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Full Length Article

Physical, Chemical and Microbiological Characteristics of Powdered Kefir Grains using Skimmed Milk as Encapsulant

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Abstract

This study was aimed at to make powdered kefir grains that are easier to use and apply. Variations in the concentration of skim milk (20, 25, 30 and 35%) used in the manufacture of powdered kefir grains were measured, which influence physical characteristics such as rehydration power and rehydration time, chemical characteristics such as yield, water content, water activity, solubility, pH, protein content and total dissolved solids. In addition, microbiological characteristics such as total microbes, total lactic acid bacteria and yeast were also determined. The results showed that variations in the concentration of skim milk significantly affected all physical, chemical and microbiological parameters except for protein content. The best added concentration was 35% of skim milk powder.

Keywords: Freeze Drying; Microencapsulation; Powdered kefir grains; Skimmed milk

Introduction

Kefir is a type of probiotic drink. Kefir is made through a fermentation process by incubating kefir grains in milk. Kefir contains stain microorganisms that can help relieve digestive disorders in the digestive tract (Shen *et al.* 2018). Kefir grain is a collection of bacterial microorganisms consisting of three dominant types of microorganisms, namely lactic acid bacteria (LAB), yeast and acetic acid bacteria (AAB) (Purutoğlu *et al.* 2020). LAB and yeast contribute to the distinctive taste of kefir because they can produce lactic acid, carbon dioxide, acetaldehyde, ethanol and acetone (Setyawardani and Sumarmono 2015). The sour taste of kefir drinks comes from lactic acid produced by LAB and acetic acid produced by AAB.

Kefir grains have the characteristics of irregular grain shape, yellowish-white, also wet and sticky texture (Barãoa *et al.* 2019). Kefir grains in the form of wet granules must be refreshed periodically to maintain the life of the microorganisms in them. In addition, kefir grains with wet characteristics must be stored at low temperatures to inhibit the metabolism in microorganisms in order to maintain the quality of kefir grains (Putri *et al.* 2020). Kefir grains stored at low temperatures cause the microorganisms in the kefir grains to go into a dormant state. Therefore, kefir grains must be activated before being used in making kefir drinks. Kefir grains activation can be done by inoculating it into sterile milk and incubating it for 24 h (Ebner *et al.* 2015).

Kefir grains have the disadvantage of having stored at low temperatures. Kefir grain damage during storage can be minimized by applying the microencapsulation. The microencapsulation is a method of preparing kefir grains in powder form. This process involves several coating materials to protect the core material during drying (Sumanti *et al.* 2016). A key factor in the microencapsulation process is maintaining the stability of the active ingredients during the shelf life. Starch, lipids, and proteins can be used as coating materials (Pradipta 2017).

One type of coating material that can be used is skim milk. Skim milk has delicate pores to reduce contact between microorganisms and air during drying (Pratana *et al.* 2019). The amino acids found in whey protein contain sulfur to prevent lipid oxidation, which can cause cell damage (Pratiwi and Mulyanti 2021). This research predicts that the use of skim milk with different concentrations may have a significant effect on the physical, chemical, and microbiological characteristics of powdered kefir grains. To test this hypothesis, rehydration power, rehydration time, yield, water content, water activity, solubility, pH value, protein content, total dissolved solids (TDS), viable count of microbe, viable count of LAB, AAB and viable count of yeast have been measured in this study.

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Materials and Methods

Preparation of kefir grains

A 20 g of kefir grains was put into 60 mL of full cream Ultra High Temperature milk and then incubated at 25ºC for 24 h. The process of preparing kefir grains is carried out for 24 h in 3 days (Kondong *et al*. 2017; Barãoa *et al.* 2019 modified).

