Chemical and Microbiological Characteristics of Fermented Cheese Whey Beverages With Soymilk Powder Addition

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Abstract. This research was aimed to determine the chemical and microbiological characteristics of fermented cheese whey beverages which was made with soymilk powder addition. The research was done experimentally using Completely Randomized Design (CRD), three treatments and replicated six times. Parameters taken were total plate count, pH, lactic acid content, lactose content and viscosity. The impact of the treatments significance was determined by ANOVA and the difference between treatments was determined by Tukey test. The best soymilk powder addition that produced desirable fermented cheese whey beverages properties was 12.5 % with properties as follows : the total plate count of 175 x 10⁹ colonies/g and pH of 3; Lactic acid content of 0.675 %; Lactose content of 7.2623 %; and Viscosity of 24 823.33 cP.

1. Introduction
Cheese whey is byproduct obtained from the precipitation and elimination of milk casein during the production of cheese [1]. Whey represents 85 – 95 % of the milk volume and preserve 55 % of the milk nutrients. The composition of bovine whey are fats 0.1 %, casein protein <0.1 %, whey protein 0.7 %, ash 0.5 %, lactose 4.9 % and total solid 6.3 % [2,3]. The green-yellowish color observed of whey is caused by the presence of riboflavin (vitamin B2) [4].Every 1 kg of cheese made, it will produce 9 kg of whey [5]. The production of cheese whey reaches 800 tons per year and considered as byproduct because of its low concentration of milk components. As an example, one of Gouda and Cheddar cheese producer which located in Sumedang, West Java, produces 3,000 kg of cheese whey per month and it’s never been utilized. Meanwhile, the utilization of whey could reduce disposal high organic matter into stream such as biochemical oxygen demand (BOD) of 30-50 g/L, chemical oxygen demand (COD) of 60-80 g/L and minimizing wastes up to 62.1% [6,7].

Cheese whey can be utilized into food products through fermentation to produce yoghurt. Low concentration of total solid from cheese whey produces yoghurt with undesirable viscosity. As an alternative, addition of high amount of total solid substances is needed. Soy milk powder, a source of galactooligosaccharides (GOS) namely raffinose (3.04 %) and stachyose (8.87 %); also most importantly, it contains 92 % protein content of 22% (it is not clear what the author mean?) [8,9]. Galactooligosaccharides in soy milk in the form of stachyose and raffinose consist of prebiotic components that can be used as the source of carbon and energy for soygurt starter bacteria [9].Thus, the purpose of the research was to determine the characteristics of fermented cheese whey beverages with soy milk powder addition, the properties examined were lactose concentration, total lactic acid bacteria, pH, lactic acid content and viscosity.

2. Materials and Methods

2.1 Cheese Whey Beverages Fermentation
Liquid whey cheese (6.13 % with 2.27 % of protein and 3.27 % of lactose) was preheated at 60 °C, Melilea Soy Powder (DM ??? 92 % with 22 % of protein, 12 % of lipid and 60 % of carbohydrate) was added in various concentration: A1 (7.5 %), A2 (10 %), A3 (12.5 %). 5 % (w / v) of skimmed milk
powder was added while stirring and heated it up to 90-95 °C for 5 minutes. The skim milk was then cooled down to 37 °C and bulk culture of Freeze-dried Yogurmet yoghurt starter (L. bulgaricus, S. thermophilus, L. acidophilus) with ratio 1:1:1 as much as 5 % (v/v). Was inoculated and then incubated in 37 °C for 16 hours. Fermented whey cheese beverages were stored in refrigerator at 3-5 °C for 24 hours (Modification of [10]).

2.2. Total Lactic Acid Bacteria
Lactic acid bacteria were isolated and enumerated by pour plate method on Plate Count Agar (PCA). Serial dilutions of $10^{-1}$ to $10^{-6}$ were prepared by diluting 1 mL of sample into 9 mL of sterilized distilled water. One milliliter from each of the dilutions were inoculated into petri dish which were previously added with PCA. The plates were then incubated at 37 °C for 24 hours. After incubation, viable colonies were counted and recorded, which expressed as colony forming unit per gram (cfu·g⁻¹).

2.3. Lactose Concentrations
Samples was diluted up to 1:200 dilution, then 2.5ml of sample was poured into capped test tube 0.2 mL of ZnSO₄ 5 % and Ba(OH)₂ 4.5 % was added. Samples were then centrifuged at 1000 rpm until the pellet appears. 1 mL of supernatant was poured into a capped test tube with addition of Telles Reagent, and boiled in water bath for 6 minutes. Aquades was added to the mixture until the volume became 12.5 mL continued by homogenizing the mixture [11].

Samples result was read into spectrometer using the formula below:

$$Lactose\ concentration = \chi \frac{g_{ram}}{B(gram)}$$

Information: \(\chi\) : Result of conversion absorbant sample
\(B\) : weight of sample
It is not clear

2.4. pH Values
pH values of samples were measured by pH-meter (MP-120, Mettler-Toledo, Switzerland). Samples were poured into respective tubes, measured for pH, and recorded.

2.5. Viscosity
Viscosity measurement for all samples were done using Brookfield DV-E Viscometer : Spindle No 3 at 20 rpm [12].

The experiment was done by using Randomized Complete Design (CRD) with 3 different amount of soybean powder addition, those are 7.5 %, 10 %, and 12.5 % respectively. The significance of treatment was tested by Analysis of Varian and the difference between treatment was tested by Tukey Test.

