**INTRODUCTION**

Whey is the liquid cheese waste serum or milk produced from the cheese-making process after being separated from the curd. Cheese production waste containing carbon sources one of which lactose as a source of energy in the development of biotechnology fermentation, in particular, the manufacture of probiotics for livestock. The content of lactose (4-7%) and protein (0.6-1%) which can be used as media growth of bacteria in the process of biotechnology fermentation very favorable for livestock health and productivity of livestock that is organic. Cheese Whey can be used as media growth of bacteria in the produce secondary metabolites such as organic acids, nutrients, and antioxidants that can be applied as an additive to cattle feed nutraceutical that provides medical benefits or health, including the prevention and treatment of disease as well as improves the performance of livestock. The purpose of this research is to know the influence of addition of kefir grain 1-5% into whey cheese on quality chemical and antioxidant. Whey cheese obtained CV. Margo Utomo around July–June 2018 added molasses to 10% and then fermented according to preferential treatment. The method of trial well-designed Randomized Complete Design with six treatments and three replicates each of 3 i.e. 0%, 1%, 2%, 3%, 4%, 5%. The results showed that the addition of kefir grains 1-5% was significant (P <0.01) increased lactic acid, alcohol, titratable acidity, antioxidant activity, and decreased kefir biomass grain, lactose, sucrose, acetic acid, pH compared to control treatment. The conclusion of this study that the addition of kefir grain into whey cheese was added 10 molasses affect the increase in lactic acid, alcohol, titratable acidity, antioxidant activity and can reduce pH, lactic acid, biomass grain kefir.

**Keywords:** fermentation, kefir starter, molasses, whey cheese.

**Abstract:** Whey is cheese waste liquid or serum milk produced from the cheese-making process after being separated from the curd. Cheese production waste containing carbon sources one of which lactose as a source of energy in the development of biotechnology fermentation, in particular, the manufacture of probiotics for livestock. The content of lactose (4-7%) and protein (0.6-1%) which can be used as media growth of bacteria in the process of biotechnology fermentation very favorable for livestock health and productivity of livestock that is organic. Cheese Whey can be used as media growth of bacteria in the produce secondary metabolites such as organic acids, nutrients, and antioxidants that can be applied as an additive to cattle feed nutraceutical that provides medical benefits or health, including the prevention and treatment of disease as well as improves the performance of livestock. The purpose of this research is to know the influence of addition of kefir grain 1-5% into whey cheese on quality chemical and antioxidant. Whey cheese obtained CV. Margo Utomo around July–June 2018 added molasses to 10% and then fermented according to preferential treatment. The method of trial well-designed Randomized Complete Design with six treatments and three replicates each of 3 i.e. 0%, 1%, 2%, 3%, 4%, 5%. The results showed that the addition of kefir grains 1-5% was significant (P <0.01) increased lactic acid, alcohol, titratable acidity, antioxidant activity, and decreased kefir biomass grain, lactose, sucrose, acetic acid, pH compared to control treatment. The conclusion of this study that the addition of kefir grain into whey cheese was added 10 molasses affect the increase in lactic acid, alcohol, titratable acidity, antioxidant activity and can reduce pH, lactic acid, biomass grain kefir.

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Protects against atherosclerosis and plaque formation in the arteries. Keskenaş, et al. [11], Xiao and Dong [17] stated that oral administration of kefir grain produced from milk or soy milk results in significant inhibition of tumor growth and there is a positive effect on reducing cholesterol metabolism.

Materials and Methods
Cheese Waste Fermentation Method
Whey cheese obtained CV. Margo Utomo around July-2018 was mixed with 10% molasses then analyzed proximate (Table 1). The stages of cheese waste fermentation include whey extraction then heated with 80°C temperature for 10 minutes then added molasses (sugarcane) 10%. Decrease the temperature until the temperature of the 35-40 oC by soaking a beaker glass contains water and molasses mixture into the cold water. The next step is the addition of kefir grain inoculation according to treatment. After the inoculation process, the process was continued with the inclusion of anaerobic kefir fermentation for 24 hours with a temperature between 35-40°C [8, 11, 18]. Then do the analysis of chemical and liquid antioxidant quality whey cheese that's been fermented.