Microencapsulation of kefir grains

Kefir grains that had been activated for 24 h in 3 days were taken as much as 5% (w/v) to be added to each skim milk solution with concentrations of 20, 25, 30 and 35% (w/v). The solution was mashed using a blender 6 times for 3 sec (Sainz *et al*. 2020; Obradovic *et al*. 2022 modified). Kefir grains solution then homogenized (12.000 rpm for 3 min) then served in several plastic cup of 12.5 mL for each cup, then incubated at 25ºC for 24 h (Gul and Altar 2019; Rizqiati *et al.* 2021). After 24 h, samples were put into the freeze dryer (Tefic Biotech, China) at a condenser temperature of -40ºC, pressure 0.1 mbar for 24 h, until the sample temperature reached 27–28ºC. The dry kefir starter sample was mashed into a powder, kept in zip-lock plastic bag with silica gel, stored at room temperature (Chranioti and Tzia 2013).

Physical analysis

Analysis of rehydration power: As described by Palijama *et al.* (2020), 1 g of the sample was put into 10 mL of distilled water and mixed well in the vortex and left at room temperature for 20 min; then centrifuged (3500 rpm, 30 min). Rehydration power was calculated with the following formula:

$$\text{Rehydration power (mL/g)} = \frac{A - B}{C} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 1}$$

Analysis of rehydration time: A 2 g of the sample was mixed with 50 mL distilled water and stirred it with a magnetic stirrer (800 rpm). The time required for the sample to be completely dispersed was recorded as rehydration time (Schuck *et al.* 2012).

Chemical analysis

Water content: Analysis of water content of the samples was carried out using the thermos-gravimetric method Legowo *et al.* (2005). The moisture content of the sample can be calculated using the following formula:

$$\text{Water content (\%)} = \frac{\text{Sample fresh weight} - \text{Sample dry weight}}{\text{Sample fresh weight}} \times 100 \ldots \text{Eq. 2}$$

Crude protein content: Analysis of crude protein content was carried out using the Kjeldahl method where protein content was calculated using the formula (Legowo *et al.* 2005):

$$\text{Protein content (\%)} = \frac{(\text{sample titrant} - \text{blank}) \times \text{N HCl} \times 14.008}{\text{sample weight x } 1000} \times 100 \ldots \text{Eq. 3}$$

Measurement of solubility: A 1 g (a) will be dissolved in 20 mL of distilled water at 50ºC and then filtered through filter paper that has been baked in oven (b). The filter paper is then heated again in the oven until a constant weight is obtained (c) (Rizqiati *et al.* 2020). The solubility of powdered kefir starter is calculated by the formula:

$$\textit{S}\text{olubility (\%)} = 1 - \left(\frac{(c-b)}{a}\right) \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 4}$$

Measurement of yield: The weight of the wet kefir starter is weighed as the weight of the initial product, then the weight of the dry sample is considered as the weight of the final product (Rizqiati *et al.* 2020). The yield is then calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{powdered kefir grains weight}}{\text{kefir grains weight}} \times 100 \quad \ldots\ldots\ldots\ldots \text{Eq. 5}$$

Measurement of TDS: A 1 g of sample is dissolved in 20 mL of distilled water and then filtered. The filtrate is then dripped into the hand refractometer and the results are recorded and multiplied by the dilution factor (Siagian *et al.* 2017; Rizqiati *et al.* 2020).

Microbial analysis

Viable microbial count: For this purpose, 1 g of sample was dissolved in 9 mL of 0.85% NaCl and made up until 10^{-6} dilution level. Three level of last dilution were cultured on plate count agar (PCA; Merck, Germany) and incubated at 37°C for 48 h (Hafsan 2014). The number of viable colonies will be calculated using the following formula:

$$\Sigma \text{ Colonies} = \frac{1}{\text{dilution factor}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 6}$$

Analysis of the viable number of LAB: Analysis the viable numbers of LAB by plate count method (Mandang *et al*. 2016). To do this, 1 g of sample was dissolved in 9 mL of 0.85% NaCl and made up until 10^{-6} dilution level. 3 level of last dilution were cultured in MRSA (Merck, Germany) then incubated at 37°C for 24 h. The number of viable colonies were calculated using Eq. 6.