3. Results and Discussion
The amount of powdered soybean addition affected the growth of lactic acid bacteria. As the amount of powdered soybean increases, total lactic acid bacteria were also increased (Table 1).
Table 1. Total Lactic Acid Bacteria in Various Amount of Powdered Soybean

| Treatment            | Number of Lactic Acid Bacteria (colonies·g⁻¹) |
|----------------------|---------------------------------------------|
| 7.5% Soy powder      | 28.33 x 10⁸c                                |
| 10.0% Soy powder     | 20.86 x 10⁹b                                |
| 12.5% Soy powder     | 175 x 10⁹a                                  |

*Description: Different letters in the direction of the columns show significantly different

The increase might be caused by the higher amount of nutrients contained such as lactose, protein, and amino acid that is enough to be used for starter bacteria growth and activities. Lactose as the main carbohydrate found in milk is well utilized by starter bacteria as a source of carbon and energy. Skim milk added acted as the source of protein β-casein for the growth of starter bacteria [13].

The addition of powdered soymilk resulting significantly increased the number of lactic acid bacteria in fermented cheese whey beverages. This was in line with the opinion of [9], the prebiotic elements of GOS contained in soybeans in the form of stachyose and raffinose are a handy energy for the activity and growth of lactic acid bacteria in the fermentation process.

Stachyose and raffinose can be selectively exploited by Lactobacillus acidophilus as the source of carbon into glucose, galactose, and fructose with the help of α-galactooidase enzyme, so it can be utilized by *S. thermophilus* and *L. bulgaricus* bacteria to increase the population of lactic acid bacteria in the product [14].

The addition of powdered soymilk in the making of fermented cheese whey beverages can increase the growth of starter bacteria and the breakdown of lactose in raw material using lactase enzyme produced by lactic acid bacteria to form glucose and galactose.

Prior to incubation, the lactose content of the product was 10.53 % while during incubation, it decreased due to the breakdown of lactose by lactic acid bacteria. So, the average lactose content available ranged from 7.0069 % - 7.2623 %. It happened by glycolysis, where lactose is converted into glucose and galactose, then both monosaccharide were converted into pyruvate and forms lactic acid and acetaldehyde. One of the factors that can affects the amount of lactose, such as the activity of starter bacteria in an optimal growth conditions [15]. It is suspected that lactic acid bacteria not only breakdown lactose, but also breakdown other sugar components such as galactooligosaccharides that existed in the product of fermented cheese whey beverages.

The pH value achieved in a fermentation product was influenced by several factors such as time, starter amount, the optimum condition of bacterial growth and number of bacteria [16]. From Table 2., it is shown that the pH of the fermented whey cheese beverages increases with the higher concentration of powdered soymilk added. pH value of fermented cheese whey beverages increased with the increasing of soymilk powder concentration dissolved in the product [17]. It was known that soymilk powder has basic properties, so the higher amount of powdered soybean added can produce a higher pH of fermented beverages [18].

The fermentation process which involves lactic acid bacteria could accumulate organic acids such as lactic acid, formic acid, pyruvic acid and acetic acid followed by the decreasing of pH into 3.65 - 4.40 [8]. The acidity of fermented milk is caused by the rate of in lactic acid production with the decreased pH [19].

Table 2. Various Soybean Powder Concentration Towards Product Properties

| Treatment  | pH     | Lactic Acid (%) | Lactose levels (%) | Viskositas (cP) |
|------------|--------|-----------------|--------------------|-----------------|
| 7.5% Soy powder | 4.27a  | 0.558c          | 7.0069a            | 8685.33c        |
| 10.0% Soy powder | 4.28b  | 0.634b          | 7.1146b            | 15294.16b       |
| 12.5% Soy powder | 3.00c  | 0.675a          | 7.2623a            | 24823.33a       |

*Description: Different letters in the direction of the columns show significantly different
Production of lactic acid in yoghurt is associated with lactose hydrolysis by the activity of lactic acid bacteria or the ability of yogurt starter to be used. Lactose were hydrolyzed by starter into lactic acid and other organic acids such as acetic acid and pyruvic acid.

Table 2. showed that the viscosity of the fermented cheese whey beverages increased along with the increasing amount soymilk powder added. Soybean contains a globulin protein that has an isoelectric pH that is almost equal to the isoelectric pH of milk protein which is 4.5 [20]. Protein from cheese whey raw materials, skim milk, and soymilk powder that contained in the fermented cheese whey raw materials, will curdled due to the increased of lactic acid levels and the formation of acidic atmosphere with pH range between 4.27 to 4.30; Which in turn the concentration of hydrogen ions in the raw material approaches the isoelectric point of the protein and when the isoelectric point is reached the protein will be completely curdled, wherein the contained protein has zero electrical charge. In addition, the presence of exopolysaccharides (EPS) from bacterial fermentation that breakdown lactose into glucose and galactose increasingly EPS formation activity results in higher viscosity of the resulting fermented beverage [21]. This is in accordance with the opinion of [20] that the higher protein content and presence of EPS will increase the viscosity of fermented whey cheese beverages in all treatments.

4. Conclusion
The addition of 10 % and 12.5 % powdered soymilk in the production of the fermented cheese whey beverages resulted in the desired product with criteria as follow an average number of lactic acid bacteria of 208.58 x 10^8 colonies·g^-1 and 175 x 10^9 colonies·g^-1, pH 4.28 and 4.30, and viscosity 15294.16 cP and 24823.33 cP, respectively.

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