Table-1: Chemical composition of whey

<table>
<thead>
<tr>
<th>Item</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>76.96</td>
</tr>
<tr>
<td>Ash</td>
<td>4.645</td>
</tr>
<tr>
<td>Protein</td>
<td>0.2</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>17.95</td>
</tr>
<tr>
<td>Sucrose</td>
<td>8.38</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.261</td>
</tr>
<tr>
<td>Lactose</td>
<td>7.85</td>
</tr>
<tr>
<td>pH</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Analysis of quality chemistry fermentation of whey
Kefir’s chemical analysis included pH with the Potentiometry method [19, 20], lactose [21], sucrose [20], alcohol with the Conway method [22], lactic acid [20], Titratable Acidity [23], antioxidants [24].

Statistics
Statistical Analysis System (version 9.3, SAS) was used to perform data analysis using the general linear model (GLM). Duncan multiple range test was used for comparison of mean of treatments. Correlation coefficient was also established to investigate relationships between parameters [25].

Results and Discussion
Biomass Grain Kefir
The effect of adding Grain kefir into whey cheese which was added with molasses had a very significant effect (P <0.01) on reducing biomass grain kefir compared to the control (Table 2). The decrease in biomass kefir grain of each treatment is influenced by the high carbon content found in molasses (sugarcane drops) in the mixture of each treatment solution. in each treatment, whey solution added 10% molasses has a high carbon source. According to Razack, et al. [26] optimal kefir biomass grain by adding 2% carbon molasse and rice bran sources and decreasing when a higher concentration of carbon sources was used. The increase in the carbon source cause the high osmotic pressure on the network of cells that causes plasmolysis can experience a decrease in the number of microbial cells [10]. Influence of the decrease in biomass can also influence the high lactose which is in the solution of whey approximately 7.85%. According to Utami, et al. [10] addition of 2.4% lactose concentrations can increase the biomass kefir grain. Furthermore, according to Cheirsilp and Radchabut [3] the addition of lactose concentration of more than 2% can affect the reduction of kefir grain biomass. The decrease of kefir grain biomass was also influenced by the low protein content in the treatment which resulted in the release of total polysaccharides including dissolved exopolysaccharides (EPS) from kefir grains [7, 11, 27]. Factors that can affect biomass include, the time interval for replacing kefir grains growth media, temperature, the concentration of the right addition of nutrients in the growth media, the microbial composition of kefir grains, and the type of bacterial strain on kefir gains [7, 10, 11, 27-29]. According to Guzel-Seydim et al. [27] that adding 2% whey protein can increase kefir grain biomass.
Lactose and Sucrose

The addition of kefir grains was very significant (P < 0.01) to decrease lactose and sucrose levels compared to controls (Table 2). The higher the kefir grain concentration will reduce lactose and sucrose levels if (Tables 2 & 3). This decrease in lactose and sucrose concentration is due to an increase in the concentration that has an impact on lactose and sucrose requirements as an energy source (carbon) in increasing the activity of microorganisms (Table 3). The activity of microorganisms from kefir grains such as lactic acid bacteria, yeast and acetobacter requires energy sources derived from carbohydrates, one of which is lactose, sucrose, and lactic acid (Table 2). In this study, the lower the lactose produced, the higher the activity of bacteria in breaking down lactose and vice versa. Lactose functions as a substrate that will be broken down into lactic acid with the help of the enzyme Beta-galactosidase [11, 28, 32-34]. The beta-galactosidase enzyme is produced by bacteria lactic acid in kefir grain as lactose hydrolyzing enzyme to glucose and galactose so that lactose will decrease [7, 35, 36]. According to Nursiwi et al. [7] lactose is metabolized by lactic acid bacteria to glucose-6-phosphate or fructose-6 phosphate and then metabolized through Embden Meyerhoff Parnas (EMP) to lactic acid. The required sucrose content is also used by lactic acid bacteria and Saccharomyces to be converted into organic acid components, one of them is lactic acid, acetic acid, and alcohol [11, 28, 32, 37].