Analysis of the viable number of yeasts: Analysis the viable number of yeasts by plate count method (Subramanya *et al.* 2017). A 1 g of sample was dissolved in 9 mL of 0.85% NaCl and made up until 10^{-6} dilution level. 3 level of last dilution were cultured in SDA media (Merck, Germany) then incubated at 37°C for 72 h. The number of viable colonies were calculated using the Eq. 6.

Statistical analysis

Design of the experiments was completely randomized with five replications. Data were analyzed using the ANOVA method to determine the effect of treatment using SPSS 26.0 computer software. Analysis was performed with a significance level of 5%. If there is an influence from the treatment, then a further test is carried out with the DMRT method.

Results

Analysis of variance (ANOVA) showed that variations in skim milk concentration had a significant effect on all observed physical and microbiological parameters (Table 1, 3) and also significantly affected all chemical parameters except protein content (Table 2).

Physical analysis

Use of skim milk with different concentrations has a significant effect ($P < 0.05$) on the rehydration power and rehydration time of powdered kefir grains (Table 1). Rehydration power of 20, 25, 30 and 35% skim milk added respectively were 1.42, 1.54, 1.63 and 1.95 (mL/g). Rehydration time of 20, 25, 30 and 35% skim milk added, respectively were 11.98, 5.94, 4.07 and 3.03 sec.

Rehydration power in this experiment showed that the powdered kefir grains were influenced by the concentration of skimmed milk as an encapsulant. The addition of skim milk powder increases the rehydration power to 1.95 mL/g (Table 1). Besides rehydration power, another physical parameter that was measured was rehydration time (Table 1). Rehydration time shows the time required until the entire product is dispersed in water. Based on Table 1, showed that the rehydration time of powdered kefir grains decreased with increasing skim milk concentration.

Chemical analysis

The use of skim milk with different concentrations has a significant effect ($P < 0.05$) on the water activity (a_w), pH value, water content, solubility, yield, and TDS but not significant on the protein content (Table 2). Yield of 20, 25, 30 and 35% skim milk added respectively were 14.44, 17.14, 21.22, and 23.74% while TDS were 63.80, 74.13, 76.84 and 80.83ºBrix. Water content of 20, 25, 30 and 35% skim milk respectively were 6.39, 5.17, 3.67 and 3.15%. The a_w of added 20, 25, 30 and 35% skim milk respectively were 0.23, 0.22, 0.11 and 0.09. Solubility of 20, 25, 30 and 35% skim milk added respectively were 48.53, 51.23, 52.17 and 53.03%. The pH value of 20, 25, 30 and 35% skim milk added respectively were 4.07, 4.13, 4.20 and 4.41. Protein content of 20, 25, 30 and 35% skim milk respectively were 26.37, 26.81, 26.76 and 27.35%.

Data showed that due to higher concentration of skim milk the yield of the product increased, which ranged from 14.44–23.74% (Table 2). A high yield means that the product produces more solids. The amount of solids is inversely proportional to the amount of water contained in the product. The lowest water content was obtained at a concentration of 35%, which reached 3.15% and was accompanied by an increase in yield product up to 23.74% (Table 2). The total amount of yield is directly proportional to the TDS. The TDS shows the number of solids contained in the product.

Water in the product was relevant to a_w. The a_w of powdered kefir starter product decreased to 0.09 with increasing total solids of skim milk. The reduced water content in the product made the powdered kefir starter product more porous, which increased the solubility in water due to the number of cavities in the product.

The existence of a fermentation process and various types of microorganisms in the product makes its pH significantly different. The pH of the product was in the range of 4.07–4.41 (Table 2). At this pH, product protein can be hydrolyzed, which has no significant impact on the resulting protein content. The protein hydrolysis process produces organic acids and peptides which affect increasing the TDS of the kefir starter powder.

