Effect of Incubation Time and Sucrose Addition on the Characteristics of Cheese Whey Yoghurt

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Abstract. The effect of incubation time and concentration of sucrose addition on the characteristics of cheese whey yogurt (lactic acid content, pH, total lactic acid bacteria, antioxidant activity, viscosity) and sensory characteristics (color, odor, flavor, consistency, and overalls) were investigated. The cheese whey yogurt fermentation process was carried out for 24h and 36h with the addition of sucrose 8, 10, and 12% (w/w) of total solid, respectively. The results showed that the lactic acid content, total lactic acid bacteria, antioxidant activity, and viscosity of cheese whey yogurt were affected by the incubation time and sucrose addition. The level of pH of yogurt which was incubated at 24h and 36h were relatively in the same levels, which were 4.51 up to 4.63. Due the sensory characteristic of cheese whey yogurt the panellists gave the high score for the cheese whey yogurt which was incubated at 24h and sucrose addition 12% (w/w) of total solid. The cheese whey yogurt has 0.41% lactic acid content; pH 4.51; 7.09 log total lactic acid bacteria cells / ml; 5.78% antioxidant activity; and 5.97 cP viscosity. The best sensory and physico-chemical characteristic of cheese whey yogurt was achieved by 24h incubation time and 12% concentration of sucrose addition.

1. Introduction
Whey is a by-product from the cheese or casein manufacturing. The whey was produced from milk coagulation process in cheese making. Whey contains a high lactose content which can be degraded by lactic acid bacteria to produce lactic acid in the fermentation process. These by-products can be converted to value-added products, e.g. yogurt. Yogurt is a fermented dairy product that produced during the fermentation process coagulates milk proteins that results a gel contribute to the overall texture. During the fermentation process, starter cultures also produce flavor compounds which contribute to the flavor of yogurt [1].

The fermentation process is the most important stage of yogurt production. During this stage, the yogurt curd is formed, and its textural characteristics and specific flavor are developed. One of important factor to maintain the quality of yogurt is incubation time. Incubation time influences the flavors, texture, and appearance of yogurt [2]. During incubation, the starter culture growth results in an increase the number of bacteria. As a result, the metabolites are produced by the starter culture larger during the incubation. The concentration of sugar addition also influences the growth of the starter culture and affect to the yogurt’s flavors. In this study, the incubation time and sugar addition were investigated on the cheese whey yogurt production.
2. Materials and Methods

2.1. Materials
Mozzarella cheese whey waste was obtained from small and medium enterprises cheese industry in Central Java, Indonesia. Sucrose “Gulaku Premium” was used as material on the sugar addition treatment. Pure culture of Lactobacillus delbrueckii spp. bulgaricus FNCC 0041 and Streptococcus salivarius spp. thermophilus FNCC 0040 were obtained from the Laboratory Processing and Process Engineering of Agricultural Products, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia.

2.2. Preparation of yogurt starter
Preparation of yogurt starter culture was described by Septiani with simple modification [3]. Pure culture was inoculated into MRS broth (Merck). Then, 0.261 g MRS broth (Merck) was dissolved in 5 ml of distilled water. The media solution was sterilized at 121°C for 15 minutes. L. bulgaricus and S. thermophilus were used as pure yogurt starter culture which then was inoculated in MRS broth (Merck) and incubated at 37°C for 24 h. The inoculated media broth was centrifuge at 1000 rpm for 10 minutes. The pellet of media broth which contained L. bulgaricus and S. thermophilus was transferred into pasteurized skim milk which was prepared by mixing 20 g of skim milk powder in 200 ml of distilled water and then pasteurized at 85°C for 30 minutes. The inoculated skim milk was incubated at 37°C for 24 h. The yogurt starter culture was ready to use when the number of bacteria was 10⁸ cells/ml.

2.3. Cheese whey yogurt production
The cheese whey yogurt fermentation process was carried out for 24h and 36h with the addition of sucrose 8, 10 and 12% (w/w) of total solid, respectively. Each flask contained 200 ml cheese whey which had been pasteurized at 75°C for 30 min. The pasteurized cheese whey was added with 8, 10, and 12% (w/w) sucrose, respectively. Then, L. bulgaricus and S. thermophilus were inoculated as 1% (v/v) with a cell density 10⁸ cells/ml into the pasteurized cheese whey which was added with 8, 10, and 12% (w/w) sucrose at 37°C. The inoculated cheese whey was incubated at 37°C with 24h and 36h of incubation time. All these experiments were performed in triplicate, and the lactic acid content, pH, total lactic acid bacteria, antioxidant activity, viscosity, and sensory characteristic of cheese whey yogurt were determined after the incubation time was 24h and 36h.

