different at the 5% test level according to Duncan's Test.
Based on the results in Table 4, the average viscosity of saanen goat milk synbiotic yoghurt in all treatments is ranged from 3296.67 to 8380.00 cPs which can be classified into yoghurt sets. The yoghurt set is thick yoghurt that has a viscosity >1500 cPs \[25\]. The results showed that the more addition of sorghum flour up to 5% (b/v) the more significantly different the viscosity of saanen goat milk synbiotic yoghurt, thus increasing the viscosity of the yoghurt produced.

The viscosity contained in synbiotic yoghurt is influenced by the addition of sorghum flour. According to \[31\] sorghum starch ranging from 56-73% and the average is 69.5%. Starch in sorghum according to \[32\] is amylopectin about 70-80% and amylose about 20-30%. The starch in the pasteurization process will experience gelatinization which produces a gel. Starch is widely used in the food industry, its uses are as a binder, thickener, gel-forming, emulsifier, texture-forming, stabilizer and film-forming \[33\].

The pasteurization process also causes an increase in viscosity because the milk protein is denatured. The dominant milk protein that is casein will reach the isoelectric point at the pH of 4.6 \[34\], casein which is at the isoelectric point will become unstable and begin to denaturate.

4. Conclusions

Treatment D which is the addition of 5% sorghum flour (b/v) is the best synbiotic yoghurt with the best characteristics with the total lactic acid bacteria (LAB) of 11,41 Log CFU g\(^{-1}\), acid-resistant probiotic bacteria of 7.89 Log CFU g\(^{-1}\), bile salt-resistant probiotic bacteria of 10.30 Log CFU g\(^{-1}\), pH value of 3.9, Total Titrated Acid of 1,40%, Total Solid of 22,18% and Viscosity value of 8380 cPs.

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