Microbial analysis

The use of skim milk with different concentrations has a significant effect ($p < 0.05$) on the viable number of microbe, LAB and yeast of powdered kefir grains which indicates an increase in the viable number microbe as the concentration of skim milk increased (Table 3). Total viable number of microbes at concentrations of 20, 25, 30 and 35% skim milk added were 6.0360, 6.7960, 7.4420 and 8.2520 log CFU/g, respectively. The viable number of LAB at concentrations of 20, 25, 30 and 35% respectively were 6.8632, 7.2095, 7.8222 and 7.8711 log CFU/g. The viable number yeast at the concentration of 20, 25, 30 and 35% skim milk added respectively were 6.09, 6.29, 6.64 and 6.48 log CFU/g.

This result indicates that the increase of skim milk powder as a coating agent had a significant effect on the microbial characteristic of powdered kefir grains (Table 3). Skim milk is used as a coating material to form a protective wall to maintain the stability of microorganisms during the drying process. The higher the concentration of skim milk, the higher the protective wall formed so that the microorganisms in powdered kefir grains tend to be more stable. The stability of microorganisms is indicated by the better number of living microbial cells.

Discussion

As regards physical properties, an increase in rehydration power can increase in the porosity of the sample with an increase in the concentration of skim milk used. An increase in the concentration of skim milk causes the sample to have a higher TDS and result in a higher viscosity (Table 2). This is in accordance with the results Elsamania and Ahmed (2014) who stated that the use of powdered skim milk with concentrations of 0, 5, 10 and 15% produced peanut milk-based yogurt which had 19.7, 21.3, 25.5 and 27.09% TDS. This condition caused the sample to go through a faster drying process due to loss of water that must be evaporated, due to causing the sample to have a more porous structure (Mishra *et al.* 2017). This is because the porous structure will increase the absorption ability of the sample which is related to the rehydration power (Tamrin and Pujilestari 2016; Zhu

Table 1: Analysis of Variance of the effect of skim milk powder on rehydration power and rehydration time of powdered kefir grains

Skim milk concentration (%)	Rehydration power (mL/g)	Rehydration time (sec)
20%	1.43±0.22^{a}	11.98±0.69^{a}
25%	1.54±0.09^{a}	5.94±0.69^{b}
30%	1.63±0.08^{ab}	4.07±0.47^{c}
35%	1.95±0.12^{b}	3.03±0.18^{d}

Data shown as the mean value of 5 replicates ± standard deviation
Different lowercase superscripts show a significant effect (P<0,05)

Table 2: Analysis of Variance (ANOVA) of the effect of skim milk powder on water activity, pH value, water content, protein content, solubility, yield, and TDS of powdered kefir starter

Skim milk concentration	Yield (%)	TDS (ºBrix)	Water content (%)	Water activity (a_w)	Solubility (%)	pH value	Protein content (%)
20%	14.44±0.52^{a}	63.80±0.84^{a}	6.39±0.62^{a}	0.23±0.02^{a}	48.53±0.75^{a}	4.07±0.00^{a}	26.37±0.31
25%	17.14±0.46^{b}	74.13±0.77^{b}	5.17±0.26^{a}	0.22±0.02^{a}	51.23±0.46^{b}	4.13±0.01^{b}	26.81±0.91
30%	21.22±0.27^{c}	76.84±0.85^{c}	3.67±0.62^{b}	0.11±0.02^{b}	52.17±0.79^{bc}	4.20±0.01^{c}	26.76±0.32
35%	23.74±0.87^{d}	80.83±0.76^{d}	3.15±0.42^{c}	0.09±0.01^{b}	53.03±0.92^{c}	4.41±0.02^{d}	27.35±0.45

Data shown as the mean value of 5 replicates ± standard deviation
Different lowercase superscripts show a significant effect (P<0,05)

Table 3: Analysis of Variance of the effect of skim milk powder on total microbe, LAB, and yeast of powdered kefir starter

Skim milk powder concentration	Total microbe (log CFU/g)	Lactic acid bacteria (log CFU/g)	Yeast (log CFU/g)
20%	6.0360^{a}	6.8632^{a}	6.0898^{a}
25%	6.7960^{b}	7.2095^{a}	6.2868^{ab}
30%	7.4420^{c}	7.8222^{b}	6.6411^{c}
35%	8.2520^{d}	7.8711^{b}	6.4846^{bc}