2.4. Consumer acceptance testing
The acceptance test was carried out with 32 consumers (aged 19–21 years), preselected according to their interest and habits of yogurt consumption. Consumer evaluation was performed according to an hedonic scoring from 1 (dislike very much) to 5 (like very much) for aspect color, odor, flavor, consistency, and overall appreciation. Samples were presented under similar conditions (temperature and quantity) for all panelists, according to Setyaningsih [4].

2.5. Analytical methods
Lactic acid content was determined according to IS 1166-1973 method [5]. Total lactic acid bacteria was calculated using Petroff-Hauser Bacteria Counter. Viscosity of cheese whey yogurt was performed using Falling Ball Viscometer. All characteristic analyses were carried out in triplicate. Experimental data were subjected to one-way analysis of variance (ANOVA) using the Duncan Multiple Range Test (DMRT) test (P < 0.05). The IBM SPSS software 17.0 was used.
3. Results and Discussion

3.1. Chemical and Physical Properties of Cheese Whey Yogurt

The coagulation of the milk proteins on the yogurt production caused by the secretion of lactic acid by lactic acid bacteria cultures. The metabolism of lactose into lactic acid resulted in low pH level [1]. Lactic acid content indicates the amount of lactose has been metabolized by lactic acid bacteria. Lactic acid content of yogurt with cow dairy ingredients ranged from 0.5-1.0 %. The lactic acid produced by L. bulgaricus is the L isomer, while that by S. thermophilus is the D isomer of 50-70%. Yogurt contains L-lactic acid isomer, and this lactic acid is more rapidly metabolized by the body [6].

The lactic acid content and pH profile are shown in Table 1. The highest lactic acid content was achieved by cheese whey yogurt which was added with 12% sucrose and incubated for 36 h. The lactic acid content of cheese whey yogurt which was incubated for 24h was lower than the corresponding lactic acid of incubated yogurt for 36h. The lowest lactic acid content was obtained from cheese whey yogurt which was added with 8% sucrose and incubated for 24h. Increasing sucrose addition and longer incubation time resulted in increasing lactic acid content in yogurt. The longer incubation time resulted in the increasing of total acid of yogurt [7].

| Incubation Time | Sucreose concentration | Lactic acid content (%) | Total Lactic Acid Bacteria (log cells / ml) | Antioxidant Activity (%) | Viscosity (cP) |
|----------------|------------------------|-------------------------|-------------------------------------------|--------------------------|---------------|
| 24h            | 8%                     | 0.33^a                  | 7.255^f                                  | 3.60^c                   | 5.994^a       |
|                | 10%                    | 0.41^b                  | 7.221^d                                  | 7.46^f                   | 5.808^a       |
|                | 12%                    | 0.42^b                  | 7.093^a                                  | 5.78^e                   | 5.975^a       |
| 36h            | 8%                     | 0.49^c                  | 7.250^e                                  | 5.14^d                   | 6.651^b       |
|                | 10%                    | 0.49^c                  | 7.127^c                                  | 3.47^b                   | 5.655^a       |
|                | 12%                    | 0.53^c                  | 7.113^b                                  | 2.06^a                   | 9.033^c       |

*Notation different letters in the same column indicate significant difference at a significance level of 5%.

The lactic acid content of cheese whey yogurt was 0.33% up to 0.53%. SNI No. 2981: 2009 reported that the lactic acid content of yogurt has range from 0.5% up to 2.0% [8]. Dairy Australia also reported that the standard of lactic acid content in yogurt is 0.7-1.0% [9]. The cheese whey yogurt with 12% sucrose addition and 36h time incubation has the lactic acid content which is allowed by SNI No. 2981: 2009 and Dairy Australia Standard.

The decreasing pH level during the fermentation of yogurt was caused by increasing lactic acid content. The carbohydrate addition on yogurt can be degraded by lactic acid bacteria and produce organic acid [6]. As a result, the pH of yogurt was decreased from 4.6 to 4.5. The concentration of hydrogen ions increases during the fermentation of yogurt due to a decreasing pH level [10]. The synergism effect between S. thermophilus and L. bulgaricus is based on their individual characteristics, and as a result higher lactose metabolism and lactic acid production is attained compared to each one acting individually [1]. The pH of the yogurt is not more than 4.5. Fermented milk products have standard maximum pH 4.5 [6]. The cheese whey yogurt which was incubated for 24h and 36h has the pH level 4.63-4.52 and 4.56-4.45, respectively.