Tabel-3: Correlation coefficients (r) between Biomass, Alcohol, sucrose, lactose, acetic acid, pH lactic acid and titratable acidity (%)

<table>
<thead>
<tr>
<th>Items (%)</th>
<th>0 (Kontrol)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>0,00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-11,70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-12,00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-16,28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-17,96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-18,43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,64</td>
</tr>
<tr>
<td>Lactose</td>
<td>7,02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5,24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4,89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4,47&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3,95&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3,59&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0,27</td>
</tr>
<tr>
<td>Sucrose</td>
<td>8,27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4,59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4,23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4,05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3,82&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3,62&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0,39</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0,17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2,73&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2,90&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3,18&lt;sup&gt;g&lt;/sup&gt;</td>
<td>3,32&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3,46&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0,27</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0,26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0,25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0,23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0,21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0,19&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0,18&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0,01</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0,19&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2,22&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2,55&lt;sup&gt;h&lt;/sup&gt;</td>
<td>2,93&lt;sup&gt;i&lt;/sup&gt;</td>
<td>3,08&lt;sup&gt;j&lt;/sup&gt;</td>
<td>3,50&lt;sup&gt;k&lt;/sup&gt;</td>
<td>0,11</td>
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<tr>
<td>pH</td>
<td>4,87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3,87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3,77&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3,70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3,67&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3,5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0,11</td>
</tr>
<tr>
<td>TA</td>
<td>0,150&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0,525&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0,525&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0,579&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0,577&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0,582&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0,05</td>
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<tr>
<td>Antioxidants</td>
<td>16,23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39,39&lt;sup&gt;d&lt;/sup&gt;</td>
<td>39,19&lt;sup&gt;e&lt;/sup&gt;</td>
<td>38,91&lt;sup&gt;f&lt;/sup&gt;</td>
<td>38,69&lt;sup&gt;g&lt;/sup&gt;</td>
<td>38,07&lt;sup&gt;h&lt;/sup&gt;</td>
<td>2,05</td>
</tr>
</tbody>
</table>

SEM: Standard error of mean. TA: titratable acidity **Means within a row with different superscripts are significantly different (P < 0.01).

Alcohol

The increase in alcohol content per treatment was very significant (P <0.01) with the addition of kefir grain concentration (Table 2). An increase in the number of concentrations has an effect on increasing alcohol according to the correlation test (Table 3). This increase in alcohol is due to the high levels of kefir grain in degrading carbon sources, one of which is lactose and sucrose derived from whey cheese and molasses to be converted into ethanol (Alcohol) by microorganism activity [10, 28, 38]. Microorganisms that have a very important role in the breakdown process of glucose make Alcohol is Saccharomyces (yeast) [11, 39, 40]. Saccharomyces (yeast) in the kefir grain catalyze glucose, fructose, and sucrose to be converted into cellular energy and also produce ethanol and carbon dioxide as by-products [38, 41]. This resulting in the longer fermentation and kefir grain increases then the alcohol will generate will be optimized and will eventually be decreasing concentrations of glucose [38, 42].