Data shown as the mean value of 5 replicates
Different lowercase superscripts show a significant effect (P<0,05)

et al. 2018). Decrease in sample rehydration time is related to the water content of the sample. Samples with a higher concentration of skim milk have a lower water content, which causes a faster rehydration time. This is because samples with lower water content and lower water activity have a less sticky texture. These conditions cause a greater surface area of the sample in contact with water so that it can reduce the rehydration time. In this context Fontes *et al.* (2014) showed that prebiotic fruit drinks made using 10 and 20% maltodextrin drying agents with an inlet air temperature of 180°C had a water activity of 0.211 and 0.191 and a rehydration time of 120.56 and 90.34 sec.

Regarding chemical properties, it is known that different concentrations of skim milk gave different results on the chemical characteristics of powdered kefir starter (Table 2). Every product processing process must have a yield value which is interpreted as the efficiency value of a product. The yield value in this research increases with increasing skim milk concentration variations (Table 2). An increase in yield is due to skim milk acting as a solids enhancer in a product. The TDS in powdered skim milk are protein, lactose and minerals. The process of making powdered kefir starter products can be said to be efficient because the yields produced increase (Liu *et al.* 2018). According to Chuacharoen (2020), higher the yield obtained, better the processing because there is no significant product loss during the powder kefir starter manufacturing process.

The efficiency of powdered kefir starter products is also supported by low water content. Freeze-dried products have a low moisture content because most of the water has sublimed. Skim milk with different concentrations had a significant effect ($p<0.05$) on the water content of powdered kefir starter which indicates a decrease in water content along with an increase in skim milk concentration. Increased concentration of skim milk caused an increase in TDS (Table 2). This is due to an increase in the number of dispersed particles along with an increase in the concentration of skim milk (Tsermoula *et al.* 2021). Higher TDS cause the sample to have a higher viscosity. An increase in viscosity can occur because the protein content in skim milk has can bind water (Botrel *et al.* 2014). Therefore, a sample with a lower water content will be obtained.

In addition, the low water content makes the product also have low water activity. This is because the water content is directly proportional to the water activity. Skim milk acts as a binder, so it can increase the ability to bind water to the product (Yuanita and Silitonga 2014). Lactose in skim milk is hygroscopic and can absorb or release water in the product (Sansone *et al.* 2018). The results of microencapsulation products are in line with the recommendations of Fauziyyah *et al.* (2022), who stated that the recommended a_w value <0.6 for microencapsulated products aims to maintain product stability during the shelf life.

The product resulting from drying becomes porous, lighter and can be easily crushed, which affects its solubility in water. The solubility of powdered kefir starter also increased with increasing skim milk concentration (Table 2). Skimmed milk is a coating material with high solubility (Ji *et al.* 2016). The process of protein hydrolysis in skim milk increases solubility because the molecular weight decreases.

Small molecular weight makes it easier for the solvent to diffuse through the material so that the solubility of the product increases. The small particle size also supports increased solubility (Colombo *et al.* 2017). The slow freezing process carried out during product manufacture causes the formation of more porous cavities in the product. According to Laokuldilok and Kanha (2015) freeze drying also makes the product have a higher hygroscopicity.