During the yogurt fermentation, the symbiotic culture induces changes in the native components of the milk that are responsible for the physicochemical and sensory characteristics of yogurt [1]. In this study, S. thermophilus and L. bulgaricus were used as culture starters. S. thermophilus produces significant levels of lactase, which catalyzes the hydrolysis of lactose to glucose and galactose due to producing of pyruvic acid, formic acid, CO₂ and folic acid. The presence of lactose degradation...
product by *S. thermophilus* in the lowered oxygen tension and formic, then this condition stimulates the growth of *L. bulgaricus*. When the pH of the yogurt reaches up to 5.0, the activity of *S. thermophilus* subsides and *L. bulgaricus* gradually dominates the overall fermentation process until the fermentation process finishes [1]. *L. bulgaricus* grow rapidly after *S. thermophilus* reach stationary phase [11]. *L. bulgaricus* produces the amino acid valine, glycine and histidine required by *S. thermophilus* [12].

The highest total lactic acid bacteria were achieved by cheese whey yogurt which incubated for 24h and added 8% sucrose. The lowest total lactic acid bacteria were obtained by cheese whey yogurt with 12% sucrose addition and incubation for 24h. In this study, the increasing of sucrose addition causes the decreasing of the total lactic acid bacteria.

Milk and dairy products can be useful as an antidote to oxidation with particular mechanism. Milk and bioactive peptides are released by hydrolysis of the milk during the fermentation process can showed antioxidant activity. The antioxidant activity of whey protein caused by the presence of lactoferrin, α-lactalbumin and β-lactoglobulin contained in the milk also contributes to add the antioxidant activity of yogurt [13].

The content of DPPH on plain yogurt on the market is around 60 up to 80% [14]. Previous study reported that the addition of yogurt cultures increase the antioxidant activity up to 45.17% [15]. The complex reaction between peptides, amino acids and enzymes in yogurt causes the antioxidant activity. As a result, shelf life of yogurt is longer. In Table 1 showed that the antioxidant activity of cheese whey yogurt was highest in 24h incubation time and the concentration of sucrose 10%, *i.e.* 7.46%. The lowest antioxidant activity had showed by cheese whey yogurt that incubated of 36h and 12% sucrose concentration, *i.e.* 2.06%.

Viscosity is the consistency of a product that shows the resistance of a fluid to flow and stirring. During the fermentation process, sucrose and lactose is degraded by yogurt starter culture into lactic acid. The residual sucrose, lactose and organic acids which are not degraded will be counted as total dissolved solids. The higher total dissolved solids in solution will increase the viscosity level [16]. The viscosity of cheese whey yogurt with a 24h incubation time and the addition of sucrose 8, 10, and 12% were lower than that of yogurt with a 36h incubation time and the addition of sucrose 8%, 10% and 12%. The coagulation of casein that was caused by the pH of the yogurt reaches isoelectric point in an increasing of the viscosity of yogurt. The lactic acid produced by lactic acid bacteria cause an increasing the acidity of yogurt. As a result, the point of negative ion charge isoelectric occurs in milk. A part from casein and calcium binds with negative ionic charge, causing clotting [17].

### 3.2. Sensory Evaluation of Cheese Whey Yogurt

Cheese whey yogurt was evaluated for sensory attributes using acceptance test after production (Figure 1). The higher scores for cheese whey yogurt color value were found for cheese whey yogurt with 24h incubation time and sucrose addition 10%. The corresponding value was 3.31. The color of cheese whey yogurt is depend on the riboflavin content on whey that gives a greenish yellow color [16]. In this study, the color value of cheese whey yogurt was not affected by the incubation time and sucrose addition level. The higher scores for cheese whey yogurt odor value were found for cheese whey yogurt with 24h incubation time and sucrose addition 8%. The specific flavor of cheese whey yoghurt occurs during the fermentation process of yogurt that is produce non-volatile acid, volatile acid, carbonyl compounds and other compounds [17]. In this study, the higher concentration of sucrose addition produced the lower acceptability value of flavor characteristic.

Acceptance tests showed that no significant difference (*P* > 0.05) for the flavor and consistency of the cheese whey yogurt. In general, these results showed the good acceptance of the cheese whey yogurt. As shown in figure 1a,b, the cheese whey yogurt which was incubated for 24h had a higher number for color, flavor, and overall acceptance than that of the cheese whey yogurt with 36h incubation time. The cheese whey yogurt with 36h and 8% sucrose addition had the lowest score for
overall acceptance (figure 1b). The panelists of the acceptance test give a highest overall score on cheese whey yogurt with 24h incubation time and 12% sucrose addition.

Figure 1. Graphical representation of sensory acceptance of the cheese whey yogurt with 24h (a) and 36h (b) incubation time and sucrose addition 8, 10, and 12%.

4. Conclusions
The characteristic of cheese whey yogurt, i.e lactic acid levels, total lactic acid bacteria, antioxidant activity, and viscosity was affected by incubation time and sucrose addition of cheese whey yogurt. Sensory acceptance test of all parameters of yogurt with 24h incubation time and sucrose addition 12% was most preferred.

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