Acetic acid dan lactic acid

The effect of 1-5% gran kefir concentration was very significant (P <0.01) to decrease acetic acid and increase lactic acid (Table 2). In this study, the interactive effects of acetic acid and lactic acid at a certain level were evaluated based on acid
concentration by reviewing the decrease in Biomass kefir grain. The decrease in acetic acid is caused by a decrease in the activity of acetic acid bacteria contained in the kefir grain [42]. This can be seen from the data: increasing the alcohol content of each treatment, acetic acid is produced from the overhaul of alcohol (ethanol) by acetic acid bacteria and generally, an increase in acetic acid production is closely related to acetic acid bacteria activity (Table 3). From these results that all Alcohol is not used by acetic acid bacteria because of decreased activity. According Kustiyawati and Setyani [43], the growth rate of acetic acid bacteria will increase after the availability of oxygen and alcohol resulting from the transformation of lactic acid bacteria and yeast (yeast), alcohol is oxidized to acetic acid and acetic acid is oxidized to CO2 and water.

pH dan TA (Titratable acidity)

In Table 2 the addition of kefir grain concentration is also able to increase the concentration of lactic acid in lactose overhaul, so that a high kefir grain concentration will increase the production of lactic acid and alcohol [11, 13, 37, 44, 45]. Increased lactic acid production is affected by enzyme activity produced from lactic acid bacteria to convert sugar (lactose) to lactic acid [13]. Changes in chemical composition (including lactic acid) in whey fermentation are influenced by the ability of kefir grain to produce lactic acid which is determined by the number and type of starter used. The more number of bacteria inoculated into whey, the greater the chemical changes that occur in it, the emergence of H+ ions can be caused by the decomposition of lactose to produce volatile acids and the breakdown of organic phosphate contained in casein which can produce acid [46]. Furthermore Utami, et al. [10] explained the results of the trend of this pH value in accordance with the concentration of lactic acid obtained where the pH and lactic acid values were inversely proportional, the higher the level of lactic acid showed the lower pH value (Table 3).

### Tabel-4: Correlation coefficients (r) between antioxidants, and lactic acid (%)

<table>
<thead>
<tr>
<th>Items</th>
<th>Antioxidants</th>
<th>Lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidants</td>
<td>-</td>
<td>0.006**</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.006**</td>
<td>-</td>
</tr>
</tbody>
</table>

** Pearson correlation is very significant (P <0.01).

The effect of the addition of 1.5-5% kefir grain has a very significant (P <0.01) on decreasing pH and increasing titratable acidity (TA). This is consistent with the increase in lactic acid which affects the decrease in pH in the whey cheese which is from 4.87 to 3.5. The titratable acidity value in the treatment is considered as the value of lactic acid formed by lactose fermentation by lactic acid bacteria [47]. Lactic acid yang terbentuk akan menurunkan pH menyebabkan terbentuknya gel akiat koagulas protein whey, Relationship The total value of titrated acid will be inversely proportional to the pH value and associated with lactic acid (Table 3)[48]. The activity of lactic acid bacteria that exist in the kefir grain will change the lactose into lactic acid that total lactic acid increased rapidly and titrated the acid value, too.

Antioxidants

The effect of increasing kefir grain concentration was very significant (P <0.01) increasing antioxidant activity (Table 3). The treatment of the addition of 1% - 5% kefir grains was very significant (P <0.1) increasing antioxidant activity compared to controls (0% kefir grain) of 38.07-39.39%. Increased antioxidants activity is due to the increase in organic acids, one of which is lactic acid produced by lactic acid bacteria in the kefir grain (Table 2). According to Oktaviani [49] organic acids produced by lactic acid bacteria during the fermentation process, one of which is lactic acid. Increased antioxidants activity due to the formation of lactic acid, during the fermentation process of lactic acid concentration also continues to increase [11, 13, 50]. Lactic acid in kefir contains α-hydroxy acids (AHA) which function as antioxidants [51]. Apart from lactic acid, there is an increase in antioxidant activity due to the presence of secondary metabolites from bacterial metabolism, one of which is vitamin C and vitamin E [29, 49].

CONCLUSION

Adding the kefir grain to the whey cheese added with 10% molasses affects the increase in lactic acid, alcohol, titratable acidity (TA), antioxidants activity and can reduce pH, lactic acid, biomass kefir grain.

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