Skim milk used as a coating material increased pH of the kefir powder starter (Purbosari 2019). Skimmed milk has a pH closer to neutral, so greater amount of skim milk used can increase the pH of the powdered kefir starter. The starter's pH value is low because fermentation occurs during sample preparation. The amount of lactic acid produced is reflected as pH value of the product (Oktaviana *et al.* 2015). The pH value of powdered kefir starter corroborated with that reported by Prabhurajeshwar and Chandrakanth (2017). Product pH conditions in the range of 4.00 was close to the isoelectric point, so it can weaken the protein structure, and eventually the protein content (Febriana *et al.* 2021). Besides, the protein contained in powdered kefir starter can come from the fermentation by microorganisms. The fermentation process with kefir grains produces several proteolytic enzymes that play a role in protein hydrolysis (Chua *et al.* 2021). The protein content in powdered kefir starter was calculated based on the amount of nitrogen in the product (Table 2). LAB can use peptides resulting from protein hydrolysis as nitrogen source thereby affecting the protein content in powdered kefir starter products (Dallas *et al.* 2017). Several factors make the protein content of powdered kefir starter non-significant. The protein hydrolysis during fermentation makes free amino acids and peptides easily soluble in water to increase TDS (Sukkhown *et al.* 2018). Skimmed milk is high in protein, which can increase the TDS of the product. Furthermore, the breakdown of lactose by LAB also affects the TDS (Barus *et al.* 2019).

An increase in viable amount of a microbes (Table 3) is related to the role of skim milk components such as lactose, casein protein, and whey protein (Liu *et al.* 2018). Lactose contained in skim milk acts as a protective agent during the microencapsulation process using freeze drying. Lactose can maintain the viability of microorganisms in the sample due to low molecular weight and can penetrate the cells of microorganisms to prevent cell damage and increase cell stability during drying (Moody *et al.* 2019; Wu *et al.* 2021). Lactose can form a 'wall material' in a microencapsulation product. Increasing the coating material can form a more substantial protective wall to maintain the condition of the bacterial cells during the drying process (Sumanti *et al.* 2016). Casein protein forms an interaction between the amino and carboxyl groups to create a more stable plasma membrane structure (Teijeiro *et al.* 2018; Wu *et al.* 2021). Whey protein plays a role in film formation to protect the active ingredients in the form of microorganism during the microencapsulation process (Amila *et al.* 2016).

The freeze-drying is done at temperatures between 27–28ºC so that the microorganisms present in the kefir starter can still survive (Darvishzadeh *et al.* 2021). Addition of skim milk as a coating material can form emulsion. A good emulsion in microencapsulation process can lead to higher viability microorganism (Cao *et al.* 2019). During the drying process there is a drastic decrease in pH of the product, while skim milk plays an important role as a buffer to maintain the pH within range. A drastic decrease in pH can lead to microbial cell death (Niamah *et al.* 2018). The viable number of yeasts was lower than LAB because yeast experienced cold shock thereby reducing the metabolic activity and cell death during the freezing process at the product preparation (Niamah *et al.* 2018; Sainz *et al.* 2020). LAB were more resistant to damage because of their ability to survive in the extreme condition (Sieiro *et al.* 2016). During slow freezing process, intracellular crystals form in microorganisms, leading to cell death (Polo *et al.* 2017; Cao *et al.* 2019).

As regards microbial analysis, the total microbial count is a reflection of the number of LAB, yeast and AAB present in kefir grains as the main raw material for making samples. Kefir contain three types of dominant microorganisms consisting of 60.5% LAB, 30.6% yeast and 8.9% AAB (Sulmiyati *et al.* 2019). *Lactobacillus*, *Leuconostoc* and *Lactococcus* as dominant LAB genera in the sample utilize lactose derived from skim milk as a growth substrate. Meanwhile, the yeast does not utilize lactose as a growth substrate but utilizes glucose resulting from the breakdown of lactose by LAB (Hilmi *et al.* 2019). *Acetobacter*, as a dominant genus in AAB, utilizes the results of the breakdown carried out by LAB and yeast. *Acetobacter* uses ethanol produced by yeast and lactic acid produced by LAB as growth substrates (Moens *et al.* 2014).

Conclusion

A higher concentration of skim milk can increase the rehydration power, solubility, yield, TDS, total LAB, total yeast, total microbes, pH, reduce water content, a_w and starter rehydration time of powdered kefir. However, variations in the concentration of skim milk did not affect the protein content. The best treatment was 35% concentration skim milk.

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Author Contributions

HR, AML, ASP, RCM, and WAY planned the experiment, interpreted the results, made the write-up, statistically analyzed the data, and made illustrations.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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